PROCISE® 49X cLC

Protein Sequencing System

User's Manual

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

Contents

1 Introduction

Contents

About This Manual

This user's manual provides detailed instructions on the use and maintenance of the Procise 49X cLC Protein Sequencing System. The manual is organized into the following sections:

Section Title	Contents
Introduction	Contains important safety information, a description of user attention words, information on how to get help, and a brief system description.
System Setup	Describes how to prepare the sequencer, standards, solutions, pump and detector for a run.
Pre-Sequencing Sample Preparation Guidelines	Contains important pre-sequencing sample preparation guidelines to help ensure you obtain optimal sequencing results.
System Operation	Describes important sequencing terms and concepts, and contains instructions for loading samples, performing leak tests, and starting a run.
Troubleshooting Guide	Provides instructions for troubleshooting most of the problems you may encounter while using this system.
Optimization	Provides guidelines for optimizing sensor functions, the injector percentage and flask dry times, sequencer chemistry, chromatography, and PTH-amino acid separation.
Tests and Procedures	Includes general test and procedure information, instructions on running tests and procedures, and a description of the various tests and procedures included with this system.
Custom Functions, Cycles, Methods and Gradients	Provides instructions on creating custom functions, cycles, methods and gradient programs to improve sequencing results for your particular samples.
Maintenance	Contains recommendations and instructions for the routine maintenance and replacement of system components, repair instructions, and idle time recommendations.
User Bulletins	Serves as a place holder for user bulletins that may be issued by Applied Biosystems for this system.
Appendix A	Lists the standard functions supplied with this system.
Appendix B	Lists the standard cycles supplied with this system.
Appendix C	Lists the standard sequencing methods supplied with this system.
Appendix D	Lists the standard gradient programs supplied with this system.
Appendix E	Contains the warranty statement.
Appendix F	Contains a table of amino acid abbreviations and symbols.

Safety Issues

Two types of *user attention words* dealing with operator safety appear throughout this manual. These user attention words are:

Caution

and

WARNING

These words are used in the format shown above to alert you to procedures that must be carefully followed to prevent personal injury and damage to the instruments. Refer to page 1-7 for more information on these and other user attention words.

The Safety Summary

Before operating the Procise 49X cLC Protein Sequencing System, we strongly recommend you thoroughly read the safety summary provided for this system—Procise 49X cLC Protein Sequencing System *Safety Summary,* P/N 904201.

You received two copies of this document. One copy was included with the pre-installation manual; the other copy is included as part of this manual.

The safety summary includes:

- An explanation of the safety symbols affixed to each instrument in the system.
- General safety procedures to be followed while operating or moving the system.
- Recommendations for avoiding various potential hazards such as chemical, heat, and compressed gas hazards.
- Laboratory ventilation recommendations and guidelines.
- Computer setup and use guidelines.
- A Material Safety Data Sheet for each chemical supplied with this system.
- A waste profile describing the waste produced by this system.

Chemical Safety

WARNING	***The Procise 49X cLC Protein Sequencing System produces toxic vapors. Therefore, the sequencer must always be connected to a properly functioning ventilation system. The fume hood must be ON. Do not operate a vented instrument unless it is connected as described under "Laboratory Ventilation" in the "Procise 49X cLC Protein Sequencing System Safety Summary".***

WARNING	***The waste produced by certain chemicals used in Applied Biosystems instruments are hazardous. Handle all liquid, solid and gaseous waste products from the instruments as potentially hazardous. Read all applicable Material Safety Data Sheets and Waste Profiles. Dispose of wastes in accordance with all applicable health and safety regulations and laws. Always mix and prepare hazardous materials in a fume hood.***

A Material Safety Data Sheet (MSDS) is provided in the safety summary for each reagent supplied with this system. Each MSDS provides the following information about the reagent:

- Chemical product, trade names/synonyms
- Composition and information on ingredients
- Hazard identification
- First aid measures
- Fire fighting measures
- Accidental release measures
- Handling and storage
- Exposure controls/personal protection
- Physical and chemical properties
- Stability and reactivity
- Toxicological information
- Ecological information
- Disposal consideration
- Transport information

- Regulatory information

The waste produced by this system is a complex mixture of reagents which may have properties of greater hazard than the individual waste components by themselves. The Waste Profile provides:

- The approximate percent composition of the waste
- Physical data
- Fire and explosion data
- Health hazards
- Effects of acute exposure
- Emergency first aid
- Reactivity
- Spill and leak procedures
- Special protective equipment
- Special precautions

The section, “Laboratory Ventilation”, in the safety summary provides guidelines for connecting the vent line from the common vent manifold of the Procise 49X cLC Protein Sequencing System to an appropriately ventilated hood. Gaseous wastes produced by certain chemicals are hazardous. We strongly recommend you follow all the guidelines listed in the safety summary for this system.

User Attention Words

Four user attention words appear in the text of all Applied Biosystems user documentation. Categorically, each word implies a particular level of observation or action as follows.

Note	*This word is used to call attention to information.*
IMPORTANT	*This information is necessary for the correct operation of the instrument.*
Caution	***This word informs the user that damage to the instrument could occur if the user does not comply with this information. It also indicates a potentially hazardous situation which could result in minor or moderate injury to the user.***
WARNING	***Serious physical injury to the user or other persons could occur if these required precautions are not taken.***

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section "To Obtain Documents on Demand" following the telephone information below).

To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers	tsupport@appliedbiosystems.com
Applied Biosystems/MDS Sciex	api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

Hours for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

Product	Hours
Chemiluminescence	8:30 a.m. to 5:30 p.m. Eastern Time
Framingham support	8:00 a.m. to 6:00 p.m. Eastern Time
All Other Products	5:30 a.m. to 5:00 p.m. Pacific Time

To Contact Technical Support by Telephone or Fax

In North America

To contact Applied Biosystems Technical Support, use the telephone or fax numbers given below. (To open a service call for other support needs, or in case of an emergency, dial **1-800-831-6844** and press **1**.)

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM® 3700 DNA Analyzer	1-800-831-6844, then press 8	1-650-638-5981
DNA Synthesis	1-800-831-6844, then press 21	1-650-638-5981
Fluorescent DNA Sequencing	1-800-831-6844, then press 22	1-650-638-5981
Fluorescent Fragment Analysis (includes GeneScan® applications)	1-800-831-6844, then press 23	1-650-638-5981
Integrated Thermal Cyclers (ABI PRISM®877 and Catalyst 800 instruments)	1-800-831-6844, then press 24	1-650-638-5981
ABI PRISM® 3100 Genetic Analyzer	1-800-831-6844, then press 26	1-650-638-5981
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Peptide Synthesis (433 and 43X Systems)	1-800-831-6844, then press 31	1-650-638-5981
Protein Sequencing (Procise® Protein Sequencing Systems)	1-800-831-6844, then press 32	1-650-638-5981
PCR and Sequence Detection	1-800-762-4001, then press 1 for PCR, 2 for the 7700 or 5700, 6 for the 6700 or dial 1-800-831-6844, then press 5	1-240-453-4613
Voyager™ MALDI-TOF Biospectrometry and Mariner™ ESI-TOF Mass Spectrometry Workstations	1-800-899-5858, then press 13	1-508-383-7855
Biochromatography (BioCAD® Workstations and Poros® Perfusion Chromatography Products)	1-800-899-5858, then press 14	1-508-383-7855
Expedite™ Nucleic acid Synthesis Systems	1-800-899-5858, then press 15	1-508-383-7855
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	1-800-899-5858, then press 15	1-508-383-7855
PNA Custom and Synthesis	1-800-899-5858, then press 15	1-508-383-7855

Product or Product Area	Telephone Dial...	Fax Dial...
FMAT™ 8100 HTS System and Cytofluor® 4000 Fluorescence Plate Reader	1-800-899-5858, then press 16	1-508-383-7855
Chemiluminescence (Tropix)	1-800-542-2369 (U.S. only), or 1-781-271-0045	1-781-275-8581
Applied Biosystems/MDS Sciex	1-800-952-4716	1-650-638-6223

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Africa (English Speaking) and West Asia (Fairlands, South Africa)	27 11 478 0411	27 11 478 0349
South Africa (Johannesburg)	27 11 478 0411	27 11 478 0349
Middle Eastern Countries and North Africa (Monza, Italia)	39 (0)39 8389 481	39 (0)39 8389 493
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	61 3 9730 8600	61 3 9730 8799
China (Beijing)	86 10 64106608	86 10 64106617
Hong Kong	852 2756 6928	852 2756 6968
Korea (Seoul)	82 2 593 6470/6471	82 2 593 6472
Malaysia (Petaling Jaya)	60 3 758 8268	60 3 754 9043
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Taiwan (Taipei Hsien)	886 2 22358 2838	886 2 2358 2839
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Austria (Wien)	43 (0)1 867 35 75 0	43 (0)1 867 35 75 11
Belgium	32 (0)2 712 5555	32 (0)2 712 5516
Czech Republic and Slovakia (Praha)	420 2 61 222 164	420 2 61 222 168
Denmark (Naerum)	45 45 58 60 00	45 45 58 60 01
Finland (Espoo)	358 (0)9 251 24 250	358 (0)9 251 24 243
France (Paris)	33 (0)1 69 59 85 85	33 (0)1 69 59 85 00
Germany (Weiterstadt)	49 (0) 6150 101 0	49 (0) 6150 101 101
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Norway (Oslo)	47 23 12 06 05	47 23 12 05 75
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Portugal (Lisboa)	351 (0)22 605 33 14	351 (0)22 605 33 15

Region	Telephone Dial...	Fax Dial...
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South East Europe (Zagreb, Croatia)	385 1 34 91 927	385 1 34 91 840
Spain (Tres Cantos)	34 (0)91 806 1210	34 (0)91 806 1206
Sweden (Stockholm)	46 (0)8 619 4400	46 (0)8 619 4401
Switzerland (Rotkreuz)	41 (0)41 799 7777	41 (0)41 790 0676
The Netherlands (Nieuwerkerk a/d IJssel)	31 (0)180 331400	31 (0)180 331409
United Kingdom (Warrington, Cheshire)	44 (0)1925 825650	44 (0)1925 282502
All other countries not listed (Warrington, UK)	44 (0)1925 282481	44 (0)1925 282509
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	81 3 5566 6006	81 3 5566 6505
Latin America		
Del.A. Obregon, Mexico	305-670-4350	305-670-4349

To Reach Technical Support Through the Internet

We strongly encourage you to visit our Web site for answers to frequently asked questions and for more information about our products. You can also order technical documents or an index of available documents and have them faxed or e-mailed to you through our site. The Applied Biosystems Web site address is

http://www.appliedbiosystems.com/techsupp

To submit technical questions from North America or Europe:

Step	Action
1	Access the Applied Biosystems Technical Support Web site.
2	Under the Troubleshooting heading, click Support Request Forms, then select the relevant support region for the product area of interest.
3	Enter the requested information and your question in the displayed form, then click Ask Us RIGHT NOW (blue button with yellow text).
4	Enter the required information in the next form (if you have not already done so), then click Ask Us RIGHT NOW. You will receive an e-mail reply to your question from one of our technical experts within 24 to 48 hours.

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by phone for fax delivery	a. From the U.S. or Canada, call 1-800-487-6809, or from outside the U.S. and Canada, call 1-858-712-0317. b. Follow the voice instructions to order the documents you want. **Note** There is a limit of five documents per request.

To order documents...	Then...
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Getting Started

If you are familiar with the sequencer operation

1. Read the Procise 49X cLC Protein Sequencing System *Safety Summary*. Many of the chemicals used on this system are hazardous, and must be handled properly to avoid personal injury.
2. Thoroughly read Section 3, “Pre-Sequencing Sample Preparation Guidelines”. Proper sample preparation is critical when sequencing samples at very low picomole levels. This section provides guidelines for preparing samples on various types of sample supports to help ensure you obtain optimum sequencing results with this system.
3. Then, proceed to Section 4, “System Operation”.

If you are not familiar with the sequencer operation

1. Read the Procise 49X cLC Protein Sequencing System *Safety Summary*. Many of the chemicals used on this system are hazardous, and must be handled properly to avoid personal injury.
2. Thoroughly read Section 3, “Pre-Sequencing Sample Preparation Guidelines”. Proper sample preparation is critical when sequencing samples at very low picomole levels. This section provides guidelines for preparing samples on various types of sample supports to help ensure you obtain optimum sequencing results with this system.
3. Perform test runs of all standards provided with this system until satisfactory results are obtained. Instructions for preparing the standards and Procise 49X cLC Protein Sequencing System are provided in Section 2, “System Setup”. Instructions for loading samples and starting a sequencing run are in Section 4, “System Operation”.
4. Always perform a cartridge leak test on each cartridge you have loaded before starting a run.

System Description

The Procise 49X cLC Protein Sequencing System:

- Sequentially cleaves amino acids from the N-terminus of a protein or peptide.
- Separates and identifies the cleaved amino acids.
- Later analyzes the data.

Cleavage and separation of the amino acids occurs during what is commonly referred to as a *sequencing run,* or a *run*. The following information includes a brief description of:

- What occurs during a sequencing run.
- The main system components:
  - Procise 49X cLC Protein Sequencer (the sequencer)
  - ABI 140D Microgradient Delivery System (the pump)
  - ABI 785A UV/VIS Detector (the detector)
  - Macintosh computer

The system and the sequencer are referred to as the 49X. X represents the number of reaction cartridges on the sequencer. The sequencer will have 1 (491), 2 (492) or 4 (494) reaction cartridges.

The Sequencing Run

To execute a sequencing run, your sample is first applied to a solid support, such as a PVDF membrane or a glass-fiber disk. The sample on the support is then placed inside one of the reaction cartridges on the sequencer. During a run, Edman degradation is carried out inside the reaction cartridge. At the end of each degradation cycle, the N-terminal amino acid is cleaved as an anilinothiazolinone (ATZ) derivative.

The ATZ derivative is then transferred from the reaction cartridge to the flask on the sequencer. Inside the flask, the ATZ-amino acid is further derivatized to a more stable phenylthiohydantoin-amino acid (PTH-AA). The PTH-AAs are then transferred from the flask to the injection valve for subsequent injection, separation and quantitation on the chromatographic system.

The Procise 49X cLC Protein Sequencer

The Procise 49X cLC Protein Sequencer sequentially cleaves N-terminal amino acids from protein and peptide chains. The sequencer controls precise delivery of up to twelve different solvents and reagents. Solvents and reagents are transferred to and from the reaction cartridge, the flask, and the sample injection loop by a microprocessor-controlled, electromechanical, pressure-driven chemical delivery system.

Chromatographic Components

The chromatographic components of this system used to detect the PTH-amino acids (PTH-AAs) are:

- The *ABI 140D Microgradient Delivery System*—a dual-syringe, programmable capillary liquid chromatography system.
- The *ABI 785A UV/VIS Detector*—a low-noise, high-sensitivity, variable wavelength UV/VIS detector.
- A *reversed-phase analytical column* in a temperature-controlled heating block that separates the PTH-AA.

Because the different PTH-AAs have unique relative affinities for the column, the PTH-AAs exit the column at different times.

The Macintosh® Computer

The Macintosh computer controls and monitors the Procise 49X cLC Protein Sequencing System. The Macintosh is equipped with two types of software:

- Procise cLC control software
- ABI 610A Data Analysis software

Procise cLC Control Software

The Procise cLC control software controls and coordinates the operation of all the instruments in the system. The software also constantly monitors each sequencing run, and overall system operation.

Standard automated functions, cycles, sequencing methods and gradient programs are included in this software. Via the Macintosh user interface, you can select various combinations of cycles, methods and gradients for sequencing runs. In addition, you can create custom functions, cycles, methods and gradients. Refer to Section 4, “System Operation”, and Section 8, “Custom Functions, Cycles, Methods, and Gradients”, for more information on creating your own functions, cycles, methods and gradients.

ABI 610A Data Analysis Software

The ABI 610A Data Analysis software (610A software) collects, stores, analyzes and reports protein and peptide sequence data. The output from the 785A UV/VIS detector is collected by the Procise cLC control software. A 24-bit analog-to-digital (A/D) converter is located inside the sequencer. The A/D converter converts the analog signal to a digital signal, and transmits the digital signal to the 610A software. Refer to the 610A software user's manual for more information on this product.

2 System Setup

Contents

Guidelines for Preparing Standards and Solvents

We strongly recommend you follow the guidelines listed below when preparing all standard and other solutions for use on this system.

- Dedicate a low-traffic area in your lab for all solution preparation. This will make it easier to keep the area and your samples as clean as possible.
- Always wear non-powdered gloves.
- Thoroughly clean all work surfaces with straight methanol before preparing samples.
- Clean pipette tips and all other glass receptacles with a solution of 50% methanol in D.I. water with 0.1% TFA before use.
- Clean all forceps and other tools with straight methanol, then dry them before use.

Preparing the Sequencer for a Run

Setting Pressures and Temperatures

Guidelines for Setting Pressures

- If the sequencer loses pressure, or if the pressures and temperatures have been modified via functions such as the automatic leak test, click Default to restore the default settings.
- Pressures and temperatures for the sequencer are set and adjusted from the Pressures & Temperatures dialog box.
- Appropriate pressure values range from 0 to 5 psi, selectable in 0.1 psi increments.
- Regulator pressures can be changed during a sequencing run.

Procedure for Setting Pressures

1. Open the Pressures and Temperatures dialog box from the dialog box menu (Figure 2-1).
2. Highlight the value in the *Set* column you wish to change.
3. Enter the new value.
4. Click Execute.
5. If necessary, click Revert to restore the original setting.

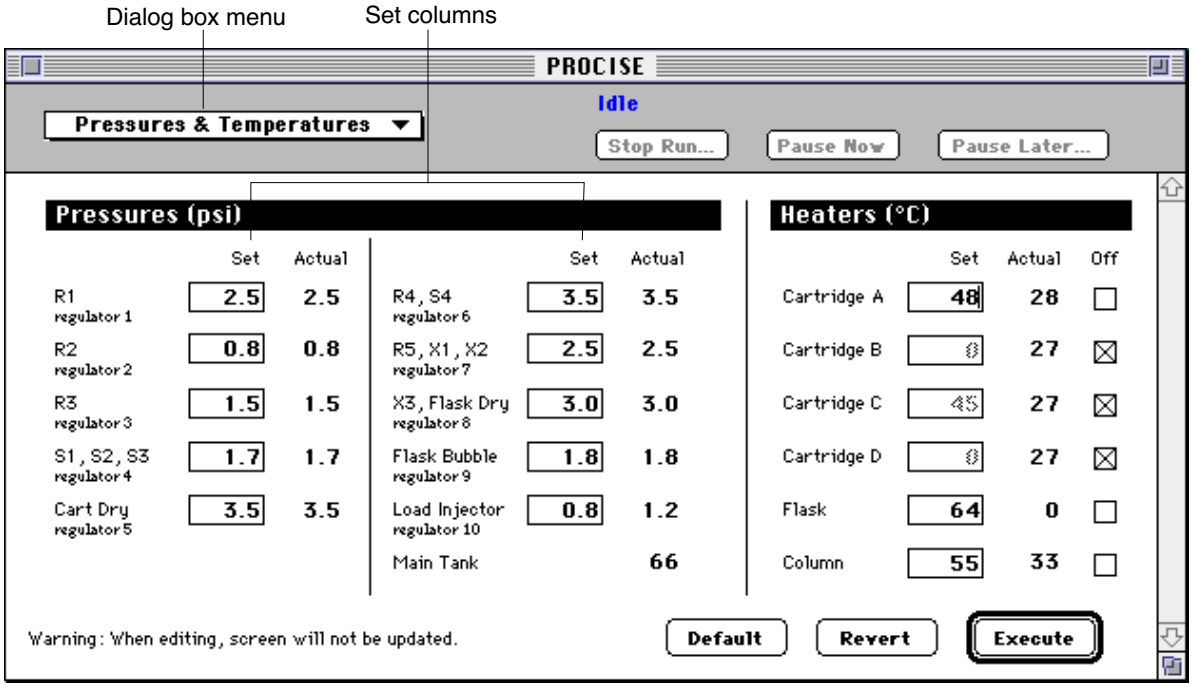

Figure 2-1. Pressures & Temperatures dialog box

Guidelines for Setting Temperatures

Appropriate temperature values are integer values ranging from:

- 30 °C to 70 °C for the column and cartridge heaters.
- Up to 78 °C for the flask heater.

Procedure for Setting Temperatures

1. Open the Pressures and Temperatures dialog box from the dialog box menu.
2. Highlight the value in the *Set* column you wish to change.
3. Enter the new value.
4. Click Execute.
5. If necessary, click Revert to restore the original setting.

Guidelines for Activating Heaters

- Heaters are turned on and off by selecting or deselecting the appropriate box in the *Off* column.
- A heater is off if the box is checked and the numerical value in the *Set* column is grayed.
- Only one cartridge heater at a time can be activated.

Procedure for Activating Heaters

1. Click the appropriate box in the *Off* column to remove the check.

Sequencer Reagent, Solvent and Standard Descriptions

All reagents and solvents supplied by Applied Biosystems are highly purified and tested to ensure optimal performance. The reagents, solvents and standards supplied for the sequencer are listed in Table 2-1. Storage conditions are also included in this table.

Table 2-1. Procise cLC reagents, solvents and standards

Bottle Position	Reagent/Solvent	Part Number	Storage Conditions
1	R5, acetonitrile with 0.001% DTT—40 mL	400315	RT[a]
2	R4A, 25% TFA in water with 0.01% dithiothreitol (DTT)—40 mL	400028	4 °C[b]
3	R3, Trifluoroacetic acid (TFA), neat—40 mL	400003	RT[a]
4	R1, 5% phenylisothiocyanate (PITC) in n-heptane—40 mL	400208	–20 °C[b]
5	R5, acetonitrile with 0.001% DTT—40 mL	400315	RT[a]
6	X1, Methanol—450 mL (Must be transferred to a 40 mL for installation on the sequencer)	400470	
7	S2B, ethyl acetate—450 mL	400854	RT[a]
8 & 10	S3, n-butyl chloride—2 bottles, 200 mL each	400008	RT[a]
9	S4C, 10% acetonitrile in water—200 mL	402051	RT[a]
11	R2B, N-methylpiperidine/water/methanol (MeOH)—40 mL	401535	4 °C[b]
12	X3, n-Heptane—200 mL	400079	
—	20 Amino Acid PTH Standard	400879	–20 °C[b]
—	Beta-lactoglobulin Sequencing Standard	400979	4 °C[b]
—	BioBrene Plus	400385	4 °C[b]

a. RT (Room temperature) = 15 to 20 °C in a dark, dry place.

b. Allow these chemicals to reach room temperature before opening. If these bottles are opened while still cold, water can condense inside. Check bottle caps for tightness after placing these bottles at either 4 °C (2 to 8 °C), or –20 °C (–15 to –20 °C).

WARNING	***CHEMICAL HAZARD. Consider each sequencer chemical potentially harmful. Completely familiarize yourself with the MSDSs provided for each hazardous chemical in the safety summary for this system. When using hazardous chemicals, wear the appropriate safety attire listed in the MSDSs. Prevent inhalation of chemicals. Do not leave chemicals uncapped. Work under a well-ventilated hood when disposing of waste chemicals. Dispose of waste in accordance with all applicable local, state and federal laws and regulations.***

Preparing the PTH-Amino Acid Standard

Note	*Use the R5 acetonitrile reagent for all PTH-amino acid standard dilutions. This reagent contains a small amount of DTT (0.001%), which increases PTH-amino acid stability.*

IMPORTANT	*Read "Guidelines for Preparing Standards and Solvents" on page 2-3 before preparing the PTH-amino acid standard.*

Procedure for Preparing Stock Solutions
(1 nmol of each component/10 mL)

1. Uncap each of the 3 vials. PTH-PE-Cys can be omitted from the standard.
2. Add 1.0 mL of R5 reagent to each vial.
3. Blanket the vials with inert gas.
4. Cap the vials and vortex thoroughly. Allow 20 min for the contents to dissolve, mixing several times during this period.
5. Store the stock solution vials at –20 °C.

Procedure for Preparing a Fresh Working Solution
(1 pmol each PTH-amino acid/mL)

1. Transfer 100 µL from each stock solution vial to a clean, dry 10 mL volumetric flask or graduated cylinder.
2. Add R5 reagent to bring the total volume to 10 mL.
3. Mix thoroughly.
4. Transfer the dilution to a clean, dry sequencer reagent bottle.
5. Store the working solution at –20 °C.

Determining the Amount and Concentration Required

Run the R5 Large Loop Cal cLC procedure:

1. Select the Bottle Change dialog box (Figure 2-3 on page 2-13) from the dialog box menu.
2. Select and run the R5 bottle change procedure. The bottle change procedure is listed on page 2-12.
3. When the procedure pauses, remove the R5 bottle, and replace it with a bottle of D.I. water.
4. Click Continue, and proceed through the end of the bottle change procedure.
5. Remove the line at port 42 in the sequencer, and replace it with a stub line.
6. Place the free end of the stub line into a tared tube of ~1 mL volume.
7. From the Test dialog box (Figure 2-2), select Flow.

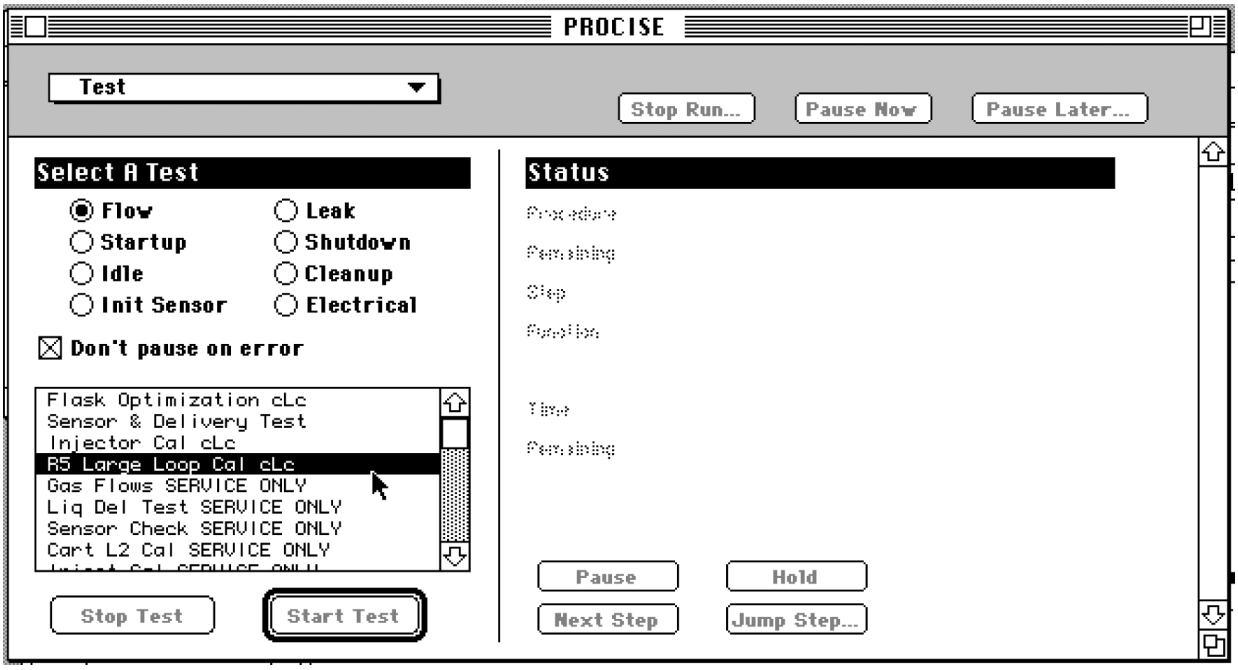

Figure 2-2. Test dialog box

8. Then select the R5 Large Loop Cal cLC procedure.
9. Click Start Test.

Calculate the amount required:

1. When the procedure is finished, weigh the tube and perform the following calculation:

   The R5 large loop volume in µL = (M_T / 5) X 1000, if M_T is in grams.

   (M_T = the mass of the tube in grams)

   Example: If M_T = 0.200 g, then (.200 / 5) X 1000 = 40 µL.

2. Remove the stub line, and reinsert the fitting into port 42.
3. Run the R5 bottle change procedure, and install the correct concentration of standard.

Determine the concentration required:

1. Choose the desired standard amount.
2. Divide the standard amount by the R5 loop volume for the concentration.

   Example

   For 1 pmol standard and a 44 µL loop:

   1000 fm / 44 µL = 22.7 fm/µL

   227 µL of working solution at 1 pmol/µL diluted to 10 mL, or 90.8 µL per 4 mL of R5 reagent

Storing the PTH-Amino Acid Standard Solutions

- Store the stock solutions at –20 °C for up to six months.
- Store the working solutions at –20 °C for up to three months.
- The standard can be used for peak identification on the system for one week.

Note	*Several of the PTH-amino acids, such as PTH-Ser, PTH-Thr, PTH-Arg, and PTH-PE-Cys, are less stable in solution than the others at room temperature. Change the standard more frequently if accurate quantitation of these residues is desired.*

Preparing the ß-lactoglobulin Standard

ß-lactoglobulin (ßLG) is used as a standard for evaluating sequencer performance. Follow the instructions listed below to prepare ßLG solutions.

IMPORTANT *Read "Guidelines for Preparing Standards and Solvents" on page 2-3 before preparing the ß-lactoglobulin standard.*

Procedure for Preparing the Dilution Solvent

1. Aliquot 40 mL of S4C (10% acetonitrile/water) into a clean 2 ounce bottle.
2. Add 40 µL of R3 (trifluoroacetic acid) to the bottle and mix well.

Procedure for Preparing a Stock Solution

1. Add 500 µL of dilution solvent to the vial of ßLG.
2. Vortex and/or sonicate the vial to dissolve the protein. This may require 20 min of intermittent mixing.

The yield is 50 pmol/µL.

Procedure for Preparing Dilutions

1. Rinse a clean Eppendorf tube 3 times with 50/50 methanol/D.I. water with 0.1% trifluoroacetic acid.
2. Dry the tube.
3. For 1 pmol ßLG/1 µL diluted solvent, add 2 µL of the stock solution, and 98 µL of dilution solvent to the clean tube.
4. Gently vortex the tube until thoroughly mixed.

Storing the ß-lactoglobulin Solutions

- Store the dilution solvent and dilutions at 4 °C or below.
- Store the stock solution at –20 °C.
- Discard the stock solution after 6 months.
- Discard any dilutions of the stock solution after one week.

Changing Bottles on the Sequencer

Overview

Use the following procedure to load fresh chemicals onto the sequencer. The sequencer automatically depressurizes and backflushes the bottles to ensure operator safety during the procedure.

Note	*Once Argon is supplied to the Procise 49X cLC Protein Sequencer, the electronic pressure system will attempt to pressurize all bottles to the settings in the Pressures & Temperatures dialog box. All bottle positions must have a bottle installed to prevent excessive Argon consumption.*

WARNING	***CHEMICAL HAZARD. Consider each sequencer chemical as potentially harmful. When using hazardous chemicals, wear appropriate safety attire as listed in the Material Safety Data Sheets located in the Procise 49X cLC Protein Sequencing System Safety Summary (P/N 904201). Prevent inhalation of chemicals. Do not leave chemicals uncapped. Work under a well-ventilated hood when disposing of waste chemicals.***

Guidelines

- The sequencer must be idle or paused before you can change a bottle.
- To pause a cycle, Select the pause function at the top of the screen. Click Pause Now or Pause Later.

Procedure

Remove the old bottle:

1. Open the Bottle Change dialog box (Figure 2-3 on page 2-13) from the dialog box menu.
2. Click the bottle to be changed in the Bottle/Chemical list.
3. Choose the appropriate bottle change procedure by opening the Bottle Change Procedure pop-up menu, and selecting the correct procedure.
4. Enter the lot number of the new bottle in the Lot Number window.
5. Click Change Bottle, and wait until you are prompted to remove the old bottle.
6. When prompted, remove the old bottle and bottle seal.

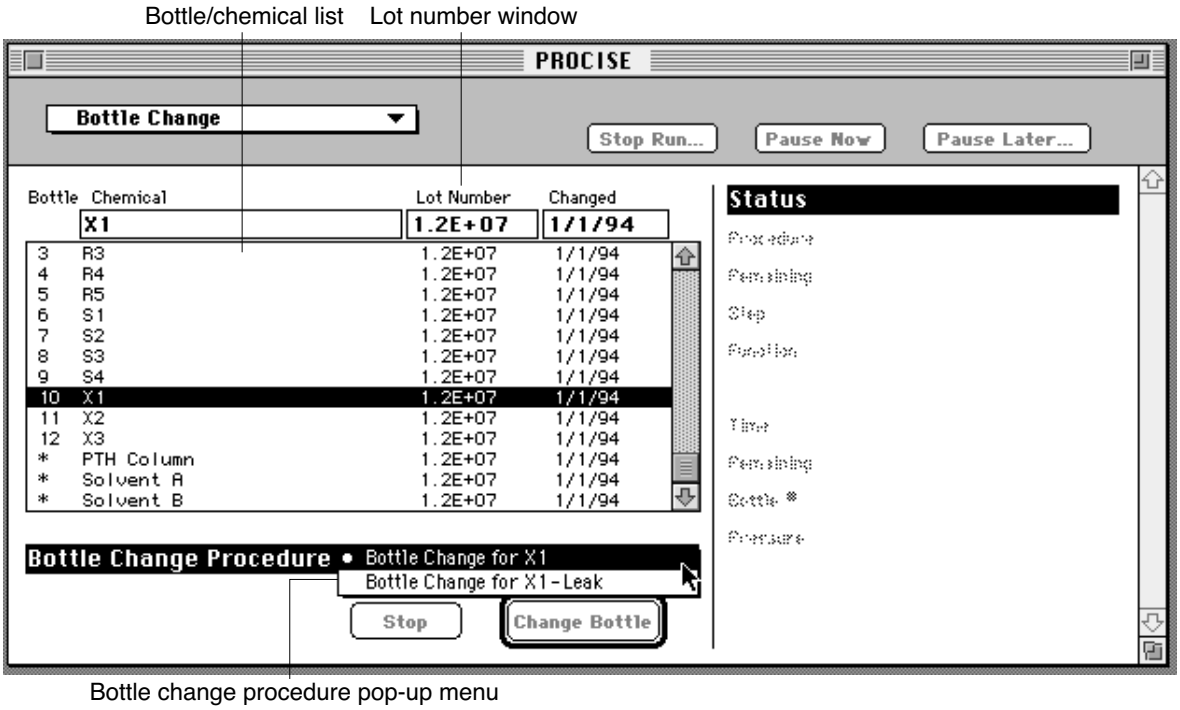

Figure 2-3. Bottle Change dialog box

Install the new bottle:

1. Place a new seal on the rim of the new bottle.
2. Screw the new bottle into the bottle cap assembly until the bottle seal contacts the top of the assembly.
3. Tighten the bottle approximately 1/4-turn more.

IMPORTANT *Do not tighten bottles until a snapping sound is produced by the bottle cap assembly. Ratcheting the bottle cap assembly will cause premature wear and may crack the bottle seal.*

4. Click Continue to execute the remaining steps in the bottle change procedure.
5. Repeat steps 3 through 4 for each additional bottle you wish to change.
6. When you are finished changing bottles, select Save from the File menu to save the new chemical data you entered. The main bottle change menu is then displayed.
7. If the run was paused, click Resume to continue the run.

Emptying the Waste Bottle

Guidelines

Empty the waste bottle when the waste level is 2 in. from the top of the bottle. Do not empty the waste bottle while a run is in progress.

WARNING	***CHEMICAL WASTE HAZARD. Waste produced by the sequencer can be hazardous and can cause injury, illness, or death. Only operate a vented instrument if it is connected in accordance with all the requirements. Handle all liquid, solid and gaseous waste as potentially hazardous. Sequencer waste must be disposed of properly and carefully in accordance with all state, local, and federal requirements. Refer to the Waste Profile in the Procise 49X cLC Protein Sequencing System safety summary to classify sequencer waste for proper disposal. When handling the waste for disposal, wear gloves and use eye protection. Avoid inhalation and skin contact.***

Procedure

To empty the waste bottle:

1. Raise the black bar above the waste bottle, so that the cap assembly disengages fully from the top of the bottle.
2. Carefully pull the bottle out, keeping the bottle level at all times. ***Immediately cover the bottle to contain the vapors.***
3. Refer to the Waste Profile in theProcise 49X cLC Protein Sequencing System Safety Summary (P/N 904201) to classify sequencer waste for proper disposal.
4. Add approximately 1 in. of water to the waste bottle.
5. Raise the black bar, re-install the waste bottle, and release the bar.
6. Inspect the top of the bottle where it seals against the o-ring on the waste manifold. The entire seal should be inside the bottle. The o-ring should be flattened against the bottle surface.

Caution	***Besides collecting waste, the waste bottle assists venting by acting as a low pressure area. Chemical deliveries flow from high pressure (reagent or solvent bottle) to low pressure (vent or waste). Therefore, for flow to occur, the waste bottle and its associated delivery and exhaust lines must be open to the vent only. If the waste bottle is not effectively vented, gas and liquid deliveries will be impeded.***

Emptying the Trap Bottle

Overview

A polypropylene bottle is mounted on the rear of the sequencer. This bottle traps condensate from the waste bottle.

Guidelines

- Empty the trap bottle when it is 40% to 50% full.
- The trap bottle can be left empty, or you can place approximately 0.5 in. of sodium or potassium hydroxide pellets in the bottom of the bottle to neutralize the waste.

WARNING	***CHEMICAL WASTE HAZARD. Waste produced by the sequencer can be hazardous, and can cause injury, illness, or death. Handle all liquid, solid and gaseous waste as potentially hazardous. Sequencer waste must be disposed of properly and carefully in accordance with all state, local, and federal requirements. Refer to the Waste Profile in the Procise 49X cLC Protein Sequencing System safety summary to classify sequencer waste for proper disposal. When handling the waste for disposal, wear gloves and use eye protection. Avoid inhalation and skin contact.***

Preparing the 140D, 785A and Column for a Run

Overview

Routine operation of the ABI 140D and 785A is controlled by the Procise cLC control software via the Macintosh. Gradient programs are downloaded from the Macintosh as part of function 227, *Prepare Pump*.

Solvent gradient programming changes the retention time of sample species automatically during the course of a single chromatographic run. Both gradient programs and changes to the composition (ionic strength) of solvent A3 are used to optimize the retention times of PTH-amino acids. One standard gradient program, *Normal 1 cLC,* is included with this system. You can use this program as a template to create custom gradient programs for special requirements.

Refer to Section 8, "Custom Functions, Cycles, Methods and Gradients" for a brief overview of solvent gradient programming, and information on creating custom gradient programs. Instructions for optimizing the PTH-amino acid separation are in Section 6, "Optimization".

The *mobile phase* for this system is a controlled combination of:

- Solvents A3 and B2
- Premix buffer concentrate

The mobile phase elutes the PTH-amino acids from the column. Table 2-2 describes a typical mobile phase for this system.

Table 2-2. Typical mobile phase composition for this system

Chemical	Quantity	Part Number
Solvent A3 (3.5% aqueous tetrahydrofuran/water)	450 mL	401887
Solvent B2 (12% isopropanol in acetonitrile)	450 mL	401886
Premix Buffer Concentrate*	5 mL	401446
Column Temperature = 55°C Column temperature may vary slightly for optimum separation.		
* The amount of Premix buffer concentrate added must be properly adjusted to achieve optimal separation of PTH-Histidine and PTH-Arginine from other PTH-amino acids. Refer to page 2-21 for more information.		

When to Change the Mobile Phase

- If your system is idle for more than one week, prepare fresh solvents, and optimize the separation before sequencing.
- If any of the following indicators of an aged mobile phase occur, replace the solvents.
  - Changes in peak shape such as broadening or tailing.
  - Increased baseline noise, or an unusual baseline rise.
  - Decreased peak resolution which cannot be corrected by minor adjustments in mobile phase composition.
  - Precipitate is present in the mobile phase.

Procedure for Preparing Solvents A3 and B2

IMPORTANT *Read "Guidelines for Preparing Standards and Solvents" on page 2-3 before preparing the solvents.*

To prepare solvent A3:

1. Add 11.25 mL of Premix buffer concentrate to the bottle of solvent A3.
2. Invert the bottle several times to mix the contents.
3. Optional step—adding acetone:

   Adding acetone to solvent A3 increases the UV absorbancy of the solvent. This, in turn, reduces the baseline rise observed with increasing concentrations of solvent B2 during gradient elution.

   Incrementally add small amounts of HPLC-grade 1% acetone in H_2O (up to 1000 μL) to solvent A3. Check your baseline after each addition.
4. Enter the date and lot number of the new solvent in the Bottle Change dialog box (Figure 2-3 on page 2-13), and in sequencer logbook.

To prepare solvent B2:

- Use solvent B2 as supplied by Applied Biosystems. No additives are required.
- Enter the date and lot number of the new solvent in the Bottle Change dialog box (Figure 2-3 on page 2-13), and in sequencer logbook.

Changing Solvents A3 and B2

Overview

Changing solvents involves:

- Purging the 140D
- Changing the solvent bottles
- Purging the 140D again
- Equilibrating the column

Purging the 140D rapidly expels solvents and trapped gases from the pump's syringes. The 140D is equipped with an automatic purge valve to divert the flow of solvent to waste. Everytime a solvent is changed, equilibrate the column with the new solvent(s) until the baseline is stable before sequencing or evaluating a separation. Refer to the *ABI 140D Microgradient Delivery System User's Manual* for additional information on changing solvents and purging the pump.

WARNING ***The Waste Profile in the Safety Summary describes safe handling and percent composition of waste. Always dispose of all chemicals according to all local, federal and state requirements.***

Procedure for Changing Solvents A3 and B2

The following procedure for changing solvents A3 and B2 is performed via the 140D control panel. The keys F1, F2, F3, and F4 are referred to as *soft keys*, and are followed by the > symbol (PURGE> for example). The prompts for which you must enter values are shown in all capitals (for example NUMBER OF PURGES). For more information on this procedure and the 140D control panel, refer to the *ABI 140D Microgradient Delivery System User's Manual*.

Before starting this procedure, prepare fresh solvents. Instructions for preparing the solvents are on page 2-17.

Purge the old solvent from the 140D:

1. Remove the old solvent bottle(s).
2. Check the solvent lines for obstructions or salt deposits. If the lines are not clear, clean or replace them.
3. Check all fittings for salt deposits or indications of leakage. Clean or replace as necessary.
4. From the Ready Screen (Figure 2-4; also referred to as the main menu) on the 140D control panel, press the PURGE> soft key to display the Purge Screen (Figure 2-5).

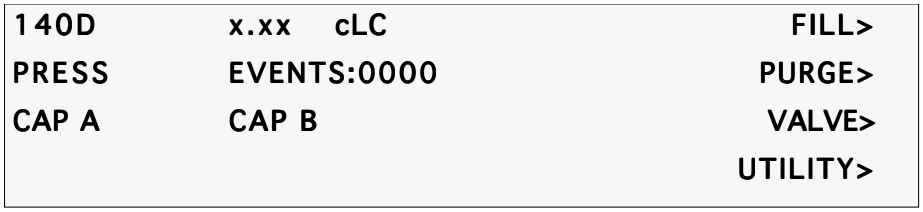

Figure 2-4. Ready Screen

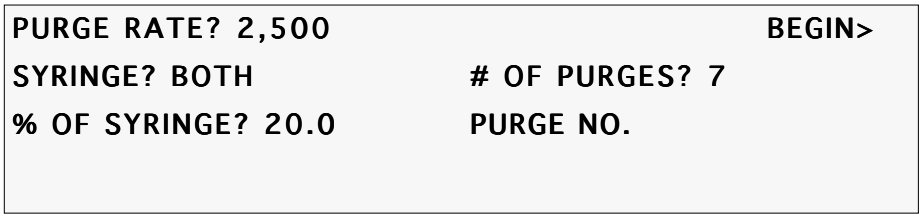

Figure 2-5. Purge Screen

5. Use the arrow keys and numeric keypad to enter 2500 for the PURGE RATE. This is the rate in µL/min at which the cylinders empty. The maximum value is 2500. The smaller the value entered, the longer the purge takes.
6. Use the arrow keys to move the cursor to the SYRINGE prompt. Then use the Prev./Next keys to select BOTH.
7. Move the cursor to NUMBER OF PURGES, and use the numeric keypad to enter 7.
8. Move the cursor to PERCENT OF SYRINGE, and enter 20 or more. This is the percent of the syringe to empty, refill and empty again.

Purge the 140D with fresh solvent:

1. Place the solvent inlet line into the new bottle, attach the cap, and place the bottle in the bottle holder. Repeat for each new bottle.
2. Press the BEGIN> soft key to start the purge procedure. The 140D and lines are rinsed with fresh solvent. Any air bubbles in the system are removed as well.

   The status of the procedure is displayed along the bottom of the screen on the 140D. To stop the purge procedure, press the Stop key.
3. Press the Manual key to enter the manual mode of operation and display the Manual Status screen. The syringes will fill with new solvent.
4. Press the FLOW> soft key. Type 40 to change the flow rate to 40 μL/min. Then press the Enter key.
5. Press the %B> soft key, and type 50 to change the composition to 50 %B. Then press the Enter key.
6. Press the PRESS> soft key, and type 3500 to change the maximum operating pressure to 3500 psi. Then press the Enter key.
7. Allow the 140D to flow at this rate and composition for 10 min to equilibrate the column.
8. Run at least 4 Flask Standard cLC cycles to check PTH-AA separation efficiency and reproducibility before sequencing an unknown sample. If the separation is essentially the same as with the old buffers, begin sequencing.

   If the separation changes significantly with the new buffers, you may need to optimize the separation. Compare and evaluate the results of the last two cycles to determine if optimization is required. If so, follow the guidelines listed under “Optimizing the PTH-Amino Acid Separation” in Section 6, “Optimization”.

Effect of Premix Buffer Concentrate

Premix Buffer Concentrate employs an ion-pairing additive to improve both peak shape and retention time reproducibility for the PTH-derivatives of histidine, arginine and the pyridylethyl derivative of cysteine.

PTH-derivatives with positively-charged side-chain groups interact with underivatized silanol groups on the silica particles in a column. This causes peak broadening and retention time shifting. By adding an ion-pairing modifier to the mobile phase, the interaction of the basic derivatives with free silanol is significantly reduced through preferential interaction with a strongly acidic ion-pairing additive.

Guidelines for Using Premix Buffer Concentrate

- For a separation in which
  - PTH-His elutes before PTH-Ala,
  - PTH-Arg elutes before PTH-Tyr, and
  - PTH-PE-Cys elutes before PTH-Pro.

  Add approximately 5 mL of Premix buffer concentrate to 200 mL of solvent A3. Cap and mix well.
- If PE-Cys is not a derivative of interest, you can position His *after* Ala, and Arg *after* Tyr by using less Premix buffer—approximately 3 mL (see Figure 2-6).

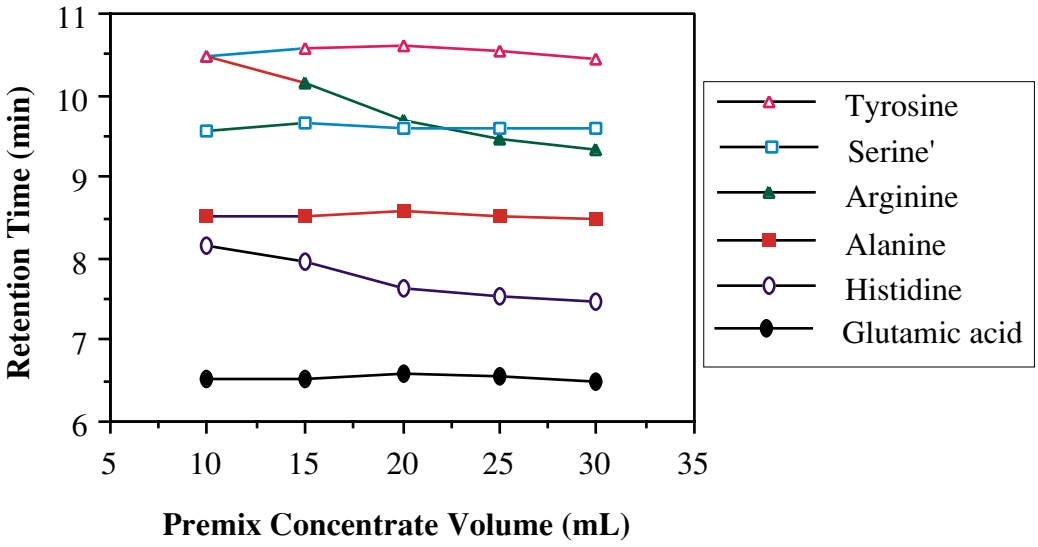

Figure 2-6. Effect of Premix Buffer Concentrate on retention times

Basic System Connections

During installation, all the physical connections between the instruments in this system are made by your Applied Biosystems Service Representative. If the system is moved, or is shut down for an extended period of time, review this section to ensure that all connections are properly made before restarting the system.

Electrical Connections

Four power connections are required for the Procise 49X cLC Protein Sequencing System. Additional connections may be needed for additional modules, such as a chart recorder. We recommend a dedicated electrical line with a circuit breaker for this system. The outlet must be located within 2.5 m (8 ft) of the system. For additional details, refer to the Procise 49X cLC Protein Sequencing System *Pre-installation Manual* (P/N 904203).

The system has an automatic line-switching power supply that will accept an AC voltage between 90 and 264 VAC at a frequency of 50 or 60 Hz. The Macintosh computer is equipped with an automatic switching power supply, and will operate between 90 and 264 VAC at a frequency of 50 or 60 Hz.

The 140D and 785A are shipped from Applied Biosystems with the voltage set for 120 VAC. However, the system is shipped with a universal voltage kit which contains the fuses and power cords necessary to reconfigure these instruments for most other voltage requirements (100, 120, 220, or 240 VAC).

Communication Connections

- Connect the sequencer to the modem port on the Macintosh.
- Connect the printer to the printer port on the Macintosh.
- If a chart recorder with an external paper feed control is being used, connect the respective pins to the two Event 1 terminals on the rear connection strip of the 140D.
- Set the chart recorder to auto-paper feed with a chart speed of 5 mm/min.
- An illustration of the connections between the instruments in this system is on page 2-23, Figure 2-7.

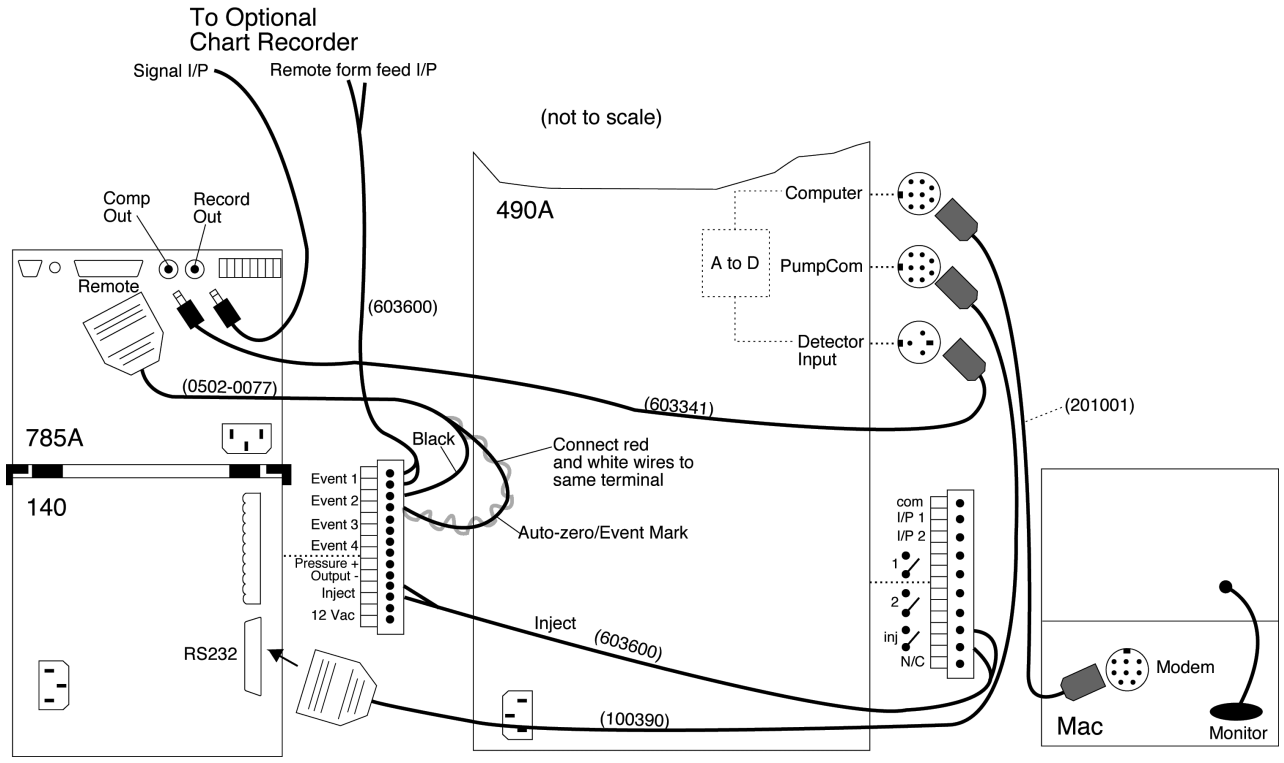

Figure 2-7. Instrument interconnections

Argon Supply Connections

Requirements	Specifications
Minimum of 1 Argon cylinder	• Size 1A • Pre-purified; 99.998% purity or greater
Regulator	• One for each cylinder • Swagelok-type end fittings on exit side for connection to 1/4-in. (6.355 mm) o.d. tubing. • Set between 65 and 75 psi (448 and 517 kPa)
CGA 580 cylinder-adaptor	• One for each cylinder

If the input pressure drops below 60 psi during sequencing, the system will pause.

WARNING ***EXPLOSION HAZARD. Ensure that the pressurized gas cylinder is safely attached to the wall or cylinder truck by means of approved brackets or clamps. Failure to do so could cause the cylinder to fall over and explode, which could cause physical hazard. Always turn off, cap, and secure any cylinder that is not in use. Keep cylinders away from electrical circuits and excessive heat.***

System Plumbing Diagram

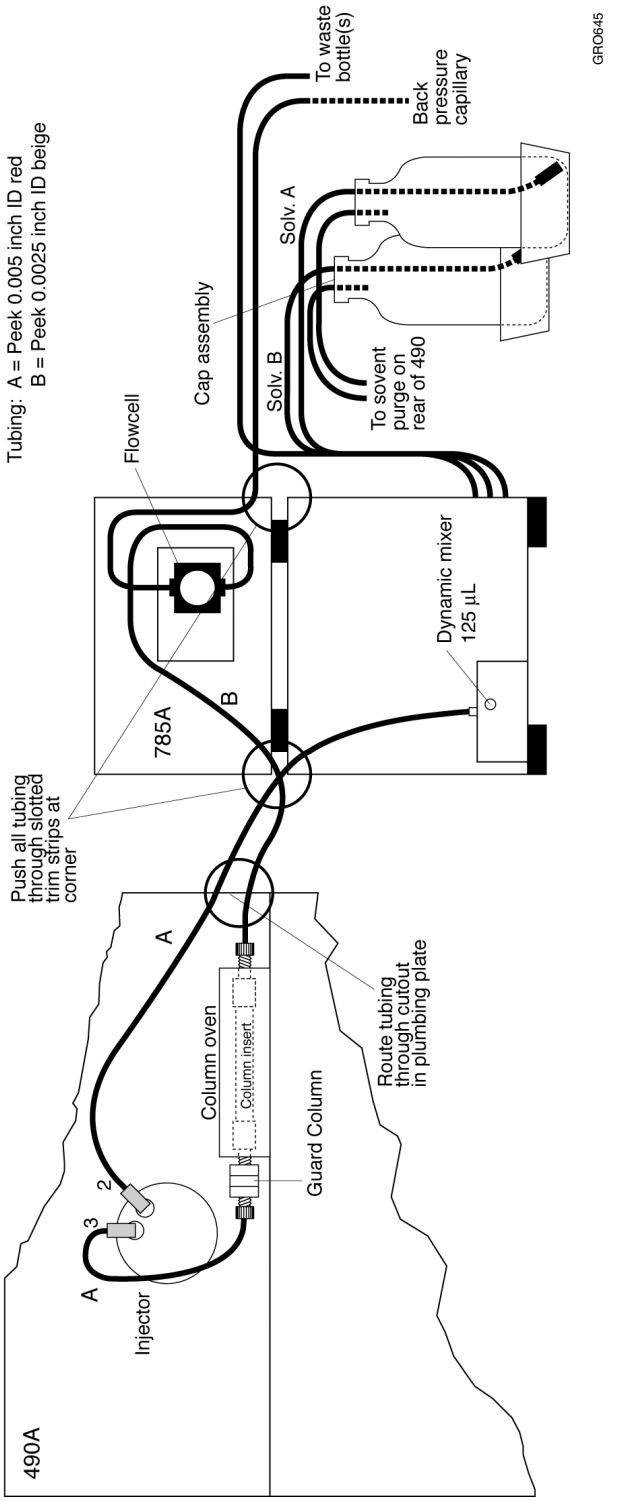

Figure 2-8. System plumbing diagram

3 Pre-sequencing Sample Preparation Guidelines

Contents

Introduction

This section contains instructions and recommendations for preparing protein and peptide samples for N-terminal sequencing on the Procise 49X cLC Protein Sequencing System.

We recommend you follow these guidelines to ensure optimum system performance and sequencing results.

Guidelines for All Sample Preparation Techniques

Sample purity is critical when sequencing samples at very low picomole levels. Therefore, we strongly recommend you adhere to the following guidelines when preparing your samples for sequencing on the Procise 49X cLC Protein Sequencing System.

- Dedicate a low-traffic area in your lab for sample handling and preparation. This will make it easier to keep the area and your samples as clean as possible.
- Always wear non-powdered gloves.
- Thoroughly clean all work surfaces with straight methanol before preparing samples.
- Clean pipette tips and all other sample receptacles with a solution of 50% methanol in D.I. water with 0.1% trifluoroacetic acid before use.
- Clean all forceps and other sample handling devices with straight methanol, then dry them before use.
- Do **not** wipe implements or pipette tips to dry them. Dry them with clean, dry compressed gas, or allow them to air dry.

Samples Prepared with ProSorb Cartridges

IMPORTANT *Read "Guidelines for All Sample Preparation Techniques" on page 3-4 before preparing your samples.*

Materials Required

- ProSorb holders (P/N 401950)
- ProSorb filters (P/N 402050)
- ProSorb sample reservoir inserts (P/N 402052)
- Membrane removal punch (P/N 401397)
- Forceps (P/N 402011)
- Methanol (HPLC grade or better)
- 0.1% trifluoroacetic acid

The holders, filters, inserts, punch tool and forceps are all contained in the ProSorb Starter Kit (P/N 402139).

Procedure for Preparing Samples in ProSorb Cartridges

Prepare the ProSorb cartridge:

1. Rinse all work surfaces and tools with methanol, and dry.
2. Rinse pipette tips with a solution of 50% methanol and 0.1% trifluoroacetic acid. Dry with clean, dry compressed gas, or allow them to air dry.
3. Slide the filter into the holder.
4. Pipette 10 µL of 0.1% trifluoroacetic acid onto the filter (Figure 3-1 on page 3-6).
5. Apply 10 µL of methanol to the underside of the PVDF membrane in the sample reservoir insert (Figure 3-2).
6. Place the insert partially into the holder, leaving a small space between the PVDF membrane and the filter.

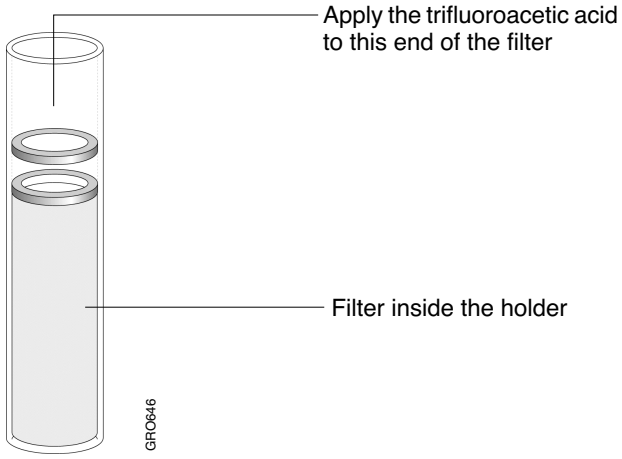

Figure 3-1. Applying 0.1% trifluoroacetic acid to the filter

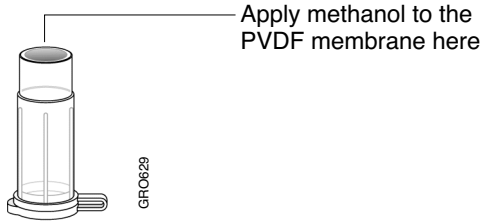

Figure 3-2. Sample reservoir insert

Load your sample into the insert:

1. If your sample volume is greater than 100 µL, pipette your sample into the insert. Remember that the total volume of the sample reservoir is 400 µL.

IMPORTANT Pipette at least 100 µL of sample into the insert at a time.

2. If your sample volume is less than 100 µL, subtract the sample volume from 100. The remainder is the amount of 0.1% trifluoroacetic acid you must add to the insert before loading your sample.

   Pipette the appropriate amount of 0.1% trifluoroacetic acid into the insert. Then pipette your sample into the insert. Your total sample volume should now be 100 µL.

   Example: If your total sample volume is 85 µL, first pipette 15 µL 0.1% trifluoroacetic acid into the insert. Then, pipette your sample into the insert.

3. Close the cap on the insert, and push the insert into the holder as far as it will go.
4. Allow all the fluid to pass through the membrane into the filter.
5. Remove the insert from the holder, and allow the PVDF membrane to dry completely.

IMPORTANT	*Keep the insert as free of contaminants as possible while the PVDF membrane dries. We suggest placing a beaker or similar clean container over the insert while it dries.*

6. Discard the filter, and set aside the holder for cleaning and reuse.

IMPORTANT	*To help prevent sample contamination, leave the PVDF membrane inside the insert until you are ready to load it for sequencing.*

The PVDF membrane can be left inside the sample reservoir insert until you are ready to load it onto the sequencer. This will help keep your sample free of contaminants.

When you are ready to sequence the sample, you will:

- Punch the PVDF membrane into the insert
- Remove the membrane from the insert
- Apply a BioBrene solution to the membrane
- Load the membrane into a reaction cartridge

Instructions for these procedures are in Section 4, "Loading Samples Prepared in ProSorb Cartridges".

Effect of BioBrene on Peptide Sequencing

Experiments also showed that adding BioBrene to the PVDF membrane after sample application can greatly improve the sequencing performance of peptides. A series of peptides (5 to 20 pmol) were prepared in ProSorb cartridges, and subjected to sequencing in the absence and presence of 100 µg BioBrene. Based on the results listed in Table 3-1, the addition of BioBrene dramatically improved the sequencing of peptides.

Table 3-1. Peptide sequencing results with and without the addition of BioBrene

Peptide	**Without BioBrene** residues/residues	**With BioBrene** residues/residues
DRVYHIPF	4/8	8/8
KRQHPGKR	7/8	8/8
VHLTPVEK	8/8 (repetitive yield = 67%)	8/8 (repetitive yield = 84%)
LEHFRKGIQVNY	10/12	12/12
Insulin A chain	16/21	21/21
Atrial Natriuretic Factor	13/29	29/29
Insulin B chain	18/30	28/30

General Guidelines for Using ProSorb Cartridges

Overview

IMPORTANT *Read "Guidelines for All Sample Preparation Techniques" on page 3-4 before preparing your samples.*

The ProSorb sample preparation cartridge rapidly concentrates and desalts dilute protein and peptide samples onto a matrix suitable for sequencing. The cartridge (Figure 3-3) consists of three parts:

- A sample reservoir insert with PVDF membrane (400 µL volume)
- A disposable, absorbent filter (750 µL capacity)
- A holder into which the sample reservoir insert and filter are inserted

Unlike the centrifugal field used in devices such as ProSpin™, the absorbent filter draws sample solutions through the PVDF membrane by capillary action. The membrane binds proteins and peptides, but allows buffer components that could potentially interfere with sequencing to pass through.

Once the protein or peptide has been immobilized, wash solutions can be introduced into the sample reservoir insert to further remove sample components that can interfere with sequence analysis. Samples or washes larger than 400 µL are accommodated by loading multiple aliquots into the insert.

IMPORTANT *Do **not** allow the PVDF membrane to dry between aliquots.*

Small Sample Volumes

Small amounts of protein or peptide can irreversibly bind to glass and/or plastic tubes. To minimize this loss with small volume samples, add at least 100 µL of diluent to the pre-wetted membrane before adding the sample. Then add your sample directly to this solution.

Effects of Detergents

Many protein samples submitted for sequence analysis contain detergents that were used in their preparation. These detergents can dramatically effect the binding of proteins to PVDF membrane. Table 3-2 summarizes the results of experiments which examined the effect of common detergents on the binding of bovine serum albumin (BSA) to the PVDF membrane in ProSorb cartridges. The detergent concentrations listed in Table 3-2 are the maximum concentrations that did not inhibit the binding of BSA to the membrane.

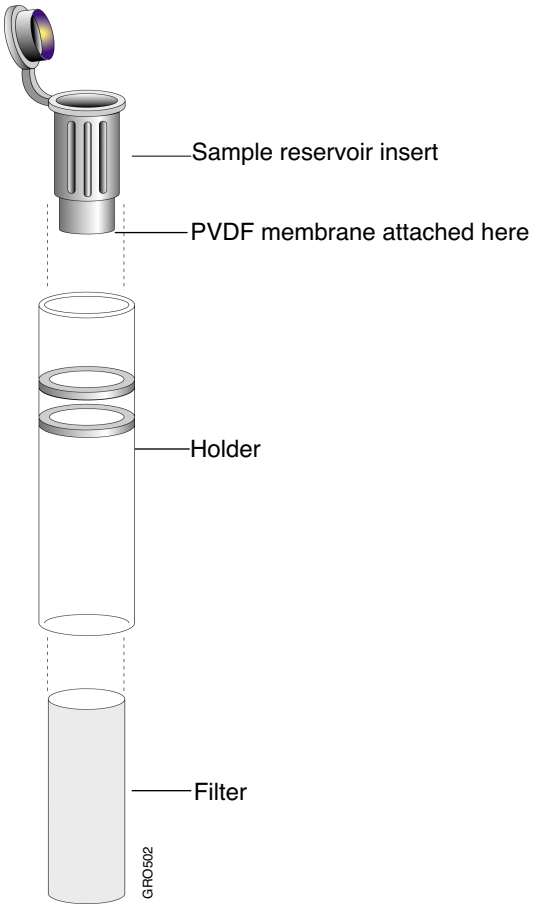

Figure 3-3. ProSorb Sample Preparation Cartridge

Many samples contain significantly more detergent than the amounts listed in Table 3-2. One way to deal with higher amounts of detergent is to add a small amount of methanol (20% to 30% by volume) to the sample before loading it into the ProSorb cartridge. This will weaken sample interaction with the detergent.

Table 3-2. Maximum detergent concentrations

Detergent	Maximum Concentration (%) without interference to binding on ProSorb PVDF membranes (v/v or w/v)
Triton X-100 (reduced)	0.01
Tween 20	0.01
SDS	0.02
Octyl Glucoside	0.25
Brij 35	0.02

For example, we diluted BSA into two solutions: Tris-glycine buffer containing 0.2% SDS, and arginine-phosphate buffer containing 0.05% Triton X-100 (reduced). When these solutions were directly loaded into the ProSorb cartridge, no detectable protein was observed during sequence analysis. When methanol was added to similar solutions prior to loading into the cartridge, 35% of the protein in the SDS buffer was bound, and 55% of the protein in the Triton X-100 buffer was bound. Experiments with other proteins in these buffer systems resulted in relative sequencing yields both greater and lesser than those observed for BSA, indicating that results are dependent upon the particular protein being analyzed.

Sample Matrix Comparison

Another example of how loading conditions can affect protein binding to PVDF membranes is displayed in Table 3-3. In these experiments, large amounts of BSA (approximately 750 pmol) were loaded into ProSorb cartridges using the sample solutions (400 µL total volume) listed in the table.

Table 3-3. Various loading conditions for large sample amounts of BSA

Sample Matrix	Initial Yield (pmol)
0.1% trifluoroacetic acid	140
Deionized water	130
0.25 M NaCl	350
0.2 M Ammonium Bicarbonate	330
7 M Guanidine HCl	290

The amount of BSA that bound to the membrane was clearly dependent upon the sample solution. Twice as much protein bound to the membrane in the presence of higher ionic strength solutions. This effect was observed only with large sample loads of 750 pmol. When smaller amounts of BSA were loaded (approximately 15 pmol), the dependence on sample matrix was not observed.

Effect of Acetonitrile on Peptide Binding

An application for ProSorb cartridges is the preparation of peptides purified by reversed-phase HPLC. The effect of acetonitrile on peptide binding to the PVDF membrane in ProSorb cartridges was investigated using angiotensin II (8 residues) as a model system. Approximately 10 pmol of the peptide was mixed with the appropriate solution, and passed through the cartridge. The yield of the third amino acid, valine, was used to quantitate the amount of peptide bound to the membrane. The yield of valine that resulted from 10 pmol of peptide spotted onto a pre-cycled glass fiber filter was used as the zero volume control.

The results of experiments (Figure 3-4) indicate that high concentrations of acetonitrile(>10%) inhibited the binding of angiotensin II to PVDF membranes. Therefore, we recommend that samples prepared by reversed-phase HPLC be diluted with deionized water or 0.1% trifluoroacetic acid until the acetonitrile concentration is less than 10% before introduction into the ProSorb cartridge.

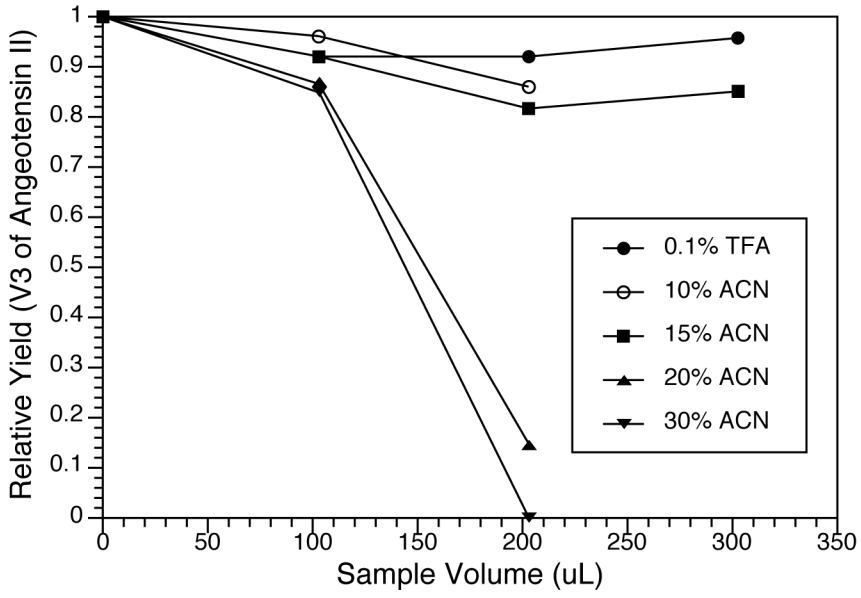

Figure 3-4. Effect of acetonitrile on peptide binding

The PVDF membrane can be left inside the sample reservoir insert until you are ready to load it onto the sequencer. This will help keep your sample free of contaminants.

Troubleshooting Guide for ProSorb Cartridges

Table 3-4 contains information provided to help you determine the cause of low initial yields when sequencing samples prepared in ProSorb cartridges.

Table 3-4. ProSorb Sample Preparation Troubleshooting Guide

Problem	Considerations	Recommendation
Low initial yields.	Did you use Beta-lactoglobulin?	• If not, we recommend you perform a run with Beta-lactoglobulin. This standard can help diagnose potential instrument problems, and may help separate instrument from sample issues.
	What buffer was used?	• This can effect protein binding. Refer to page 3-9 for more information.
	Did you lose proteins due to absorption to tubes?	• Proteins can stick to snap-cap tubes (supplier dependent). Dilute protein solutions are unstable; do not allow them to sit around. We recommend performing dilutions in the ProSorb sample reservoir insert after wetting it with methanol.
	Did you follow all of the steps in the protocol?	• Prewet membrane with methanol. • Minimum volume of 100 µL sample required. • Did the sample dry out, thereby requiring the reapplication of methanol.

Samples Prepared on the 173A MicroBlotter System

IMPORTANT *Read "Guidelines for All Sample Preparation Techniques" on page 3-4 before preparing your samples.*

Sample Preparation Guidelines

When preparing your samples, we strongly recommend you:

- Never inject samples that contain visible particulates. Particulates can block the column and damage it beyond repair.
- Use only the highest quality reagents for digestions.
- Avoid or minimize the use of reagents that may interact with solvents, or react to temperature changes and precipitate inside the system. Precipitation occurring after sample injection can block the capillary tubing or column.
- Centrifuge every sample for at least 5 min before injection.
- Use a clean, sharp razor blade to excise the area of PVDF membrane with the peak to be sequenced.
- Limit the size of the membrane to be sequenced to less than 3 x 3 mm. If the piece is too big, other peptides might be present on the membrane.
- Load approximately 50 µg BioBrene onto each piece of PVDF membrane.

Recommended Protein Digestion Protocols

To minimize the occurrence of blockages, we recommend you use one of the following protein digestion protocols to prepare your samples. The use of volatile digestion buffers minimizes the possible occurrence of blockage due to salt elimination.

Protocol For Large Proteins with Multiple Disulfide Linkages
Example: Bovine Serum Albumin

The following procedure is based on preparing a 500 ng to 100 µg solution.

Initial sample preparation:

1. In an Eppendorf tube, dissolve the protein to be analyzed in a 100 to 200 µL solution of 250 mM Tris/HCl (pH 8.0) containing 2 M Guanidine HCl.
2. Add 10 µL of 10% b-mercaptoethanol or DTT.
3. Flush the tube with argon for 1 min.

Incubation periods:

1. Incubate the solution for 2 h at room temperature in the dark.
2. Add 2 µL of 4-vinylpyridine.
3. Incubate the solution for an additional 2 h at room temperature in the dark.
4. Dialyze against DI water using a microdialysis technique for 4 h to remove excess reagents and salts.
5. Add 1 M NH_4HCO_3 to bring the final concentration to 200 mM NH_4HCO_3 (pH 8.0).
6. Add trypsin or Lys-C in an enzyme/substrate ratio of 1:30 (w/w).
7. Incubate the solution at 37 ˚C for 20 h in the dark.
8. Centrifuge the sample for at least 5 min immediately prior to injection onto the 173A system.

Protocol For Small Proteins with Few or no Disulfide Bridges
Example: Apomyoglobin

1. In an Eppendorf tube, prepare a 10 μg solution of protein in approximately 50 μL of a 200 mM solution of NH_4HCO_3 containing:
   - 10% acetonitrile
   - 1% hydrogenated Triton X-100 (pH 8.0)
2. Add trypsin or Lys-C in a 1:20 w/w ratio of enzyme/substrate.
3. Incubate the solution at 37 ˚C for 20 h in the dark.
4. Centrifuge the sample for at least 5 min immediately prior to injection onto the 173A system.

Samples Prepared for Loading onto Glass Fiber Filters

IMPORTANT *Read "Guidelines for All Sample Preparation Techniques" on page 3-4 before preparing your samples.*

Follow the guidelines for all sample preparation techniques listed on page 3-4.

Samples Prepared by Electroblotting

IMPORTANT *Read "Guidelines for All Sample Preparation Techniques" on page 3-4 before preparing your samples.*

Optimal Sample Amount

- 0.5 µg of protein is more than sufficient for sequencing on the Procise 49X cLC Protein Sequencing System.

General Recommendations

- Better sequencing results are usually obtained from samples that are concentrated on a small surface area of the PVDF membrane. The tighter the band on the membrane, the better the sequencing results.
- For samples that are overloaded (1 µg blotted sample, for example), cut the PVDF membrane and sequence part of the sample only.
- Avoid loading oversized pieces of PVDF membrane into the reaction cartridge. Remember, the chamber i.d. is only 6 mm.

Using BioBrene

BioBrene is not required for the routine analysis of blotted samples.

However, applying a small amount of BioBrene (approximately 5 µL) onto a blotted PVDF membrane may improve the sequencing results for some samples.

Procedure for Preparing Samples by Electroblotting

1. Perform SDS-polyacrylamide gel electrophoresis in a tris-glycine or tris-tricine buffer system[1,2].
2. Electroblot protein samples from the gel to a PVDF membrane in CAPS or tris-glycine buffer systems[2].
3. Stain the blotted PVDF membrane with conventional staining techniques, such as Coomassie Brilliant Blue, Ponseau S, or Amino Black.
4. Destain the PVDF membrane with a 50% methanol destaining solution.
5. Rinse the membrane thoroughly with D.I. water.
6. Excise the bands of interest with a clean razor blade.
7. Apply the appropriate amount of BioBrene onto the membrane (discussed in Section 4, "System Operation").
8. Sequence the sample on the Procise 49X cLC Protein Sequencing System.

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1. Laemmli, U. K. (1970) *Nature* 227, p. 680-686.

2. Applied Biosystems User Bulletin #42 (1991).

Samples Prepared by Reverse-Phase HPLC

IMPORTANT	*Read "Guidelines for All Sample Preparation Techniques" on page 3-4 before preparing your samples.*

Note	*For low or sub-picomole sample amounts, we strongly recommend using the ABI 173A MicroBlotter system for sample preparation.*

Reverse-phase HPLC is mainly used for separating peptide samples obtained from solution or the in-situ (in-gel or on-membrane) digestion of proteins.

General Recommendations[1]

- Always use the highest grade and purity of water, solvent and reagents.
- Use a microbore column that is 1 mm or smaller, if available.
- The preferred peak fraction volume is < 50 µL. The amino acid background peaks of the first cycle of sequencings (gly/ser/ala) are directly correlated to the sample volume applied.
- Prewash the polypropylene or polyethylene tubes with D.I. water.
- Avoid a complete dry down of the membrane.
- Be aware of sample loss upon storage at low pmol levels, even at –70 ˚C.
- Add neat trifluoroacetic acid in a 1:4 ratio (trifluoroacetic acid/sample; vol/vol) just prior to loading samples onto glass fiber disks.
- Rinse pipette tips prior to sample transfer.

1. Paul Tempst et.al., *Methods: A Companion to Methods in Enzymology* 6, p. 248-261 (1994).

Suggested Sample Preparation Protocol

1. Run SDS-PAGE for protein separation and purification.
2. Electroblot the separated proteins from the gel to a PVDF membrane.
3. Perform an in-situ digestion as described under "In-Situ Digestion Protocol", on page 3-21.
4. Run reverse-phase HPLC for peptide mapping, and for collecting peptides separated for direct sequencing.

In-Situ Digestion Protocol

1. Stain the gel with Coomassie Brilliant Blue G-250 solution.
2. Using a clean razor blade, excise the bands of interest.
3. Place each band in a separate Eppendorf tube.
4. Dry the bands down completely in a Savant Speed Vacuum.
5. Rehydrate the gel bands with 50 to 100 µL of digestion buffer containing 10% acetonitrile, 0.05% of reduced Triton X-100, and an appropriate enzyme (enzyme/substrate ratio of 1:2 to 1:10 is recommended).
6. Digestion[1] at 38 ˚C for 6 to 20 h.
7. Extract the digested gel with extraction solvent (2 x 100 µL of 50% acetonitrile and 5% trifluoroacetic acid) using a sonicate (20 min each extraction).
8. Pool the digestion buffer with extracts, and dry down to an appropriate volume.
9. Inject the sample onto the column.

On-Membrane Digestion Protocol

1. Stain the blotted membrane with Coomassie Brilliant Blue G-250.
2. Rinse the membrane thoroughly with D.I. water.
3. Destain the membrane with destaining buffer (50% methanol).
4. Excise the stained bands of interest.
5. Digestion[2,3] of the sample bands with an appropriate enzyme in digestion buffer at 38 ˚C for 10 to 20 h. Use the same digestion buffer and S/E ratio as for in-gel digestion.
6. Extract the digested gel with extraction solvent (2 x 100 µL of 50% acetonitrile and 5% trifluoroacetic acid) using a sonicate (20 min each extraction).
7. Pool the digestion buffer with extracts and dry down to an appropriate volume.
8. Inject the sample onto the column.

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1. Jeno, Paul et. al. (1995) *Anal Biochem* 224, p. 75-82.
2. Fernandez, J. (1992) *Anal Biochem* 201, p. 255-264.
3. Fernancez, J. (1995) *Techniques in Protein Chemistry* VI, p. 135-142.

4 System Operation

Contents

Important Sequencing Terms for Users

A clear understanding of the following terms is important for the proper use of this system.

Term	Description
Valve	• A mechanical device which opens and closes to provide a flow path for the transfer of gas, solvent, or reagent. • Three types of valves: – Delivery valves for liquid, vapor or gas delivery. – Three-way valves for gas delivery only. – Bottle vent valves for venting chemical bottles.
Function	• Activates a valve or set of valves to deliver a chemical. • Activates or deactivates a relay. • Defines or increments a setpoint. • Directs the 140D to start and stop. • Can have a fixed or global time associated with it. • Can be customized. Refer to Section 8, "Custom Functions, Cycles, Methods and Gradients", for information on creating functions.
Step	• A function that has been incorporated into a cycle. • The building blocks of cycles.
Cycle	• A series of steps that accomplishes a specific chemical process in the reaction cartridge or flask. • Can be customized. Refer to Section 8, "Custom Functions, Cycles, Methods and Gradients", for information on creating functions.
Method	• A grouping of cycles designed to sequence a peptide or protein. • Typically begins with one cycle, followed by several repetitions of another cycle. • Includes starting temperatures for the cartridge, flask and column. • Includes the gradient program to be run by the 140D. • Can be customized. Refer toSection 8, "Custom Functions, Cycles, Methods and Gradients", for information on creating functions.
Gradient	• A programmed run for the HPLC components of the system that defines flow rate and solvent composition changes over a specified period of time. • Downloaded to the 140D via the Macintosh. • Can be customized. Refer to Section 8, "Custom Functions, Cycles, Methods and Gradients", for information on creating functions.

Purpose and Types of Valves Inside the Sequencer

Overview

Gas and chemical deliveries inside the sequencer are controlled by three types of valves:

- Three-way valves
- Bottle vent valves
- Delivery valves

Valves are opened (activated) and closed (deactivated) electronically to create pathways to a particular destination, such as a reaction cartridge. Each valve is assigned a number. The valve diagram on page 4-6 illustrates the position of each valve.

Three-way Valves

Three-way valves are used exclusively for argon delivery. They control argon input to valve positions 15, 24, and 44, and provide two different argon pressures (high and low) from the same manifold inlet line. Standard pressures for these valves are listed in Table 4-1.

Table 4-1. Standard pressures for three-way valves

Valve Status	Function(s)	Pressure
Valve 46 off	Cart dry	3.5 psi
Valve 46 on	All cart block flushing	Internal manual regulator pressure
Valve 47 off	Flask dry; all flask flushing	3.0 psi
Valve 47 on	Load injector	0.8 psi
Valve 48 off	Flask bubble; low pressure sample loop flushing	1.8 psi
Valve 48 on	High pressure sample loop flushing	Internal manual regulator pressure

Bottle Vent Valves

Bottle vent valves control the flow of argon, which is required for bottle pressurization and flushing. The Procise 49X cLC Protein Sequencer has twelve vent valves, one for each chemical bottle. The valves are activated by the pressure control system to maintain proper bottle pressurization.

During chemical delivery, the bottle vent valves remain closed. During venting or flushing the valves are opened. This allows the argon in the bottle headspace to flow to waste.

Delivery Valves

Delivery valves are grouped into valve blocks. Seven valve blocks, interconnected with Teflon tubing, comprise the chemical delivery system.

Delivery Valve Block	Description
Cartridge Reagent Block	• Controls delivery of the reagents R1, R2, X1(liquid and gas), and X3 to the Cartridge Input Block and to waste.
Cartridge Solvent Block	• Controls delivery of: – one reagent—R3 (liquid and gas) – solvents S2B, S3, and S1, and – argon to the Cartridge Reagent Block, Cartridge Input Block, Cartridge Output Block, and to waste.
Cartridge Input Block	• Controls the transfer and metering of reagents, solvents, and argon from the Cartridge Reagent Block and Cartridge Solvent Block into or out of the active cartridge, and to waste.
Cartridge Output Block	• Controls the transfer of reagents, solvents and argon from the Cartridge Reagent Block and Cartridge Solvent Block into or out of the active cartridge, and to waste.
Flask Reagent Block	• Controls the delivery and metering (small loop) of: – reagents R4, R5, X2 (liquid), X3 – solvent S4C, and – argon to the Flask Input Block.
Flask Input Block	• Controls the delivery of X2 (gas). • Controls the transfer and metering (large loop) of reagents, solvents, and argon from the Flask Reagent Block to the conversion flask, and to waste.
Flask Output Block	• Controls the delivery of argon to the conversion flask for bubbling, and for flushing the sample loop. • Controls the transfer of the conversion flask contents to the sample loop, and to waste.

The design of the valve blocks minimizes any holdup volume following chemical delivery. Delivery lines feed into each valve block, and connect to the common pathway (manifold) inside the block through a manifold inlet line and a solenoid-controlled valve. Delivery from the inlet line into the manifold occurs only when the appropriate valve is activated. The manifold zig-zags through the valve block to bypass closed valves. The direction of the flow is determined by the pressures on both sides of the pathway.

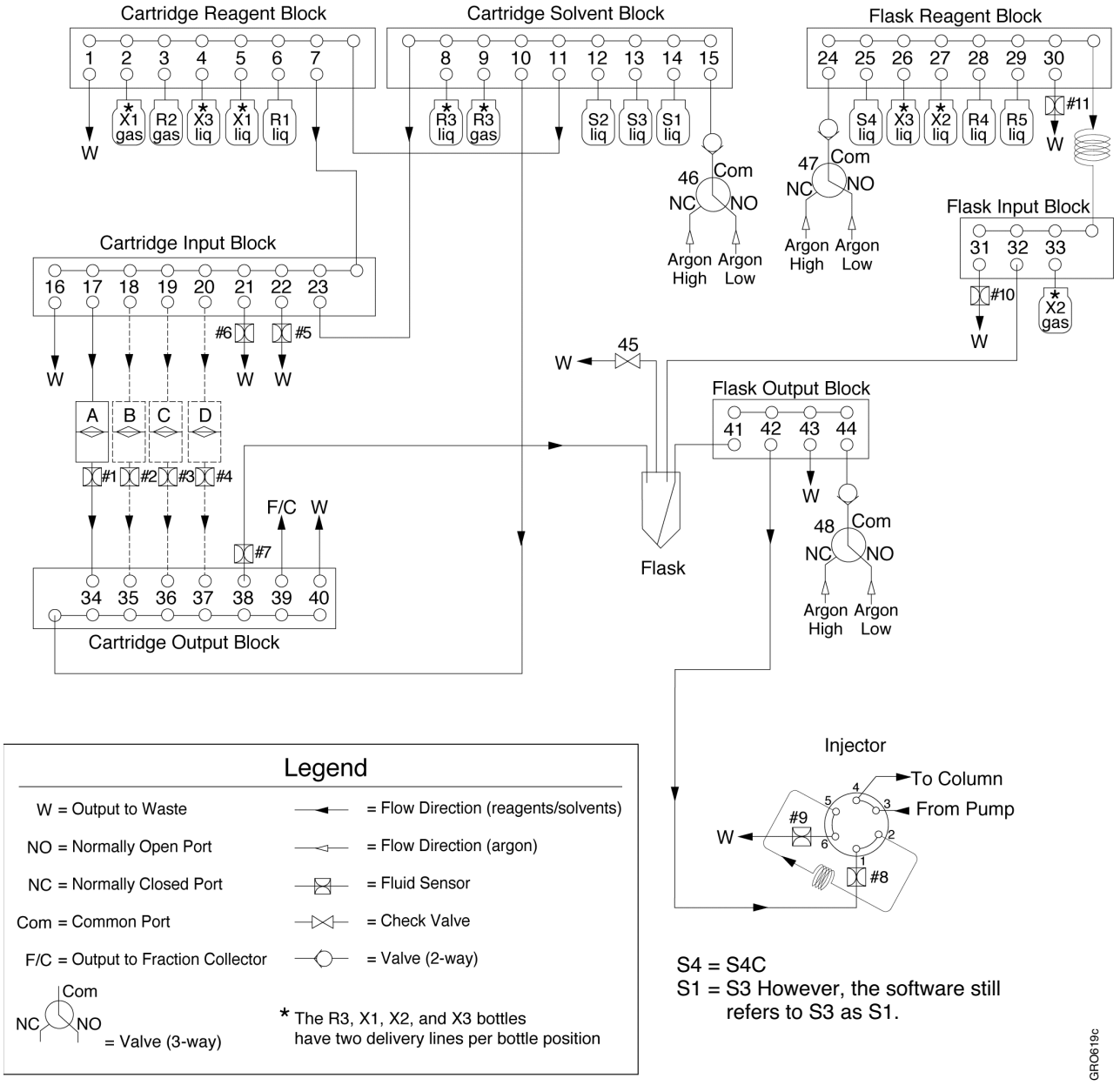

Figure 4-1. Procise 49X cLC Protein Sequencer valve diagram

Purpose and Types of Functions

Functions are the building blocks of cycles. Each step in a cycle is a function. In general, functions are used to:

- Activate and deactivate the valves inside the sequencer.
- Activate the sensors that control valve operation.
- Signal the start and end of a cycle.
- Facilitate the transfer of sample from the reaction cartridge to the flask, and from the flask to the column.
- Set the cartridge, flask and column temperatures.
- Download and start the gradient program run by the 140D.

A function grouping and numbering scheme (Table 4-2 on page 4-8) simplifies the programming and operation of the Procise 49X cLC Protein Sequencing System. Each function is assigned:

- A name that describes its purpose.
- A number based on the function grouping and numbering scheme.

A list of the standard functions supplied with this system is located in Appendix A. You can also open the Functions dialog box from the dialog box pop-up menu on the Macintosh to view the available functions.

The information on pages 4-9 through 4-11 describes each of the following function types:

- Valve control functions
- Sensor functions
- Cycle-synchronizing functions
- Required cartridge and flask functions
- User-defined functions

Table 4-2. Function grouping and numbering format

Standard functions are numbered 1 to 400. Numbers 401 to 450 are reserved for user-defined functions.	
1-150	Cartridge functions
151-250	Flask and HPLC functions
251-259	Cartridge and flask transfer functions
260-360	Cartridge and flask test and procedure functions
361-400	Cartridge and flask reserved
401-450	Reserved for user-defined functions
Ten standard cartridge functions are available for each reagent or solvent. A two digit numbering scheme is used for these functions. The first digit indicates the reagent/solvent being used; the second digit refers to the action that occurs. For example, function 41: the 4 indicates S1 is used; the 1 indicates an N1 action. Therefore, function 41 = Deliver S1, Cartridge (top).	
01-10 = R1 functions	N1 = Deliver <. . .>, Cart (top)
11-20 = R2 functions	N2 = Deliver <. . .>, Cart (bottom)
21-30 = R3 functions	N3 = Deliver <. . .>, Cart (sensor)
31-40 = R3 (gas) functions	N4 = Deliver <. . .>, Waste
41-50 = S1 functions	N5 = Deliver <. . .>, Cart (sm loop)
51-60 = S2 functions	N6 = Deliver <. . .>, Cart (lg loop)
61-70 = S3 functions	N7 = Vent <. . .>
71-80 = X1 functions	N8 = Flush <. . .>
81-90 = X1 (gas) functions	N9 = Backflush <. . .>
91-100 = X3 functions	NO = Reserved [except Fxn 30, Transfer R3, Cart (gas)]
Ten standard flask functions are available for each reagent or solvent bottle. A three digit numbering scheme is used for these functions. The first two digits indicate the reagent/solvent used; the third digit indicates the action that occurs. For example, function 163. The 16 indicates R5 is used. The 3 indicates an NN3 action. Therefore, function 163 = Load R5, Flask (large loop).	
151-160 = R4 functions	NN1 = Deliver <. . .>, Flask
161-170 = R5 functions	NN2 = Load <. . .>, Flask (sm loop)
171-180 = S4 functions	NN3 = Load <. . .>, Flask (lg loop)
181-190 = X2 functions	NN4 = Vent <. . .>
191-200 = X2 (gas) functions	NN5 = Flush <. . .>
201-210 = X3 functions	NN6 = Backflush <. . .>
	NN7 = Deliver <. . .>, Waste
	NN8, 9 and 0 are Reserved

Valve Control Functions

Valve control functions activate (open) and deactivate (close) a valve or set of valves simultaneously to deliver a chemical or gas. These functions are also referred to as *time-dependent* functions because the valves are opened for a fixed period of time, then closed at the end of the step. The time is specified as a parameter in a cycle, or by the operator via manual control mode.

To trace the flowpath created by a valve-controlling function, refer to the valve diagram (Figure 4-1 on page 4-6), or the operator assistance card inside of the front cover of the sequencer.

Sensor Functions

Sensor functions control the activation and deactivation of fluid sensors, which in turn control the activation and deactivation of certain valves inside the sequencer. When a sensor function begins, the sensor *looks* for fluid. When fluid is detected, the reagent or solvent delivery valve is turned off, or the injector is triggered to switch positions. The remaining time allotted for the function continues to count down to zero, then the next step begins.

The duration of a sensor function must be long enough for fluid to reach the sensor. If fluid does not reach the sensor by the end of the step, an error message is sent to the Event Log, and sequencer operation is paused.

Cycle-Synchronizing Functions

- Cycle-synchronizing functions are used to synchronize sample delivery from the cartridge to the flask during sequencing.
- Every cycle must have a *Begin* and *End* step (functions 258 and 259 respectively).
- Function 257, *Wait*, is used to pause a cycle for a specific period of time. When a cycle advances to a Wait step, it pauses while a timer counts up (increments) the specified amount of time before proceeding to the next step.
- Sample transfer from the cartridges to the flask is accomplished by two steps in the cartridge cycle, and one step in the flask cycle.

Table 4-3. Cycle-synchronizing functions

Cycle Type	Function Name	Function Number	Description
Both	Begin	258	• Must be the first step of all cycles, tests, and procedures.
Both	End	259	• Must be the last step of all cycles, tests, and procedures.
Both	Wait	257	• Keeps the cycle time running for a particular step in a cycle while all the valves are closed.
Flask	Ready to Receive*	228	• Indicates the flask is ready to accept sample from the cartridge. • The flask waits at this step until the transfer is complete.
Cartridge	Ready Transfer to Flask*	127	• Indicates the start of sample transfer from the cartridge to the flask.
	Transfer Complete	128	• Indicates the sample transfer is complete.
* Synchronization is set up such that the *Ready to Receive* step in the flask cycle occurs 5 sec before the *Ready Transfer to Flask* step in the cartridge cycle.			

Required Cartridge and Flask Functions

The functions listed in Tables 4-4 and 4-5 do not control valves, but are required for typical sequencer cycles.

Table 4-4. Required cartridge functions

Function Name	Number	Description
Set Cart Temperature	142	• Used to adjust the cartridge temperature at a fixed time during a cycle. • Acceptable temperature range is ambient to 70 °C.

Table 4-5. Required flask functions

Function Name	Number	Description
Load Position	226	• Switches the sample loop out of the HPLC flow path. • During a flask cycle, this function must precede the Load Injector step for the sample loop to be flushed, and for the sample in the flask to be transferred into the sample loop.
Set Flask Temp	230	• Used to adjust the flask temperature at a fixed time during a cycle. • Acceptable temperature range is ambient to 78 °C.
Prepare Pump	227	• Downloads a gradient program from the Procise cLC control software to the 140D. • After the download is complete (30 to 60 sec), the 140D will start, pressurize, and run at the initial gradient conditions.
Stop Pump	231	• Stops all 140D activity.
Start Gradient	232	• Used to start the gradient program in cases where no sample is injected.
Inject Position	223	• Switches the sample loop into the HPLC flow path. • Not necessary when using the Sample Loop Load sensor, which automatically activates the Rheodyne valve when fluid is detected.
Set Column Temp	229	• Used to adjust the column temperature at a fixed time during the flask cycle. • Acceptable temperature range is ambient to 70 °C.

User-defined Functions

You can create your own functions for specialized needs or applications. Fifty function numbers—401 to 450—are reserved for user-defined functions. Refer to Section 8, "Custom Functions, Cycles, Methods and Gradients", for information on creating your own functions.

Purpose and Types of Cycles

Cycles are groups of functions designed to control the chemical processes that must occur in the cartridges and the flask to sequence a protein or peptide. Once incorporated into a cycle, each function becomes a *step* in that cycle. Steps are activated for a specific period of time during the cycle.

Chemical processes that occur in cartridge blocks are referred to as *cartridge cycles*. Processes that occur in the flask are referred to as *flask cycles*. The cycles supplied byApplied Biosystems with the Procise 49X cLC Protein Sequencing System are referred to as *standard cycles*. Standard cycles cannot be deleted or modified, but can be used as templates for creating new cycles. Refer to Section 8, "Custom Functions, Cycles, Methods and Gradients", for information on creating cycles.

Standard Cartridge Cycles

The standard cartridge cycles provided with this system are described in Table 4-6. Appendix B contains a complete list of the steps in each standard cartridge cycle.

Table 4-6. Standard cartridge cycles

Cycle Name	Description
Cart Precycle cLC	• Prepares a polybrene-treated glass fiber filter for sequencing by running abbreviated coupling and repetitive cleavage reactions.
Cart Begin cLC	• Prepares a sample for pulsed-liquid sequencing by delivering an aliquot of liquid TFA to denature the sample, followed by coupling with PITC.
Cart Begin Gas-phase cLC	• Prepares a sample for sequencing by delivering TFA vapor to denature the sample, followed by coupling with PITC.
Cart-PL 6mmGFF cLC	• An Edman chemistry cycle for sequencing samples on polybrene-treated glass fiber filters. • Delivers an aliquot of liquid TFA for cleavage. • Two ATZ extractions: one with butyl chloride; one with ethyl acetate.
Cart Gas-phase cLC	• An Edman chemistry cycle for sequencing samples on polybrene-coated glass fiber filters. • Delivers TFA vapor for cleavage. • Two ATZ extractions: one with butyl chloride; one with ethyl acetate.
Cart-PL Prosorb cLC	• Edman chemistry cycle for sequencing samples on PVDF membranes. • Delivers an aliquot of liquid TFA for cleavage.

Standard Flask Cycles

The standard flask cycles provided with this system are listed and described in Table 4-7. Appendix B contains a complete list of the steps in each standard flask cycle.

Table 4-7. Standard flask cycles

Cycle Name	Description
Flask Blank cLC	• Performs conversion chemistry and reconstitution in the absence of a sample or standard. • Starts the HPLC components of the system. • Transfers flask contents to the sample loop for analysis.
Flask Standard cLC	• Performs conversion chemistry and reconstitution with the PTH-amino acid standard mixture in the flask. • Starts the HPLC components of the system. • Transfers the flask contents to the sample loop for analysis.
Flask Normal cLC	• Converts the ATZ-amino acid to a PTH-amino acid. • Starts the HPLC components of the system. • Transfers flask contents to the sample loop for analysis.
Prepare Pump cLC	• Downloads the gradient program to the 140D. • Prepares the flask blank.
Run Gradient cLC	• Used to troubleshoot chromatography problems. • Downloads the gradient program to the 140D. • Isolates the HPLC components of the system. • Equilibrates the column. • Runs the gradient. • No injection occurs.

Purpose and Types of Sequencing Methods

What is a Sequencing Method?

A method consists of a variable number of cartridge cycles, flask cycles and gradient programs grouped in a specific order to sequence a protein or peptide. Methods also include a starting temperature for the cartridge, flask and column.

Overview of Standard Sequencing Methods

The sequencing methods provided by Applied Biosystems with the Procise 49X cLC Protein Sequencing System are referred to as *standard methods*. Standard methods cannot be deleted or modified, but can be used as templates for creating new methods. Refer to Section 8, "Custom Functions, Cycles, Methods and Gradients" for information on creating methods.

The standard sequencing methods provided with this system are listed in Table 4-8 on page 4-15. A typical method begins with the cycles necessary to prepare a sample for sequencing, followed by a sequencing cycle. At the same time, a gradient program is downloaded to and run on the ABI 140D.

As shown in Table 4-8, each standard sequencing method begins with the same 3 cartridge and flask cycles (except cartridge cycle 3 in the Gas-phase cLC method). Cycles 1, 2 and 3 are followed by repetitions of the sequencing cycle, which is referred to as the *Default* cycle. Each repetition of the Default cycle yields one residue. The number of repetitions is specified by the user. Refer to the procedure and example on page 4-16 for more information on the determining the number of repetitions (cycles) required for your sequencing runs.

At the same time, the function *Prepare Pump cLC:*

- Downloads the gradient program, *Normal 1 cLC,* to the 140D.
- Instructs the 140D to prepare for a run.

The gradient program is started during cycle 2, and continues running through the end of the sequencing run.

Table 4-8. Standard Procise cLC sequencing methods

Method	Cycle #	Cartridge Cycle	Flask Cycle	Gradient Program
Pulsed-liquid cLC Cartridge Temp: 45 Flask Temp: 64 Column Temp: 55	1	None	Prepare Pump cLC	Prepare Pump cLC
	2	None	Flask Blank cLC	Normal 1 cLC
	3	Cart Begin cLC	Flask Standard cLC	Normal 1 cLC
	Default	Cart-PL 6mmGFF cLC	Flask Normal cLC	Normal 1 cLC
Gas-phase cLC Cartridge Temp: 48 Flask Temp: 64 Column Temp: 55	1	None	Prepare Pump cLC	Prepare Pump cLC
	2	None	Flask Blank cLC	Normal 1 cLC
	3	Cart Begin Gas-phase cLC	Flask Standard cLC	Normal 1 cLC
	Default	Cart Gas-phase cLC	Flask Normal cLC	Normal 1 cLC
IMPORTANT: The Gas-phase cLC method works best for sequencing samples on PVDF membranes. This method may require optimization to sequence samples on glass fiber filters treated with BioBrene. Refer to page 4-19 for more information.				
Pulsed-liquid Prosorb cLC Cartridge Temp: 48 Flask Temp: 64 Column Temp: 55	1	None	Prepare Pump cLC	Prepare Pump cLC
	2	None	Flask Blank cLC	Normal 1 cLC
	3	Cart Begin cLC	Flask Standard cLC	Normal 1 cLC
	Default	Cart-PL Prosorb cLC	Flask Normal cLC	Normal 1 cLC

Table 4-9. Other standard Procise cLC methods

Method	Cycle #	Cartridge Cycle	Flask Cycle	Gradient Program
Filter Precycle cLC Cartridge Temp: 45 Flask Temp: 64 Column Temp: 55	1	None	Prepare Pump cLC	Prepare Pump cLC
	2	Cart Precycle cLC	Flask Blank cLC	Normal 1 cLC
	3	Cart Precycle cLC	Flask Standard cLC	Normal 1 cLC
	Default	Cart-PL 6mmGFF	Flask Normal cLC	Normal 1 cLC
PTH-Standards cLC Cartridge Temp: 45 Flask Temp: 64 Column Temp: 55	1	None	Prepare Pump cLC	Prepare Pump cLC
	Default	None	Flask Standard cLC	Normal 1 cLC
Run Gradient cLC	Default	None	Run Gradient cLC	Normal 1 cLC

Determining the Number of Cycles Required

Procedure

1. Determine the number of residues you would like. Each repetition of the sequencing cycle (Default) yields one residue.
2. Add the number of residues to the number of preparation cycles in the method you are using. The standard sequencing methods (Table 4-8 on page 4-15) each have 3 preparation cycles—cycles 1, 2 and 3.

Example

You would like a sequencing yield of 7 residues from Cartridge A only, using the Pulsed-Liquid cLC sequencing method. To accomplish this, configure Cartridge A in the Start Run dialog box (Figure 4-2) as follows:

1. Select 1st for the Run Order.
2. Enter a unique file name in the File Name box.
3. Specify 10 in the Cycles box.
4. Select Pulsed-Liquid cLC for the sequencing method from the Method pop-up menu.
5. Enter the amount of sample and standard in pmol in the appropriate boxes.

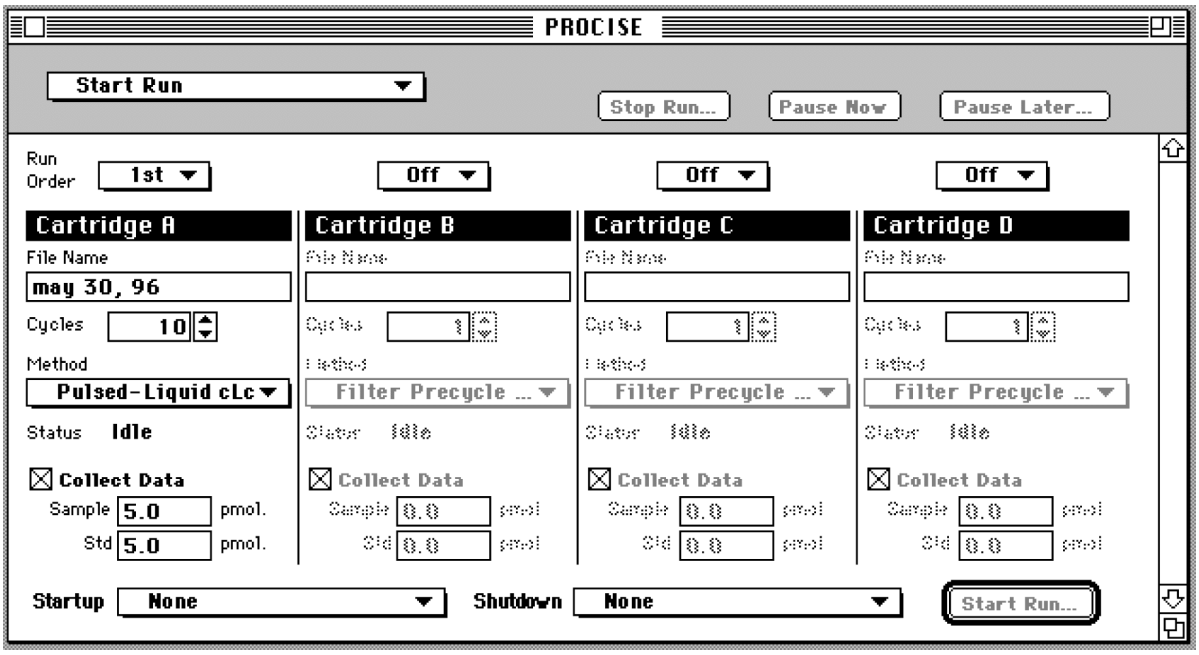

Figure 4-2. Configuring the Start Run dialog box

Sequencing Liquid Samples

Two methods are available for sequencing liquid samples:

Method Name	Description
Pulsed-liquid cLC	• Delivers a small aliquot of liquid TFA to the cartridge for cleavage after coupling. • Has a 45 min cycle time. • Offers slightly higher repetitive yields than the Gas-phase cLC method.
Gas-phase cLC	• Delivers TFA vapor for the cleavage. • Has a 45 min cycle time. • Offers lower background that the Pulsed-liquid cLC method. • May require optimization to sequence samples on glass fiber filters. Refer to page 4-19 for more information.

Precycling Glass-Fiber Filters

Before loading your sample onto a glass-fiber filter, you must:

1. Apply BioBrene to the filter.
2. Precycle the filter using the *Filter Precycle cLC* method listed below in Table 4-10.

Precycling is necessary because the BioBrene solution may contain small amounts of compounds that could interfere with sequencing. The method, Filter Precycle cLC, washes and conditions the BioBrene-coated filter by running several short cycles of Edman chemistry.

Table 4-10. Filter Precycle cLC method

Cycle #	Cartridge Cycle	Flask Cycle	Gradient
Default	Cart-PL 6mmGFF cLC	Flask Normal cLC	Normal 1 cLC
1	None	Prepare Pump cLC	Prepare Pump cLC
2	Cart Precycle cLC	Flask Blank cLC	Normal 1 cLC
3	Cart Precycle cLC	Flask Standard cLC	Normal 1 cLC
Cartridge Temp: 48 Flask Temp: 64 Column Temp: 55			

Sequencing Blotted/Membrane-bound Samples

Two methods are available for sequencing blotted samples:

Method Name	Description
Pulsed-liquid Prosorb cLC	• Delivers a large aliquot of liquid TFA to the cartridge for cleavage after coupling. • Has a 45 min cycle time. • Offers slightly higher repetitive yields than the Gas-phase cLC method.
Gas-phase cLC	• Delivers TFA vapor for the cleavage. • Has a 45 min cycle time. • Offers lower background than the Pulsed-liquid Prosorb cLC method.

Optimizing the Gas-Phase cLC Sequencing Method

As is, the standard Gas-Phase cLC sequencing method works best when sequencing samples on PVDF membranes. This method may require optimization if used to sequence samples on glass fiber filters.

If optimization is required, the following two parameters in the Gas-phase cLC sequencing method must be changed:

- R3 bottle pressure
- R3 delivery time

The procedure for optimizing this method is located in Section 6, "Optimization", on page 6-18.

BioBrene Plus Storage, Preparation, and Use

BioBrene Plus is a dehydrated compound which must be reconstituted with distilled water. Adding BioBrene to your sample is optional; however, we strongly recommend its use. Refer to "The Effect of BioBrene on Peptide Sequencing" on page 4-21 for information on the benefits of using BioBrene.

Guidelines for Using BioBrene Plus

- We strongly recommend storing the reconstituted BioBrene in small volumes of 20 µL each in Eppendorf tubes.
- Always use fresh BioBrene when preparing a methanolic dilution; otherwise, sequencing yields may be reduced due to solution degradation.
- Use methanolic dilutions within 48 hours after preparation or thawing for best results.

Reconstituting and Storing BioBrene Plus

1. Follow the instructions enclosed with the BioBrene Plus (Applied Biosystems P/N 400385) to prepare your stock solution.
2. Split the stock solution into one-time use amounts by aliquotting 20 µL of BioBrene into individual Eppendorf tubes.
3. Freeze the tubes of BioBrene.

Preparing the BioBrene Solution

1. Thaw one of the tubes containing 20 µL of reconstituted BioBrene.
2. Add 70 µL methanol, and 10 µL 0.1% TFA to the BioBrene (100 µg/µL).
3. Vortex the solution for 5 sec.

The Effect of BioBrene on Peptide Sequencing

BioBrene is a cationic polymer used to immobilize the sample on the filter during Edman chemistry. Adding BioBrene to the PVDF membrane after sample application can greatly improve the sequencing performance of peptides.

At Applied Biosystems, a series of peptides (5 to 20 pmol) were prepared in ProSorb cartridges, and subjected to sequencing in the absence and presence of 100 μg BioBrene. The results of these tests (listed in Table 4-11) show that the addition of BioBrene dramatically improved the sequencing of these particular peptides.

Table 4-11. Peptide sequencing results with and without the addition of BioBrene

Peptide	**Without BioBrene** (residues/residues)	**With BioBrene** (residues/residues)
DRVYHIPF	4/8	8/8
KRQHPGKR	7/8	8/8
VHLTPVEK	8/8 (repetitive yield = 67%)	8/8 (repetitive yield = 84%)
LEHFRKGIQVNY	10/12	12/12
Insulin A chain	16/21	21/21
Atrial Natriuretic Factor	13/29	29/29
Insulin B chain	18/30	28/30

Sample Loading Overview and Requirements

Overview

The following pages describe how to load various sample types onto 6 mm reaction cartridges (Figure 4-3). Separate loading instructions are provided for:

- Samples prepared using ProSorb sample preparation cartridges
- Liquid samples
- Electroblotted samples

In each case, you are instructed to add a certain amount of BioBrene solution to your sample support. Instructions for preparing this solution are provided on page 4-20.

Materials Required to Load Samples onto the Sequencer

- Procise cartridge seals (Applied Biosystems P/N 401950)
- BioBrene solution (preparation instructions on page 4-20)
- ProSorb membrane punch tool (if using ProSorb Sample Preparation cartridges; Applied Biosystems P/N 401397)
- Self-closing forceps

Loading Samples Prepared in ProSorb Cartridges

Instructions for preparing your samples in ProSorb sample preparation cartridges are in section 2, “Sample Preparation Guidelines”. Before loading your sample onto the reaction cartridge, we strongly recommend you apply a small amount of BioBrene solution to the PVDF membrane. Instructions for preparing this solution are on page 4-20.

WARNING	***Some components on the sequencer may be hot! Use caution when working around hot components to avoid injury.***

IMPORTANT	*Always wear gloves and use forceps when handling seals and sample supports. All forceps, pipette tips, glassware and other hardware used should be clean and dedicated for sample preparation. Sample and cartridge contamination must be minimized to ensure optimal sequencing results.*

Procedure

Remove, disassemble and clean the reaction cartridge(s):

1. Unscrew and remove the reagent inlet cap connected to the sequencer.
2. Remove the reaction cartridge from the holder.
3. Unscrew and remove the cartridge block holder cap (Figure 4-3 on page 4-24).
4. Slowly invert the cartridge block holder until the upper and lower glass cartridge blocks slide out.
5. Discard the used Procise cartridge seal and sample support from the previous run.
6. Clean the upper and lower glass cartridge blocks by rinsing the inner surfaces of both blocks with methanol.
7. Place each block in the cartridge block drying assembly on the sequencer, and dry them with a stream of argon.
8. Referring to Figure 4-4 on page 4-25, place the telfon seal back into the cartridge block holder if it came out during disassembly.

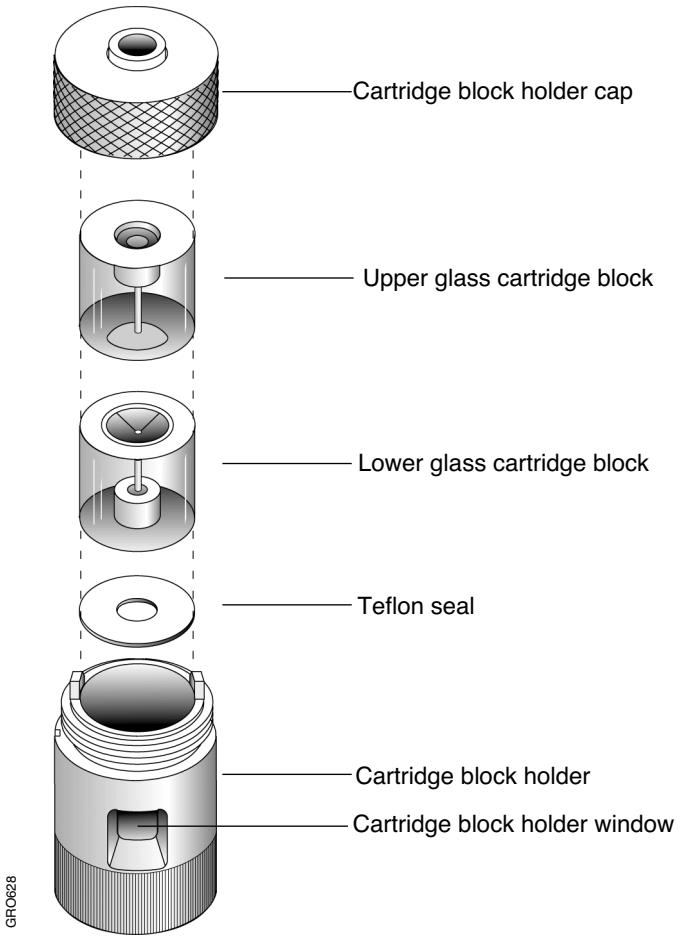

Figure 4-3. 6 mm reaction cartridge components in upright position

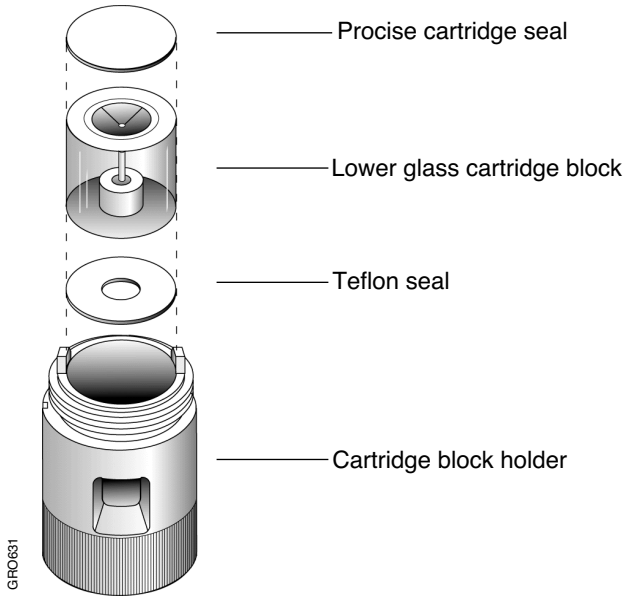

Figure 4-4. Reassembling the lower portion of the reaction cartridge

Load the sample:

1. Insert the lower glass cartridge block into the cartridge block holder.
2. Using forceps, place a new cartridge seal on top of the lower glass cartridge block.
3. If you have not already punched the PVDF membrane into the ProSorb sample reservoir insert, remove the insert from the holder now.

   If you have already punched the membrane into the insert, proceed to step 7.

IMPORTANT *Do not allow the membrane to touch anything except the ProSorb insert, the forceps, and the glass cartridge block.*

4. Discard the filter, and set the holder aside for cleaning and reuse.
5. Keeping the cap closed, carefully push the PVDF membrane into the sample reservoir insert using the punch tool (Figure 4-5).
6. Using self-closing forceps, remove the membrane from the ProSorb insert.
7. *Optional step:* Apply 5 µL of BioBrene solution to the membrane, and allow it to dry. Instructions for preparing this solution are on page 4-20.
8. Using forceps, place the PVDF membrane in the well of the upper glass cartridge block. Center the membrane in the well as accurately as possible (Figure 4-6).

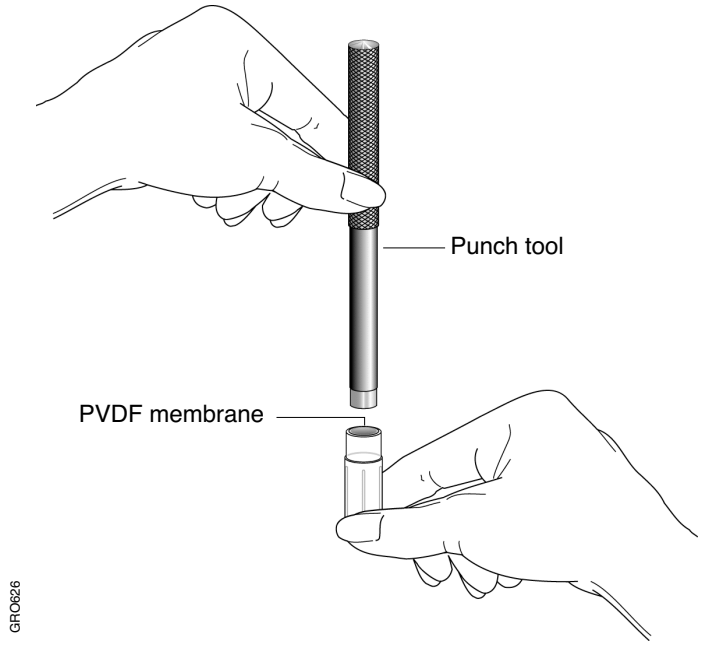

Figure 4-5. Punch the PVDF membrane into the ProSorb sample reservoir insert

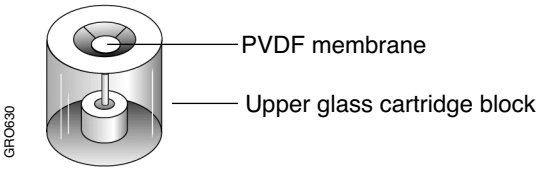

Figure 4-6. PVDF membrane centered in well of upper glass cartridge block

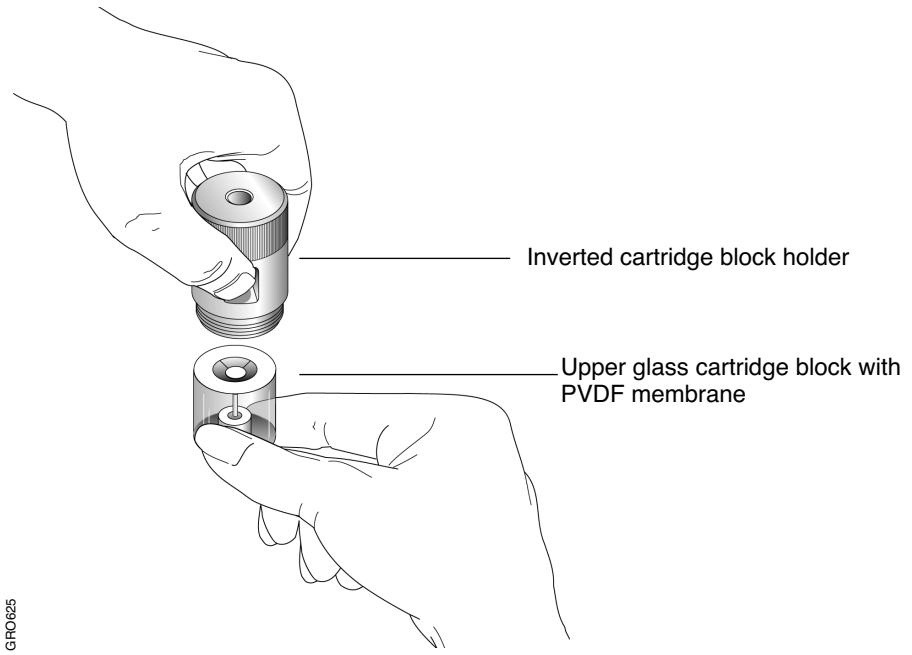

Figure 4-7. Slide the upper glass cartridge block with PVDF membrane up into the cartridge block holder

Reassemble and leak test the reaction cartridge(s):

1. Hold the lower glass cartridge block and Procise cartridge seal in place inside the holder by placing your fingers in the cartridge block holder windows. Invert the holder as shown in Figure 4-7.
2. Slide the upper glass cartridge block with the sample up into the cartridge block holder until it is flush against the lower cartridge block.
3. Invert the holder once again so it is upright, and screw on the cartridge block holder cap until snug.
4. Place the reaction cartridge back into the cartridge assembly on the sequencer.
5. Screw the reagent inlet cap onto the top of the reaction cartridge until it stops. Do not overtighten the cap.

IMPORTANT	*The seal between the cartridge blocks and the KEL-F ferrules is made by spring force. Overtightening the reagent inlet cap will not increase the sealing force.*

6. Perform a cartridge leak test by following the instructions on page 4-35.

Now you are ready to sequence your sample. Turn to page 4-37 for instructions on starting a run.

Loading Electroblotted Samples

Before loading your sample onto the reaction cartridge, we strongly recommend you apply a small amount of BioBrene solution to the sample. Instructions for preparing this solution are on page 4-20.

WARNING	***Some components on the sequencer may be hot! Use caution when working around hot components to avoid injury.***

IMPORTANT	*Always wear gloves and use forceps when handling seals and sample supports. All forceps, pipette tips, glassware and other hardware used should be clean and dedicated for sample preparation. Sample and cartridge contamination must be minimized to ensure the best sequencing results.*

Procedure

Remove, disassemble and clean the reaction cartridge(s):

1. Unscrew and remove the reagent inlet cap connected to the sequencer.
2. Remove the reaction cartridge from the holder.
3. Unscrew and remove the cartridge block holder cap (Figure 4-3 on page 4-24).
4. Slowly invert the cartridge block holder until the upper and lower glass cartridge blocks slide out.
5. Discard the used Procise cartridge seal and sample support from the previous run.
6. Clean the upper and lower glass cartridge blocks by rinsing the inner surface of both blocks with methanol.
7. Place each block in the cartridge block drying assembly on the sequencer, and dry them with a stream of argon.
8. Referring to Figure 4-4 on page 4-25, place the teflon seal back into the cartridge block holder if it came out during disassembly.
9. Insert the lower glass cartridge block into the cartridge block holder.

Load the sample:

1. Using forceps, place a new cartridge seal on top of the lower glass cartridge block in the holder.
2. *Optional step:* Apply 5 µL of BioBrene solution onto the membrane, and allow it to dry. Instructions for preparing this solution are provided on page 4-20.
3. Using self-closing forceps, place the sample you have excised into the well of the upper glass cartridge block. Center the membrane in the well as accurately as possible (Figure 4-6 on page 4-26).

Reassemble and leak test the reaction cartridge(s):

1. Hold the lower glass cartridge block and Procise cartridge seal in place inside the holder by placing your fingers in the cartridge block holder windows. Invert the holder as shown in Figure 4-7 on page 4-27.
2. Slide the upper glass cartridge block with the sample up into the cartridge block holder until it is flush against the lower cartridge block.
3. Invert the holder once again so it is upright, and screw on the cartridge block holder cap until snug.
4. Place the reaction cartridge into the cartridge assembly on the sequencer.
5. Screw the reagent inlet cap onto the reaction cartridge until it stops. Do not overtighten.

IMPORTANT	*The seal between the cartridge blocks and the KEL-F ferrules is made by spring force. Overtightening the reagent inlet cap will not increase the sealing force.*

6. Perform a cartridge leak test by following the instructions on page 4-35.

Now you are ready to sequencer your sample. Turn to page 4-37 for instructions on starting a run.

Loading Liquid Samples onto Glass Fiber Filters

Overview

- Liquid samples must be loaded onto BioBrene-treated glass fiber filters for sequencing.
- The treated filter must be precycled before the sample is loaded.
- Because the reaction cartridge is disassembled twice, the cartridge leak test must be run twice.

Procedure

The general steps required for loading a liquid sample are:

Step	Action
1	Load a glass fiber filter into a reaction cartridge, and treat it with BioBrene solution. Instructions for preparing this solution are on page 4-20.
2	Run a cartridge leak test.
3	Precycle the glass fiber filter. Precycling takes 2.5 h.
4	Load the sample onto the treated, precycled filter.
5	Run a cartridge leak test.

Detailed instructions for each step are provided on pages 4-31 through 4-34.

WARNING ***Some components on the sequencer may be hot! Use caution when working around hot components to avoid injury.***

IMPORTANT *Always wear gloves and use forceps when handling seals and sample supports. All forceps, pipette tips, glassware and other hardware used should be clean and dedicated for sample preparation. Sample and cartridge contamination must be minimized to ensure the best sequencing results.*

Step 1—Load and Treat the Glass Fiber Filter

Remove, disassemble and clean the reaction cartridge(s):

1. Unscrew and remove the reagent inlet cap connected to the sequencer.
2. Remove the reaction cartridge from the holder.
3. Unscrew and remove the cartridge block holder cap (Figure 4-3 on page 4-24).
4. Slowly invert the cartridge block holder until the upper and lower glass cartridge blocks slide out.
5. Discard the used Procise cartridge seal and sample support from the previous run.
6. Clean the upper and lower glass cartridge blocks by rinsing the inner surface of both blocks with methanol.
7. Place each block in the cartridge block drying assembly on the sequencer, and dry them with a stream of argon.

Load and treat a glass fiber filter:

1. Referring to Figure 4-4 on page 4-25, place the teflon seal back into the cartridge block holder if it came out during disassembly.
2. Insert the lower glass cartridge block in the cartridge block holder.
3. Using forceps, place a new cartridge seal on top of the lower glass cartridge block.
4. Center a new glass fiber filter in the well of the upper glass cartridge block (Figure 4-6 on page 4-26).
5. Gently press the filter in place with the tamper tool.

Note	*An off-center filter can cause cartridge sealing problems. Rips or holes in the filter will reduce sequencing efficiency.*

6. Load 7.5 µL of BioBrene solution onto the center of the filter. Instructions for preparing this solution are on page 4-20.

Note	*The volume of BioBrene solution applied to the filter must be sufficient to wet the entire filter. The maximum liquid capacity of a dry filter is approximately 7.5 µL. Additional fluid can be loaded if the filter is dried between loadings.*

7. To dry the filter, place the upper cartridge block in the cartridge drying assembly with the filter facing up.

8. Lower the drying arm. The filter will dry automatically in 5 min.
9. If not completely dry, raise and lower the drying arm again for an additional 5 min.

Reassemble and leak test the reaction cartridge(s):

1. Hold the lower glass cartridge block and Procise cartridge seal in place inside the holder by placing your fingers in the cartridge block holder windows. Invert the holder as shown in Figure 4-7 on page 4-27.
2. Slide the upper glass cartridge block up into the cartridge block holder until it is flush against the lower cartridge block.
3. Invert the holder once again so it is upright, and screw on the cartridge block holder cap until snug.
4. Place the reaction cartridge into the cartridge assembly on the sequencer.
5. Screw the reagent inlet cap onto the top of the reaction cartridge until it stops. Do not overtighten the cap.

IMPORTANT *The seal between the cartridge blocks and the KEL-F ferrules is made by spring force. Overtightening the reagent inlet cap will not increase the sealing force.*

Step 2—Perform a Cartridge Leak Test

1. Perform a cartridge leak test by following the instructions on page 4-35.

Step 3—Precycle the Glass Fiber Filter

1. Select the Start Run dialog box from the dialog box pop-up menu on the Macintosh (Figure 4-8).
2. Set the run order of your cartridges.
3. Enter a unique file name for each cartridge.
4. Enter 5 for the number of cycles.

   If additional BioBrene is used, more cycles are required. For example, at least 6 cycles are required to precycle a filter loaded with 15 µL of BioBrene.
5. Select Filter Precycle cLC for the method.
6. Select Collect Data.
7. Click Start Run.

The filter precycle procedure will take approximately 2.5 h. When complete, the status line on your monitor will say idle. Now you are ready to load your sample.

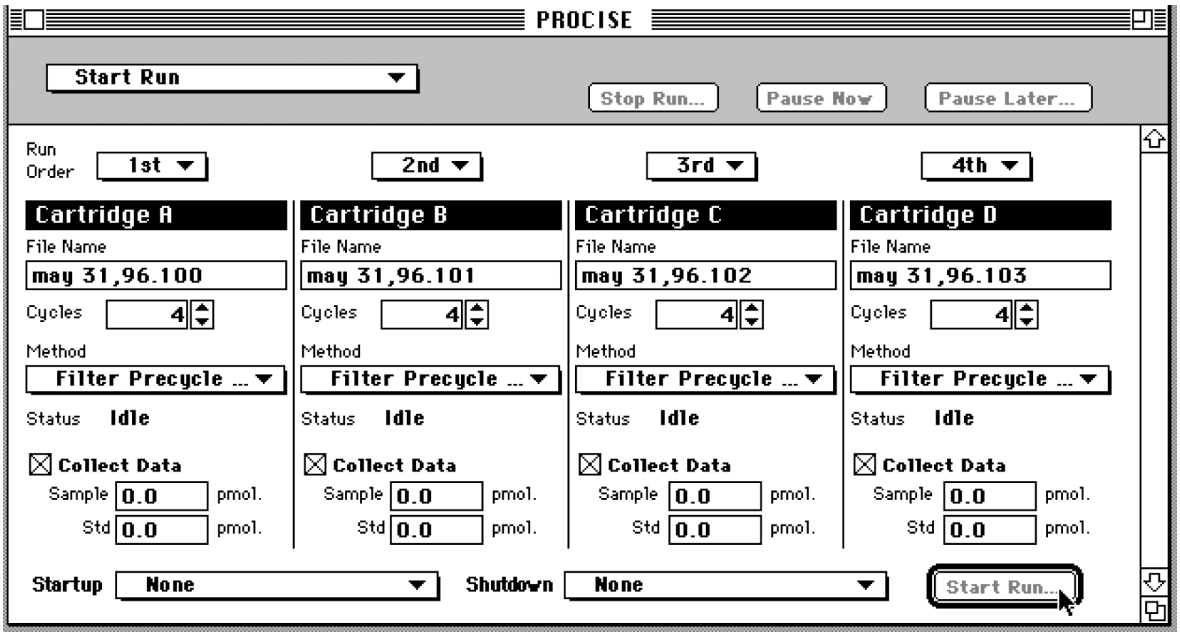

Figure 4-8. Precycling a glass-fiber filter treated with BioBrene

Step 4—Load Sample onto the Glass Fiber Filter

Remove, disassemble and clean the reaction cartridge(s):

1. Unscrew and remove the reagent inlet cap connected to the sequencer.
2. Remove the reaction cartridge from the holder.
3. Unscrew and remove the cartridge block holder cap (Figure 4-3 on page 4-24).
4. Slowly invert the cartridge block holder until the upper and lower glass cartridge blocks slide out.
5. Place the upper cartridge block on a clean, dry surface with the precycled filter facing up.
6. Discard the used Procise cartridge seal.
7. Clean the *lower glass cartridge block only* by rinsing the inner surface with methanol.
8. Place the lower glass cartridge block in the cartridge block drying assembly on the sequencer, and dry it with a stream of argon.

Load the sample:

1. Load your sample onto the center of the treated glass fiber filter, so it distributes evenly across the filter. A maximum aliquot of 5 µL is suggested.

Note	*The maximum liquid capacity of a dry filter is approximately 7.5 µL. With very dilute samples, you may need to load more than 7.5 µL total volume. Additional fluid can be loaded by drying the filter between loadings.*

Reassemble and leak test the reaction cartridge(s):

1. Referring to Figure 4-4 on page 4-25, place the teflon seal back into the cartridge block holder if it came out during disassembly.
2. Insert the lower glass cartridge block in the cartridge block holder.
3. Using forceps, place a new cartridge seal on top of the lower glass cartridge block in the holder.
4. Hold the lower glass cartridge block and Procise cartridge seal in place inside the holder by placing your fingers in the cartridge block holder windows. Invert the holder as shown in Figure 4-7 on page 4-27.
5. Slide the upper glass cartridge block with the sample up into the cartridge block holder until it is flush against the lower cartridge block.
6. Invert the holder once again so it is upright, and screw on the cartridge block holder cap until snug.
7. Place the reaction cartridge into the cartridge assembly on the sequencer.
8. Screw the reagent inlet cap onto the reaction cartridge until it stops. Do not overtighten.

IMPORTANT	*The seal between the cartridge blocks and the KEL-F ferrules is made by spring force. Overtightening the reagent inlet cap will not increase the sealing force.*

Step 5—Perform a Cartridge Leak Test

1. Perform another cartridge leak test by following the instructions on page 4-35.

Now you are ready to sequence your sample. Turn to page 4-37 for instructions on starting a run.

Performing a Cartridge Leak Test

A cartridge leak test should be performed prior to every run to verify that the cartridge assembly is leak tight. During the test, the cartridge is pressurized to 3.5 psi, and the pressure drop is monitored for 20 sec. Test results are reported in the Event Log at the end of the test.

Procedure

The sequencer must be idle to perform a leak test.

1. Select the Test dialog box from the dialog box pop-up menu (Figure 4-9).

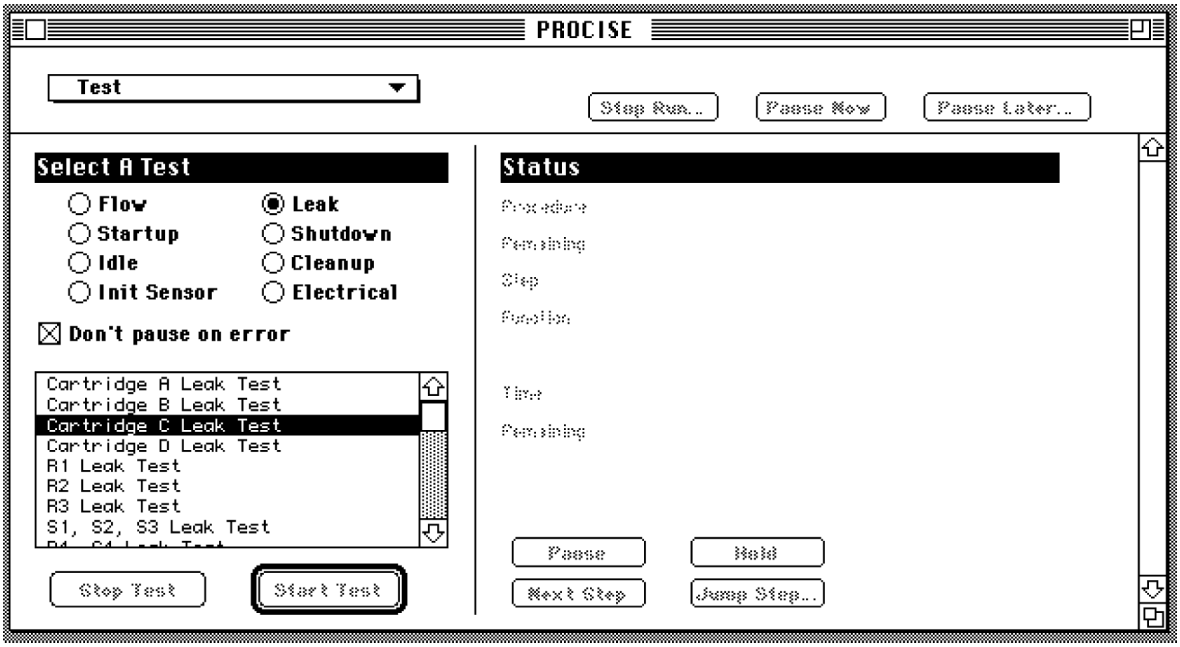

Figure 4-9. Test dialog box

2. Click Leak.
3. Scroll through the test menu, and select the cartridge(s) to be tested. Hold down the Command or shift key to select more than one cartridge.
4. Click Start Test.

IMPORTANT *Interrupting this procedure can invalidate the test results. In addition, the pressure regulator may not be reset to the correct pressure. User intervention commands, such as Jump Step and Pause, should never be used during a leak test.*

If a Reaction Cartridge Fails a Leak Test

Remove and disassemble the reaction cartridge:

1. Remove the reagent inlet cap.
2. Remove the reaction cartridge from the holder.
3. Unscrew and remove the cartridge block holder cap (Figure 4-3 on page 4-24).
4. Slowly invert the cartridge block holder until the upper and lower glass cartridge blocks slide out.

Inspect the sample matrix and cartridge seal:

1. Check the position of the sample. Is it centered in the well of the upper glass cartridge block? If not, recenter the sample.
2. Check the Procise cartridge seal for tears or unevenness in the sealing impression. Even if the seal appears ok, discard the seal and insert a new seal.

Reassemble and leak test the reaction cartridge:

1. Reassemble the reaction cartridge.
2. Check the KEL-F ferrules on the reagent inlet cap for damage or foreign materials. Repair or clean the cap if necessary.
3. Place the reaction cartridge into the cartridge assembly on the sequencer.
4. Screw the reagent inlet cap onto the reaction cartridge until it stops. Do not overtighten.

IMPORTANT *The seal between the cartridge blocks and the KEL-F ferrules is made by spring force. Overtightening the reagent inlet cap will not increase the sealing force.*

---

5. Repeat the cartridge leak test.

Starting a Run

WARNING ***CHEMICAL WASTE HAZARD. Waste produced by this system can be hazardous and can cause injury, illness, or death. Only operate a vented instrument if it is connected in accordance with all the requirements. Handle all liquid, solid and gaseous waste as potentially hazardous. Sequencer waste must be disposed of properly and carefully in accordance with all state, local, and federal requirements. Refer to the Waste Profile in the Procise 49X cLC Protein Sequencing System Safety Summary for classification of waste before disposal. When handling the waste for disposal, wear gloves and use eye protection. Avoid inhalation and skin contact.***

Before proceeding with the following instructions, you should have:

- The sample(s) loaded onto the sequencer.
- Leak tested all loaded reaction cartridges.

To start a run, you will now execute the following general steps:

Step	Action
1	Perform the checks listed in the Pre-run Checklist.
2	Purge the ABI 140D.
3	Set up the sequencer for a run.

Detailed instructions for these steps are on pages 4-38 through 4-41.

Step 1—Pre-run Checklist

- Check the quantities of sequencing chemistry and HPLC solvents. Replace chemistry and solvents as necessary, to ensure sufficient quantities are present for the entire run. The bottle change procedure is listed in Section 2, page 2-12.

IMPORTANT *Changing HPLC solvents during sequencing can cause retention times to shift, and make peak identification difficult.*

- Check the sequencer and 140D waste bottle levels. Empty the bottles if the waste level is close to 2 in from the top of the bottle.

WARNING ***Do not empty the waste bottle when the sequencer is running.***

- Check the argon supply. Enough argon must be present for the entire run. Change the argon tank if necessary.

Step 2—Purge the 140D

Always purge the 140D before you start a run. Purging (sometimes referred to as priming) the 140D removes old solvent from the pump cylinders, and clears any air bubbles in the solvent supply lines that may have formed while the instrument was idle. You will use the control panel on the 140D to purge this instrument.

The steps involved in purging the 140D are:

- Configure and run the purge cycle 7 times.
- Make adjustments if air bubbles emerge during the last purge cycle.
- Fill the cylinders.

Configure and run the purge cycle 7 times:

1. From the Ready Screen (Figure 4-10 on page 4-39) on the 140D, press the **PURGE>** soft key to display the Purge Screen (Figure 4-11 on page 4-39). Soft keys are F1, F2, F3 and F4.
2. Using the arrow keys, move the cursor to each field, and enter the values shown in Table 4-12 on page 4-39.

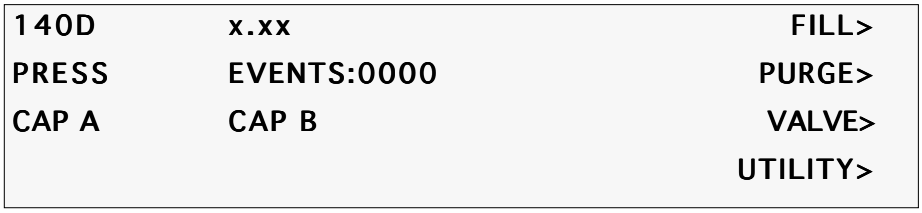

Figure 4-10. Ready Screen

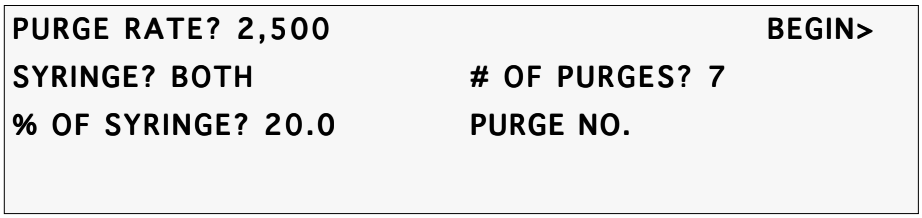

Figure 4-11. Purge Screen

Table 4-12. Purge Screen settings

Parameter	Choices	Setting
Purge Rate[1,2]	1 to 2,500 µL/min	2,500
Syringe	A, B or BOTH	BOTH
# of Purges[3]	1 to 100	7
% of Syringe[1]	0 to 100	20
1. The system must be purged before operation. Purging is best accomplished with fast fill and purge rates using 20% of the syringe volumes.		
2. Different solvents may require different purge rates and a different number of purges for optimization.		
3. Generally, 7 cycles are sufficient for initial purging.		

3. Press the **BEGIN>** soft key to begin purging the 140D.

   The purge cycle will repeat 7 times, ending with the pump syringes in the full-forward position. Throughout this procedure, messages will appear on the bottom line of the screen to indicate the status of the procedure (for example, FILLING PUMPS, VALVE OPENING, VALVE CLOSING, PURGING). After the fourth or fifth purge cycle, no air bubbles should emerge from the waste line.

If air bubbles still emerge during the last purge cycle:

1. Confirm that the inlet lines are immersed in solvent.
2. Check the solvent manifold connections. Tighten if necessary.
3. Repeat the purge procedure.
4. If air in the pump continues to be a problem, check the solvent lines at the bulkhead for leaks.

Once the purge cycles have finished, the Ready Screen is displayed automatically. Finish purging the 140D by filling the cylinders.

Fill the cylinders:

1. Press the **FILL>** soft key to display the Fill Screen (Figure 4-12).

Figure 4-12. Fill Screen

2. Using the arrow keys, move the cursor to each field and enter the values shown in Table 4-13.

Table 4-13. Fill Screen settings

Parameter	Choices	Setting
Fill Rate	1 to 2,500 µL/min	2,500
Syringe	A, B or BOTH	BOTH

3. Press the **BEGIN>** soft key. Both syringes will retract to completely fill the cylinders.

The 140D is now ready for operation. Refer to your 140D user's manual for more information on purging the 140D. In the 140D user's manual, this procedure is referred to as *Priming the 140D*.

Step 3—Set Up the Sequencer for a Run

A cartridge leak test should always be performed on each cartridge you have loaded before starting a run. This test is included as part of the sample loading procedures on the preceding pages of this section. Therefore, if you have loaded your sample(s), but have not yet run this test, turn to page 4-35 now, and run the cartridge leak test.

To setup the sequencer for a run:

1. If the system has been idle for one or more days, refer to the Section 8, "Maintenance", for information and instructions on the procedures you should run before sequencing a sample.
2. Select the Start Run dialog box from the dialog box pop-up menu (Figure 4-13).
3. Select the cartridge run order. Cartridges can be run in any order. Selecting the run order for a cartridge activates the File name, Cycles and Methods fields.
4. Enter a unique file name for each sample.
5. Enter the number of cycles to be run by highlighting each cycle field and typing the number, or by using the scroll up/down button. For filter precycling, enter 5 or more cycles as appropriate. When sequencing samples using the standard methods, the first 3 cycles prepare the sequencer and sample for sequencing. Therefore, if 20 residues are required, enter 23 in the Cycles box.

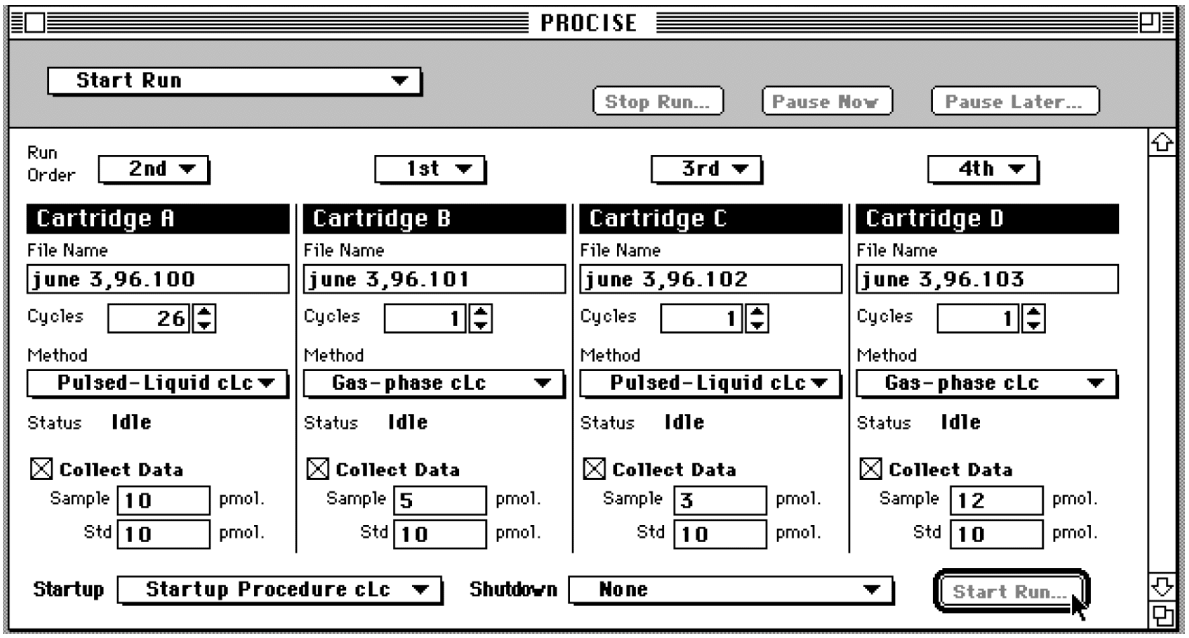

Figure 4-13. Configuring the sequencer for a run

6. Open the Method pop-up menu, and select the appropriate method for each cartridge.
7. Select the collect data boxes if they are not already selected. An X will appear in the box when selected.
8. Enter the sample and standard amounts to be run for each cartridge.
9. Click Start Run, or press Return.

Sequencing parameters are downloaded to the sequencer and the 140D. The Monitor Run window is displayed.

How Data is Collected During a Run

The Procise 49X cLC Protein Sequencer contains a virtual analog-to-digital (A/D) converter. The maximum storage capacity of the converter is 75 min of data. Data collection from the converter is controlled by the Procise cLC control software. When a sequencing run begins, a virtual A/D file is created in the sequencer folder located in the system folder.

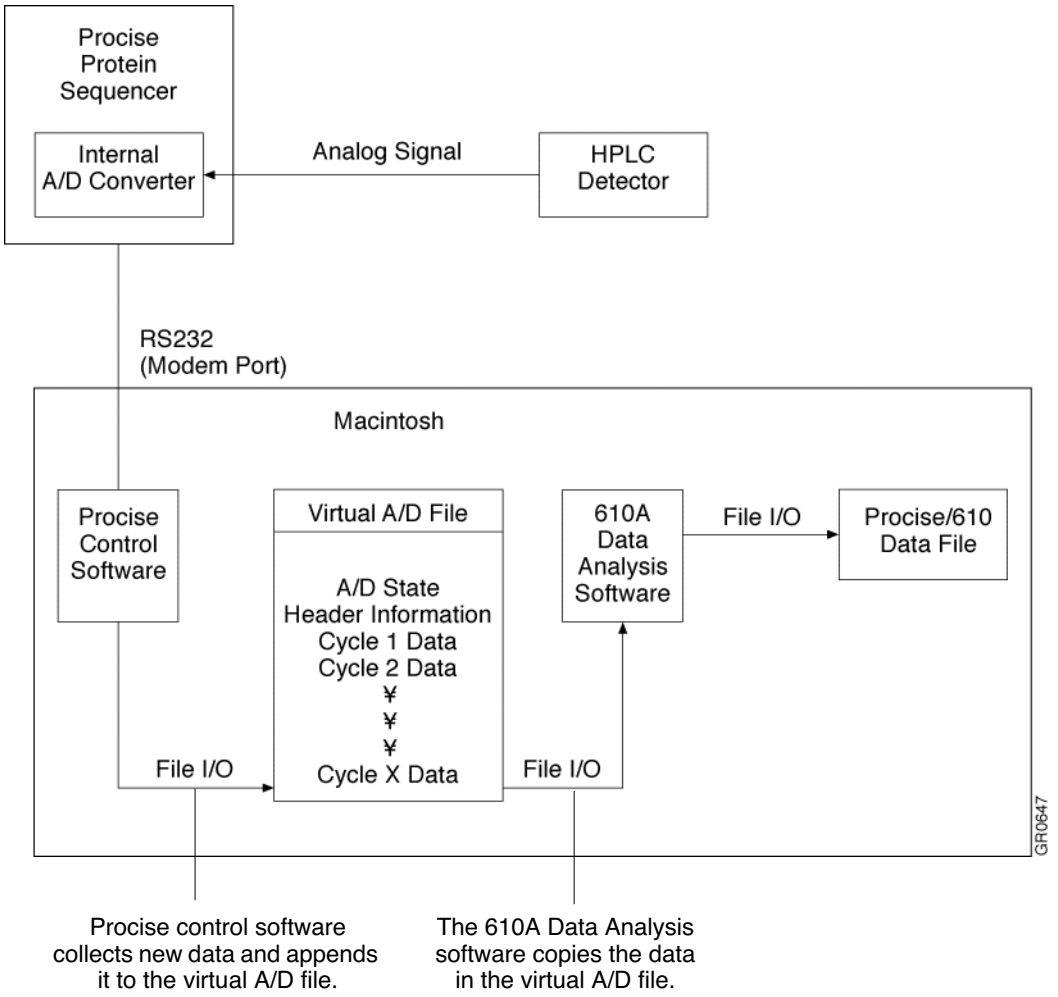

Figure 4-14. Virtual A/D data collection

Throughout the run, data is collected and appended to the virtual A/D file. When the run is complete:

- The control software turns on the *run finished* flag in the virtual A/D file to indicate that the file contains a complete set of sequencing data.
- The virtual A/D file contains:
    - Complete header information (sequencer name; sample name; run data and time; sample and standard amount).
    - The raw data for each cycle in the sequencing run for the specified cartridge and sample.

You can start the 610A Data Analysis software at any time. The 610A software continuously monitors the sequencer folder for new virtual A/D files.

A chromatogram display window is opened for each virtual A/D file, and the data is stored in a Procise/610A data file. The data file and the chromatogram are updated every 15 sec until the run is finished and all the data is collected. When the run is finished, the virtual A/D file is deleted, and the data collected by the 610A software is stored in the Procise *f* folder.

If the sequencer determines that the control software has stopped collecting data from converter, the sequencer pauses automatically at the end of the current cycle, and waits until data collection resumes. This mechanism helps prevent data loss.

Sequencer Idle Time

When the Procise 49X cLC Protein Sequencing System is not in use, oxygen diffuses slowly into the system causing solvents and reagents to decompose and form by-products. These by-products can interfere with sequencing efficiency.

To minimize sequencing problems due to chemical decomposition during an inactive period, follow the appropriate recommendations provided in section 9, “Maintenance”.

5 Troubleshooting Guide

Contents

Troubleshooting Table

Overview

The following table is designed to help you troubleshoot most of the problems you may encounter while using the Procise 49X cLC Protein Sequencing System. The information in the table is arranged by category as follows:

- Chemistry & chromatography problems
- Event log error messages
- Software and communication problems
- Pump and detector error messages

Each category contains subcategories, followed by a brief description of the symptoms you might encounter. To use this table, look for the symptom you are experiencing. The reference number associated with the symptom corresponds to a description of the possible cause(s) and recommended action(s) for that particular problem. The causes and recommended actions are listed by reference number in numerical order after this table.

Table

Category	Symptom	Reference Number
Chemistry & Chromatography Problems ***Baseline disturbances and anomalies***		
	Noise (high frequency) on baseline, even when pump is off.	1
	Stepping of baseline intermittently in chromatograms.	2
	Cycling of baseline (low frequency).	3
	Spikes on baseline.	4
	Noise (medium frequency) on baseline.	5
	Slope (negative-going) on which early eluting amino acids ride.	6
	Humps or dips on baseline.	7
	Deflection (small) at consistent retention time in all chromatograms.	8
	Deflection (off scale) in chromatogram.	9
	Additional peaks in blank, standard & residue chromatograms anywhere after injection artifact.	10

Category	Symptom	Reference Number
Chemistry & Chromatography Problems *continued*		
Missing Peaks		
	Flat baseline with no injection artifact. No errors in Event log.	11
	All residue cycles resemble a blank chromatogram. Maybe some aniline. No Event log errors.	12
	All residue cycles resemble a blank chromatogram. Transfer sensor error in Event log.	13
	Deflection at about 6 minutes. Injection artifact. No other peaks. Injector full sensor error in Event log.	14
	Reduced number of peaks elute early in chromatogram. The peaks are broad.	15
Poor recovery, standard chromatogram		
	All peak heights reduced and peak width increased.	16
	All peak heights reduced.	17
	All peak heights reduced intermittently.	18
	Low lysine.	19
	Low lysine & PE cysteine.	20
Poor recovery of residue amino acids		
	Low lysine.	19
	Low lysine & PE cysteine.	20
	Low serine & threonine.	21
	Low asparagine or glutamine, and high aspartate & glutamate respectively.	22
	Low glycine.	23
	Low histidine & arginine.	24
Low repetitive yield		
	Low repetitive yield, high lag.	25
	Low repetitive yield, no lag.	26
Artifact peaks		
	DTT-PITC adduct close to proline.	27
	High aniline.	28
	High oxidized DTT.	29

Category	Symptom	Reference Number
Chemistry & Chromatography Problems *continued*		
Retention time problems		
	General retention time instability during the run.	30
	Most peaks miscalled even though peak retention times reasonably stable.	31
	Only early eluting peaks miscalled.	32
	Retention time shift only in occasional cycles.	33
	All peaks, including injection artifact, delayed by same amount.	34
Event Log Error Messages		
Cartridge load, cartridge outlet, flask load and transfer fluid sensor error messages		
	Fluid never reaches sensor (average wet reading = 0).	36
	Too many bubbles in liquid slug (average wet reading ≠ 0).	37
Injector Sample Loop Fluid Sensor Errors		
	Sample loop full errors, and no amino acid peaks.	38
	Sample loop full errors with partial injection.	39
	Sample loop full and load errors; no injection.	40
Other event log messages generated during a run		
	Argon tank pressure too low.	41
	Cannot reach set temperature.	42
	Communication with HPLC system lost.	43
	Event buffer overrun.	44
	Injector position error and no corresponding sample loop sensor errors.	45
	Insufficient data collection memory for cycle.	46
	Invalid sensor dry reading.	47
	Power failure.	48
	Vacuum assist activated more than once every 8 hours.	49

Category	Symptom	Reference Number
Leak test error messages		
	Actual pressure above 5.5 psi.	50
	All leak tests fail.	51
	Failing vent test.	52
	Flask failing vent test.	53
	Leak test fails because pressure too high.	54
	Cartridge leak test fails.	55
Software and Communication Problems *Lock-ups*		
	Frequent Macintosh lock-ups.	56
	Macintosh locks up during data collection.	57
	No communication between Macintosh and sequencer.	58
Procise, 610A and Macintosh operating system errors		
	File error every time 610A is launched.	59
	File or disk error every time Procise is launched. Usually [PROCISE error -48].	60
	File missing error when Procise launched.	61
	File already exists error generated by the 610A.	62
	“No data has been collected for 12 hours” dialog box message generated.	63
	610A does not print all cycles.	64
Pump and Detector Error Messages *785A*		
	Detector (785A) beeping.	65

Chemistry & Chromatography Problems

Baseline Disturbances and Anomalies

1. Noise (high frequency) on baseline, even when pump is off.

High frequency noise is usually electronic noise from the UV detector, and is always present at some level.

Possible Causes

- Flow and mixing problems produce a slower baseline response.
- As the UV lamp ages, lamp energy is reduced, and electronic noise is amplified.
- Wavelength set lower than 269 nm. The baseline profile may also change due to different absorbance characteristics of the HPLC solvents.
- Rise time on the detector is not set to 1.0 sec.
- A small air bubble is trapped in the flowcell, and is interfering with the UV transmission. In this situation, the noise tends to come and go throughout the cycle and the run.

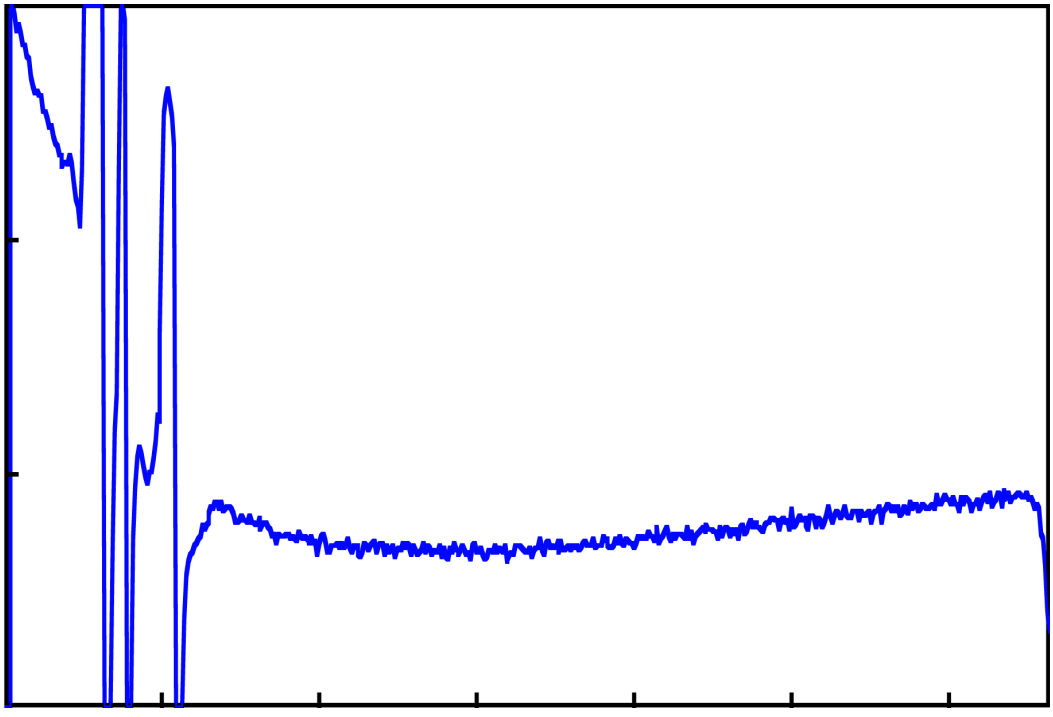

Figure 5-1. High frequency noise (the scale of the diagram has been expanded to exaggerate the noise)

Baseline Disturbances & Anomalies *continued*

Recommended Actions

If every chromatogram is affected to same extent:

- Has the lamp in the 785A exceeded its recommended lifespan (>1000 h)? If so, replace the lamp. Refer to Section 9, "Maintenance", for replacement instructions.
- Is the detector rise time less than 1.0 sec? If so, set the rise time to 1 sec.
- Is the detector wavelength set to 269 nm? If not, noise will be amplified with possible baseline profile changes. Set the wavelength to 269 nm.
- Perform the dry cell test on page 9-53 in Section 9, "Maintenance". Isolate the problem, and fix as recommended.

If not all chromatograms or sections of the baseline are affected to the same extent:

- An air bubble is probably in the flowcell. Perform the following checks and appropriate maintenance.

  a. Is the back-pressure PEEK tubing correctly installed onto the flowcell outlet line? If not, properly reinstall the back-pressure line.

  b. Are there any leaks before or after the flowcell? If so, repair the leaks.

  c. Clean the flowcell by following the flushing procedure listed on page 9-47 in Section 9, "Maintenance".

Baseline Disturbances & Anomalies *continued*

2. Stepping of baseline intermittently in chromatograms.

Possible Causes

As a UV lamp ages, the electrodes burn and the arc becomes less stable. The arc then has a tendancy to jump to another location for a while, then back again intermittently. This results in a step on the baseline (Figure 5-2). In rare cases, this condition can also be caused by an unstable electronic component.

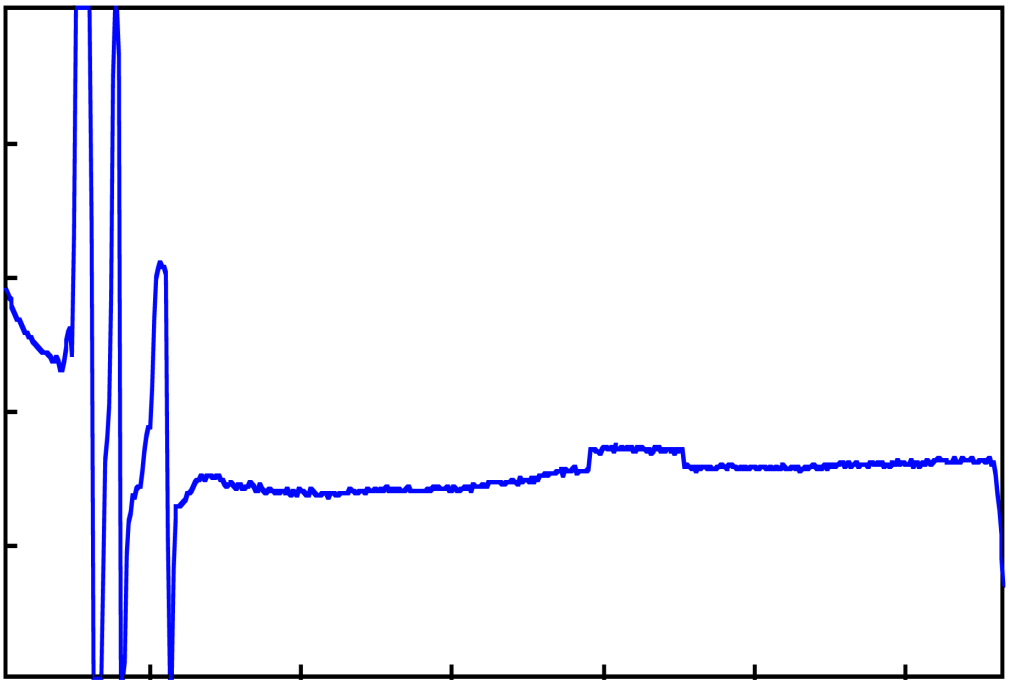

Figure 5-2. Baseline stepping

Recommended Actions

- Has the lamp in the 785A exceeded its recommended lifespan (>1000 h)? If so, replace the lamp. Refer to page 9-49 in Section 9, "Maintenance" for replacement instructions.
- Perform the dry cell test on page 9-53 in Section 9, "Maintenance". Correct any problems found as recommended.

Baseline Disturbances & Anomalies *continued*

3. Cycling of baseline (low frequency).

Possible Causes

- Slow current variation due to the effects of heating and cooling at bad connections.
- Environmental signals are not effectively screened out, for example incorrect or poor grounding.

Recommended Actions

- Has the lamp in the 785A exceeded its recommended lifespan (>1000 h)? If so, replace the lamp. Refer to page 9-49 in Section 9, "Maintenance", for replacement instructions.
- Are all system instruments plugged into the same power source? If not, plug all the instruments into the same power source. For example, if a universal power supply (UPS) is being used, all of the instruments should be powered from this one source.
- Check the detector fan in the lamp compartment. If not functioning, call Applied Biosystems.
- Is the signal cable shielding grounded correctly? If not, ground the shielding correctly.
- Perform the dry cell test on page 9-53 in Section 9, "Maintenance", to isolate faulty electronic components or loose connections. Repair any problems found as recommended.

4. Spikes on baseline.

Possible Causes

- Line voltage disturbances (Figure 5-3).
- Column is losing packing material (silica). A build up of white material in the flowcell is an indicator of this problem.
- Tiny air bubbles due to inadequate back-pressure on the flowcell.

Whether the problem is being caused by a loss of column packing material or by bubbles, the spikes will be seen only when the 140D is running.

Baseline Disturbances & Anomalies *continued*

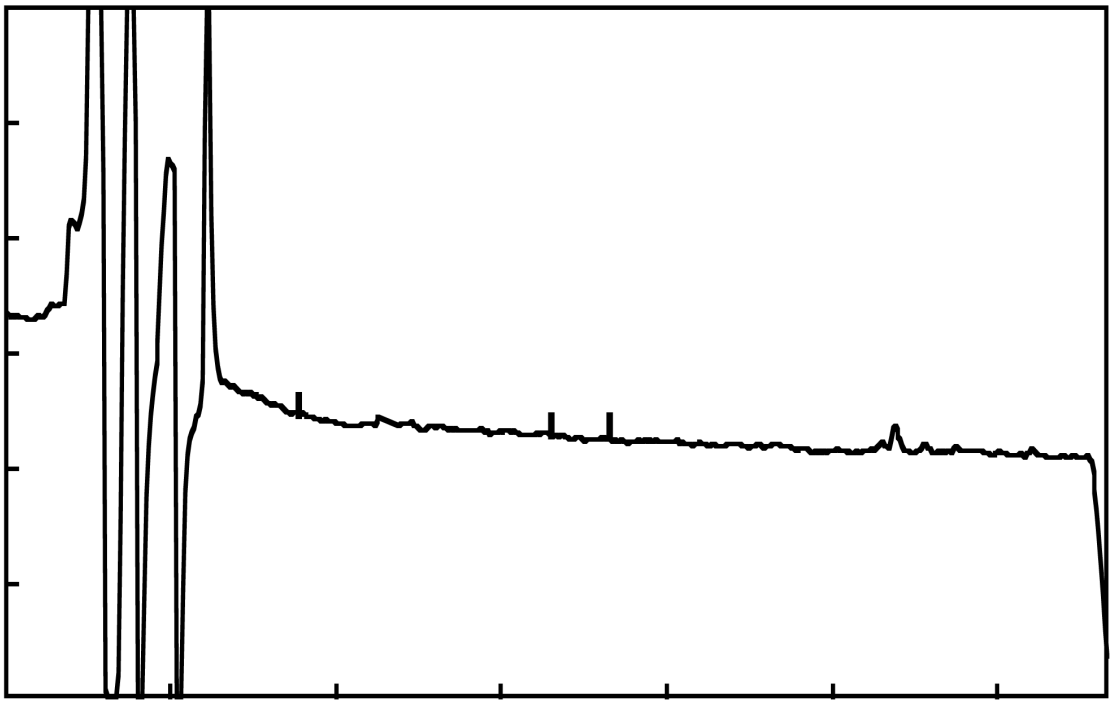

Figure 5-3. Spikes on baseline

Recommended Actions

- Are all the instruments in this system plugged into the same power source? If not, plug all the instruments into the same power source. For example, if a universal power supply (UPS) is being used, all of the instruments should be powered from this one source.
- Is the power supply unstable? A dry cell test (page 9-53 in Section 9, "Maintenance") can help reveal disturbances. If the power is unstable, plug the system into a different, stable circuit.
- If you suspect an air bubble in flowcell, set the detector wavelength to 656 nm, and look for an air bubble in flowcell. If a bubble(s) are found, increase the solvent composition to 90 %B at 50 μL/min to flush out the bubbles.
- Check for leakage at the flowcell outlet. Repair if necessary.
- Inspect the column outlet. Precipitate at the outlet indicates the column is losing packing material. If precipitate is present, replace the column, and clean the flowcell by flushing it with 90% B or methanol. Refer to Section 9, "Maintenance", for column replacement instructions.

Baseline Disturbances & Anomalies *continued*

5. Noise (medium frequency) on baseline.

Possible Causes

- Inefficient mixing.

  Normally, a mixing problem will have less impact on retention time variation, and is characterized by a dip at the front end of the chromatogram (Figure 5-4).

- Leaks.
- The introduction of air into the system.
- A bad seal can cause a retention time shift and baseline abnormalities (Figure 5-5 on page 5-13).

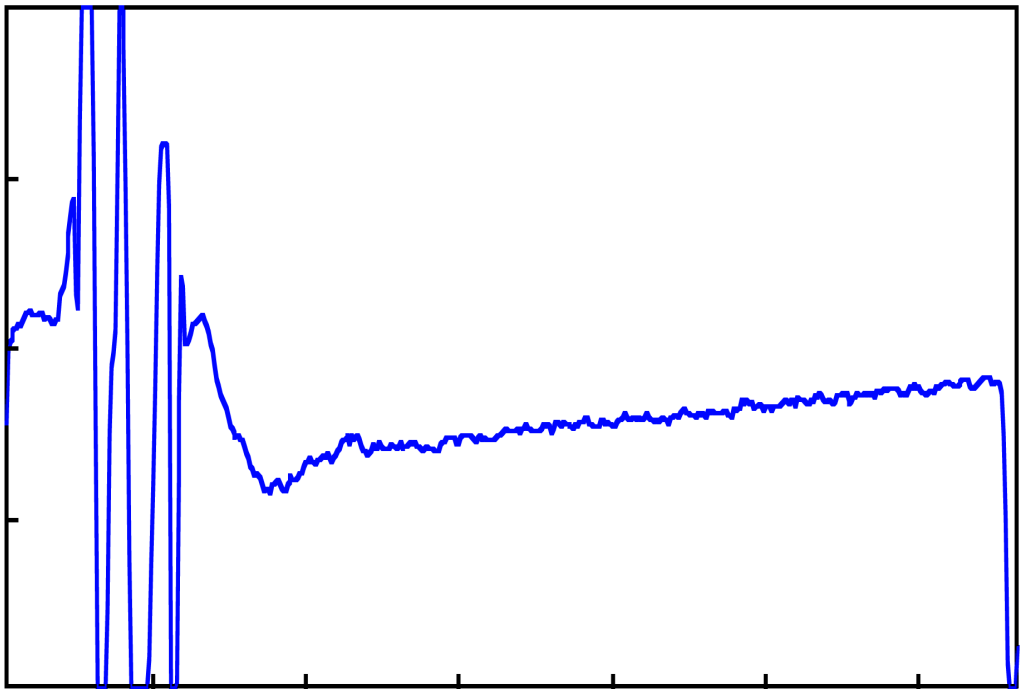

Figure 5-4. Dynamic mixer not turning

Baseline Disturbances & Anomalies *continued*

Recommended Actions

If retention times are stable:

- Check the 1A fuse in the 140D. Replace if necessary. Refer to the 140D user's manual for instructions.
- Has the lamp in the 785A exceeded its recommended lifespan (>1000 h)? If so, replace the lamp. Refer to page 9-49 in Section 9, "Maintenance", for replacement instructions.

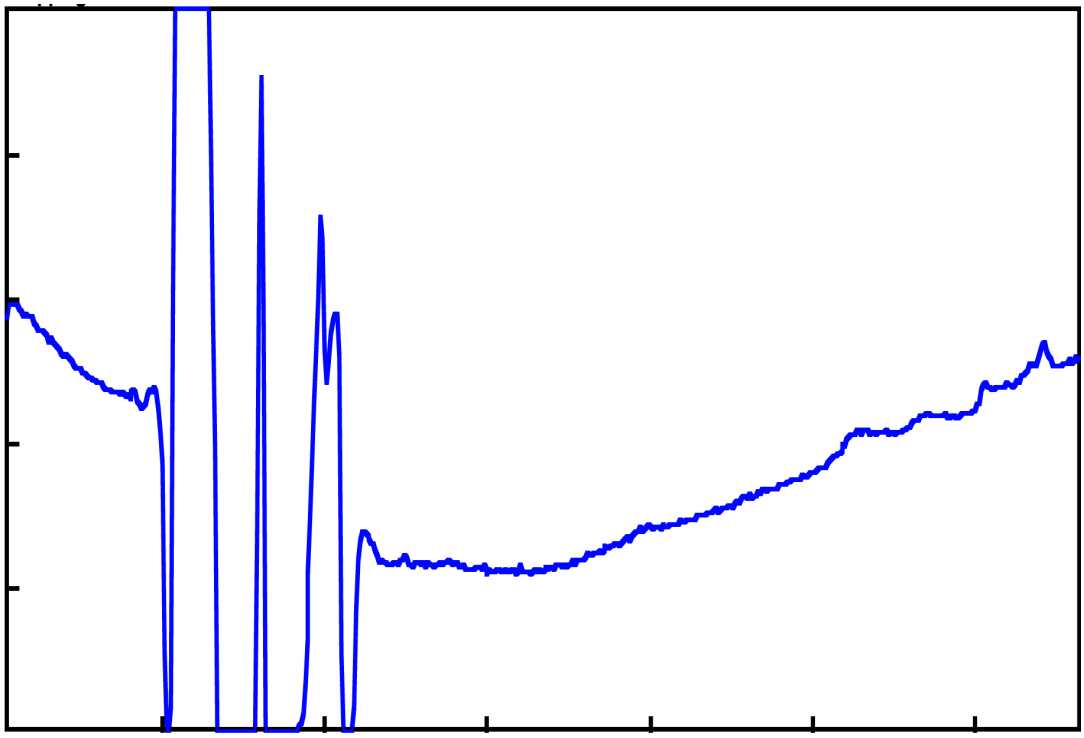

Figure 5-5. Leaking pump seal

Baseline Disturbances & Anomalies *continued*

If retention times are unstable:

Procedures for the following tests and repairs are located in the Troubleshooting and Maintenance sections of the 140D user's manual.

- Check the pump manifold fittings for leaks. Replace all leaking seals.
- Check for leakage at all fittings, and at the pump seal leak points (Figure 5-6). Repair all leaks.
- If there are no obvious leaks, perform the static pressure test, and monitor the system pressure during a run to determine the source of the leak. Repair any leaks that are found.

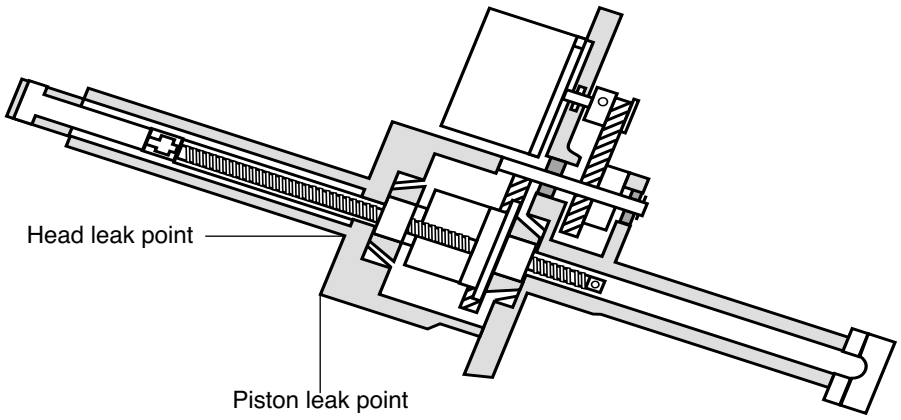

Figure 5-6. Pump seal leak points

Baseline Disturbances & Anomalies *continued*

6. Slope (negative-going) on which early eluting amino acids ride.

Possible Causes

A sloping front end (Figure 5-7) is normally caused by a UV-absorbing contaminant in solvent A, or inadequate column equilibration.

A hump early in the chromatogram usually indicates the presence of a contaminant in the pumping system. This is common after replacing a system component such as a pump seal, and usually disappears on its own over time.

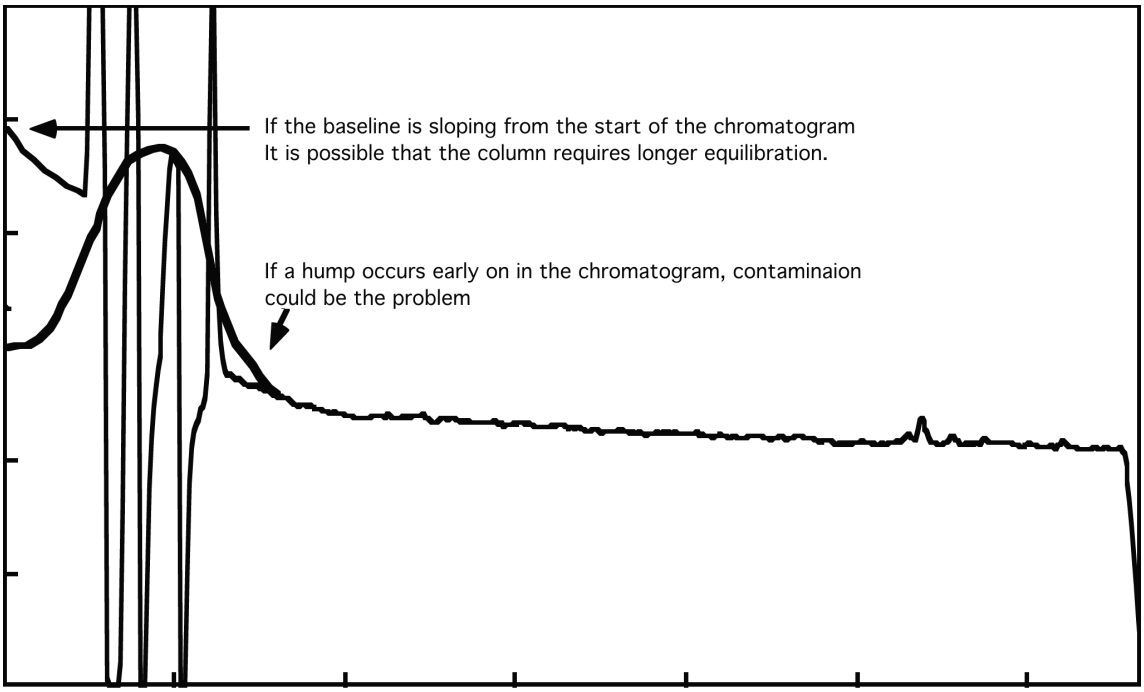

Figure 5-7. Slope or hump at start of chromatogram

Recommended Actions

- If you suspect a contaminated pumping system, wash the column and pumping system. Refer to pages 9-41 and 9-43 in Section 9, "Maintenance", for wash instructions. To minimize the slope, add sodium phosphate monobasic to solvent A.
- Is the column equilibration time at least 18 min? This is the time between the *Prepare Pump* and *Load Injector* steps in all Flask cycles. If the time is less than 18 min, increase the time.
- Is the 140D configured to “Fill between runs”? If not, reconfigure the 140D to fill between runs.

- Configure the 140D to continue pumping after completion of the gradient. To configure the 140D, press Run on the front panel of the instrument, then select Y for Manual.

Baseline Disturbances & Anomalies *continued*

7. Humps or dips on baseline.

Possible Causes

- Solvent A or B. Solvent A is usually the cause, since it degrades more quickly.
- The detector flowcell is not flush with the monochromator. Consequently, refractive index effects are exaggerated.

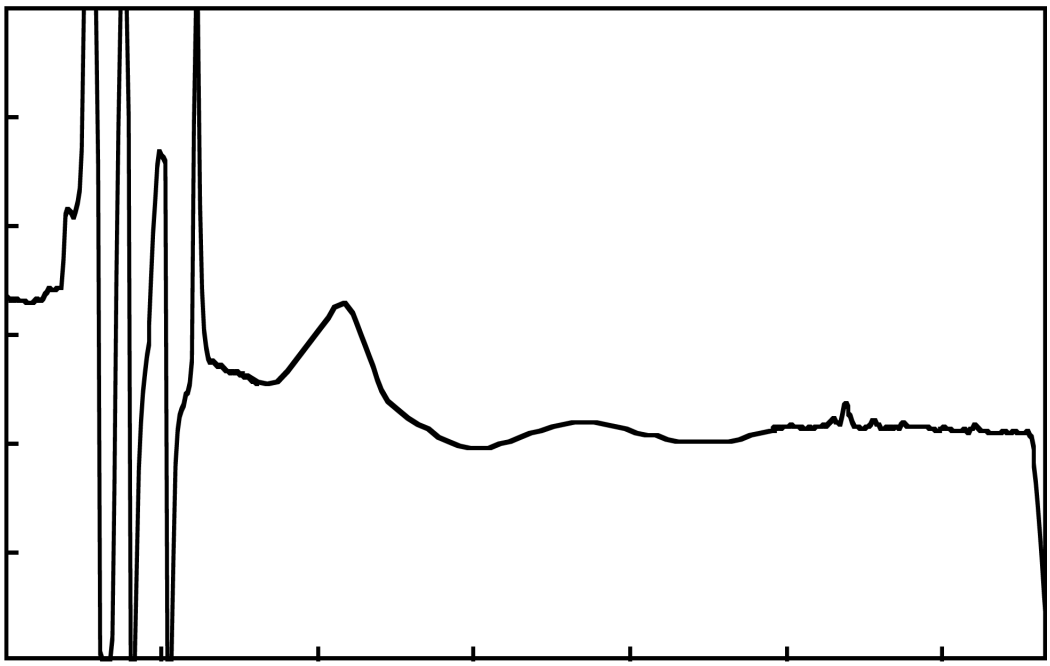

Figure 5-8. Hump on baseline

Recommended Actions

- Run the Run Gradient cLC method to isolate the HPLC components of the system from the sequencer. If the profile looks normal, there is a problem with the sequencer. Clean or replace the flask, and replace R4A and S4. Cleaning instructions are in Section 9, "Maintenance".

  If the profile is not normal, there is a problem with the pumping system. Replace solvents A & B, and purge the system 3 times. Instructions for changing solvents and purging the 140D are in Section 9, "Maintenance".

- Is the flowcell flush with the monochromator? If not, reposition the flowcell.

Baseline Disturbances & Anomalies *continued*

8. Deflection (small) at consistent retention time in all chromatograms.

Possible Cause

- A scratched cylinder in the 140D typically causes this problem.

Recommended Action

- Monitor the pressure as the system is running. If there is a sudden change in pressure consistent with the deflection on the chromatogram, remove and inspect the cylinders in the 140D. Replace damaged cylinders. Refer to the 140D user's manual for instructions.

9. Deflection (off scale) in chromatogram.

Possible Causes

An air bubble trapped in the flowcell can make the absorbance so high it will go off the scale (Figure 5-9). Air bubbles tend to occur with high concentrations of aqueous solvent.

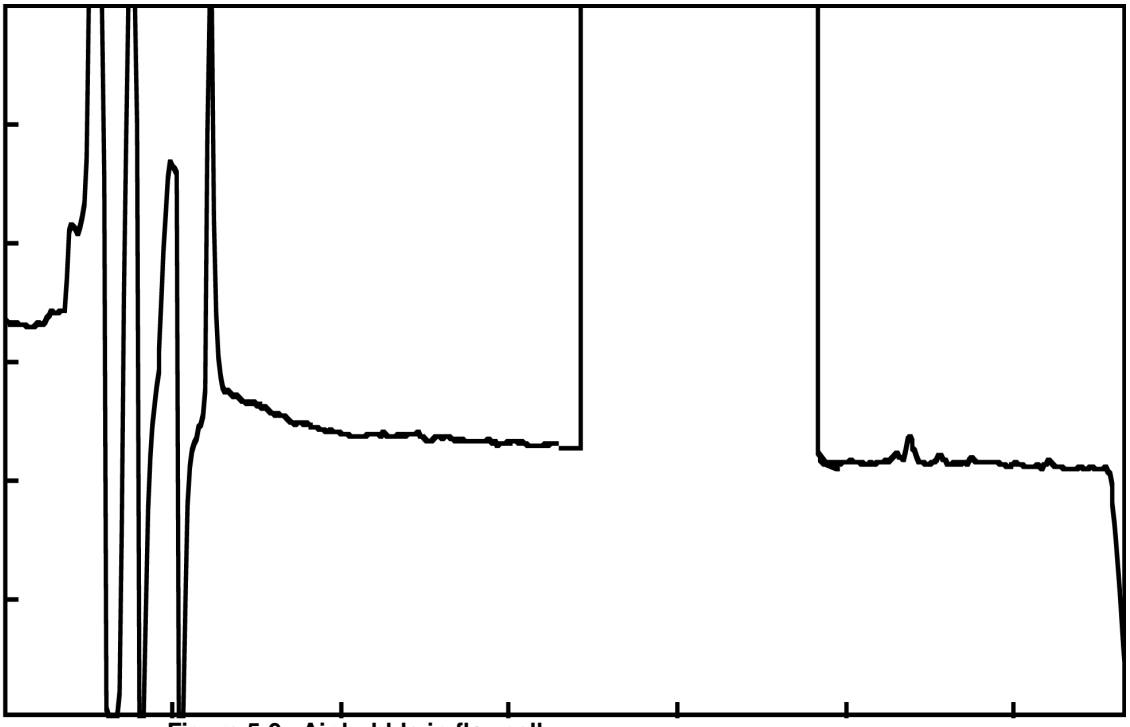

Figure 5-9. Air bubble in flowcell

Baseline Disturbances & Anomalies *continued*

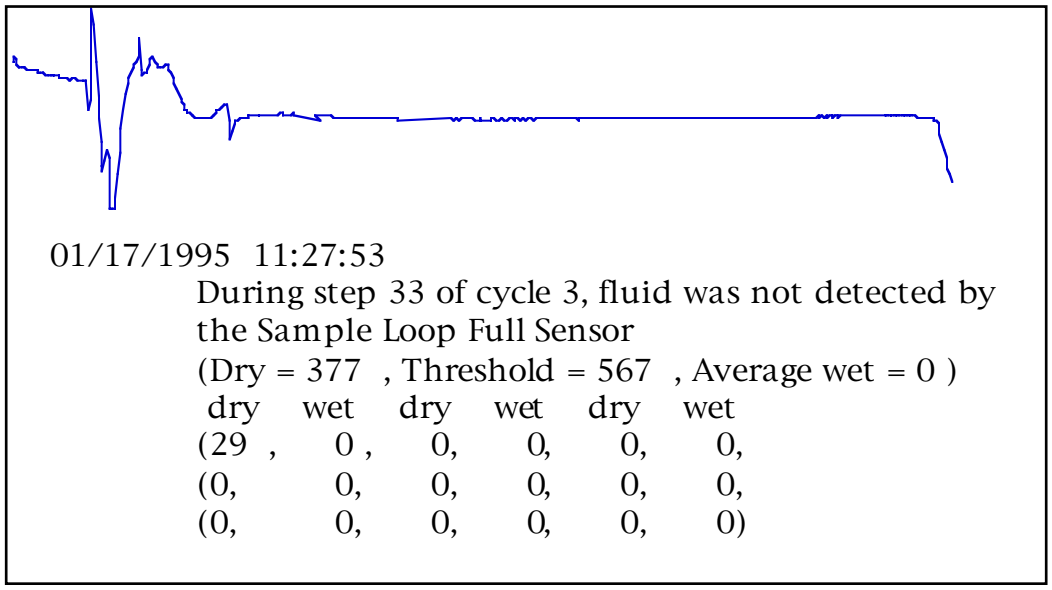

Figure 5-10. Air injection

Recommended Actions

- Check for air bubbles in flowcell by setting the wavelength to 656 nm and looking into flowcell. If air bubbles are present, the red light will be defracted. To clear air bubbles, wash out the flowcell by increasing the solvent composition to 90%B at 50 µL/min.
- Is the back pressure line properly installed? If not, reinstall it.

  A properly installed back-pressure line (48 in. of 0.0025-in. i.d. PEEK tubing) is very effective at preventing the formation of bubbles, making it unnecessary for users to degas the solvents. To demonstrate this, the resulting chromatogram of an air injection is shown in Figure 5-10. Even though the 50 µL loop was completely full of air when the injection took place, there is no indication that an air bubble is trapped in the flowcell.
- Are adequate quantities of solvents A and B present? If not, replenish the solvents.

Baseline Disturbances & Anomalies *continued*

10. Additional peaks in blank, standard & residue chromatograms anywhere after injection artifact.

Possible Causes

A contaminant is present in solvents A or B, the flask reagents, the flask system, or the pumping system.

Recommended Actions.

Run the Run Gradient cLC method to isolate the HPLC components of the system from the conversion flask.

- If the peaks are still present, replace solvents A and B. Instructions are in Section 9, "Maintenance".
- If the peaks are no longer present, replace the flask reagents (R4A, S4 and R5).
- Clean or replace the conversion flask. Cleaning instructions, which include removal, are in Section 9, "Maintenance".
- Replace the injector loop.

Missing Peaks

11. Flat baseline with no injection artifact. No errors in Event log.

Possible Causes

- The 140D
- The A/D convertor in the Macintosh
- The 785A UV/VIS detector

Since data collection occurred, and there were no errors in the Event log, an injection took place.

Recommended Actions

- Is the UV lamp lit? Check it by pressing the UTIL> key on the front panel of the detector. The reference reading should fall between –0.25 and –0.4. If the reading is incorrect, replace the lamp by following the procedure on page 9-49 in Section 9, "Maintenance".

12. All residue cycles resemble a blank chromatogram. Maybe some aniline. No Event log errors.

Possible Cause

All liquid deliveries and transfers took place, since there were no fluid sensor errors in the Event log. Therefore, the R2 vapor did not deliver.

Recommended Actions

- Are the R2 *Set* and *Actual* pressures the default values (0.8 psi)? It is normal for the Actual pressure to float a little higher than the Set pressure when there is no R2 delivery. If the pressures are not correct, go to the Pressures & Temperatures dialog box, and click Default to restore the default settings.
- Is the R2 bottle empty? If so, replace the bottle.
- Perform a bottle leak test on the R2 bottle. Repair any leaks. Leak test instructions are located in Section 7, "Tests and Procedures".

Missing Peaks *continued*

13. All residue cycles resemble a blank chromatogram. Transfer sensor error in Event log.

Possible Causes

- The transfer from the cartridge to the flask did not take place. This could be due to a blockage in the transfer line, or an incorrect pressure.
- If the respective cartridge outlet sensor determines that it is sensing liquid when, in fact, it is not, S2 extraction and transfer will not occur.

Recommended Actions

- Are the regulator pressures set correctly? If regulator #5 is set to 0, a cartridge leak test may have been aborted before the operating pressure was saved. Never abort a leak test. If the regulator pressures are not correct, adjust the pressures, or click Default in the Pressures & Temperatures dialog box to restore the default settings.
- Check the cartridge outlet lines for crimps. If found, call Applied Biosystems.
- Watch a “Deliver S2, cart sensor” function. You should see liquid reach the cartridge before the “fluid sensed” light turns on. If the light turns on as soon as the function is executed, reinitialize the sensors by running the Init Sensor Procedure in Section 7, "Tests and Procedures", and try again. If a problem still exists, either the cartridge line flushing function is failing, or the sensor is faulty.
- Check the transfer line for blockages by running the Sensor & Delivery test (a Flow test). A transfer sensor error indicates a transfer line is blocked. Call Applied Biosystems.

14. Deflection at about 6 minutes. Injection artifact. No other peaks. Injector full sensor error in Event log.

Possible Causes

Some form of injection took place, since an injection artifact is present. The small dry reading of 29 (0.15 sec) in the sample loop full sensor error indicates that the injector was actuated almost immediately after the *Load Injector* step started, and before any liquid could reach the sample loop. The result was an air injection. Either the sample loop load sensor was incorrectly initialized, or residual liquid was not completely flushed out of the injection system prior to the *Load Injector* step.

Recommended Actions

- Run the Post-Run Valve Block Wash X1-X2 procedure. This procedure is run from the Test dialog box, and is listed under Shutdown procedures.

Missing Peaks *continued*

15. Reduced number of peaks elute early in chromatogram. The peaks are broad.

Possible Causes

The pumping system may not be delivering an adequate concentration of organic solvent, since all the peaks are not eluting. Possible causes could be a leak, or a problem with the gradient program.

Recommended Actions

- Is the cable between the inject output of the sequencer and the inject input of the 140D connected correctly? If not, reconnect the cable.
- Is the correct gradient being used?
- Check for leaks in the HPLC components of the system. Repair any leaks. Refer to the 140D user's manual for instructions on detecting and repairing leaks.

Poor Recovery, Standard Chromatogram

16. All peak heights reduced and peak width increased.

Possible Causes

- The column is losing plate count.
- Severe contamination is present in the pumping system.

Recommended Actions

- Replace the column. Replacement instructions are on page 9-34 in Section 9, "Maintenance".
- Wash the column and pumping system with phosphate. Washing instructions are in Section 9, "Maintenance".

17. All peak heights reduced.

Possible Causes

- Wrong detector output.
- A dirty phone plug connected to the detector.
- Aged solvents.

Recommended Actions

- Is the sequencer signal cable plugged into the COMP output on the detector? Correct if necessary.
- Remove the signal cable from the back of detector, and clean the plug.
- Replace solvents A and B with fresh solutions, and purge the 140D. Instructions are in Section 9, "Maintenance".

18. All peak heights reduced intermittently.

Possible Cause

- Some liquid is flowing to waste through the flask vent valve because it is bubbling too much in the flask.

Recommended Action

- Is the flask bubble pressure 1.8 psi? If the pressure is not correct, click Default in the Pressures & Temperature dialog box to restore the default settings.

Poor Recovery, Standard Chromatogram *continued*

19. Low lysine.

PTH-hydroxylysine elutes just after PTH-valine. PTH-methyllysine elutes just after PTH-leucine. PTH-succinyllysine elutes midway between DMPTU and PTH-alanine.

Possible Causes

- Lysine is extremely sensitive to metal contamination and the peroxides that can form in solvent A as a result of THF oxidation. Generally speaking, if the lysine is normal height in the PTH-amino acid standard chromatogram (taller than leucine), but not in the residue cycles, the HPLC components of the system and solvents are not responsible for lysine degradation. Instead, there may be a problem with the reaction cartridge chemistry or delivery system.
- Metal contamination.
- Contaminated S2, which can destroy lycine.

Recommended Actions

- If you suspect S2 contamination, replace the S2.
- Replace solvent A.
- Check the vacuum tubing at the valve block manifold. If the tubing is discolored, metal contamination has occurred. Replace the valve block.

20. Low lysine & PE cysteine.

Authentic PTH-cysteine is not usually recovered in sufficient yield to be seen. PTH-dehydroalanine, generated by loss of H2S from the side chain, can be seen as the DTT derivative, although the recovery of this compound is less with cysteine than with serine.

Possible Causes

- Metal contamination. Both lysine and cystine are sensitive to metal contamination and peroxide, which can form in solvent A over a period of time.

Recommended Actions

- Replace solvent A if you suspect it is contaminated.
- Wash the column and pumping system with phosphate, or add phosphate to solvent A so that the final concentration is 100 mmol. Washing instructions are in Section 9, "Maintenance".

Poor Recovery of Residue Amino Acids

21. Low serine & threonine.

A significant amount of serine dehydrates during cleavage to form dehydroalanine, which is very reactive and unstable. The DTT added to R4 reacts with this derivative and has a stabilizing effect. This DTT-dehydroanaline derivative, commonly called delta-serine (ΔS), elutes between PTH-alanine and PTH-tyrosine. It can be used to help identify a serine residue.

A significant amount of threonine dehydrates during cleavage to form dehydro-alpha-aminoisobutyric acid. This product subsequently reacts with the DTT added to R4 to produce two to four derivatives. These derivatives elute midway between PTH-tyrosine and PTH-proline.

Possible Cause

- The Pre-Conversion Dry step is too long. This is the most critical step for these amino acids. Some liquid must still be present in the flask at the end of this step. It is better to have too much liquid left, even though this will dilute the R4. Plenty of TFA must be present for conversion to take place.

Recommended Action

- Shorten the length of function 236, *Pre-conversion Dry*. Instructions for modifying functions are on page 8-4 in Section 8, "Custom Functions, Cycles, Methods and Gradients". Run the Flask Optimization method to determine the correct pre- and post-conversion dry times. Instructions are listed in Section 6, "Optimization".

22. Low asparagine or glutamine, and high aspartate and glutamate respectively.

Possible Cause

Under typical conversion conditions, approximately 10% of PTH-asparagine and PTH-glutamine are degraded by deamidation to yield PTH-aspartate and PTH-glutamate respectively in the conversion flask. Severe deamidation is more likely the result of improper sample storage or handling.

Recommended Actions

- Review your sample handling and storage techniques. Make improvements wherever possible. Refer to Section 3, “Pre-Sequencing Sample Preparation Guidelines”, for recommendations.

Poor Recover of Residue Amino Acids *continued*

23. Low glycine.

Possible Cause

ATZ-glycine converts to PTH-glycine somewhat slowly. The reaction is only 80–85% complete during normal conversion conditions. The remaining 15–20% elutes as PTC-glycine near the end of the solvent front. The flask temperature may be too low, or the flask heater may have failed.

Recommended Actions

- Check the flask temperature. It should be 64 °C.

24. Low histidine and arginine.

Possible Causes

- Histidine and arginine are adhering to glass. Both histidine and arginine are positively charged amino acids. As such, they have an affinity for glass. For example, this problem is encountered when the PTH-amino acid (PTH-AA) Standard is stored in a glass bottle. If the bottle is clean, histidine and arginine will stick to the glass, and appear much smaller in the PTH-AA Standard chromatogram. This effect will decrease over time as the surface of the glass becomes coated. To eliminate this problem, the Applied Biosystems R5 bottle is made of polyethylene.
- Histidine and arginine dried completely on the glass fiber filter. Histidine and arginine are very difficult to extract from glass fiber filters, especially if allowed to dry completely. As such, the most critical step for histidine/arginine recovery is the post-cleavage *Dry Cartridge* step right before the *Ready Transfer to Flask* step.
- Histidine and arginine are sticking to the sides of a new flask.

Recommended Actions

- Reduce the time of the post-cleavage *Dry Cartridge* step before the *Ready Transfer to Flask* step. Reduce it 10 sec at a time, until histidine and arginine recovery is improved. Remember, however, if this step is too short, and an excessive amount of TFA (R3) remains, the sample will wash out.
- If the flask is new, run the PTH–Standards cLC method for three or four cycles to coat the sides of the flask.

Low Repetitive Yield

25. Low repetitive yield, high lag.

Lag is due to incomplete coupling, or incomplete cleavage of the N-terminal amino acid. Lag is nominally 1.5% of the residue in the previous cycle.

Possible Causes

- In Pulsed-liquid cycles, the TFA (R3) is metered by a fluid sensor. Therefore, the base (R2g) delivery should be suspected if there are no sensor errors.
- The R3 valve is partially stuck open, and TFA is constantly leaching into the system. This will neutralize the basic environment required for coupling.

Recommended Actions

- Check the R2 delivery/pressurization path for restrictions by running the R2 Leak Test Bottle Change procedure. If the vent test portion of the procedure fails, there is a blockage in the R2 pressurization path. The expected flowrate for R2 measured at the waste line at the back of the instrument during the *Del R2g, Cart (top)* function is 23 sccm at 1 psi. Use a flowmeter, or the following procedure to determine if there is a restriction. The clicking frequency of the pressure valve is used for this procedure.
  a. Set the R2 pressure to 0.3 psi.
  b. Activate function 11, *Del R2g, Cart (top),* and allow it to equilibrate for 1 min.
  c. The pressure valve clicking frequency should be approximately 2.5 clicks/sec.

26. Low repetitive yield, no lag.

Possible Cause

Wash out may have occurred. Since there is no lag, coupling and cleavage are OK. The height of PMTC, DPTU and DPU is reduced when wash out occurs.

Recommended Actions

- Is the S2 pressure set to 1.7 psi? If not, click Default in the Pressures & Temperatures dialog box to restore the default setting.
- Ensure that the BioBrene applied to glass-fiber filters is completely dry before precycling.
- Try a new lot of BioBrene.

Artifact Peaks

27. DTT-PITC adduct close to proline.

Possible Cause

- Solvent contaminated with DTT. Applied Biosystems does not add DTT to solvents S1, S2 or S3. Therefore, this adduct should not be seen.

Recommended Action

- Replace the solvent to which DTT was added to eliminate this artifact peak.

28. High aniline.

You can expect to see aniline at the sub-pmol level. Aniline elutes between PTH-asparagine and PTH-serine, and can interfere with either derivative if you are working at high sensitivity.

Possible Cause

- Dead volumes in the reaction plumbing where unreacted PITC is being trapped.
- If the size of the peak is larger for a particular cartridge, the glass blocks may need to be cleaned, or the cartridge line may not be flush with the end of the ferrule.

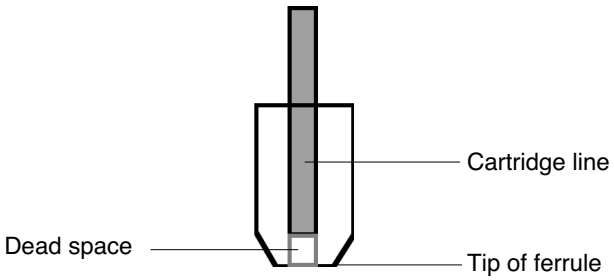

Figure 5-11. Incorrectly installed cartridge ferrule

Recommended Actions

- Thoroughly clean the glass cartridge blocks. Cleaning instructions are in Section 9, "Maintenance".
- Reinstall the cartridge line, making sure the end of the line is flush with the tip of the ferrule.
- Increase the time of function 237, *Post-conversion Dry*. As a general rule, after the flask is visibly dry, continue to dry an additional 180 sec. Increasing the time of this function will not effect amino acid recovery, since the amino acids are in the stable PTH form at this time. Instructions for modifying functions are in Section 8, "Custom Functions, Cycles, Methods and Gradients".

Artifact Peaks *continued*

29. High oxidized DTT.

Possible Cause

- R4 is bad. DTT is added to both R4 (25% TFA in water) and R5 (acetonitrile) during the manufacturing process. DTT is an oxygen scavenger, and the oxidized DTT reaction product appears as a peak immediately after the injection artifact.

Recommended Actions

- If the DTT peak is so high that PTH-aspartate rides on its shoulder, replace the R4.

Retention Time Problems

30. General retention time instability during the run.

Possible Cause

Since all peaks are eluting late, there could be a leak anywhere in the pumping system. The effective flowrate is reduced.

Recommended Actions

Procedures for detecting and repairing leaks are located in the 140D user's manual.

- Visually inspect all fittings for leaks and repair accordingly.
- Check the pump seal leak points (Figure 5-6 on page 5-14) for liquid. Repair any leaks found.
- Perform the static pressure test, and repair any leaks accordingly.

Retention Time Problems *continued*

31. Most peaks miscalled even though peak retention times reasonably stable.

Possible Causes

- A fluctuating laboratory temperature.
- A non-suitable reference peak was chosen.

Recommended Action

- Add more PMTC to the PTH-amino acid standard working solution than what is recommended in the PTH-Standard Kit product insert. PMTC tends to sublimate during flask dry-downs.
- Stabilize your laboratory temperature.
- Is your reference peak a suitable one based on the following guidelines? If not, change your reference peak.

Reference Peak Guidelines

A reference peak enables the 610A to compensate for similar shifts of all peaks in the same direction. The reference peak must be:

- Present in all residue (sequencing) cycles and the PTH-amino acid standard cycle.
- Far from amino acid peaks (± 0.25 minutes).
- The largest peak, if part of a group of non-amino acid peaks.

The PTH-standard mixture currently includes four peaks that are not amino acids: DMPTU, DPTU, DPU and PMTC. The suitability of these and other reference peaks is described below:

DMPTU is not suitable as a reference peak because it is not produced as a by-product of the N-methylpiperidine chemistry.

DPTU is only useful as a reference peak if it appears larger than the PMTC peak in the residue cycles.

PMTC is normally a larger peak than the DPTU in the residue cycles, and is an ideal candidate for a reference peak.

DPU is the oxidation product of DPTU. It can be used as a reference peak if an adequate amount is generated in each cycle.

A suitable Amino Acid can also be used as a reference peak if there is significant background in each cycle. In this case, the peak type code is *rc*.

None. If laboratory temperatures are stable, and the column has settled down, there may be no need to use a reference peak at all.

Retention Time Problems *continued*

32. Only early eluting peaks are miscalled.

Possible Cause

- TFA injected onto the column. If TFA is injected onto the column, it tends to affect the retention times and resolution of early eluting amino acids. The flask must be completely dry after the Post-conversion dry step.

Recommended Action

- Modify function 237, *Post-conversion Dry,* so the flask is completely dry upon completion of this step.

33. Retention time shift only in occasional cycles.

Possible Cause

- Air sucked into the cylinders due to a leak at the pump inlet manifold.
- A partially blocked solvent filter.

Recommended Action

- Check the fittings at 140D inlet manifold and Rheodyne valve for leaks. Repair any leaks that are found. Refer to the 140D user's manual for instructions on detecting and repairing leaks.
- Sonicate the solvent filters in nitric acid.

Retention Time Problems *continued*

34. All peaks, including injection artifact, delayed by same amount.

01/17/1995 21:26:40
When the 'Load Injector' function is finished, the Rheodyne valve must be in the inject position. When finishing step 37 cycle 15.

Figure 5-12. Injector position event log message

Possible Causes

- If the message in Figure 5-12 appears in the Event log, and the chromatogram is delayed, the injector failed to turn during the *Load Injector* step. Instead, it turned during the subsequent *Inject Position* step.

  Data collection starts as soon as liquid is sensed at the Sample loop load sensor (approximately 10 sec after the *Load Injector* step begins). However, the gradient does not start until the injector moves to the inject position, and opens a mechanical switch (approximately 30 sec after the load injector step is executed). This is caused by a bug in the Procise 1.0 firmware. The predicted frequency of occurrence is extremely low (1 in 1500 injections).

- Bad guard column.

Recommended Action

- Monitor the system pressure during a run. The pressure profile should be consistent from cycle to cycle. If it is not, replace the guard column.

Event Log Error Messages

Cartridge Load, Cartridge Outlet, Flask Load & Transfer Fluid Sensor Error Messages

36. Fluid never reaches sensor (average wet reading = 0).

In Figure 5-13, the Average Wet value is 0 because no readings were detected above the Threshold. Consequently, there are no Wet values.

Because there are so many possible causes for this type of error, the recommended action will be given directly after each possible cause. The possible causes are shown in boldface type.

Dry = 716		Threshold = 1075		Average Wet = 0	
Dry	Wet	Dry	Wet	Dry	Wet
3846	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
Load R1 Cart (lg loop)					

Figure 5-13. Sample event log message—fluid never reaches the sensor

Empty bottle.

When a bottle is close to empty, the fluid in the delivery line becomes segmented with argon bubbles, causing delivery to slow until it eventually stops.

- When replacing an empty bottle, be sure to run the respective bottle change procedure so that the delivery line is backflushed before the line is primed. The bottle change procedure is in Section 7, "Tests and Procedures".

Incorrect Set pressure.

If the Set pressure is too low, the delivery will slow down and may stop altogether. If it is too high, the chemical will be subject to increased outgassing. A Set pressure of 0 may be the result of an aborted leak test.

- Check the *Set* and *Actual* pressures in the Pressures & Temperatures dialog box. A Set pressure of 0 may be the result of an aborted leak test. Always allow a leak test to finish, or click Next Step to jump to the end of a step.

Cartridge Load, Cartridge Outlet, Flask Load & Transfer Fluid Sensor Error Messages *continued*

- Click Default in the Pressures & Temperatures dialog box to restore the default operating pressures for the system. Turn the heaters back on if necessary. Click Execute. The pressure and vent valves will actuate, causing the Actual bottle pressures to reach the Set pressures. Non-bottle Actual pressures may remain higher than Set pressures until an associated function is activated.

Note	*If you are using version 1.00 firmware, the R3 default operating pressure is 1.2 psi. The R3 liquid-phase delivery may be more reliable at 1.5 psi. The R3 gas-phase delivery is optimal at 0.8 psi.*

If the Actual bottle pressure does not follow the Set pressure within ± 0.1 psi after clicking Execute, there is a problem with the pressure management system. Before replacing parts such as the respective pressure transducer, ensure that the tubing connected to the pressure transducer is unrestricted and free of crimps.

Corrupted RAM.

- Reset the sequencer as follows:
  a. Shutdown the Macintosh.
  b. Power-down the sequencer.
  c. Unplug the Melcard (firmware).
  d. Power-up the sequencer.
  e. Power-down the sequencer.
  f. Plug in the Melcard.
  g. Power-up the sequencer.
  h. Reboot the Macintosh.

Manual regulator pressure too low.

- The manual regulator gauge should be set to 5.5 psi. Check the pressure by lowering the plumbing plate and looking at the manual regulator pressure gauge. If necessary, set the regulator to 5.5 psi.

Bottle pressure leak.

- Remove the leaking bottle and examine the bottle seal. Replace the seal if it is cracked.
- Run a leak test on the bottle from the Bottle Change dialog box. Make repairs as necessary.

Cartridge Load, Cartridge Outlet, Flask Load & Transfer Fluid Sensor Error Messages *continued*

Restricted pressurization path.

The delivery pressures are monitored at the pressure management printed circuit board (PCB). A flow restriction between the PCB and a bottle can result in reduced bottle pressure during a delivery.

- Run a Leak test on the appropriate bottle, for example the R3 leak test. The actual pressure reported in the Event log after the venting portion of the test should be no greater than 0.1 psi. If it exceeds this value, determine the source of the restriction. The restriction will be located between the pressure transducer and the vent valve for the respective bottle. Start with the check valve.

Restricted fluid delivery path.

If there is no problem with bottle pressurization, the fluid delivery path may be blocked.

- First determine whether any other deliveries are affected by examining Event log and sensor data. Concentrate on flow paths that are common to other affected deliveries. If the problem is unique to a single chemical, check the appropriate delivery line for crimps or restrictions.

Insufficient vacuum assist.

- Check the vacuum gauge. The vacuum should be no less than 12 in. Hg. If less than 12 in., adjust the vacuum switch located on the vacuum manifold assembly. This procedure is located in Section 9, "Maintenance", on page 9-33.

Restricted waste lines.

- Check the line from the waste bottle to the fume hood. Remove any restrictions such as trapped liquid.

Restricted flushing path.

Before a load function, the plumbing pathway is flushed with high pressure argon to ensure it is dry. All flushing functions use the high pressure argon supply connected to the respective 3-way valve. The high pressure input to valves 46 and 48 is connected directly to the manual regulator (5.5 psi). For valve 47, it is connected to regulator 8 on the pressure management board.

- Run the procedure, "Testing Gas Flow Rates", on page 9-29 in Section 9, "Maintenance", to check for restrictions. Remove restrictions if found.

Cartridge Load, Cartridge Outlet, Flask Load & Transfer Fluid Sensor Error Messages *continued*

3-way valve mechanical failure.

If a 3-way valve fails to switch from low pressure input to high pressure (5.5 psi) input during a flush function, the effectiveness of the flush will be compromised.

- Run the procedure, "Testing 3-way Valves", in Section 9, "Maintenance", to determine whether the valve is switching correctly. If it is not, replace the valve.

Cartridge Load, Cartridge Outlet, Flask Load & Transfer Fluid Sensor Error Messages *continued*

37. Too many bubbles in liquid slug (average wet reading ≠ 0).

When too many bubbles are present, fluid is fragmented as it flows through the sensor. Fragmentation tends to slow down the delivery, resulting in longer than normal initial Dry readings. Because the sensor never detects a sufficient number of consecutive Wet readings, the function times out, and an error is posted in the Event log. All 18 fields for Dry/Wet readings will contain a non-zero value (Figure 5-14).

Because there are so many possible causes for this type of error, the recommended action is given directly after each possible cause. Possible causes are shown in boldface type.

Dry = 716		Threshold = 1075		Average Wet = 2744	
Dry	Wet	Dry	Wet	Dry	Wet
2800	180	8	98	78	165
78	183	89	173	78	79
84	187	167	25	74	171
Load R1 Cart (lg loop)					

Figure 5-14. Sample event log error message—too many bubbles in liquid slug

Incorrect Set pressure.

If the Set pressure is too low, the delivery will slow down and may stop altogether. If it is too high, the chemical will be subject to increased outgassing.

- Check the *Set* and *Actual* pressures in the Pressures & Temperatures dialog box. A set pressure of 0 may be the result of an aborted leak test. Always allow a leak test to finish, or click Next Step to prematurely advance to the end of a step.
- Click Default in the Pressures & Temperatures dialog box to restore the default operating pressures for the system. Turn the heaters back on if necessary. Click Execute. The pressure and vent valves will actuate, causing the Actual bottle pressures to reach the Set pressures. Non-bottle Actual pressures may remain higher than Set pressures until an associated function is activated.
- At high altitudes, a set pressure lower than the default pressure may be required. For example, if R1 is failing due to overgassing, reduce the set pressure from 2.5 psi to 2.0 psi.

Cartridge Load, Cartridge Outlet, Flask Load & Transfer Fluid Sensor Error Messages *continued*

- If the Actual bottle pressure does not follow the Set pressure within ± 0.1 psi after clicking Execute, there is a problem with the pressure management system. Before replacing parts such as the respective pressure transducer, ensure that the tubing connected to the pressure transducer is unrestricted and free of crimps.

Fume hood vacuum too high.

If suction on the vent/waste line is too great, gas is pulled out of solution during a delivery, severely segmenting the flow.

- Ensure that the fume hood vacuum complies with the guidelines listed in the Procise 49X cLC Protein Sequencing System Pre-installation Manual (P/N 904203).

Sequencer has been sitting idle.

If the sequencer is idle for a long period of time with the default pressure settings, a significant amount of argon is absorbed by the chemicals. The argon will tend to outgas when the chemical is next delivered.

- To minimize this problem, adjust all of the bottle Set pressures to 1 psi before leaving the sequencer idle.

Injector Sample Loop Fluid Sensor Errors

38. Sample loop full errors, and no amino acid peaks.

Possible Causes

The small dry reading of 29 (0.15 sec) in the sample loop full sensor error (Figure 5-15) indicates that the injector was actuated almost immediately after the Load Injector function started, and before any liquid could reach the sample loop. The result is an air injection.

- The sample loop load sensor was incorrectly initialized.
- Residual liquid was not completely flushed out the injection system prior to the *Load Injector* step.

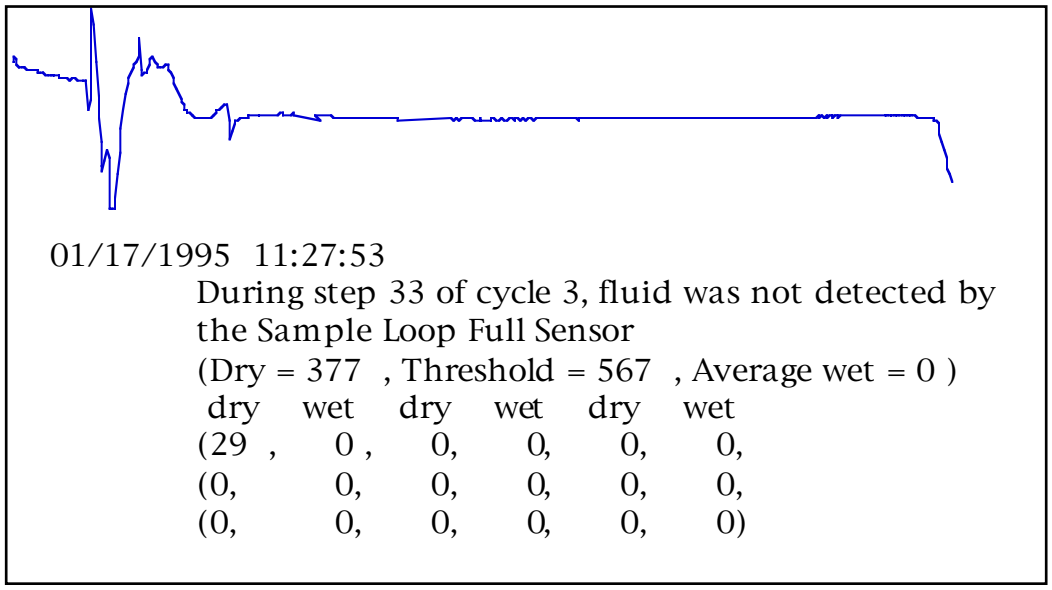

Figure 5-15. Air injection

Injector Sample Loop Fluid Sensor Errors *continued*

Recommended Actions

- Are the PEEK fittings are at ports 5 & 6 of the injector properly installed? Installation is correct if the fittings do not leak, and remain in place when you tug on them. If not, reinstall the fittings as follows:
  a. Remove the orange Teflon lines from ports 5 & 6 of the injector.
  b. Cut 1 in. off each line. If this makes the lines too short, replace the lines.
  c. Route the tubing connecting the injector to the waste bottle through the hole in the panel to the left of the injector.
  d. Reconnect the lines to the injector. Finger-tighten the PEEK fittings, then tighten them 1/4-turn more using a wrench.
  e. Slide the sensors as close to the peak fittings as possible.
  f. Gently tighten the pre-tee fittings only enough to hold the sensors in place.
- Replace the pick-up line and flask if they are dirty. Always replace the pick-up line instead of re-adjusting it to prevent multiple occlusions caused by repositioning the fitting and ferrule.

39. Sample loop full errors with partial injection.

Possible Cause

Liquid passed the sample loop full sensor prior to the injection.

Dry = 1019		Threshold = 1530		Average Wet = 3212	
Dry	Wet	Dry	Wet	Dry	Wet
430	1530	10	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0

Figure 5-16. Sample loop full sensor

Recommended Actions

- Reduce the *Concentrate Sample* step (function 238) by 5 sec.
- Ensure that two complete loads of S4 were delivered to the flask. To do this, you must analyze the Flask load loop sensor data file. This file is generated when the *Always Report Sensor Data* box is checked in the Preferences dialog box of the Sequencer pull-down menu.

Injector Sample Loop Fluid Sensor Errors *continued*

40. Sample loop full and load errors; no injection.

Possible Causes

An error such as the one shown in Figure 5-17 will pause the run at the end of the **next** flask cycle. The chromatogram data file from the last cycle will be collected as long as the injection for that cycle was OK. It will appear as the last chromatogram in the 610A file. The second-to-last chromatogram, therefore, is the data from the flask cycle in which the error occurred. If a chart recorder is being used to collect data simultaneously, it will include data from the cycle in which the error occurred.

Event log data shows that fluid arrived at the sample loop full sensor (sample loop inlet) in the normal time. However, the fluid never made it to the sample loop load sensor (sample loop outlet). This can occur when:

- The tail-end of the slug leaving the flask becomes severely fragmented, slowing delivery down to a halt.

The problem is exacerbated by:

- A flow restriction
- A leaking flask
- An improperly positioned pick-up tube

```
01/17/1995  21:26:40
          When the 'Load Injector' function is finished, the Rheodyne
        valve must be in the inject position.  When finishing step 37
        cycle 15.

01/17/1995  21:26:40
          During step 37 of cycle 15, fluid was not detected by
          the Sample Loop Load Sensor
          The sequencer will pause at end of this cycle.
          (Dry = 232  , Threshold = 349  , Average wet = 0    )
           dry    wet    dry    wet    dry    wet
          (7576 , 0,       0,     0,     0,     0,
          (  0,    0,      0,     0,     0,     0
          (  0,    0,      0,     0,     0,     0)
01/17/1995  21:26:40
          During step 37 of cycle 15, fluid was not detected by
          the Sample Loop Full Sensor
          (Dry = 377  , Threshold = 567  , Average wet = 1045 )
           dry    wet    dry    wet    dry    wet
          (334  , 7259 , 0,      0,     0,     0)
          (  0,      0,    0,     0,     0,     0)
          (  0,      0,    0,     0,     0,     0)
```

Figure 5-17. No injection

Injector Sample Loop Fluid Sensor Errors *continued*

Recommended Actions

- Are the PEEK fittings at ports 5 & 6 of the injector properly installed? Installation is correct if the fittings do not leak, and remain in place when you tug on them. If not, reinstall the fittings as follows:
  a. Remove the Teflon lines from ports 5 & 6 of the injector.
  b. Cut 1/4-in. off each line. If this makes the lines too short, replace the lines.
  c. Reconnect the lines to the injector. Finger-tighten the PEEK fittings, then tighten them 1/4-turn more using a wrench.
  d. Slide the sensors as close to the peak fittings as possible.
  e. Gently tighten the pre-tee fittings only enough to hold the sensors in place.
- Replace the pick-up line and flask if they are dirty. Always replace the pick-up line instead of re-adjusting it to prevent multiple occlusions caused by repositioning the fitting and ferrule.
- Reset the sequencer as follows:
  a. Shutdown the Macintosh.
  b. Power-down the sequencer.
  c. Unplug the Melcard (firmware).
  d. Power-up the sequencer.
  e. Power-down the sequencer.
  f. Plug in the Melcard.
  g. Power-up the sequencer.
  h. Reboot the Macintosh.

Other Event Log Error Messages

41. Argon tank pressure too low.

Argon tank pressure is too low. The sequencer is paused.

Figure 5-18. Event log message—Argon tank pressure too low

Possible Cause

The message shown in Figure 5-18 is generated when the argon supply pressure drops below 60 psi.

Recommended Actions

- Is sufficient pressure being supplied by the argon tank regulator? If not, readjust the regulator to 70 to 80 psi.
- Is the 1/4-inch tubing securely connected to the high pressure transducer on the pressure control board (visible from the top of the instrument after removing the top cover). If not, properly secure the tubing.

42. Cannot reach set temperature.

Unable to reach cartridge, flask or column temperature setpoints. The sequencer is paused.

Figure 5-19. Event log message—Unable to reach temperature setpoints

Possible Cause

During the *Begin* step of a cycle, the cartridge, flask and column temperatures are monitored, and an error message is generated if any fail to reach setpoint within 20 min.

Recommended Actions

- Are the method temperatures within the ambient to 70 °C range? If not, adjust the temperatures accordingly.
- Test the thermal fuse on the respective heater printed circuit board. Replace the fuse if necessary.

Other Event Log Messages *continued*

43. Communication with HPLC components of the system lost.

Communication with the HPLC was lost. Reset the HPLC.

Figure 5-20. Event log message—communication with HPLC components of the system lost

Possible Cause

The event log message shown in Figure 5-20 indicates the sequencer can no longer communicate with the 140D via the RS232 cable.

Recommended Actions

- Is the 140D powered-up? If not, power-up the 140D.
- Is the RS232 cable between the 140D and the sequencer properly seated? If not, reseat the cable.
- Cycle the 140D power by turning the instrument off and on.

44. Event buffer overrun.

Event buffer overrun. Some event messages may have been lost.

Figure 5-21. Event log message—event buffer overrun

Possible Cause

The message shown in Figure 5-21 indicates communication between the Macintosh and the sequencer was lost, and the sequencer posted errors in the event buffer.

Because this error is normally the result of a sequencer-Macintosh communication failure, this message will not be transferred from the sequencer to the Macintosh until communication is re-established.

Recommended Actions

- None.

Other Event Log Messages *continued*

45. Injector position error and no corresponding sample loop sensor errors.

When the “load injector” function is started, the Rheodyne valve must be in the load position. When starting step (a) of cycle (b), the Rheodyne valve was in the inject position.

Figure 5-22. Event log message—start injector position error

When the “Load injector” function is finished, the Rheodyne valve must be in the inject position. When finishing step (a) of cycle (b), the Rheodyne valve was in the load position.

Figure 5-23. Event log message—finish injector position error

Possible Cause

- The injector did not move to the correct position. Either of the event log messages shown in the figures above is generated.

Recommended Actions

- If a sample loop load error is generated, an injector position error will be generated at the same time by default. Follow the troubleshooting information provided for reference number 40 on page 5-43.
- Does your flask cycle include a *Load Position* step prior to the *Load Injector* step? If not, modify the function to include a *Load Position* step prior to the *Load Injector* step. Instructions for modifying cycles are on page 8-11 in Section 8, "Custom Functions, Cycles, Methods and Gradients".
- Check the injector actuator mechanism by moving the injector between the load and inject positions while in Manual mode. You should hear the valve switching and the vacuum out-gassing if the mechanism is working properly.

Other Event Log Messages *continued*

46. Insufficient data collection memory for cycle.

Insufficient data collection memory for cycle (a). The sequencer is paused.

Figure 5-24. Event log message—insufficient data collection memory for cycle

Possible Cause

- Insufficient memory available in the data buffer.

  During the *Begin* step of a cycle, the system control software determines whether there is enough free space is available in the data buffer to collect the chromatogram for that cycle. If the amount of memory is insufficient, the sequencer will wait at the *Begin* step for up to 4 min. If enough memory is still not available, the message shown in Figure 5-24 is sent to the event log.

  This error is typically the result of a sequencer-Macintosh communication failure. As such, the message is not transferred from the sequencer to the Macintosh until communication is re-established.

Recommended Actions

- None.

47. Invalid sensor dry reading.

Sensor (a) does not have a valid dry reading.

Figure 5-25. Event log message—invalid sensor dry reading

Possible Cause

- Init Sensor procedure not run after resetting the memory card.

  The dry reading for each fluid sensor is established automatically at the start of each sequencing run during the Init Sensor procedure. These readings are stored in battery-backed memory until overwritten during subsequent Init Sensor procedure execution.

  Dry readings are lost when the instrument is reset by pulling the memory card. If a function utilizing a sensor is executed after a reset, and prior to running the Init Sensor procedure, the message shown in Figure 5-25 is sent to the event log.

Recommended Action

- Run the Init sensor procedure to re-establish Dry readings. The procedure is in Section 7, "Tests and Procedures".

Other Event Log Messages *continued*

48. Power failure.

Power failure occurred on mm/dd/yy, at hh:mm:ssc

Figure 5-26. Event log message—power failure

A power fail occurred while sequencing. The run will be paused on the End step of the active cycle.

Figure 5-27. Event log message—power failure during a run

Possible Cause

- A power failure occurred.

Recommended Action

- If no other instruments in the lab experienced the same power failure, make sure all power cords for the system are properly connected to the instruments and power outlets.

49. Vacuum assist activated more than once every 8 hours.

Vacuum assist activated.

Figure 5-28. Event log message—vacuum assist activated too often

Possible Cause

- The vacuum system is leaking.

Recommended Action

- Inspect the valve block vacuum lines for discoloration. If one of the lines is discolored, replace the associated valve block. The replacement procedure is in Section 9, "Maintenance".

  If the vacuum lines look OK, the vacuum Clippard valve may be partially stuck open. Rebuild the vacuum Clippard valve on the vacuum assist assembly.

Leak Test Error Messages

50. Actual pressure above 5.5 psi.

Possible Cause

The Actual pressure for a particular regulator in the Pressures & Temperatures dialog box is above 5.5 psi (the manual regulator setting).

Recommended Action

- Is the manual regulator gauge reading 5.5 psi? If it is not, adjust the gauge while executing function 137, *Flush Input Block*.
- Reset the sequencer as follows:
  a. Shutdown the Macintosh.
  b. Power-down the sequencer.
  c. Unplug the Melcard (firmware).
  d. Power-up the sequencer.
  e. Power-down the sequencer.
  f. Plug in the Melcard.
  g. Power-up the sequencer.
  h. Reboot the Macintosh.
- Replace the pressure transducer for the affected position. The replacement procedure is in Section 9, "Maintenance".
- If the problem persists, the pressure control board or the I/O board may be malfunctioning. Call Applied Biosystems.

51. All leak tests fail.

Possible Causes

- Incorrect grade of argon is being used.
- The vent line is obstructed.
- Manual regulator not set to 5.5 psi.

Recommended Actions

- Is the correct grade of argon installed? If not, replace it with the proper grade of argon (99.998% purity or greater).
- Visually inspect the vent line that runs from the sequencer to the fume hood for obstructions such as condensation. Remove any obstructions.
- Is the manual regulator set to 5.5 psi? If not, adjust the regulator setting to 5.5 psi.

Leak Test Error Messages *continued*

52. Failing vent test.

Possible Causes

- A check valve malfunction.
- A vent line obstruction.
- Malfunctioning Angar vent valve.
- Blockage between the pressure transducer and the waste bottle.

Recommended Actions

- Replace the malfunctioning check valve (the one connected to the bottle failing the vent test).
- Clear obstructions from the vent line.
- Replace or repair the Angar valve.

53. Flask failing vent test.

Possible Cause

The X3 pressure check valve is malfunctioning.

Recommended Actions

- Replace the X3 pressure check valve.

54. Leak test fails because pressure too high.

Possible Cause

A Lee valve on the pressure control board is leaking.

Recommended Action

- Replace the Lee valve. Follow the procedure in Section 9, "Maintenance".

Leak Test Error Messages *continued*

55. Cartridge leak test fails.

Possible Causes

- If all cartridges fail the leak test, there is a leak somewhere before the cartridge.
- The seal and/or filter in the cartridge is worn.
- A cartridge ferrule is scratched.
- The cartridge is dirty.

Recommended Actions

- If the source of the leak is before the reaction cartridge, run the cartridge reagent block and cartridge input block leak tests listed in Section 5, "Tests and Procedures".
- Replace the seal and filter in the reaction cartridge. Be sure to centrally position the filter.
- Examine the cartridge ferrule(s) for scratches. If it is a multi-cartridge instrument, swap out cartridge components until the scratched ferrule is identified.
- Clean dirty reaction cartridges. Remove the glass cartridge blocks, and clean those as well. Instructions are in Section 9, "Maintenance". Sonicate the cartridge and cap in methanol to clean the threads.

Software & Communication Problems

Lock-ups

Guidelines for all Lock-ups

To help recover from and troubleshoot a lock-up, refer to the guidelines listed on pages 5-56 through 5-58. These guidelines describe how to:

- Gather information during a lock-up
- Gather information after a lock-up
- Recover from a lock-up

56. Frequent Macintosh lock-ups.

Possible Causes

- The RAM is too fragmented.
- The Desktop application is running too slowly. Desktop is the application that, among other things, keeps track of where files are located and which have been marked for deletion. Over time, much of this information becomes redundant. As the amount of information that must be searched grows, the computer's operation will continue to slow.
- Memory is incorrectly allocated.

Recommended Actions

- Restart the Macintosh once a day to defragment the RAM.
- Rebuild the desktop once a month. To rebuild the desktop, restart the Macintosh while holding down the OPTION and keys.
- Check the Memory allocation by choosing Control panels under the menu, and selecting the Memory control panel. The Macintosh memory should be set up as follows:

Memory Component	Quadra 650 (system 7.1)	Power PCs (system 7.5)
Disk cache	512K	512K
Modern memory manager	N/A	ON
Virtual memory	OFF	ON (10 MB)
RAM Disk	OFF	OFF

- As a last resort, reformat the hard drive. Instructions are in Section 9, "Maintenance".

Lock-ups *continued*

57. Macintosh locks up during data collection.

If the sequencer is set up to run a considerable number of cycles, set up the 610A data analysis software as follows:

1. From the Acquisition pull-down menu, select Configure.
2. In the "Collect Data from:" field (Figure 5-29), select Procise Sequencer. Do **not** select "Leave window open when done".

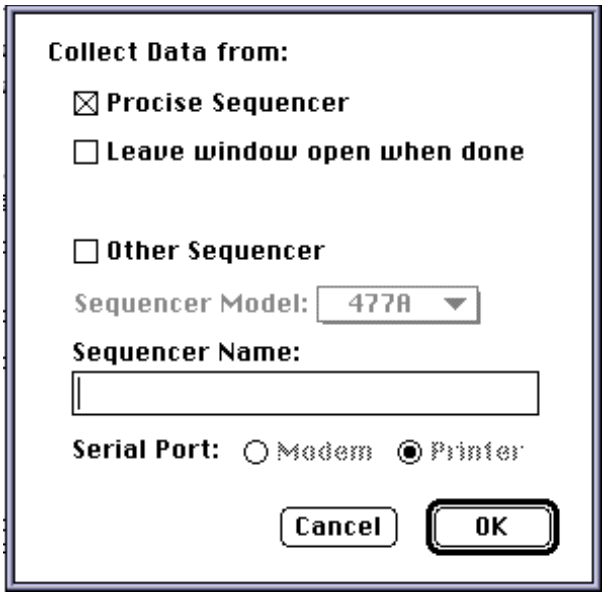

Figure 5-29. 610A configuration menu

In this mode, only the chromatogram being collected is displayed. Once collection is complete, the window closes. Therefore, once all cartridges have completed their runs, there will be no sign of a chromatogram on the screen. The data is stored in the respective file on the hard disk.

Lock-ups *continued*

58. No communication between Macintosh and sequencer.

Possible Causes & Recommended Actions

Under normal circumstances, the COM light inside the sequencer is on, indicating the Macintosh and sequencer are communicating. This light is visible through the front panel visor of the sequencer. If the light turns off, communication has been lost. Consequently, sequence data and event information cannot be loaded from the sequencer to the Macintosh. If this occurs, the run will be paused to protect your data.

- A dialog box on the Macintosh generated by the Procise application will cause the COMM light to turn off. Select one of the prompts in the dialog box to re-establish communication.

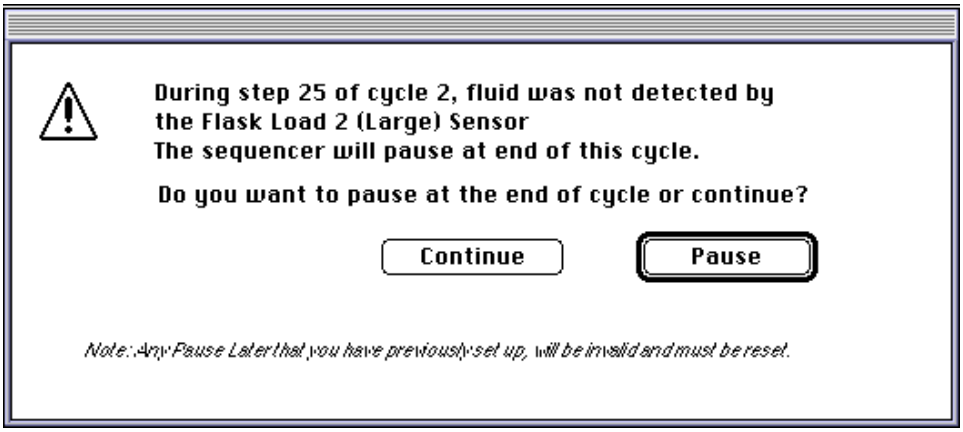

Figure 5-30. Procise dialog box

- The Macintosh has locked-up. Restart the Macintosh.
- The sequencer has locked up. Reset the sequencer as follows:
  a. Shutdown the Macintosh.
  b. Power-down the sequencer.
  c. Unplug the Melcard (firmware).
  d. Power-up the sequencer.
  e. Power-down the sequencer.
  f. Plug in the Melcard.
  g. Power-up the sequencer.
  h. Reboot the Macintosh.

The communication cable between the Macintosh and sequencer is loose. Power-down the two instruments, and reseat the cable.

Lock-ups *continued*

Gathering Information During a Lock-up

Note the answers to the following general, Macintosh computer, and sequencer questions.

IMPORTANT *Note the circumstances under which the lock-up occurs. This information is critical for determining the cause of the problem.*

General

1. What time did the lock-up occur (morning, over night etc)?
2. Did any other instrumentation experience a problem?
3. What were the sequence of events that preceded the lock-up?
4. If a system lock-up has occurred in the past, has it occurred under the same circumstances? Can it be reproduced?

Macintosh Computer

1. Does the mouse cursor move?
2. Are any of the screen functions active?
3. Is a 610A "Collecting" window open?
4. Is the step time counting down on the Monitor Run screen?
5. Which steps are displayed?
6. What is the most current information in the Event log?

Sequencer

1. Is the door panel COMM LED lit?
2. Is the door panel SEQ LED lit?
3. Is it apparent, from audible "clicking" of the valves, that the sequencer is still running?
4. Are any of the red Error LEDs on the inner panel lit?
5. Are any of the green Status LEDs on the inner panel lit?

Lock-ups *continued*

Recovering from a Lock up

Try the following suggestions, one at a time, in sequence until normal operation is restored. Resetting (cold booting) the sequencer as described below is a last resort because it will erase the sequencer memory including the current run conditions, chromatogram data and the Event buffer. The Event buffer may contain valuable information which has not yet been transferred to the Macintosh Event log file on the hard disk.

IMPORTANT	*If you wish to abort the run if communications have been re-established after step 3, wait 5 min before clicking the stop button. This will ensure that all data is transferred from the sequencer to the Macintosh.*

Reboot the Macintosh:

1. Reboot the Macintosh computer. (Re-launch the Procise application if it does not automatically launch as part of the start-up routine).
2. Power-down and power-up the sequencer.
3. Reboot the Macintosh computer.

Reset (cold boot) the sequencer:

1. Power-down the sequencer.
2. Unplug the Mel card (left-hand side, upper, rear corner).
3. Power-up the sequencer.
4. Power-down the sequencer.
5. Plug in the Mel card.
6. Power-up the sequencer.
7. Reboot the Macintosh computer. The message “Execution of Cold start (all RAM has been initialized)” should appear in the Event log. If it does not, make sure that jumper, W6, has been removed from the CPU PCB.

Lock-ups *continued*

Gathering Information after a Lock-up

Gather the following information:

1. Check and verify that the instrument has the most recent versions of software/firmware.
   - Mel card
   - Procise operating software
   - 610A software
2. What version of Macintosh Operating System is being used?
3. Print relevent sections of the Procise Event log.
4. Print the 610A Status log (in the Preferences folder).

Print 610A status log complete with service information:

1. While in the 610A application, hold down the key while selecting the menu.
2. Select Show Service info. After a short time, the 610A status log will open. The Macintosh service information will be appended to the end of the 610A status log.

- Select Print from the File menu.

Procise, 610A and Macintosh Operating System Errors

59. File error every time 610A is launched.

Possible Cause

- A corrupted virtual A/D file.

Recommended Action

Delete the corrupted file by following this procedure:

1. Restart the Macintosh.
2. Move all virtual A/D files from the Procise folder to the Desktop.
3. Launch the 610A application. Move the files back into the Procise folder, one at a time, until one of them causes the error.
4. Delete the file that caused the error.

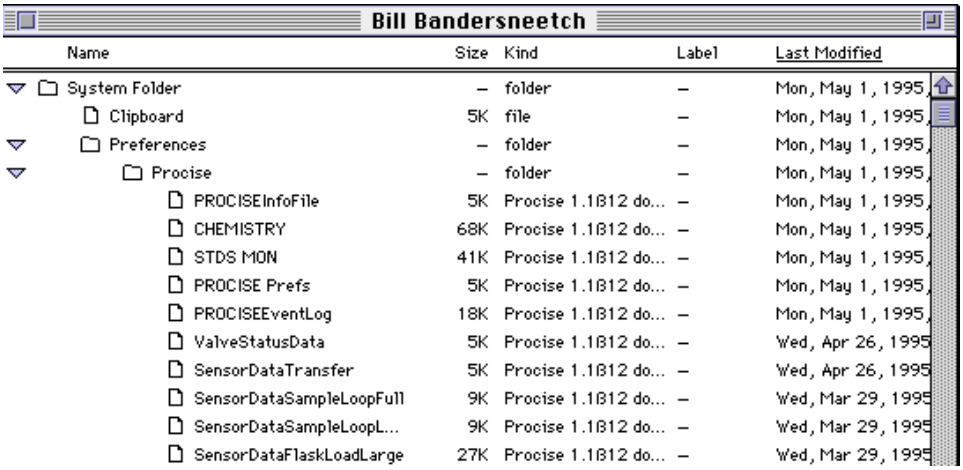

Figure 5-31. Virtual A/D file location

60. File or disk error every time Procise is launched. Usually [PROCISE error -48].

Possible Causes

- A corrupted virtual A/D file.
- No printer selected.

Recommended Actions

- If you suspect a corrupted virtual A/D file, see #59 above.
- If using the system 7.1 operating system, ensure that a printer driver is selected from the Chooser menu (under the menu). This is necessary even if a printer is not physically connected.

Procise, 610A and Macintosh Operating System Errors *continued*

61. File missing error when Procise launched.

Possible Cause

- The chemistry file is not in the Procise folder.
- The name of the chemistry file has been changed.

For Procise version 1.1, the chemistry file must be named Chemistry 1.1, and must be located in the Procise folder.

Recommended Action

- Rename the chemistry file to Chemistry 1.1 if necessary.
- If the chemistry file is missing from the Procise folder, use the Find File command from the File pull-down menu to locate it. Move the file to the Procise folder.

62. File already exists error generated by the 610A.

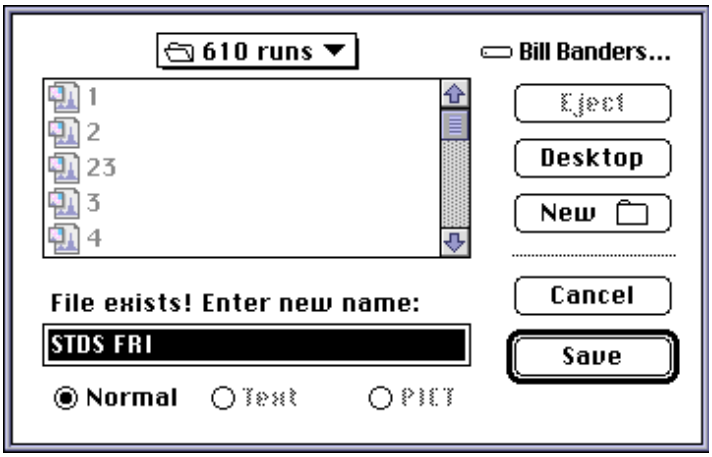

Figure 5-32. 610A file already exists

Possible Causes

- The ABI 610A Data Analysis software application displays this message to help prevent accidental file deletion. An error message like the one shown in Figure 5-32 will be generated if an attempt is made to use an existing filename.
- The operator quit the 610A application during data collection.

Recommended Actions

- We recommend including the date as part of the filename, or some other strategy to ensure exclusivity of filenames.
- Do not quit the 610A application during data collection. If you quit the 610A application during data collection, this message will be generated when the application is relaunched.

Procise, 610A and Macintosh Operating System Errors *continued*

63. "No data has been collected for 12 hours" dialog box message generated.

Possible Cause

- The *End of Run* flag was not set, probably because the system crashed.

  When a run is completed or stopped by the user, an End of Run flag is set in the virtual A/D file. The 610A constantly monitors these files, and transfers new data to a 610A file with the same name. If the End of Run flag is set, the 610A will delete the virtual A/D file, because a 610A version of the complete file now exists.

Recommended Action

- Check the PROCISE folder (located in the Preferences folder) for residual virtual A/D files after a system crash. Delete these files by dragging them to the Trash, and emptying the Trash.

64. 610A does not print all cycles.

Possible Cause

- Last chromatogram not displayed in the window before attempting to print.

Recommended Action

- Display the last chromatogram in the chromatogram window before attempting to print.

Pump & Detector Error Messages

785A UV/VIS Detector

65. Detector (785) beeping.

Possible Causes

The 785A UV/VIS detector uses a liquid sensor and beeper to indicate a possible flowcell leak.

- The flowcell is leaking.
- The sensor in the detector is not initialized correctly.
- The sensor in the detector is not positioned correctly.

Recommended Actions

- Stop the 140D, soak up the spill, and rebuild the flowcell. Follow the procedure, "Replacing the 785A Detector Flowcell Windows", in Section 9, "Maintenance", to rebuild the flowcell.
- Cycle the power on the detector.
- Reposition the sensor so it is not touching the drip tray. Cycle the power on the detector.

6 Optimization

Contents

Optimizing the Injector

Overview

During flask cycles, the sample is reconstituted in 10% acetonitrile (S4C). This ensures that hydrophobic amino acids will go into solution. For consistent chromatography, however, the percent of acetonitrile must be reduced prior to sample injection.

To reduce acetonitrile content, two *Concentrate Sample* steps are included in all flask cycles. The duration of these steps determines the percent of sample injected. Therefore, the duration of the Concentrate Sample steps must be optimized so the correct amount of sample is injected.

Optimize the injector percentage if a large amount of Sample Loop Full error messages are recorded in the Event Log.

Procedure

Setup the sequencer to run the procedure:

1. Scroll to the Functions dialog box.
2. Ensure that the global time for the *Concentrate Sample* step (step 238) is 100 sec.
3. Place a mark 1 in. from the hexagonal tip of the 5/16-in. bushing on the yellow tubing connected to valve block port 42.
4. Scroll to the Start Run dialog box.
5. Setup a run with the following conditions (Figure 6-1 on page 6-4):

Parameter	Setting
Cartridge A	1st
Filename	Your choice
Number of cycles	10
Method	Injector Optimization cLC

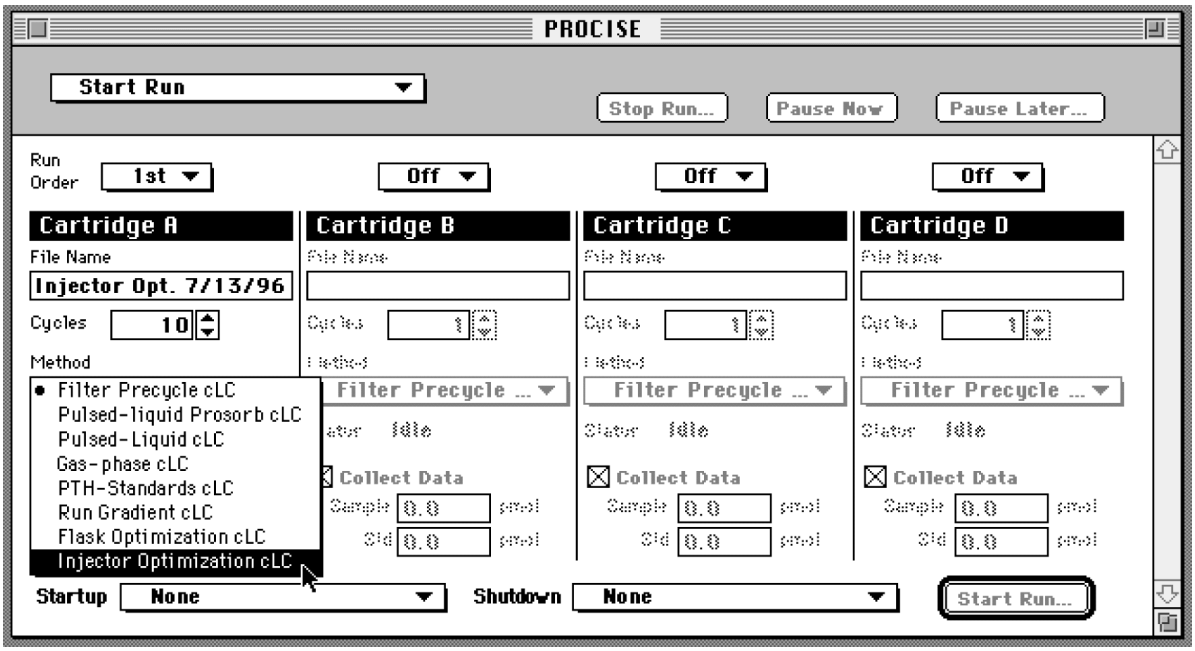

Figure 6-1. Start Run dialog box setup to optimize the injector

Run the procedure and optimize:

1. Click Start Run. The Init Sensor procedure will start running. You can click Jump to advance to the last step of this procedure.
2. If the flask temperature is 64 ˚C when the run pauses at the Begin step of the flask cycle, click Next Step to start the Injector Optimization method.
3. Click Pause Later, and configure the run to pause on Cartridge A at the end of the first cycle.
4. At the end of the first cycle, look for the injection slug in the plumbing line. Ideally, the *end* of the slug should be between the mark you made on the tubing and the valve block. It should not in the pick-up line connected to port 41.
5. If the end of the injection slug is not in the correct location, modify the Concentrate Sample time global value in the Functions dialog box. Increase or decrease the value as appropriate in **5 sec increments only**.

IMPORTANT *Do not increase the Concentrate Sample step time by more than 5 sec at a time. Otherwise, an air injection might occur. An air injection will damage the column.*

6. Click Resume.

7. Once you have determined the correct value for the Concentrate Sample step, run at least one more cycle to confirm the optimization.

Optimizing Flask Dry Times

Overview

Use the following procedure to optimize the global time values for the Pre- and Post-Conversion Dry steps in all flask cycles. We recommend you optimize these values whenever the flask and pick-up line are cleaned or replaced.

Procedure

Setup the sequencer:

1. Install a reaction cartridge in the cartridge A position on the sequencer.
2. Perform a leak test on cartridge A.
3. Scroll to the Start Run dialog box, and configure cartridge A as follows (Figure 6-2 on page 6-7):

Parameter	Setting
Cartridge A	1st
Filename	Your choice
Number of cycles	5
Method	Flask Optimization cLC

Start the run:

1. Click Start Run. The Init Sensor procedure will begin. You can click Jump to advance to the last step of this procedure.
2. Once the cartridge and flask have reached the proper temperatures, the Flask Optimization cLC method will begin.
3. At the first Pause, check the flask for liquid. The flask should not be completely dry. If the flask is not dry, proceed to step 4.

   If the flask is dry, check the pressure settings on the sequencer, which should be set to the default values. If the pressure settings are not correct, reset them to the default settings, and start the procedure over.
4. Click Resume.
5. Click Hold as soon as the Pre-Conversion step begins. When the flask contents visibly stop bubbling (between 5 µL and 10 µL of liquid remaining in the flask), note both the Time and Remaining values shown. Be sure to mark these as Pre-Conversion Dry values.

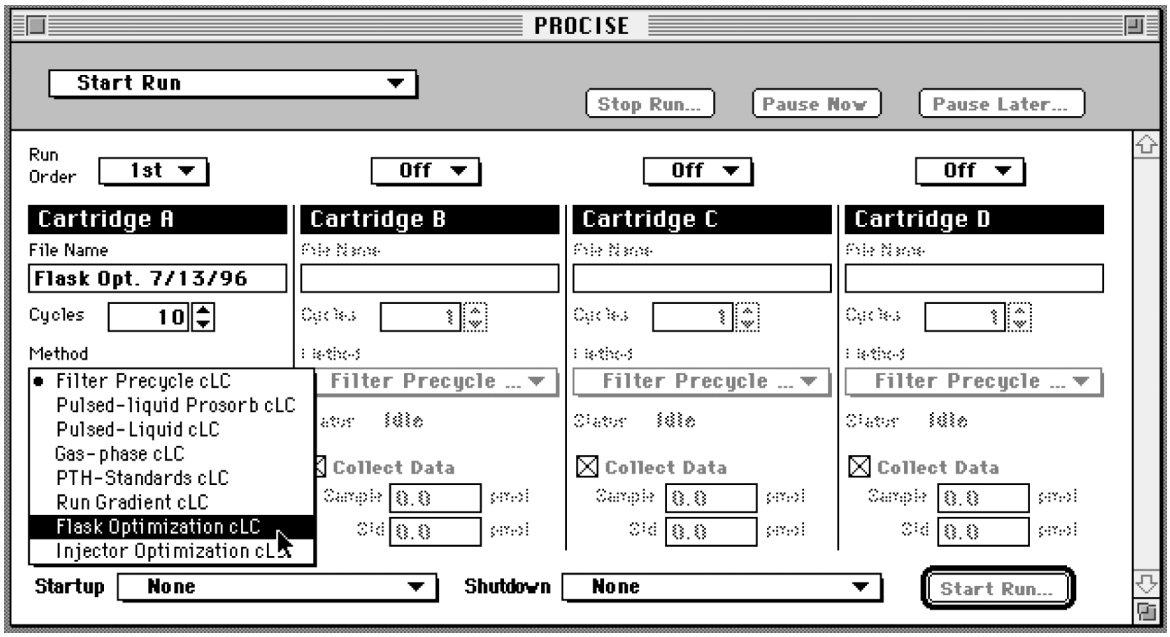

Figure 6-2. Start Run dialog box configured to optimize flask dry-downs

Continue the run:

1. Click Next Step twice.
2. At the next Pause, click Resume.
3. Click Hold as soon as the Dry Flask step begins.
4. When the flask is visibly dry, note both the Time and Remaining values. Be sure to mark these as Post-Conversion Dry values.

Calculate the optimized pre- and post-conversion dry step times:

1. Calculate the optimum time for the Pre-Conversion Dry step by subtracting the Remaining value from the Time value.

   Pre-Conversion Dry time = Time value – Remaining value

2. Calculate the optimum time for the Post Conversion Dry step as follows:

   Post-Conversion Dry time = [Time value – Remaining value] – 100

   The result must be a positive number. If it is not, ensure that the flask is set to the correct temperature, and that two full loads of S4 are being delivered to the flask during the procedure.

3. Scroll to the Functions dialog box.
4. Change the global value of function 236, Pre-Conversion Dry to the optimized value you calculated.

5. Change the global value of function 237, Post-Conversion Dry to the optimized value you calculated.

Setup the sequencer for a run:

1. Install a reaction cartridge in the cartridge A position on the sequencer.
2. Perform a leak test on cartridge A.
3. Scroll to the Start Run dialog box, and configure cartridge A as follows:

Parameter	Setting
Cartridge A	1st
Filename	Your choice
Number of cycles	1
Method	Flask Optimization cLC

Verify the optimization:

1. Click Start Run. The Init Sensor procedure will begin. You can click Jump to advance to the last step of this procedure.
2. Once the cartridge and flask have reached the proper temperatures, the Flask Optimization method will begin.
3. At the first Pause, Click Resume.
4. At the next Pause, check the flask for liquid. The flask should still contain 5 µL to 10 µL of liquid (approximately 1/3 of the conical section of the flask).
5. At the next Pause, click Resume, and continue watching the liquid dry in the flask.
6. Once the flask is visibly dry, note the amount of time that elaspes from this point to the end of the step. The elapsed time should be approximately 200 sec.
7. Click Stop Run to end the cycle.
8. If the verification was not successful, perform the optimization procedure again.

Optimizing Sensor Functions

About Sensor Functions

Sensor functions control sequencer reagent and solvent delivery valves. Each sensor function is controlled by one of the 11 optical fluid sensors inside the sequencer. Each sensor consists of an infrared emitting diode and photo-sensor receiver. Fluid is detected by increased light transmission through the Teflon tube due to the change in refractive index.

There are four types of sensor functions:

- Cartridge load functions
- Deliver to cartridge functions
- Flask load functions
- Injector load function

List of Optical Fluid Sensors in the Sequencer

The 11 optical fluid sensors in the sequencer are:

- Cartridge Load 1 (Small) Sensor
- Cartridge Load 2 (Large) Sensor
- Cartridge A Outlet Sensor
- Cartridge B Outlet Sensor
- Cartridge C Outlet Sensor
- Cartridge D Outlet Sensor
- Flask Load 1 (Small) Sensor
- Flask Load 2 (Large) Sensor
- Transfer to Flask Sensor
- Sample Loop Load Sensor
- Sample Loop Full Sensor

How Sensor Functions Work

When a sensor function is activated, the sensor begins *looking* for fluid, and the timer for the function begins counting down to zero. When fluid is detected, the reagent or solvent delivery valve is turned off, or the injector switches from the load position to the inject position. When the timer reaches zero, the next step begins. Therefore, the function must remain active long enough for fluid to reach the sensor. The period of time the function remains active is specified when the function is created.

If fluid is not detected within the specified period of time:

- A dialog box describing the failure(s) is displayed for each sensor (except the Transfer to Flask and Sample Loop Full sensors).
- The sequencer pauses at the end of the active cycle unless the operator intervenes.
- An error message is sent to the Event Log. This occurs for all sensor failures, including the Transfer to Flask and Sample Loop Full sensors.

Reading Sensor Failure Event Log Messages

```
01/01/1995    4:30:00 PM
       During step 2 of cycle 1, fluid was not detected by the Cartridge
Load 2 (large)Sensor
       The sequencer will pause at end of this cycle.
(Dry = 500, Threshold = 750, Average wet = xx)
       dry    wet    dry    wet    dry    wet
       (xx,   xx,    xx,    xx,    xx,    xx,
        xx,   xx,    xx,    xx,    xx,    xx,
        xx,   xx,    xx,    xx,    xx,    xx)
```

Figure 6-3. Typical Event Log message for a sensor failure

Figure 6-3 is a typical Event Log message for a sensor failure. The information displayed in this message is as follows:

- The date and time the failure occurred.
- The step and cycle number during which the failure occurred.
- The sensor that reported the failure.
- The status of the sequencer.
- The information conveyed by the line, `(Dry = 500, Threshold = 750, Average wet = xx)`, in Figure 6-3 is described in Table 6-1 on page 6-11.

- The 6 columns of dry and wet values represent the number of dry and wet readings taken by the sensor. A certain number of wet readings are required to discriminate the arrival of the reagent or solvent from a stray droplet of fluid in the line. If fluid never reaches the sensor, only the first dry field will have a non-zero value.

Table 6-1. Definitions of Dry, Threshold, and Average wet

Dry = 500	The empty tube transmission (dry) reading from the sensor generated during the Init Sensor procedure.
Threshold = 750	The minimum transmission value necessary for a sensor reading to be considered wet (dry reading multiplied by 1.5).
Average wet = xx	Actual transmission reading with fluid in tube. **If no fluid is detected, the average wet = 0.**

Why Sensor Failure Event Log Messages are Generated

Sensor failure event log messages are generated when:

- A bottle runs dry during a run.
- A delivery path blockage restricts the flow of a reagent or solvent.
- Air bubbles are present in the solvent or reagent stream.

If a bottle runs dry or a blockage occurs, an Event Log error message such as the one shown in Figure 6-4 is generated.

```
dry    wet    dry    wet    dry    wet
(5000, 0,     0,     0,     0,     0,...)
```

Figure 6-4. Event Log message indicating an empty bottle or restricted delivery of a reagent/solvent

When air bubbles are detected by a sensor, an Event Log message with values such as those shown in Figure 6-5 is generated.

```
dry    wet    dry    wet    dry    wet
(1500, 58,    1,     47,    2,     53,...)
```

Figure 6-5. Event Log message reporting bubbles in the solvent/reagent stream

Bubbles occur when the solvent or reagent degasses as it flows through the valve blocks. This can usually be corrected by reducing the appropriate bottle pressure.

Optimizing Cartridge Load Sensor Functions

Overview

Two load loops are available for metering reagents to the cartridge:

Loop	Description
Small	• Loads a nominal 5 µL of any cartridge reagent. • Volume of reagent delivered wets, but does not saturate, a 6 mm glass fiber filter in the reaction cartridge. • Volume of reagent delivered is appropriate for blotted samples. • May be preferred when sequencing samples on small pieces of PVDF. • A small loop load method for TFA, such as Pulsed-liquid cLC, can help prevent sample washout from occurring.
Large	• Loads a nominal 10 µL of any cartridge reagent. • Delivers a volume of reagent that saturates a 6 mm glass fiber filter in the reaction cartridge.

The standard cycles included with this system use both the large and small load loops for loading cartridge reagents.

Guidelines for Using Cartridge Load Sensor Functions in Custom Cycles

- Flush the loop for at least 15 sec before the first loading.
- Flush the loop for at least 10 sec between loadings.
- Wash and flush the loop between loadings of multiple reagents.
- Whenever the delivery pressure for a reagent is changed, load times must be changed as well.
- If the sequencer has not been run since the last cold start, run the Init Sensor procedure from the Test dialog box. Allow the procedure to run to completion. This will ensure proper sensor operation.

Procedure

To determine the duration required for a cartridge load function:

1. From the Pressures and Temperatures dialog box, set the delivery pressure for the appropriate bottle position.
2. If the reagent or solvent is not loaded on the sequencer, load it using the bottle change procedure listed in section 2, “System Setup”.
3. From the Manual Control dialog box, select the appropriate function from the cartridge function list (function 139, *Flush Large Loop,* or function 140, *Flush Small Loop).* Activate the function for 20 sec.
4. Activate the load function for the bottle and loop of choice. For example, select function 183 to load the large loop with reagent or solvent from the X2 bottle position.
5. Watch for the appearance of a check mark next to the reaction flow sensor field at the top of the screen. Note the elapsed time, and add 5 to 10 sec for the load time.
6. Enter the load time in the cycle for this function.
7. From the Functions dialog box, enter the load time in the global time field for that function.
8. Save the change by opening the File menu and selecting Save Function.

Note	*All manual control functions and valves must be deactivated before procedures or runs can be started.*

Optimizing "Deliver to Cartridge" Sensor Functions

Overview

Liquid sensors located at cartridge outlets simplify the optimization of solvent delivery to cartridges for washing and extraction. The sensors eliminate the need for timing the delivery of solvent to the midpoint of the cartridge. All washes and extractions in standard cycles are controlled by these sensors (except for the wash after cleavage).

A wash is a two part procedure. First, a delivery to the cartridge outlet sensor occurs. The delivery is followed by short pulses of solvent alternated with wait steps.

Extractions are deliveries to the cartridge outlet sensor, followed by a brief incubation period and transfer to the flask.

IMPORTANT *Whenever the delivery pressure for a reagent is changed, load times must be changed as well.*

Guidelines for Using "Deliver to Cartridge" Sensor Functions in Custom Cycles

- Flush the cartridge for at least 40 sec before the first delivery.
- Flush the cartridge for at least 40 sec between deliveries.
- If the sequencer has not been run since the last cold start, run the Init Sensor procedure from the Test dialog box. Allow the procedure to run to completion. This will ensure proper sensor operation.

Procedure

To determine the duration required for a "Deliver to Cartridge" function:

1. From the Pressures and Temperatures dialog box, set the delivery pressure for the appropriate bottle position.
2. If the reagent or solvent is not loaded on the sequencer, load the bottle using the bottle change procedure listed in section 2, "System Setup".
3. From the Manual Control dialog box, select function 131, *Dry Cart (top),* from the cartridge function list. Activate the function for 40 sec.
4. Activate the *Deliver to Cartridge (sensor)* function for the bottle or solvent of choice. For example, select function 75, *Load X1, Cart (sm loop),* to deliver reagent or solvent from the X1 bottle position to the cartridge outlet sensor.
5. Watch for the appearance of a check mark next to the reaction flow sensor field at the top of the screen. Note the elapsed time, and add 5 to 10 sec for the load time.
6. Enter the load time in the cycle for this function.
7. From the Functions dialog box, enter the load time in the global time field for that function.
8. Save the change by opening the File menu and selecting Save Function.

Note	*All manual control functions and valves must be deactivated before procedures or runs can be started.*

Optimizing Flask Load Sensor Functions

Overview

Two load loops are available for the flask. Unlike cartridge load loops, the volume of any particular reagent or solvent loaded depends on the position of that chemical on the valve block. Nominal volumes are listed in Table 6-2.

Table 6-2. Nominal volumes of reagents/solvents for the small and large loops

Reagent/Solvent	Small Loop (µL)	Large Loop (µL)
S4	25	60
X3	20	55
X2	15	50
R4	10	45
R5	5	40

Guidelines for Using Flask Load Sensor Functions in Custom Cycles

- Flush the loop for at least 10 sec before the first loading.
- Flush the loop for at least 10 sec between loadings.
- Wash and flush the loop between loadings of multiple reagents.
- Whenever the delivery pressure for a reagent is changed, load times must be changed as well.
- If the sequencer has not been run since the last cold start, run the Init Sensor procedure from the Test dialog box. Allow the procedure to run to completion. This will ensure proper sensor operation.

Procedure

To determine the duration required for a flask load function:

1. From the Pressures and Temperatures dialog box, set the delivery pressure for the appropriate bottle position.
2. If the reagent or solvent is not loaded on the instrument, load it using the bottle change procedure listed in section 2, “System Setup”.
3. From the Manual Control dialog box, select the appropriate function from the flask function list (function 217, *Flush Large Loop,* or function 218, *Flush Small Loop).* Activate the function for 20 sec.
4. Activate the load function for the bottle and loop of choice. For example, Select function 75, *Load X1, Cart (sm loop)*, to load the small loop with reagent, or solvent from the X1 bottle position.
5. Watch for the appearance of a check mark next to the reaction flow sensor field at the top of the screen. Note the elapsed time, and add 5 to 10 sec for the load time.
6. Enter the load time in the cycle for this function.
7. From the Functions dialog box, enter the load time in the global time field for that function.
8. Save the change by opening the File menu and selecting Save Function.

Note	*All manual control functions and valves must be deactivated before procedures or runs can be started.*

Optimizing the Gas-Phase cLC Method

Overview

As is, the standard Gas-Phase cLC sequencing method works best when sequencing samples on PVDF membranes. This method may require optimization if used to sequence samples on glass fiber filters.

If optimization is required, the following two parameters in the Gas-phase cLC sequencing method must be changed:

- R3 bottle pressure
- R3 delivery time

Procedure

Since the cycles, methods, procedures and gradients supplied with this system cannot be directly modified, you will create two new cycles and one new sequencing method as part of the optimization procedure.

Determine if optimization is required:

- Sequence a model compound loaded onto glass fiber filters treated with 750 µg BioBrene solution. Instructions for preparing this solution are located in Section 4 , "System Operation", on page 4-20.
- Analyze your results. If optimization is required, continue with this procedure.

Modify the R3 bottle pressure:

1. Select the Cycles and Procedures dialog box.
2. Select Cartridge Cycle from the cycle type pop-up menu, and Cart Begin Gas-phase cLC from the cartridge cycle type pop-up menu (Figure 6-6 on page 6-19).
3. Open the File menu and select Save Cycle/Procedure as.
4. Enter a unique name for the cycle, such as *Cart Begin Gas-phase cLC GFF,* and click OK.
5. Select Step 3, Set Reg Setpoint (10th psi).
6. Change the Num. Value to a value between 3 and 15 (0.3 to 1.5 psi) (Figure 6-7 on page 6-19).
7. Open the File menu and select Save Cycle/Procedure.

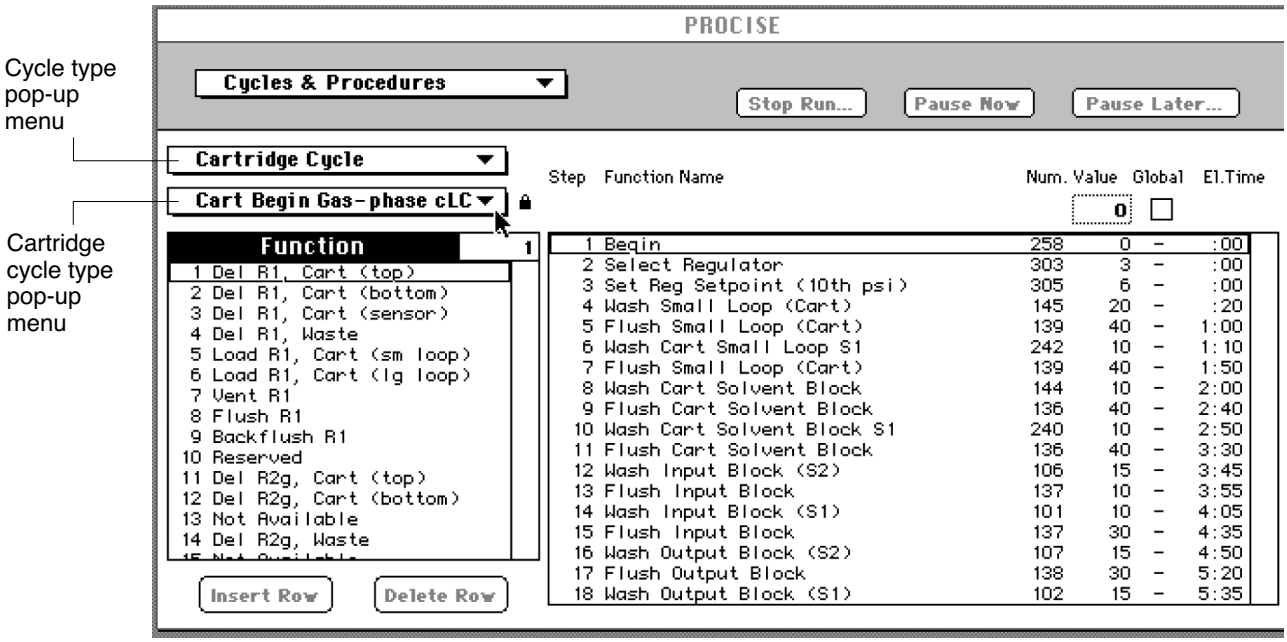

Figure 6-6. Selecting the cycle type and cartridge cycle type

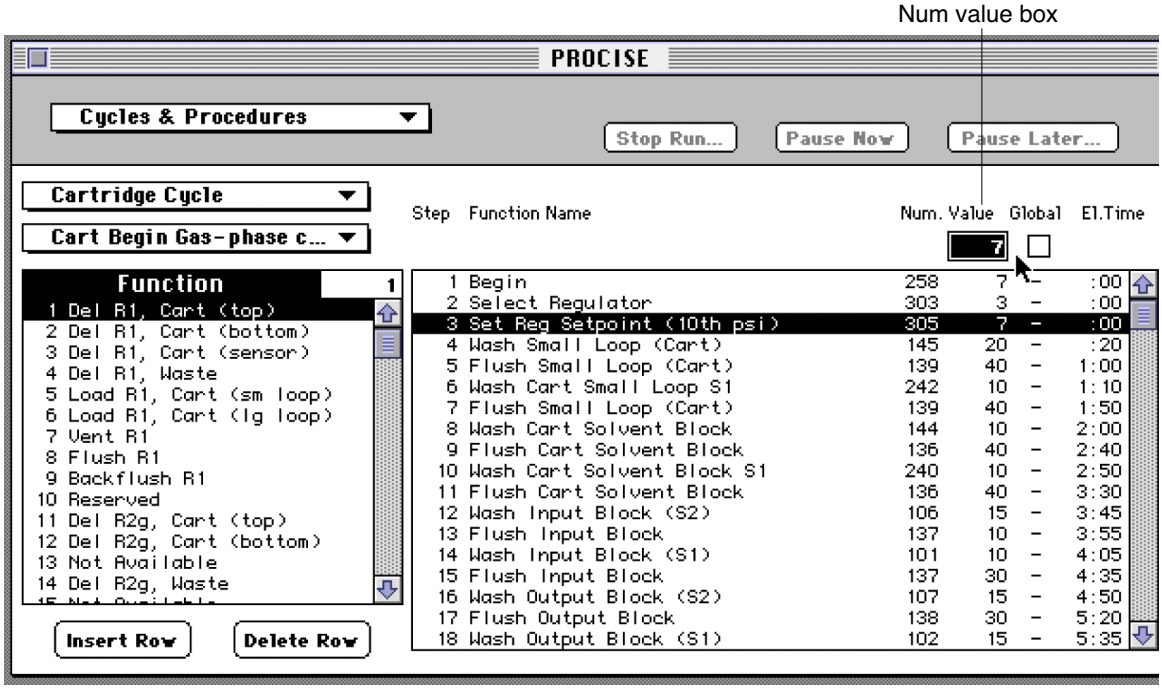

Figure 6-7. Modifying the R3 bottle pressure

Modify the R3 gas delivery time:

1. Select Cart Gas-phase cLC from the cartridge cycle type pop-up menu.
2. Open the File menu and select Save Cycle/Procedure as.
3. Enter a unique name for the cycle, such as *Cart Gas-phase cLC GFF,* and click OK.
4. Select Step 66, *Del R3g, Cart (top)*, and change the Num. Value to a value between 100 and 900. For example, this step will be lengthened for a Proline cycle (Figure 6-8).
5. Open the File menu and select Save Cycle/Procedure.

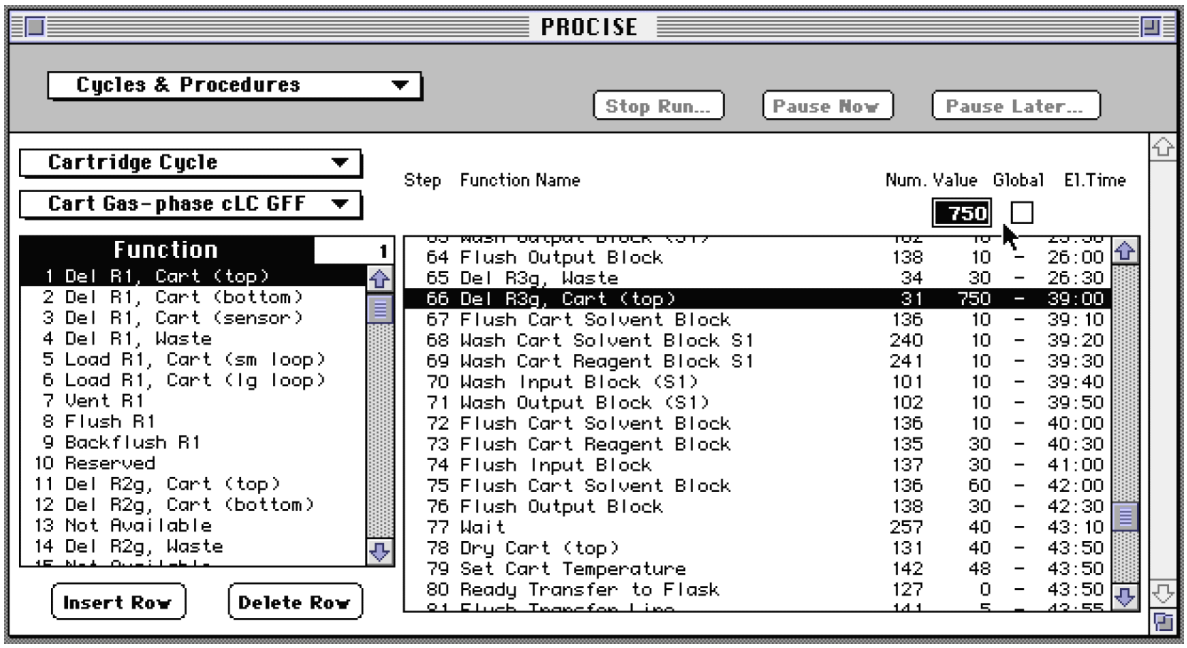

Figure 6-8. Modifying the R3 gas delivery time

Create a new method using the modified cycles:

1. Select the Sequencing Methods dialog box (Figure 6-9 on page 6-21).
2. Select Gas-phase cLC as the Method.
3. Open the File menu, and select Save Method as.
4. Enter a unique name for the new method, such as *Gas-phase cLC for GFF,* and click OK.
5. Select Cycle #3.
6. Open the cartridge cycle pop-up menu, and select the begin cycle you created.

7. Select Cycle Default.
8. Open the cartridge cycle pop-up menu, and select the Default cycle you created.
9. Open the File menu and select Save Method.

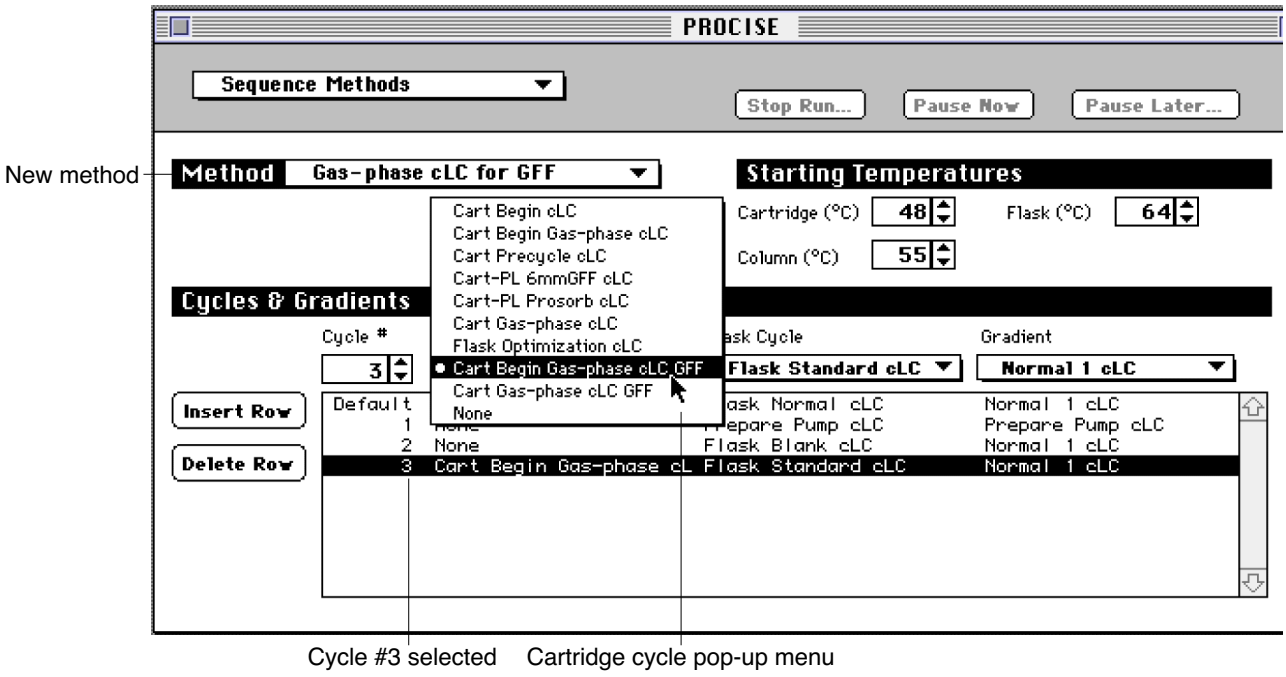

Figure 6-9. Creating a new sequencing method using the optimized cycles

Test the optimization:

1. Test the optimization by loading a model compound onto glass fiber filters treated with 750 μg BioBrene solution.
2. Execute a run(s) using the model compound and optimized method.
3. If unsuccessful, repeat the optimization procedure with new values as appropriate. Use the following guidelines for further optimization:
   - If sample washout occurs, increase the amount of BioBrene applied to the glass fiber filter. The use of additional BioBrene may result in the need for additional cycles when precycling the filter. For example, 6 cycles of the Filter Precycle cLC method are required when applying 1.5 mg of BioBrene to a glass fiber filter.
   - If lag is a problem, increase the R3 delivery time, or decrease the R3 gas pressure.

Sequencer Chemistry Optimization

N-terminal Sequencing Overview

The goal of performing N-terminal sequencing on an unknown protein/peptide sample is to unambiguously identify as many amino acids as possible using the least amount of sample. The length of the protein sequence that can be determined is limited by the chemical efficiency of the Edman degradation as well as the purity, amount, molecular weight, and conformation of the sample. Because the chemical efficiency is less than 100%, the amount of sample you can sequence decreases slightly with each successive degradation cycle.

With the exception of the initial coupling, the reaction of PITC with the amino-terminus or termini proceeds nearly quantitatively. The particular amino acid being reacted, or the local structure of the peptide chain, has little effect on the efficiency of the coupling reaction.

The cleavage reaction requires the use of a strong acid. A balance must be struck between complete cleavage of the ATZ-amino acid, and unwanted acid cleavage at other sites along the peptide chain. Consequently, cleavage efficiency is affected by the amino acid derivative being cleaved as well as the next amino acid in the chain.

Incomplete cleavage of the ATZ-amino acid is referred to as *lag*. The remaining, uncleaved portion of the current N-terminal amino acid will appear in the chromatogram in the following cycle with the next amino acid. Lag increases with each cycle in a sequencing run. Depending on the particular amino acids in the sequence, lag can be the primary reason a sample stops producing useful sequence data.

Repetitive exposure of the sample to strong acid can result in cleavage between amino acids elsewhere in the peptide chain. Each time non-specific cleavage of the peptide chain occurs, a new N-terminus is generated which can react with PITC. This will increase the *amino acid background*—the presence of other PTH-amino acids in the chromatogram which do not reflect the true N-terminal sequence.

At the start of a sequencing run, the amino acid background from non-specific cleavage is low. Background increases with each sequencing cycle. Fortunately, non-specific cleavage is sequence specific, so only peptide bonds between amino acids will be cleaved. This keeps the amino acid background rate from cycle to cycle quite low. However, for proteins with labile amino acid sequences and very large proteins, amino acid background will increase more rapidly. In practical terms, ≤10 pmol of a 100 to 200 amino acid protein may provide 40 to 50 cycles of interpretable sequence, while the same amount of a 2000 amino acid protein will typically provide only 10 to 15 cycles of sequence.

Coupling

Coupling occurs when the free amino-terminus of a protein or peptide reacts with phenylisothiocyanate (PITC) to create a phenylthiocarbamyl (PTC) protein or peptide. The coupling reaction includes:

- Delivery of PITC and base vapor to provide the basic environment necessary for coupling.
- Drying and washing to remove excess reagent and reaction by-products.

The coupling reaction used for samples bound to PVDF membrane differs slightly from the coupling for samples applied to glass fiber. Sequencing cycles have typically been written for samples applied to a hydrophilic support. The hydrophilicity of the support facilitates the absorption of a small amount of water which is necessary for the efficient coupling of PITC to the amino-terminus of the sample.

PVDF membrane is routinely used for electroblotting samples from gels. It can also be used to remove excess salt and buffers from samples prior to sequencing. The membrane binds proteins through hydrophobic interaction. Because PVDF membrane is hydrophobic, it tends to repel rather than absorb water.

Coupling Base Delivery

The first step of coupling in all chemistry cycles is the delivery of R2 vapor to the cartridge. This raises the pH of the sample, and deprotonates the free amino-groups for reaction with PITC. The length of this delivery should be at least 20 to 30 sec, but can be increased to as much as 120 sec without negative impact. The length of the base deliveries after the PITC delivery should be at least 120 sec. To minimize the modification of aspartic and glutamic acid residues, avoid making the total cumulative base delivery time longer than 700 sec.

Under the basic conditions necessary for coupling, aspartic (Asp) and glutamic (Glu) acid residues are slowly modified by the reaction of the side chain carboxylic acid group with aniline. The derivative of Asp can be found just before the DPTU peak in the chromatogram; the derivative of Glu is just after DPTU. The extent of modification of Asp and Glu residues increases slightly with each sequencing cycle. The effect is more pronounced for Glu residues. The rate of modification of Asp and Glu residues also increases with the coupling temperature, and is more noticeable with increasing amounts of sample.

PITC Delivery

The standard chemistry cycles provided with this system include three deliveries of PITC during the coupling reaction. However, customized cycles can specify more or less than three deliveries. If two PITC deliveries are used, increase the base deliveries to 270 to 300 sec each. More than three PITC deliveries might be required to sequence very large amounts of sample, or to sequence a sample on multiple pieces of PVDF where contact of reagent and membrane is a concern.

A short argon delivery occurs after each PITC delivery to evaporate heptane. Residual heptane would interfere with the coupling reaction by keeping most of the PITC in the organic phase. The drying time should be at least 20 sec to insure adequate removal of heptane. Remember, a base delivery should always precede the first PITC delivery to the cartridge.

Coupling Temperature

The temperature of the cartridge during coupling is set high enough to promote fast, efficient reaction of PITC with the amino-terminal amino group without excessive side-reactions. For example, the standard pulsed-liquid and pulsed-liquid blot cycles use a coupling temperature of 48 °C. Under the basic conditions necessary for coupling, Asp and Glu acid residues are slowly modified by reaction of the side chain carboxylic acid group with aniline.

The rate of modification of Asp and Glu residues is slightly higher on glass fiber than on PVDF. The lower coupling temperature for glass fiber provides a rate comparable to PVDF at the higher temperature. The rate of modification of Asp and Glu residues also increases with the length of coupling, and is more noticeable with increasing amounts of sample.

Drying After Coupling

Drying after coupling eliminates the water absorbed by the polybrene during the coupling reaction. Some of the reaction chemicals will also be reduced during this step, but the subsequent wash will remove the bulk of the chemistry by-products. The drying time can be extended without the loss of residues. The goal is to eliminate as much water as possible before the wash and cleavage steps. This will prevent sample washout, and hydrolysis of the peptide chain during the cleavage.

Post-coupling Wash

The post-coupling wash removes as much of the coupling reagents and reagent by-products as possible before cleavage. A combination of solvents S2B and S3 are used. The washing scheme of short solvent deliveries alternated with brief cartridge wait steps reduces the likelihood of sample washout. This scheme also results in maximum wash efficiency with minimal solvent consumption.

The first delivery of solvent to the cartridge is S3, the less polar solvent. S3 reduces the possibility of sample washout from the reaction cartridge. Increasing the volume of solvent used for this wash will reduce the chemistry background, but may increase sample loss from the cartridge due to washout, particularly if short hydrophobic peptides are being sequenced. In particular, lengthy S2B washings will aggravate sample washout.

Drying after the post-coupling wash requires no special considerations other than completely drying the sample to prevent washout. Typically there is no danger of over-drying the sample at this point.

Cleavage

Cleavage, whether pulsed-liquid or gas-phase, is the trifluoroacetic acid (TFA)-catalyzed process of removing the PTC-amino acid from the N-terminal end of the sample. Under strong acidic conditions, the peptide chain is cleaved at the peptide bond nearest the PTC-amino acid derivative, resulting in the release of an ATZ-amino acid. Cleavage is not a hydrolytic process, so ideally the sample should be as free of water as possible to minimize non-specific hydrolytic cleavage of the peptide chain.

Pulsed-liquid Cleavage

Pulsed-liquid cleavage is performed by delivering a small aliquot of TFA to the cartridge on a stream of argon. The reaction chamber is sealed off to allow the cleavage to take place. Pulsed-liquid cleavage proceeds faster than gas-phase cleavage. The standard pulsed-liquid cleavage time is 300 sec at 48 °C.

Certain samples may benefit by varying the cleavage conditions. For example, very large protein samples may sequence better using a shorter cleavage time to minimize amino acid background generated from non-specific cleavage of certain peptide bonds. Cleavage of the peptide bond after certain amino acids, particularly proline, proceeds more slowly than others, and will benefit from an extended cleavage time or increased temperature.

Cleavage for proline residues can be extended up to 600 sec, twice as long as a standard cleavage. Alternatively, the temperature of the cleavage can be increased to 55 °C. These extreme cleavage conditions should be used only when needed, since the rate of sample degradation significantly increases when they are used for every cycle.

Gas-phase Cleavage

Gas-phase cleavage is performed by delivering TFA vapor through the active cartridge for a prescribed period of time. Gas-phase cleavage requires more time than pulsed-liquid phase cleavage. As a result, the standard gas-phase cycles are approximately 600 sec—5 min longer than pulsed-liquid cycles. For optimum results, the R3 pressure setting can be reduced to 0.3 to 1.0 psi.

Too high a TFA flow rate through the cartridge will result in higher than expected lag. Reduce the R3 regulator pressure if the lag per cycle for gas-phase cleavage is higher than for pulsed-liquid. Gas-phase cleavage cycles tend to be somewhat cleaner than pulsed-liquid cycles; the level of chemistry artifact peaks is usually slightly lower. Gas-phase cleavage may also help reduce washout of hydrophobic peptides.

Drying After Cleavage

Drying times after cleavage must strike a balance between the recovery of particular residues, and excessive washout if the sample is still too acidic when extractions are done. Avoid overdrying samples after cleavage, since overdrying will drastically reduce recovery of basic residues. Overdrying can also result in the poor extraction of charged residues, and dehydration of labile residues.

A 40 sec drying time is used in the standard pulsed-liquid cycles. Incomplete drying may result in lowered repetitive yields due to sample washout. If sample washout is of greater concern than the recovery of positively charged residues, extend the drying time after cleavage.

ATZ Extraction and Transfer

After cleavage is complete, and the sample is dried, the ATZ-amino acid is extracted from the cartridge and transferred to the flask. The best method for extracting ATZ differs slightly for the various sample types. Coupling of the new amino-terminus can begin once the transfer is complete.

Liquid Samples

Samples applied to glass fiber disks with polybrene are extracted the same way, whether sequenced using gas or liquid cleavage. Each glass fiber cycle has two ATZ extractions. The first extraction is done with S3 (butyl chloride); the second with S2B (ethyl acetate). For each extraction, solvent is delivered to the cartridge outlet sensor, is allowed to incubate with the sample for 10 sec, and is then transferred to the flask with argon.

S2B, which is more polar than S3, improves the recovery of polar residues, particularly histidine, arginine, aspartic acid and glutamic acid. Using S3 for the first extraction reduces the possibility of polybrene/sample washout. The argon delivery after each extraction must be long enough to transfer the contents of the cartridge to the flask, and dry the cartridge outlet sensor. If droplets of liquid remain at the outlet sensor, incomplete transfer will occur, and low deliveries will result.

Flask Chemistry

Once cleaved, the ATZ-amino acid is extracted from the cartridge and transferred to the flask for conversion into the more stable PTH-amino acid derivative. In preparation for the transfer, a small volume of 10% acetonitrile (S4C) is delivered to the flask. The presence of S4C reduces the modification of certain amino acid residues, serine and threonine in particular.

Pre-Conversion Drying

During and immediately following the transfer, the liquid in the flask is bubbled to evaporate the S3 and S2B transferred from the cartridge. Sample volume is also reduced to 10 to 20 µL. At this point in the conversion cycle, the sample should never be completely dried. Completely drying the sample before conversion will reduce the recovery of labile residues, particularly serine and threonine. Instructions for optimizing the duration of the Pre- and Post-Conversion Dry steps is on page 6-6.

Conversion

Conversion of the ATZ-amino acid into a PTH-amino acid takes place in an aqueous acid medium. A small loop load of R4 is added to the flask and allowed to incubate with the sample for approximately 10 min. A small load of R4 can be used instead of a large load to reduce the drying time required after conversion.

Post-Conversion Drying

After conversion, the sample must be completely dried to remove all the TFA. TFA will interfere in the chromatography of early eluting PTH-amino acids. In the standard cLC flask cycles, the flask will appear dry 180 to 200 sec before the end of the standard flask dry step which follows the Post-conversion Dry step.This additional drying time will not adversely affect the recovery of the PTH-amino acids. Instructions for optimizing the duration of the Pre- and Post-Conversion Dry steps is on page 6-6.

PTH-amino Acid Solubility

The dried PTH-amino acid in the flask is dissolved in 10% acetonitrile (S4C) for subsequent transfer to the injector loop. Two large loop loads of S4C are used to dissolve the sample in the standard flask cycles. Bubbling the contents helps dissolve the sample.

Sample Transfer and Injection

Once the sample has been reconstituted in the flask, it is transferred to the HPLC injector loop. Transfer is accomplished by pressurizing the flask with argon, and driving the sample out through the pick-up line into the injector loop. When the sample loop load sensor detects fluid, the injector valve is switched from the load to the inject position, moving the sample into the HPLC solvent stream. The gradient program and data collection begin. Flask cycles must include the following steps (functions):

Step	Description
Function 227, Prepare Pump	• Downloads the gradient program to the 140D from the Procise cLC control software. • After the download is complete (30 to 60 sec), the 140D will start, pressurize and run at the initial gradient conditions.
Function 226, Load Position	• Sets the injection valve in the load position. • Must precede the Load Injector step for the sample loop to be flushed before sample transfer to the sample loop.
Function 221, Flush Injector	• Flushes the sample loop from valve 44. • Does not flush through the flask. • Must precede the Load Injector step.
Function 225, Load Injector	• Activates the sample loop sensors. • Transfers sample from the flask to the HPLC sample loop.

Sample Volume

The volume of sample transferred to the injector loop is determined by the size of the loop loads sent to the flask. The standard volume of a large loop load of S4C is 60 µL. Two loads to the flask provide a total sample volume of 120 µL.

Bubbling in the flask reduces the amount of acetonitrile in the sample, reduces the sample volume, and insures proper binding of the PTH-amino acids to the column.

Injection Percentage

The standard injection percentage for the Procise 49X cLC Protein Sequencer is 55 to 65%. This percentage was selected to provide consistent fluid detection at both the Sample Loop Load and Sample Loop Full sensors.

A procedure for optimizing the injector percentage is listed on page 6-3, "Optimizing the Injector". Optimize the injector percentage if a large number of Sample Loop Full error messages begin to appear in the Event Log.

Optimizing the Chromatography

Flattening the Baseline

To achieve a high sensitivity sequence, baseline rise must be kept to a minimum. One factor which causes baseline rise in PTH chromatograms, is the slightly higher absorbence properties of solvent B2. Eliminating this factor increases the accuracy of chromatographic peak detection and integration by the 610A Data Analysis software, especially at high sensitivity.

Acetone has a very high UV absorbence at 269 nm—an optimal wavelength for PTH amino acid analysis. When small amounts of acetone are added to solvent A3, and a linear gradient is used, the absorbence of solvents A3 and B2 will more closely match, eliminating most of the baseline rise.

To add acetone to solvent A3:

1. Make a 1% acetone/H_2O solution by mixing 1 mL of HPLC–grade acetone with 99 mL of D.I. water in a 100 mL clean bottle.
2. Add 700 µL of the acetone/D.I. water solution to 450 mL of solvent A3.
3. Mix well.
4. Additional acetone may be required to flatten the baseline. Add in increments of 50 µL each until satisfactory results are obtained. Once the proper volume has been determined for a particular system, the volume should not change significantly.

Reducing Negative Baseline Slope at the Start of the Chromatogram

Some HPLC and PTH-columns exhibit a negative slope in the baseline from DTT to Glu before flattening out in the latter part of the chromatogram. Adding a small amount of phosphate ion to solvent A will flatten the baseline over several cycles, and prevent reappearance of the slope.

To prepare a phosphate ion solution:

1. Prepare a 1.0 M stock solution of NaH_2PO_4 or KH_2PO_4 (monobasic sodium or potassium phosphate, sodium or potassium dihydrogen phosphate).
2. Add from 100 µL to 1.0 mL phosphate solution to 1 L of solvent A. The final concentration will be 0.1 to 1.0 mM phosphate.

Optimizing the PTH-Amino Acid Separation

Modifying the Standard Gradient Program

During installation, PTH-amino acid separation is optimized for the column supplied with your system using the standard gradient program, Normal 1 cLC. To maintain optimum separation, you may need to modify this program as the column ages.

Positioning Positively Charged PTH–Amino Acids

Increasing the ionic strength of the mobile phase reduces the retention time of the basic derivatives on the column. Suggested elution positions for the basic derivatives are:

- Arginine between Serine and Tyrosine
- Pyridylethyl Cysteine before Proline

For the majority of columns, these elution positions are achieved by:

1. Adding approximately 9 mL Premix buffer to 450 mL of solvent A3.
2. Making minor gradient program modifications.

Increasing the buffer concentration can cause Arginine (Arg) to elute earlier than Serine (Ser'), and Pyridylethyl Cysteine (PECys) elute earlier than Proline (Pro).

If PECys is not a derivative of interest, you can position Histidine (His) after Alanine (Ala), and Arg after Tyrosine (Tyr) by using less Premix buffer.

Histidine

- If His coelutes with Ala, increase the buffer concentration.
- To move His before Ala, add an additional 1 mL of Premix buffer per 450 mL of solvent A3.

Arginine

- If Arg coelutes with Tyr, increase the buffer concentration.
- To move Arg before Tyr, add an additional 1 mL of Premix buffer to 450 mL of solvent A3.

Pyridylethyl cysteine

- If PECys coelutes with Pro, increase the buffer concentration.
- To move PECys before Pro, add approximately 1 mL of Premix buffer to 450 mL of solvent A3.

Improving the Separation of the Aspartic Acid

- To separate Aspartic Acid (Asp) from the DTT peak, add 20 µL of neat trifluoroacetic acid to 450 mL of solvent A3.

Improving the Separation of Other Amino Acids

Methionine/Valine

- To improve the Methionine/Valine separation, increase the column temperature in 2 °C increments. Do not raise the temperature above 59 °C.

Isoleucine/Lysine

- If the peaks are more than 50% separated, decrease the %B by 2% at 22 min.
- If the peaks are less than 50% separated, decrease the %B by 4% at 22 min.

Lysine/Leucine

- If the peaks are more than 50% separated, increase the %B by 2% at 22 min.
- If the peaks are less than 50% separated, increase the %B by 4% at 22 min.

Summary of PTH-Amino Acid Separation Optimization

In Table 6-3, the arrow above an amino acid indicates the direction the peak moves after changing the variable listed in the Variable column. Left is toward the injection point.

Table 6-3. Optimization guidelines

Variable	Major Effect	Minor Effect
Increase final %B at 22 min	←←← I K L	
Decrease final %B at 22 min	→→→ I K L	
Increase column temp (2 °C)	← → M V	← ← ← H R PECys
Decrease column temp (2 °C)	→→ S' R	→→ T G
Increase molarity	← ← HA RS'	← PECys P
Decrease pH	→ DTT D	→ G E

7 Tests and Procedures

Contents

General Test and Procedure Information

- Tests and procedures cannot be run while the sequencer is active.
- All test and procedure results are reported in the Event Log.
- Some tests and procedures change the sequencer's pressure settings. Therefore, always allow tests and procedures to finish without interruption.

  If you must interrupt a test or procedure:

  - Click Next Step repeatedly until the test finishes.
  - Reset the default pressures and temperatures on the sequencer. Instructions are listed under "Restoring Default Pressures and Temperatures" on page 7-4.
- You can setup tests and procedures to pause whenever an error occurs, or you can elect to have the test/procedure run to completion without pausing for errors. Refer to "Selecting or Deselecting Don't Pause On Error" on page 7-5 for further information.

Restoring Default Pressures and Temperatures

Procedure

1. Select the Pressures and Temperatures dialog box (Figure 7-1) from the dialog box pop-up menu.
2. Click Default to restore the default pressures and temperatures recommended by Applied Biosystems.

   Under certain conditions, pressure settings other than the default settings may be desired. For example, if the instrument is being operated at a high altitude, you may need to reduce the R1 regulator pressure to 20 psi.

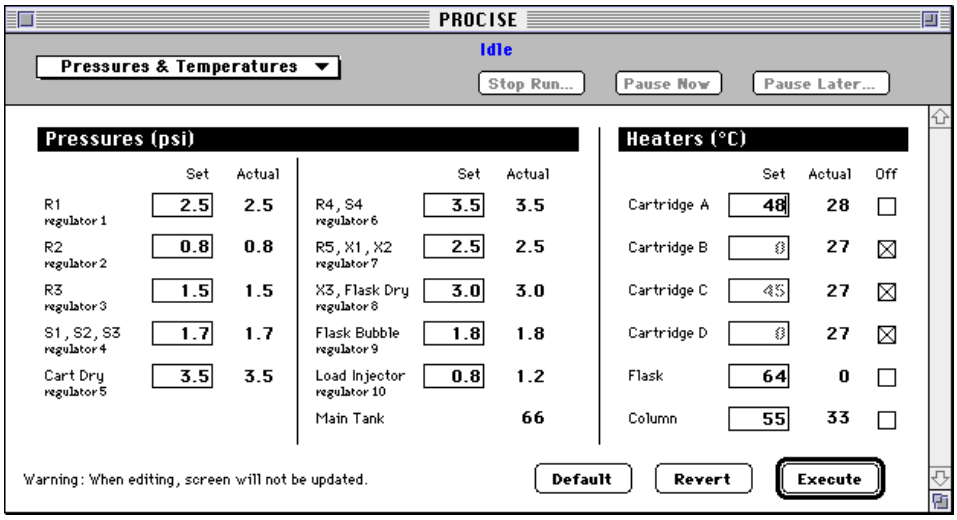

Figure 7-1. Pressures & Temperatures dialog box

Selecting or Deselecting Don't Pause On Error

When running a test or procedure, you can either select or deselect the *Don't pause on error* box in the Test dialog box.

If you deselect the *Don't pause on error* box:

- The box is empty.
- The test or procedure pauses when an error occurs.
- A dialog box noting the failure appears on the screen.
- Other tests being run sequentially are also paused.
- You must click Resume Test after each error to continue the test or procedure.

If you select the *Don't pause on error* box:

- The box has an X in it (Figure 7-2).
- The test or procedure will run to completion without pausing if an error occurs.

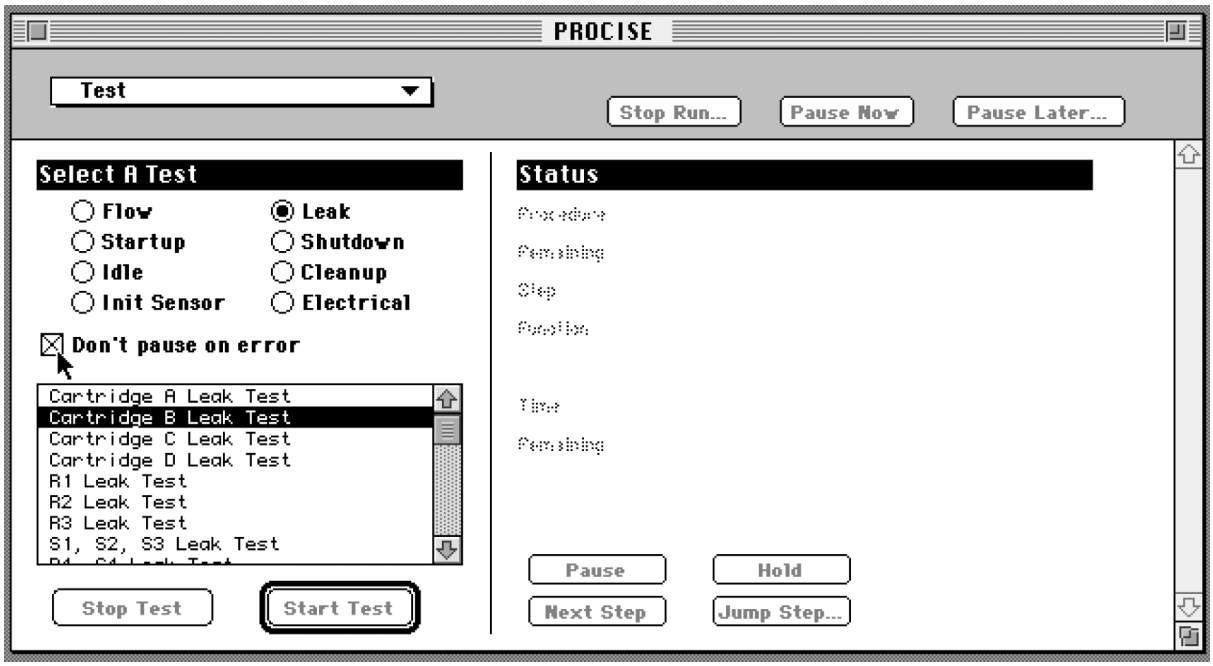

Figure 7-2. Don't pause on error box is selected

Flow Procedures

Flow Procedure Overview

- The Procise 49X cLC Protein Sequencing System has 6 flow procedures.
- The Sensor & Delivery Test can be performed by users.
- The Gas Flows, Liq Del Test, Sensor Check, and Cart L2 Cal procedures are used during instrument manufacture only.
- The R5 Large Loop Cal cLC procedure is used during system installation to determine the amount and concentration of R5 required for the PTH-Amino Acid standard. Refer to "Preparing the PTH-Amino Acid Standard" in Section 2, "System Setup", for more information on this procedure.

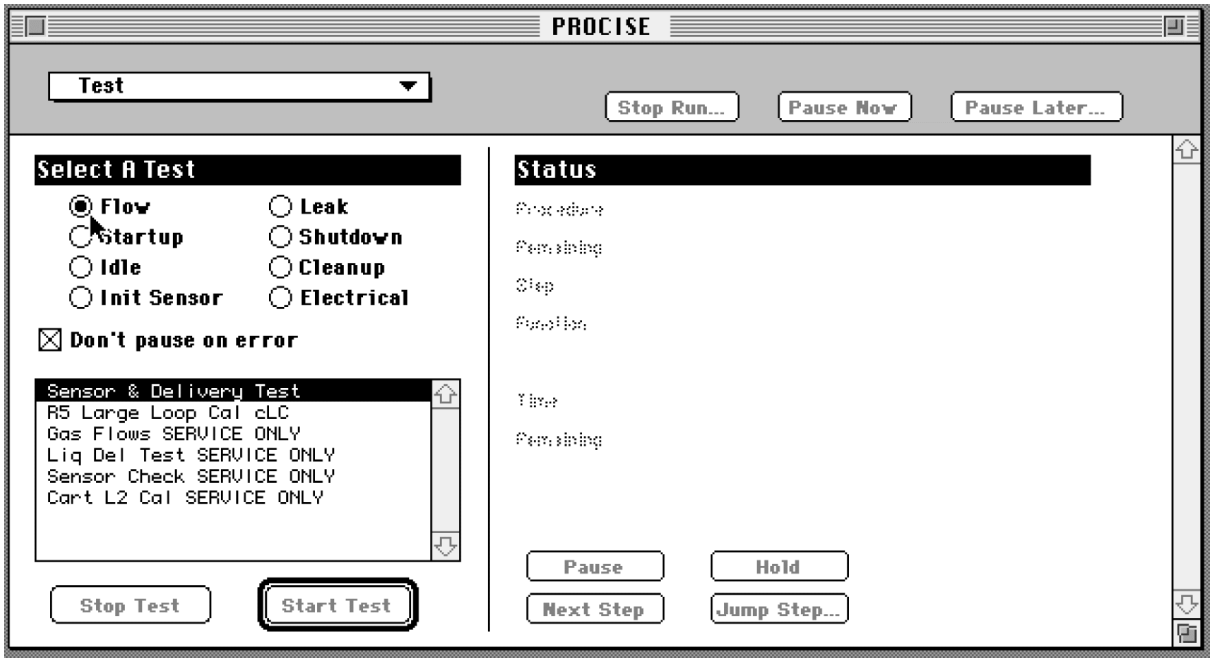

Figure 7-3. Flow procedures

Sensor and Delivery Test

Overview

The Sensor and Delivery Test verifies the operation of the fluid optical sensors in the sequencer. Chemicals are delivered from the bottles or flask through specific sensors. A check is made to determine whether or not fluid is sensed before the end of the procedure. If fluid is not sensed before the end of the delivery, either the sensor is faulty, or delivery was incomplete. Failures are reported in the Event Log.

Note	*This test should be run while the designated sequencing chemicals are loaded on the instrument. In addition, X2 must contain R5, and X3 must contain methanol.*

Procedure

1. If the sequencer has not been run since the last cold start, run the Init Sensor procedure (listed on page 7-11) before continuing with this test.
2. Select the Test dialog box from the dialog box pop-up menu.
3. Click Flow.
4. Select Sensor & Delivery Test from the test menu (Figure 7-3 on page 7-6).
5. Select or deselect the Don't pause on error box. Refer to page 7-5 for information on this option.
6. Click Start Test.
7. Allow the test to run to completion.
8. Select the Event Log dialog box from the dialog box pop-up menu.
9. Review the Event Log to determine if any delivery errors occurred.

Startup cLC Procedure

Overview

One startup procedure, Startup Procedure cLC, is included with the Procise cLC control software. This procedure flushes each reagent/solvent bottle with argon, and refreshes the reagent in the delivery line. The flask is washed with S4. No solvent or reagent is delivered through the cartridges.

The startup procedure can be included as part of a sequencing run, or it can be run independently from the Test dialog box. When included as part of a run, the procedure is executed immediately after sensor initialization. A shutdown procedure (page 7-24) can be executed after completion of the last cartridge scheduled to run.

Procedure

To include the Startup procedure in a sequencing run:

1. Select the Start Run dialog box (Figure 7-4) from the dialog box pop-up menu. At the bottom of the screen are pop-up menus for Startup and Shutdown procedures.
2. Select Startup Procedure cLC from the Startup pop-up menu.

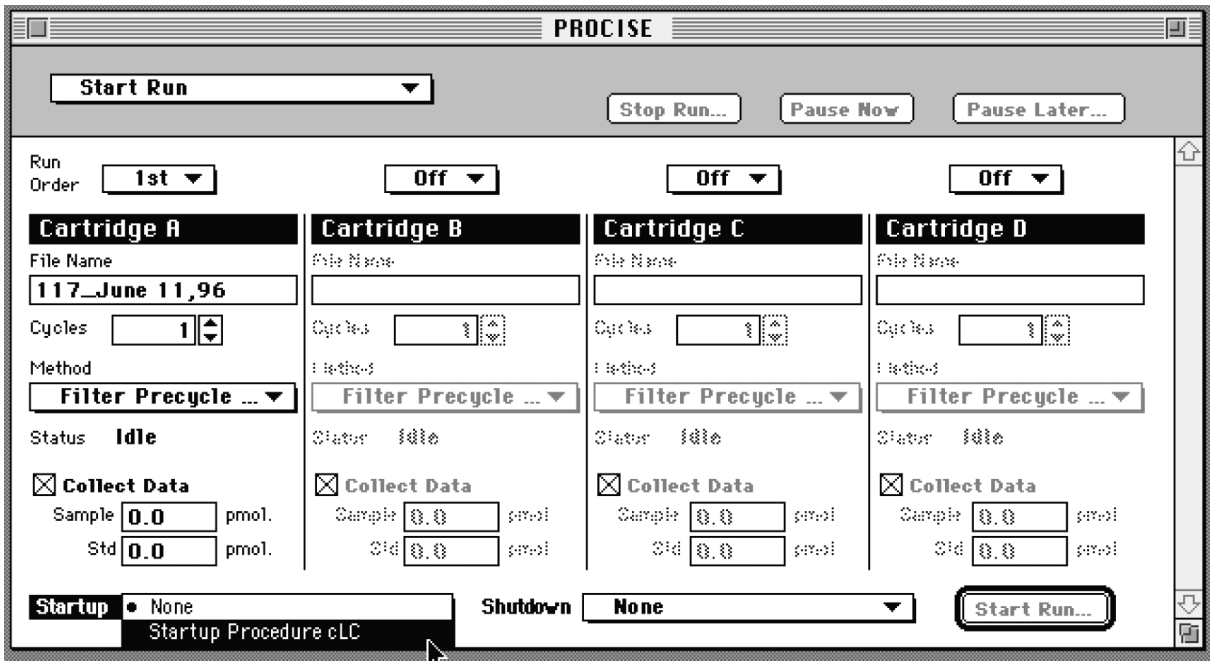

Figure 7-4. Startup procedure programmed as part of a run

To run the Startup procedure from the Test dialog box:

1. Select the Test dialog box from the dialog box pop-up menu (Figure 7-5).
2. Click Startup, and select Startup Procedure cLC.
3. Select or deselect the Don't pause on error box. Refer to page 7-5 for information on this option.
4. Click Start Test to run the procedure.
5. Allow the procedure to run to completion.

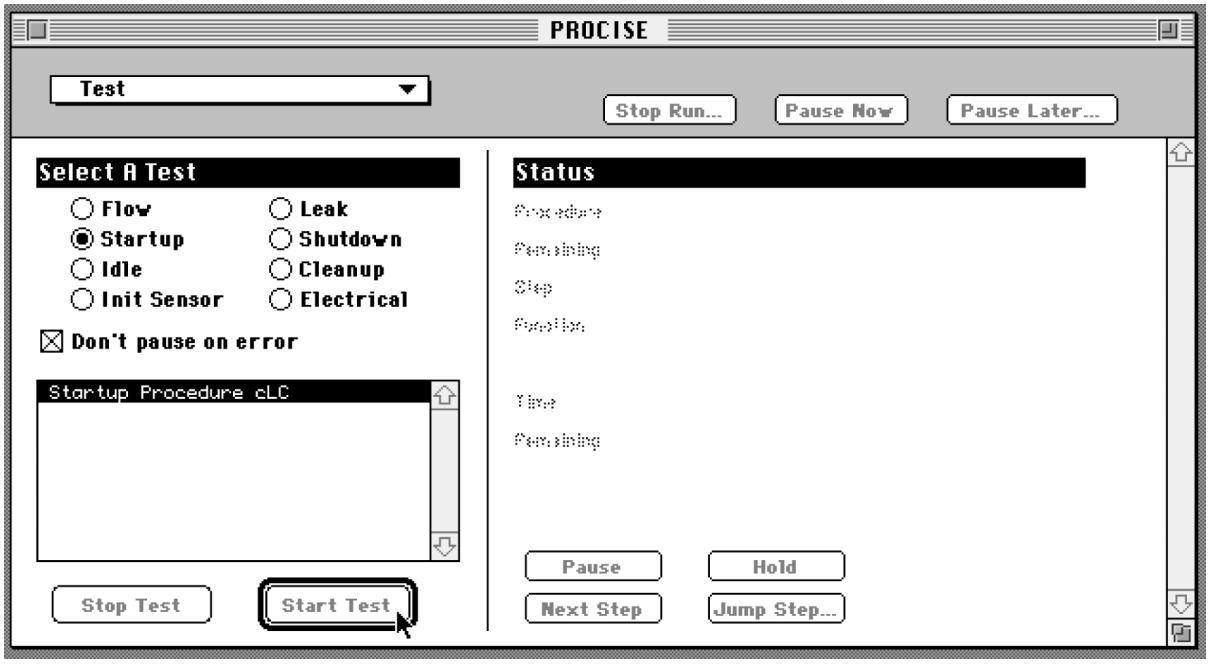

Figure 7-5. Startup procedure run from the Test dialog box

Idle Procedure

Overview

When the sequencer is not in use, oxygen slowly diffuses into the system. This causes solvents and reagents to decompose and form by-products. The idle procedure minimizes sequencing problems due to chemical decomposition during inactive periods. The procedure flushes argon gas to each reagent and solvent bottle at a user-selectable level.

Procedure

1. Select Preferences from the Sequencer pull-down menu on the upper menu bar. The Preferences box (Figure 7-6) will appear.
2. Select the box labeled “Execute Idle Procedure”.
3. Enter the frequency (in hours) that you want the procedure to run. Valid entries range from once every hour to once every 999 hours.
4. Click OK.
5. If the sequencer is active when the Idle procedure is selected, go to the Start Run dialog box, and click Update.

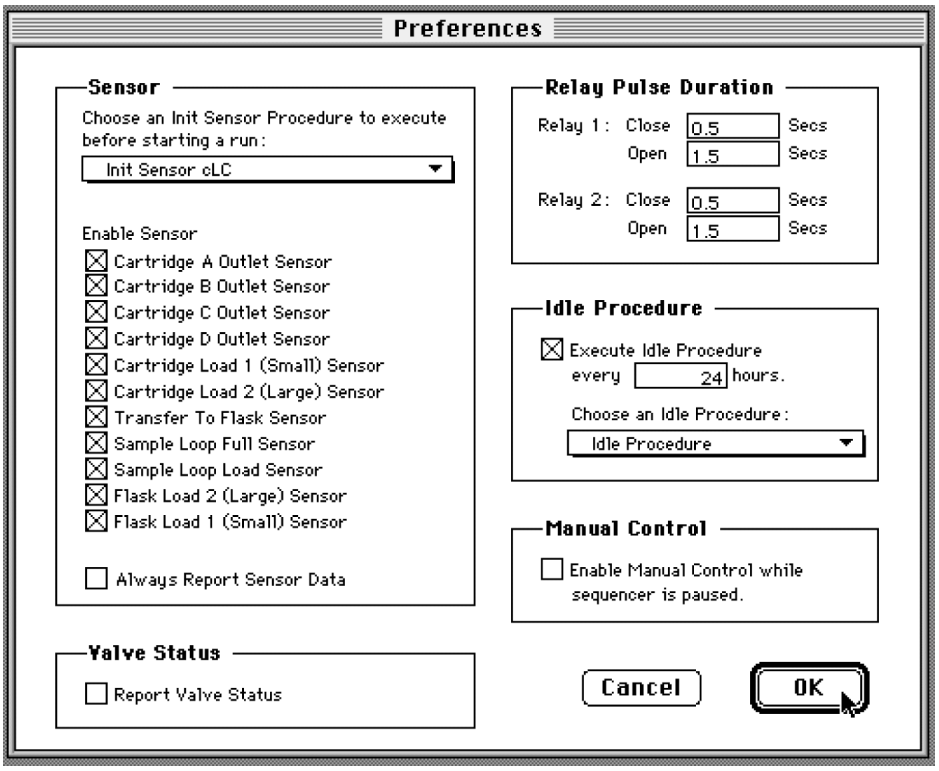

Figure 7-6. Configuring the Idle Procedure from the Preferences box

Init Sensor cLC Procedure

Overview

Depending on the number of cartridges, your sequencer has up to 11 optical sensors used to detect fluid deliveries. Every time Start Run is clicked, the Procise 49X cLC Protein Sequencing System automatically runs the Init Sensor cLC procedure. This procedure flushes the flow path through each sensor, then takes a *dry* reading for each sensor. If the sensor light path is not completely dry, the sensor will not function correctly during sequencing.

The Init Sensor cLC procedure can also be run independently from the Test dialog box (Figure 7-7).

IMPORTANT *Always allow the Init Sensor cLC procedure to run to completion. If the sequencer has been shut down, or if a sensor has been moved or replaced, the Init Sensor procedure must be run before sequencing or using manual control functions.*

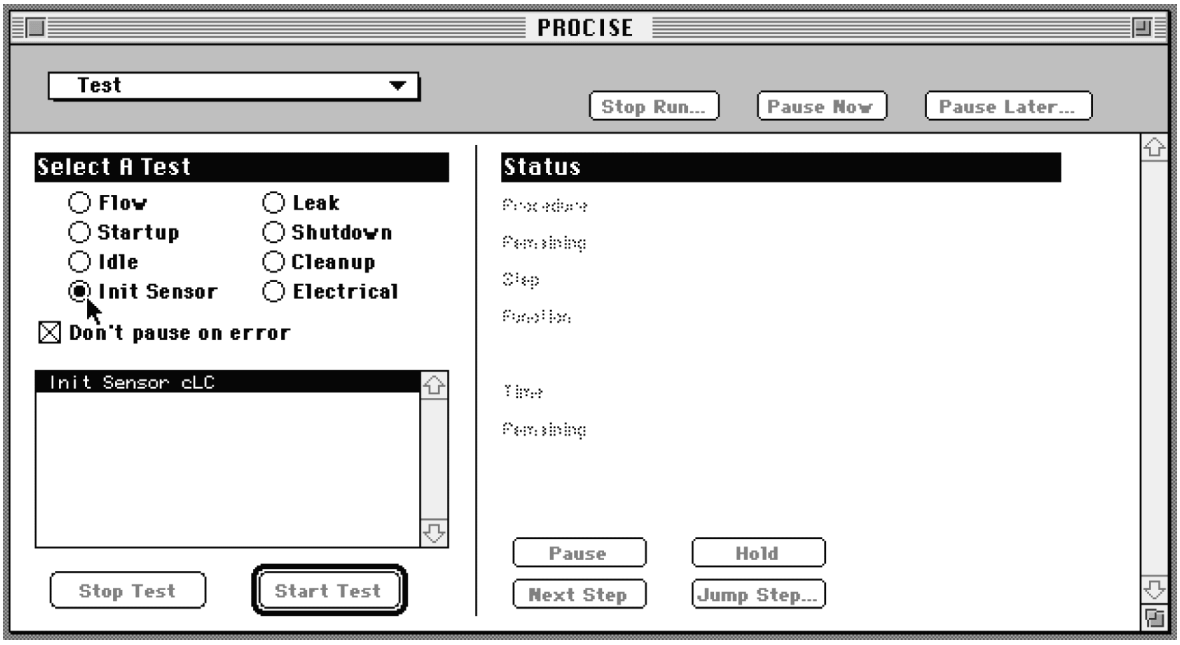

Figure 7-7. Init Sensor procedure run from the Test dialog box

Procedure

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click Init Sensor. The Init Sensor cLC procedure is automatically selected (Figure 7-7).
3. Select or deselect the Don't pause on error box. Refer to page 7-5 for information on this option.
4. Click Start Test.
5. Allow the procedure to run to completion.

Leak Test Procedures

Leak Test Procedures Overview

A variety of leak tests are included with the Procise cLC control software. Leak tests are provided for:

- Bottles
- Cartridges and cartridge blocks
- The flask and flask blocks
- The waste system
- Regulators

IMPORTANT *Leak tests alter the pressure settings for reagent, solvent, and/or gas deliveries. If a test is interrupted, pressures can remain altered. To reset the default operating pressures, select the Pressures & Temperatures dialog box, and click Default.*

Bottle Leak Test Overview

Bottle leak tests are run from the Test dialog box, or the Bottle Change dialog box by selecting the Bottle Change procedure with the suffix *-leak* (Figure 7-9). Each bottle leak test performs the following checks:

- *Pressurization*—Checks that the bottle can be adequately pressurized.
- *Monitor Leak Rate*—Measures the pressure drop with the regulator set to zero.
- *Vent*—Checks the venting capability.

Test results are reported in Event Log at the end of the test. The actual bottle pressure must be within 0.05 psi of the target pressure to pass the leak test.

Bottle Leak Test Procedure

To run a bottle leak test from the Test dialog box:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click Leak (Figure 7-8).
3. Select the appropriate bottle from the menu.
4. Click Start Test.

Refer to "Bottle Change Procedure" on page 7-32 for instructions on performing bottle leak tests as part of the bottle change procedure.

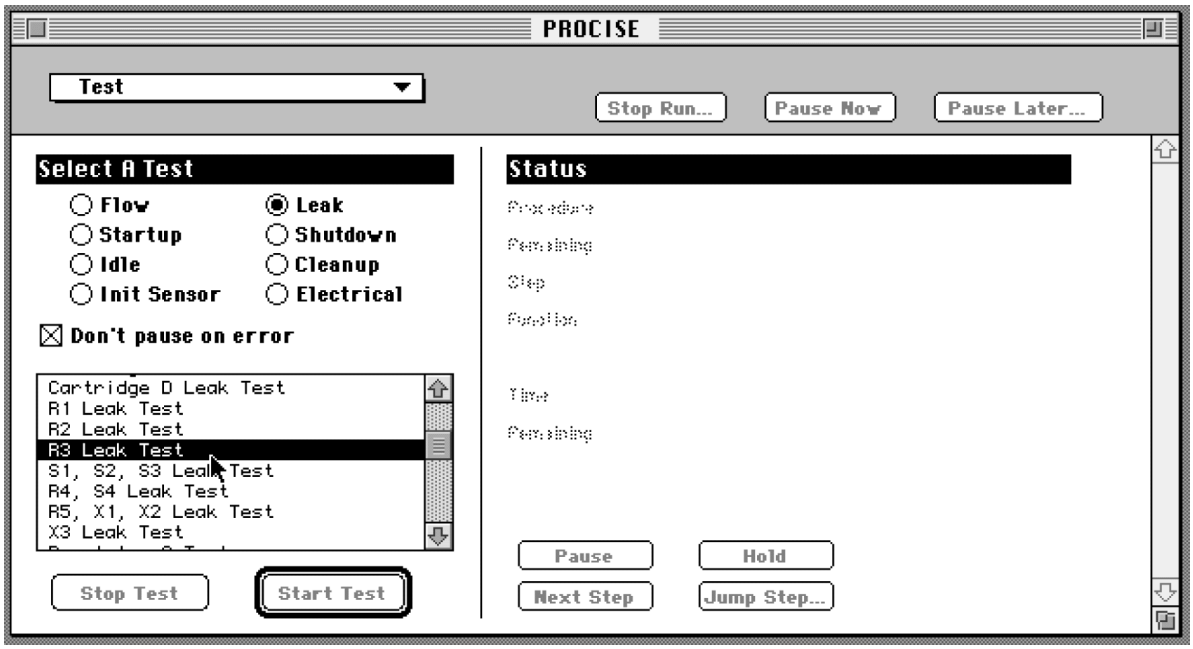

Figure 7-8. Bottle leak test run from Test dialog box

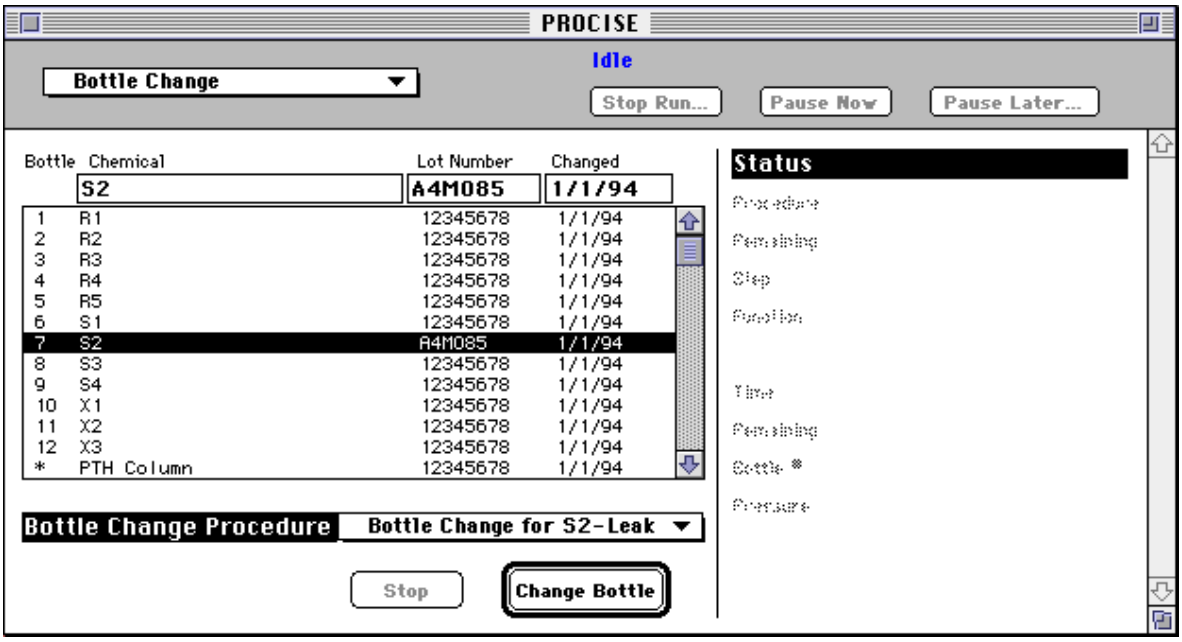

Figure 7-9. Bottle leak test selected as part of the bottle change procedure

Cartridge Leak Test Overview

The cartridge leak test ensures the leak tightness of each cartridge. The cartridge is pressurized, and the leak rate is monitored. The actual cartridge pressure must be within 0.07 psi of the target pressure to pass the cartridge leak test. The flow path for a leak test performed on cartridge A is shown in Figure 7-11 on page 7-16.

Cartridge Leak Test Procedure

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click Leak (Figure 7-10).
3. Select the appropriate cartridge leak test from the test menu.
4. Select or deselect the Don't pause on error box. Refer to page 7-5 for information on this option.
5. Click Start Test.
6. Allow the test to run to completion.

Test results are reported in the Event Log at the end of the test.

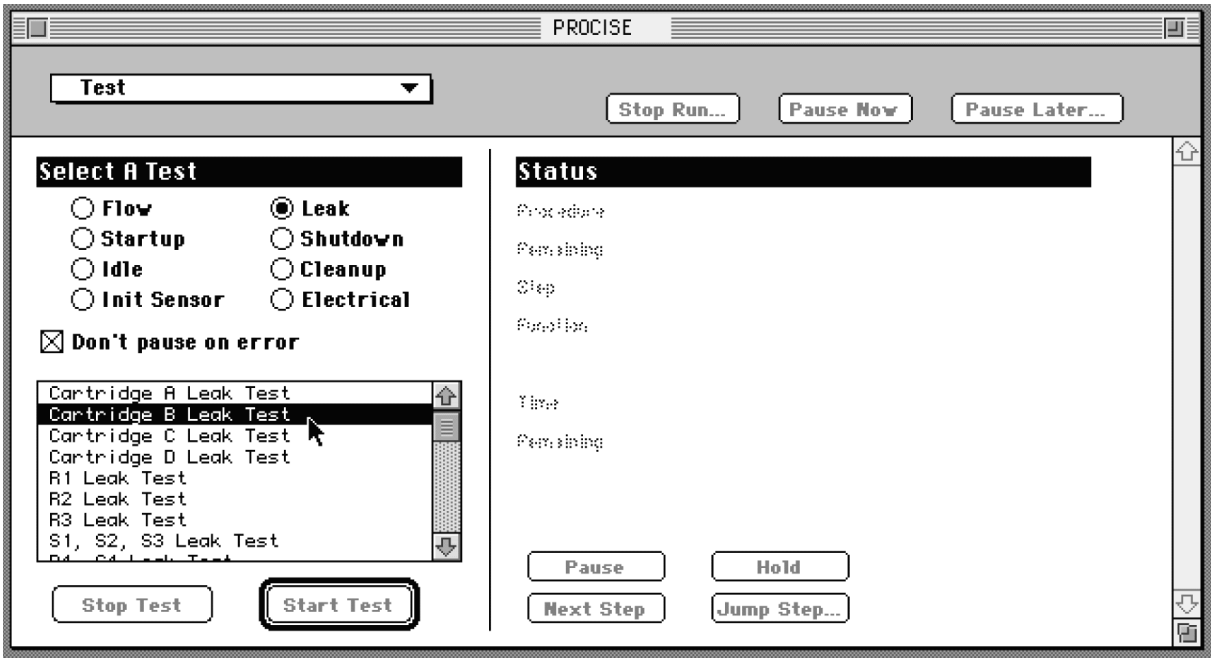

Figure 7-10. Cartridge Leak Test

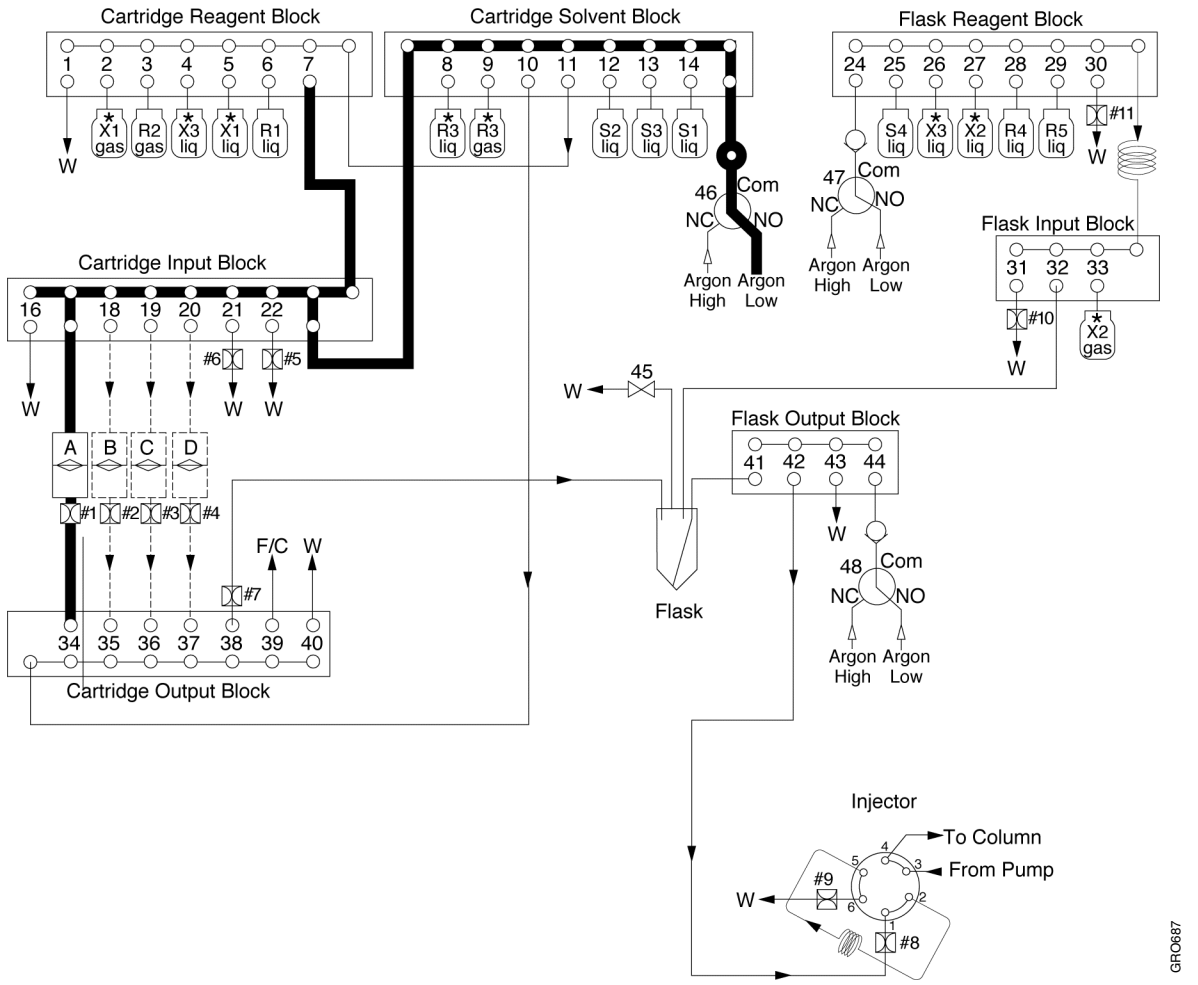

Figure 7-11. Flow path for cartridge leak test performed on cartridge A

Flask Leak Test Overview

The flask leak test checks the sealing and venting capability of the flask assembly. Test results are reported in the Event Log at the end of the test. The actual flask pressure must be within 0.05 psi of the target pressure to pass the flask leak test.

The flow path for this test is illustrated in Figure 7-13 on page 7-18.

Flask Leak Test Procedure

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click Leak.
3. Select Flask Leak Test from the test menu (Figure 7-12).
4. Select or deselect the Don't pause on error box. Refer to page 7-5 for information on this option.
5. Click Start Test.
6. Allow the test to run to completion.

Test results are reported in the Event Log at the end of the test.

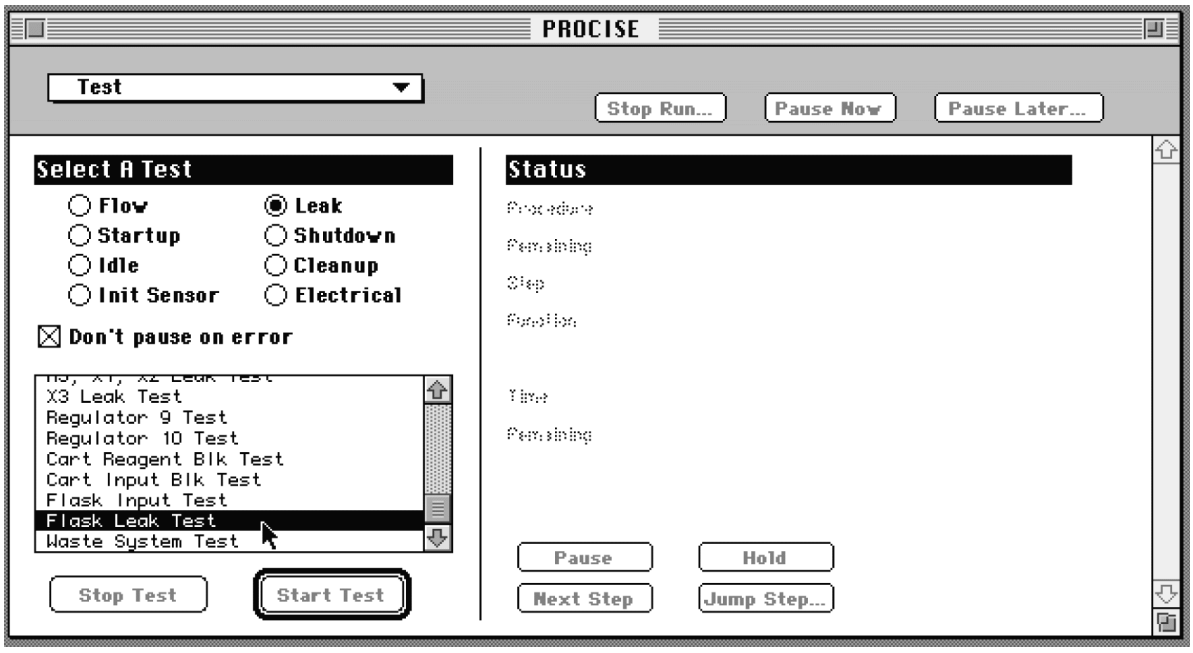

Figure 7-12. Flask Leak Test

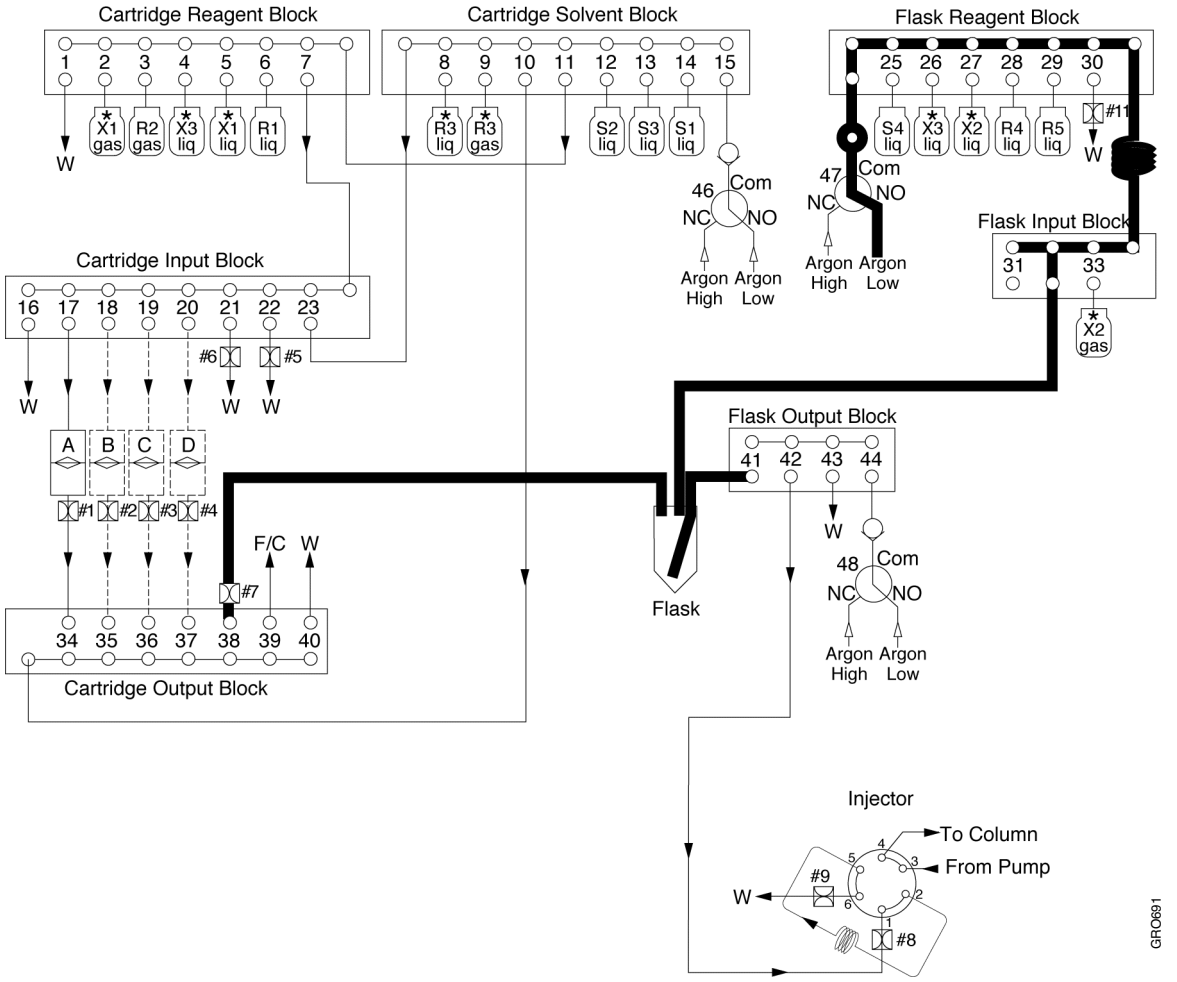

Figure 7-13. Flow path for the flask leak test

Flask Input Test Overview

The flask input test is used to leak test both the flask reagent and flask input blocks. The flow path for this test is shown in Figure 7-15 on page 7-20.

Flask Input Test Procedure

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click Leak.
3. Select Flask Input Test from the test menu (Figure 7-14).
4. Select or deselect the Don't pause on error box. Refer to page 7-5 for information on this option.
5. Click Start Test.
6. Allow the test to run to completion.

Test results are reported in the Event Log at the end of the test.

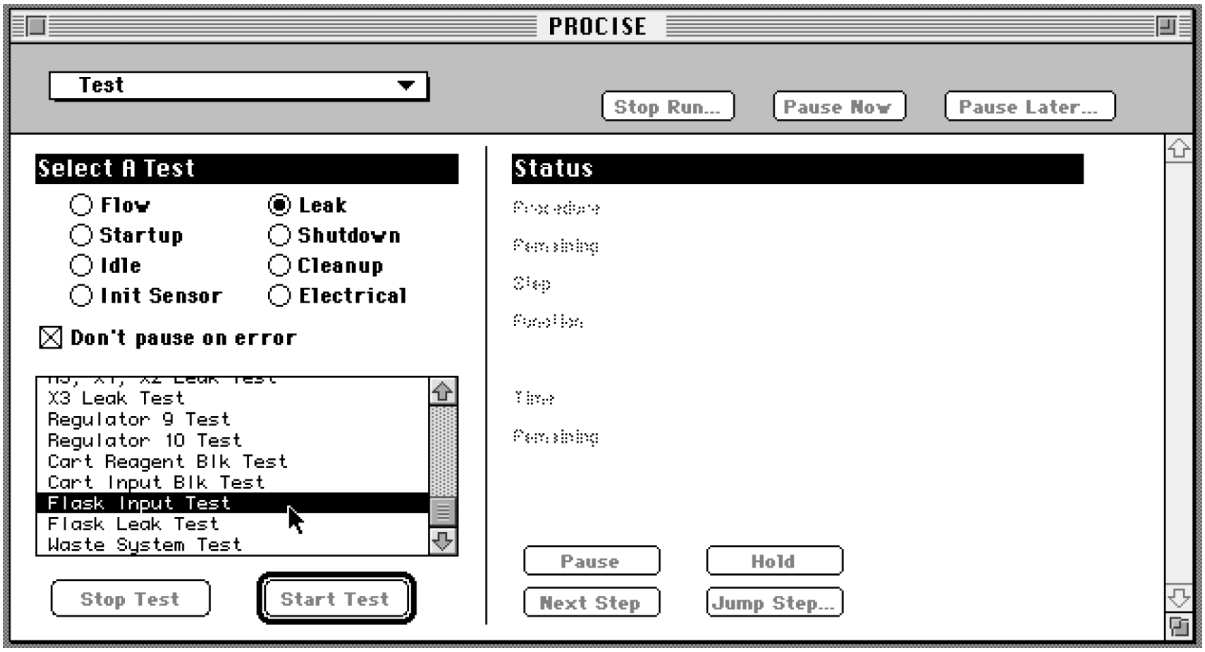

Figure 7-14. Flask input leak test

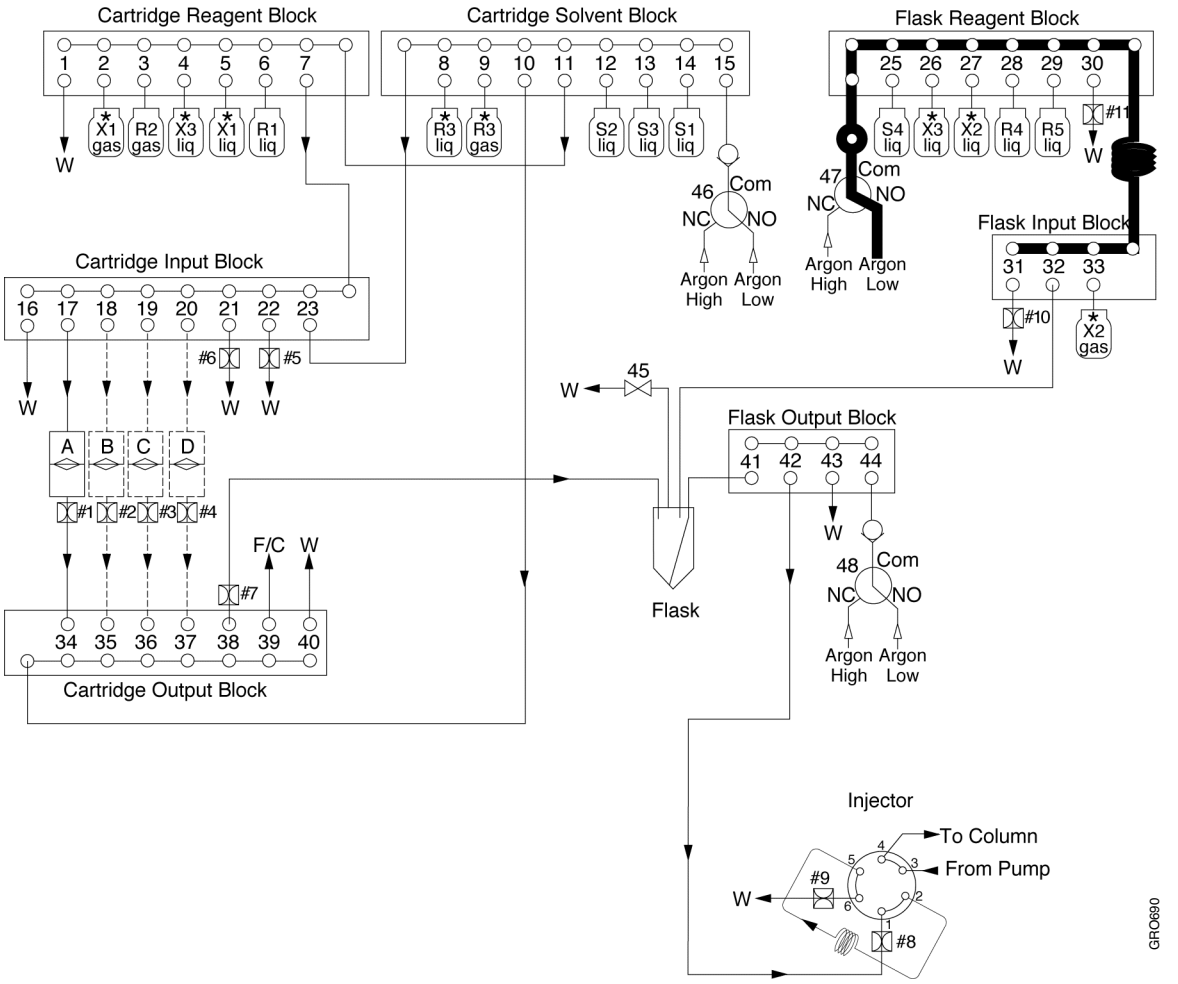

Figure 7-15. Flow path for the flask input leak test

Cartridge Block Leak Tests Overview

Two cartridge (valve) block leak tests are included with the Procise cLC control software: a cartridge reagent block test, and a cartridge input block test. These tests check the sealing and venting capability of each valve block. The actual pressure held in the valve block must be within 0.05 psi of the target pressure to pass the valve block leak test. The flow paths for these tests are illustrated on pages 7-22 and 7-23.

Cartridge Block Leak Tests Procedure

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click Leak.
3. Select either Cart Reagent Blk Test or Cart Input Blk Test from the test menu (Figure 7-16).
4. Select or deselect the Don't pause on error box. Refer to page 7-5 for information on this option.
5. Click Start Test.
6. Allow the test to run to completion.

Test results are reported in the Event Log at the end of the test.

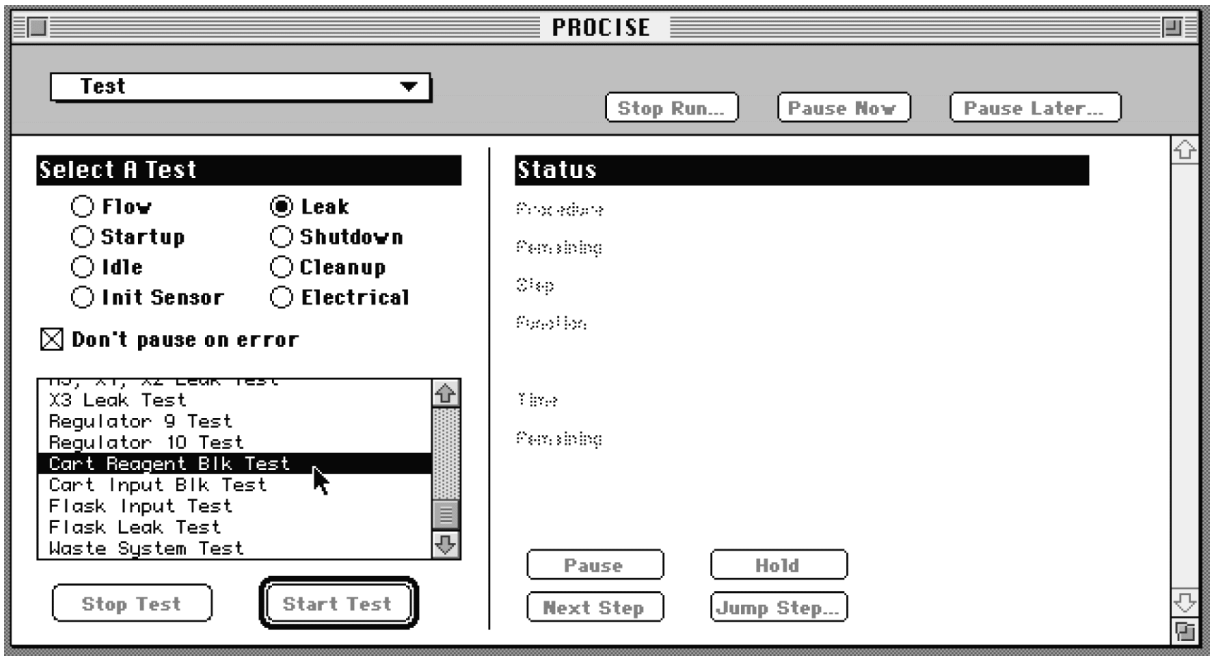

Figure 7-16. Cartridge Block Leak Tests

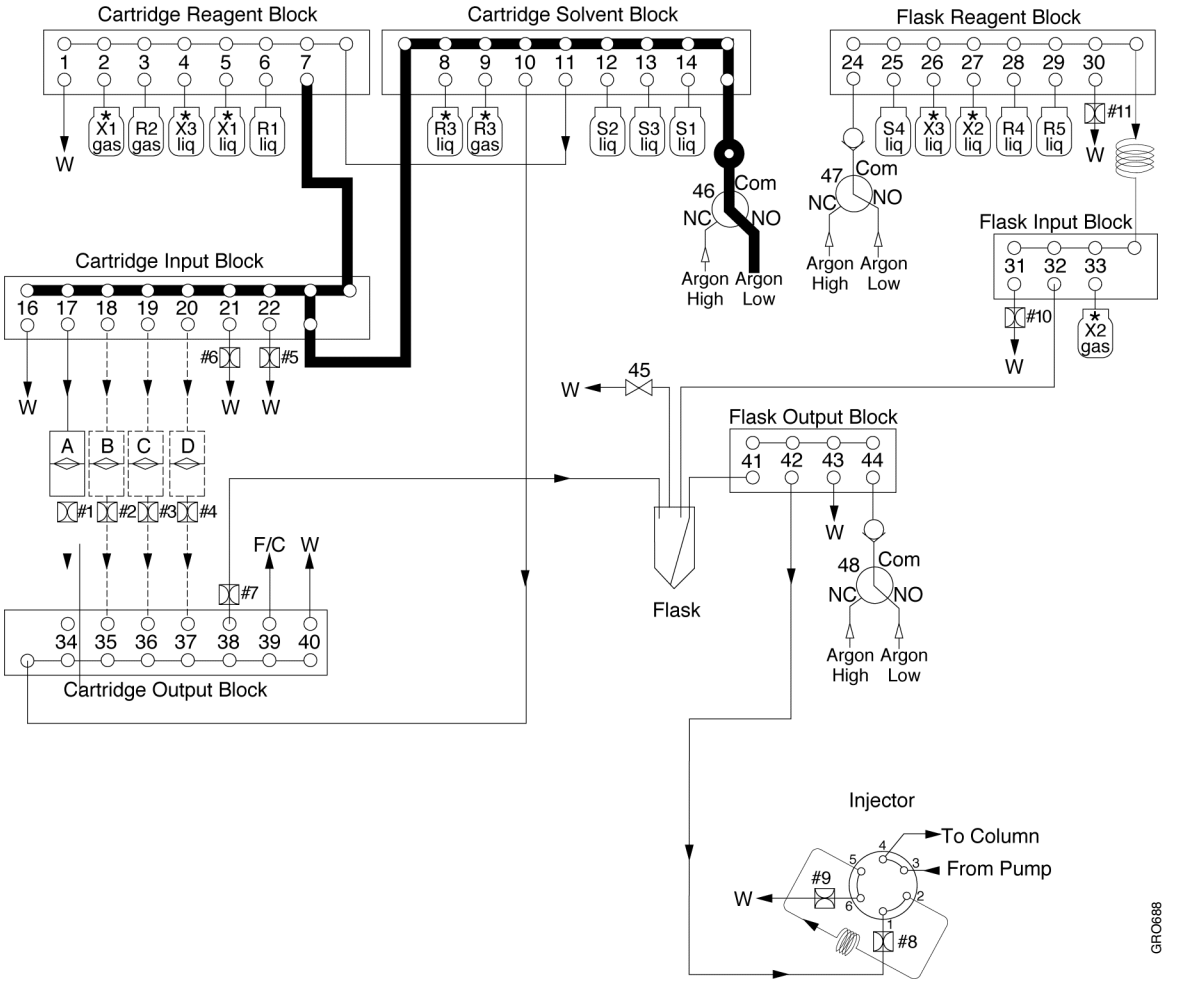

Figure 7-17. Flow path for the cartridge input block leak test

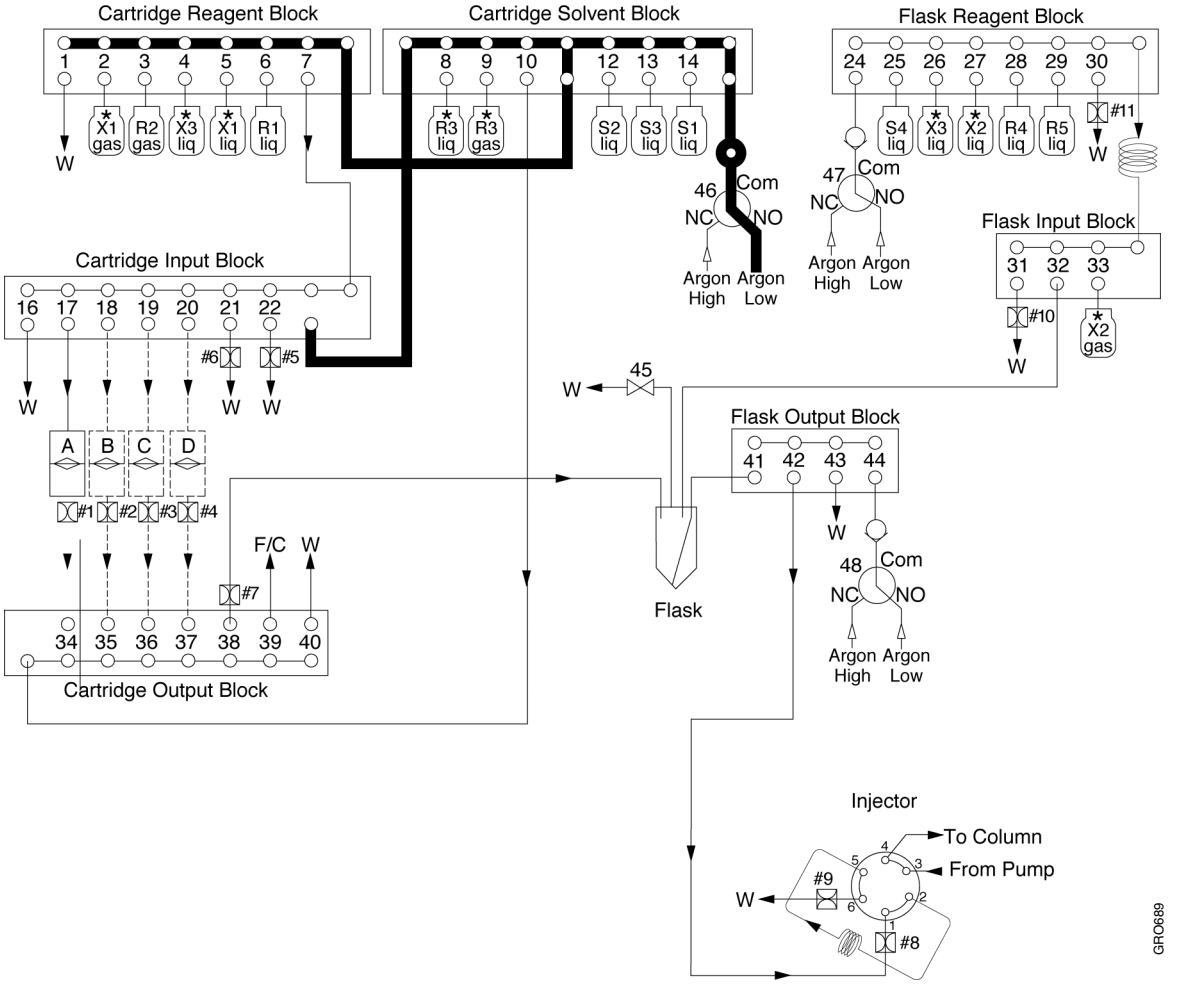

Figure 7-18. Flow path for cartridge reagent block leak test

Shutdown Procedures

Two shutdown procedures are included with the Procise cLC control software: the Post-run Valve Block Wash X1-X2, and the Short-term Shutdown cLC procedure.

Post-Run Valve Block Wash X1–X2 Overview

The Post–Run Valve Block Wash washes the system flowpaths from the X1 bottle position with methanol, and the X2 bottle position with acetonitrile. No solvent is delivered through the reaction cartridges or to other bottles.

The Post–Run Valve Block Wash can be run two different ways:

- As part of a sequencing run from the Start Run dialog box. The procedure is executed at the end of the run.
- Independently from the Test dialog box.

Post-Run Valve Block Wash X1–X2 Procedures

IMPORTANT	*Before starting this procedure, make sure methanol is loaded in the X1 bottle position, and acetonitrile is loaded in the X2 bottle position.*

To run the Post-Run Valve Block Wash X1–X2 as part of a sequencing run:

1. Select the Start Run dialog box from the dialog box pop-up menu. At the bottom of the dialog box are pop-up menus for Startup and Shutdown procedures.
2. Select Post-Run Valve Block Wash X1-X2 from the Shutdown pop-up menu (Figure 7-19).

To run the Post-Run Valve Block Wash X1–X2 independently:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click Shutdown.
3. Select Post-run Valve Block Wash X1-X2 from the test menu (Figure 7-20).
4. Select or deselect the Don't pause on error box. Refer to page 7-5 for information on this option.
5. Click Start Test.
6. Allow the procedure to run to completion.

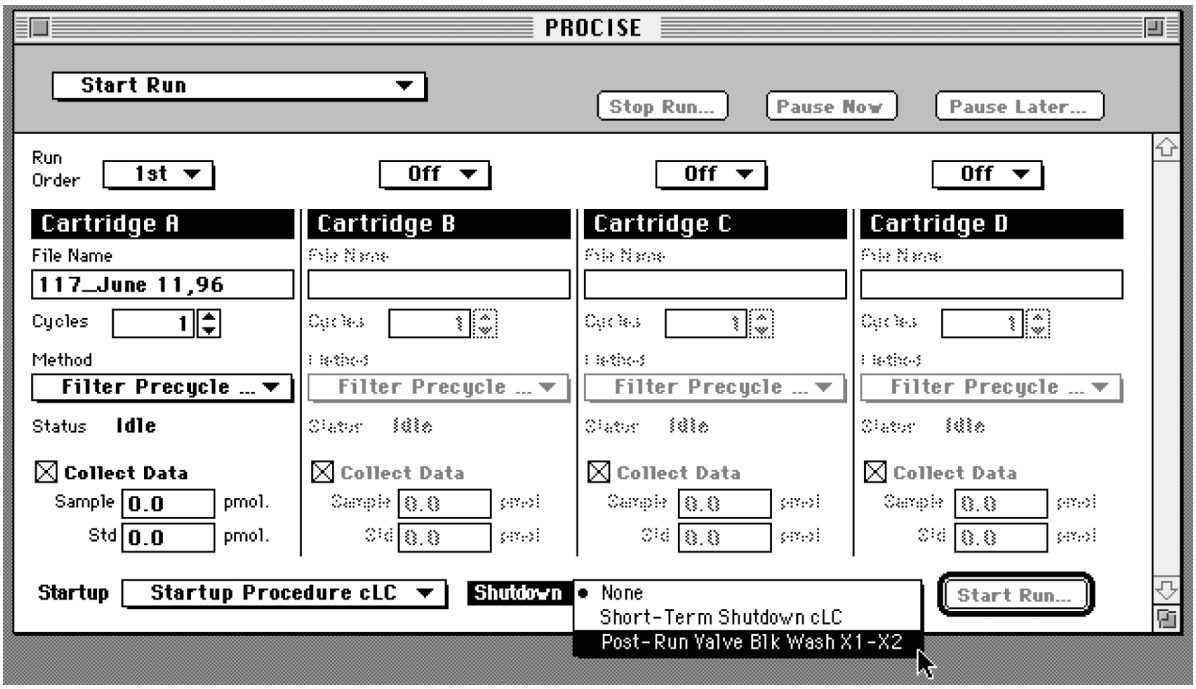

Figure 7-19. Post-Run Valve Block Wash included in a sequencing run

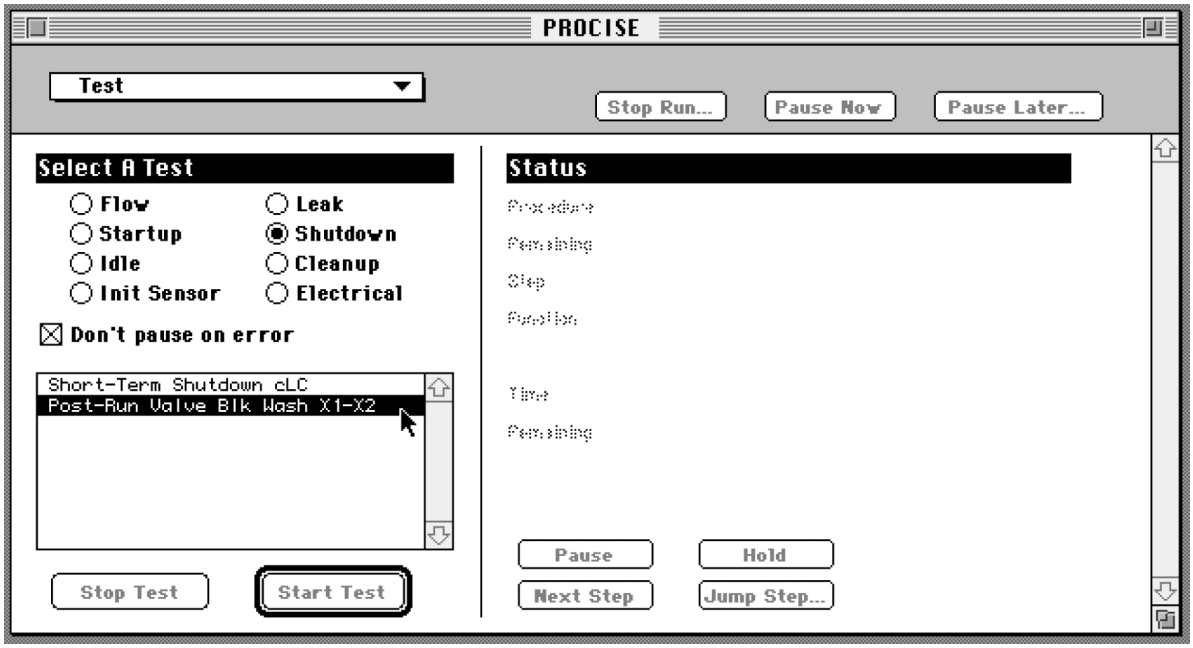

Figure 7-20. Post-Run Valve Block Wash Shutdown procedure

Short-Term Shutdown cLC Procedure Overview

If the sequencer will be idle for 1 to 2 weeks, execute the Short-Term Shutdown cLC procedure. This procedure washes all the valve blocks, delivery lines and loops with ethyl acetate (S2B). The flask and injector are washed with S4C. After common flow paths are washed and flushed with argon, each bottle is briefly backflushed with argon to remove reagents from the delivery line.

The Short-Term Shutdown cLC procedure can be included in a sequencing run from the Start Run dialog box. When included as part of a run, the procedure is executed after completion of the last cartridge scheduled to be run.

The shutdown procedure can also be executed independently from the Test dialog box.

Short-Term Shutdown cLC Procedures

To include the Short-Term Shutdown cLC procedure as part of a sequencing run:

1. Select the Start Run dialog box from the dialog box pop-up menu. At the bottom of the dialog box are pop-up menus for Startup and Shutdown procedures.
2. Select Short-Term Shutdown cLC from the Shutdown pop-up menu (Figure 7-21).

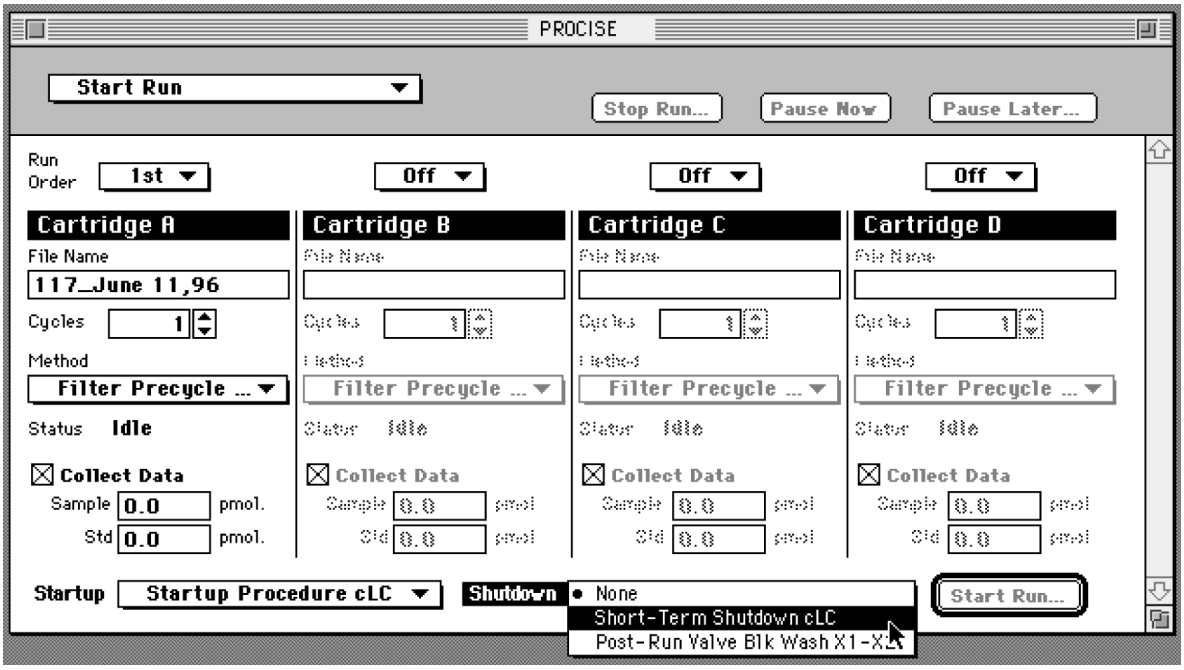

Figure 7-21. Short-Term Shutdown procedure as part of a sequencing run

To run the Short-Term Shutdown cLC procedure independently:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click Shutdown.
3. Select Short-Term Shutdown cLC from the test menu (Figure 7-22).
4. Select or deselect the Don't pause on error box. Refer to page 7-5 for information on this option.
5. Click Start Test.
6. Allow the procedure to run to completion.

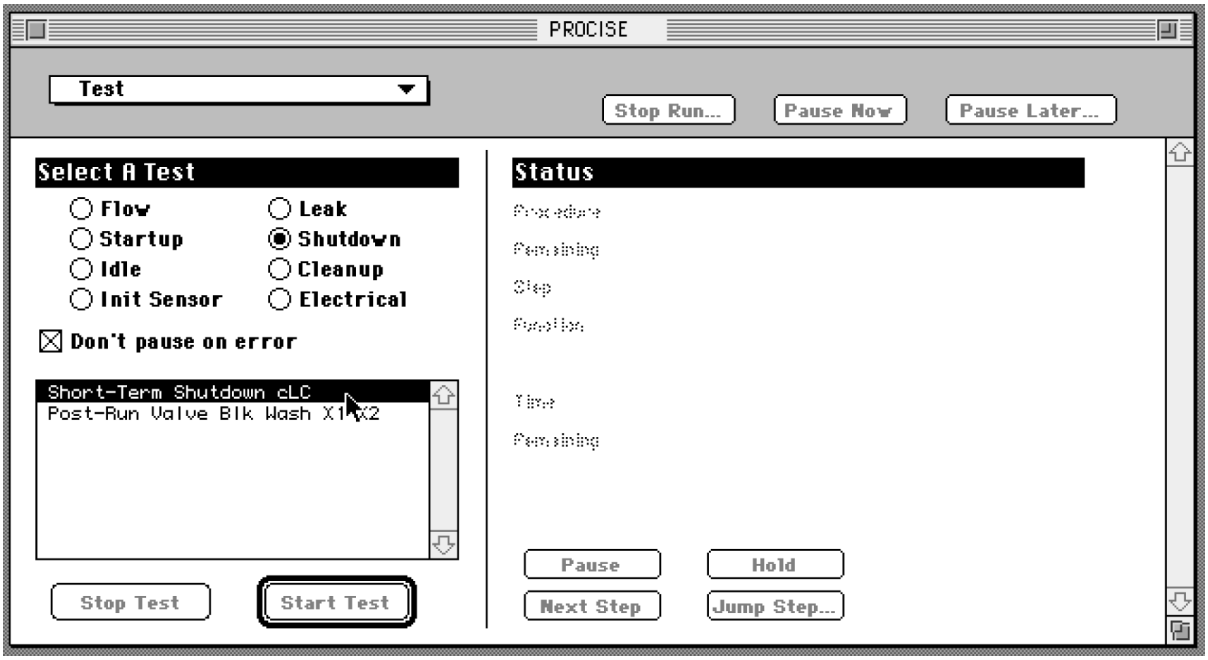

Figure 7-22. Short-term shutdown procedure run independently

Cleanup Procedures

Cleanup Procedures Overview

Five cleanup procedures for the sequencer are included in the Procise cLC control software:

- Delivery Line Backflush
- System Clean-Out – X3
- System Flush – Argon
- Cartridge Line Clean-up cLC
- Clean Transfer Line with X1

The first three procedures listed above are used only for a complete system shutdown, where all the instruments will be powered-down, disconnected and placed in storage. Refer to "Complete System Shutdown Procedure" in Section 9, "Maintenance", for further information on a complete system shutdown and the use of these procedures.

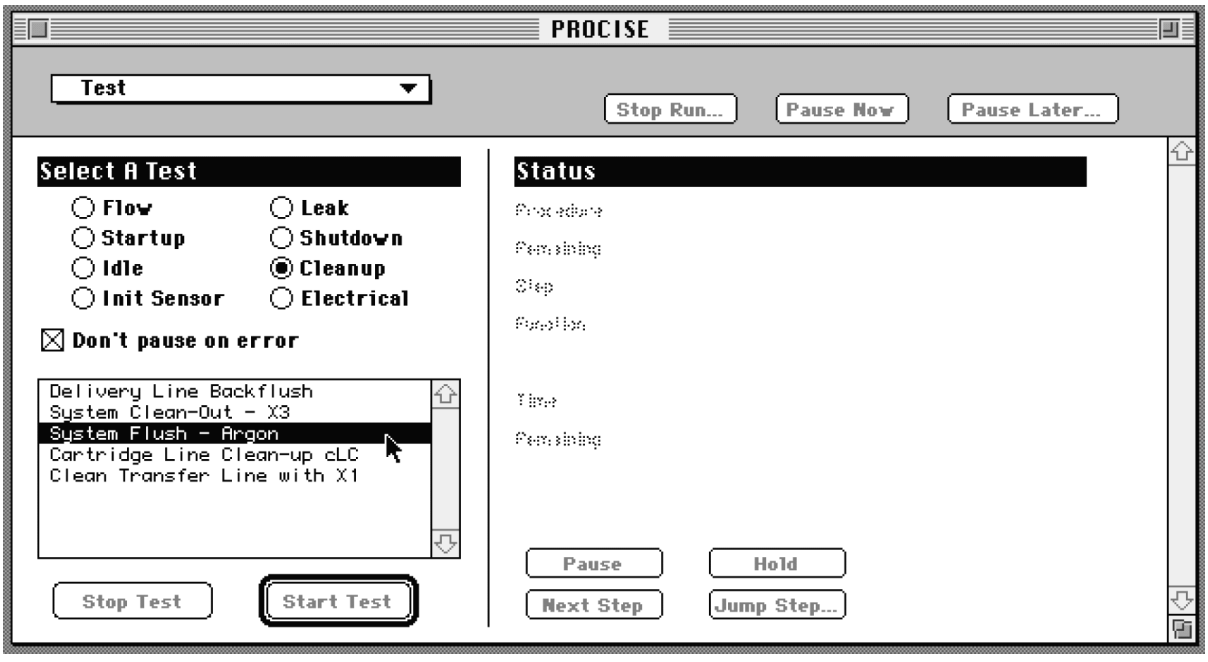

Figure 7-23. Cleanup procedures

Cartridge Line Cleanup cLC Recommendations

Run the Cartridge Line Cleanup cLC procedure:

- On a routine basis (weekly or monthly) as part of your regular sequencer maintenance.
- When chemical noise or background becomes too high, and is not due to a dirty sample(s). Verify by running a cartridge with no sample.

Cartridge Line Cleanup cLC Overview

This procedure:

- Cleans the reagent, solvent, input and output valve blocks from the S2 position with methanol.
- Thoroughly washes the cartridge inlet and outlet lines.
- Thoroughly dries the washed areas.

Note	*Do not run this procedure when samples are loaded on the sequencer.*

Cartridge Line Cleanup cLC Procedure

Install methanol in the S2 bottle position:

1. Select Bottle Change from the dialog box pop-up menu.
2. Select Bottle 7, Chemical S2 from the chemistry menu.
3. Select Bottle Change for S2 from the Bottle Change Procedure pop-up menu, and click Change Bottle.
4. When prompted, remove the S2 bottle and install a bottle of methanol.
5. Click Continue.

Run the Cartridge Line Clean-up cLC procedure:

1. Once the bottle change procedure is complete, select Test from the dialog box pop-up menu.
2. Click Cleanup.
3. Select Cartridge Line Clean-up cLC from the list of procedures.
4. Click Start Test.
5. When the clean-up procedure is complete, use the Bottle Change procedure for S2 to remove the bottle of methanol, and reinstall the S2 bottle onto the sequencer.

Clean Transfer Line with X1 Overview

This procedure removes buildup from the transfer line between the output block of the cartridges to the flask. Perform this procedure:

- When background becomes excessive.
- On a routine basis (weekly or monthly) as part of regular sequencer maintenance.

Clean Transfer Line with X1 Procedure

Install methanol in the X1 bottle position:

Perform this portion of the procedure only if methanol is not already installed in the X1 bottle position.

1. Select Bottle Change from the dialog box pop-up menu.
2. Select Bottle 10, Chemical X1 from the chemistry menu.
3. Select Bottle Change for X1 from the Bottle Change Procedure pop-up menu, and click Change Bottle.
4. When prompted, remove the X1 bottle and install a bottle of methanol if methanol is not already installed in this bottle position.
5. Click Continue.

Run the Cartridge Line Clean-up cLC procedure:

1. Select Test from the dialog box pop-up menu.
2. Click the Cleanup button.
3. Click Clean Transfer Line with X1 from the list of procedures.
4. Click Start Test.

Electrical Test Procedure

Overview

The Electrical test:

- Checks the electrical continuity of key components in the system.
- Is run automatically every time the sequencer is powered up.
- Switches the rheodyne valve from the load position to the inject position, then back to the load position during step 7 in the procedure.
- Reports failures in a dialog box on the screen, and in the Event Log.

Procedure

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click Electrical.
3. The Electrical Test Procedure is automatically selected.
4. Select or deselect the Don't pause on error box. Refer to page 7-5 for information on this option.
5. Click Start Test, and allow the test to run to completion.
6. Review the results of the test in the Event Log.

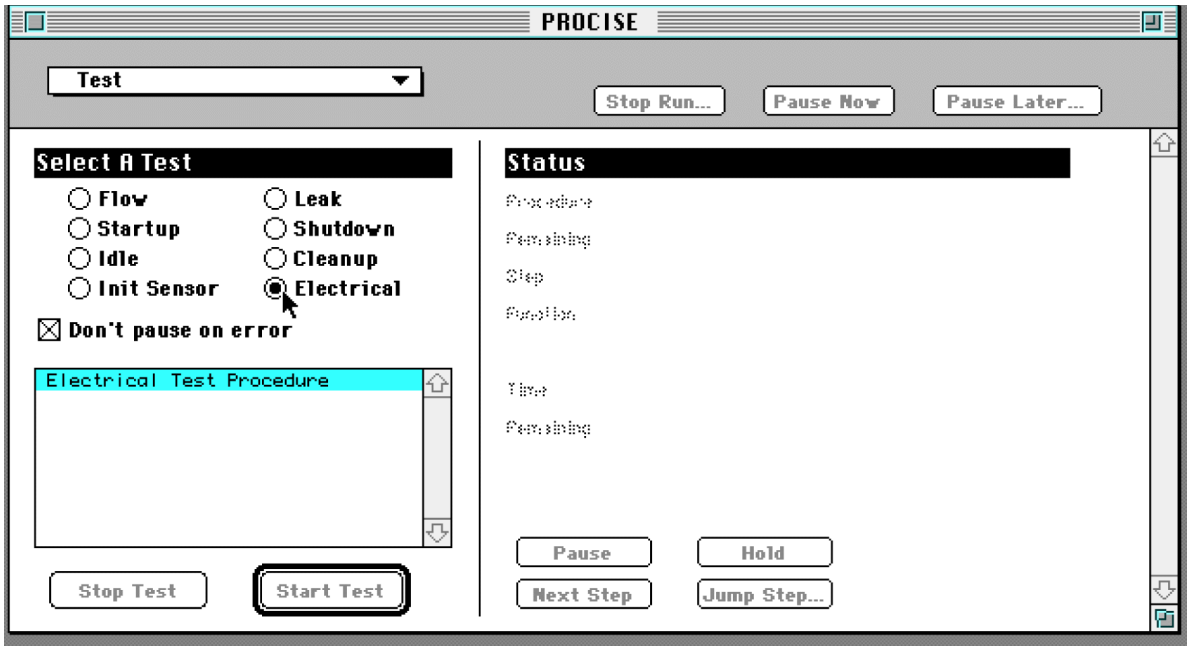

Figure 7-24. Electrical test

Bottle Change Procedure

Overview

Each bottle change procedure backflushes a specific chemical into the reagent bottle, then vents the bottle so you can change it. After the new bottle is loaded, the procedure flushes the bottle with argon gas, delivers the chemical to the waste bottle, and washes the associated valve blocks and Teflon lines.

Two types of bottle change procedures are available for each bottle position. One allows you to change the selected bottle(s) only. The other procedure performs a leak test on the bottle position as well as a bottle change. The procedures that perform leak tests are identified by *-leak* at the end of the procedure name.

The cycle time for each bottle change procedure is listed in Table 7-1.

Procedure

The system must be idle or paused to run a Bottle Change procedure.

Remove the bottle from the sequencer:

1. Select the Bottle Change dialog box from the dialog box pop-up menu. **Do not remove the bottle at this time.**
2. Select the appropriate bottle position, solvent or column from the menu.
3. Select the appropriate procedure from the Bottle Change Procedure pop-up menu (Figure 7-25 on page 7-34).
4. Place the cursor in the Chemical box and hit the tab key on the Macintosh keyboard. The cursor will move to the Lot Number box, and the lot number will be highlighted.
5. Enter the lot number of the new bottle in the Lot Number box. The date will be updated automatically.
6. Click Change Bottle.
7. When prompted, remove the old bottle and bottle seal.

IMPORTANT *Leak tests use functions that alter sequencer operating pressures. If the procedure is aborted before completion, select the Pressures & Temperatures dialog box. Click Default to restore the default settings.*

Table 7-1. Bottle Change Procedure List

Procedure	**Cycle Time** (min)
Bottle Change for R1	1:30
Bottle Change for R1 - leak	2:40
Bottle Change for R2	1:00
Bottle Change for R2 - leak	2:00
Bottle Change for R3	3:00
Bottle Change for R3 - leak	4:15
Bottle Change for R4A	1:35
Bottle Change for R4A - leak	3:00
Bottle Change for R5	1:30
Bottle Change for R5 - leak	2:55
Bottle Change for S1	1:30
Bottle Change for S1 - leak	2:55
Bottle Change for S2B	1:15
Bottle Change for S2B - leak	2:40
Bottle Change for S3	1:15
Bottle Change for S3 - leak	2:40
Bottle Change for S4C	1:15
Bottle Change for S4C - leak	2:40
Bottle Change for X1	2:40
Bottle Change for X1 - leak	4:05
Bottle Change for X2	1:55
Bottle Change for X2 - leak	3:20
Bottle Change for X3 (both)	4:20
Bottle Change for X3 (both) - leak	5:45

Install the new bottle:

1. Install a new seal on the rim of the new bottle.
2. Screw the new bottle into the bottle cap assembly, tightening until the seal contacts the top of the bottle cap assembly. Then turn the bottle approximately 1/4-turn more.

IMPORTANT *Do not tighten bottles until a snapping sound (ratcheting) is produced by the bottle cap assembly. Ratcheting the bottle cap assembly causes premature wear, and can crack the bottle seal.*

3. Click Continue. The procedure will continue through the remaining steps, which includes priming the delivery line up to the valve block.
4. Repeat the procedure to change additional bottles if necessary.
5. When you are finished, pull down the File menu from the main menu bar.
6. Select Save Chemicals.

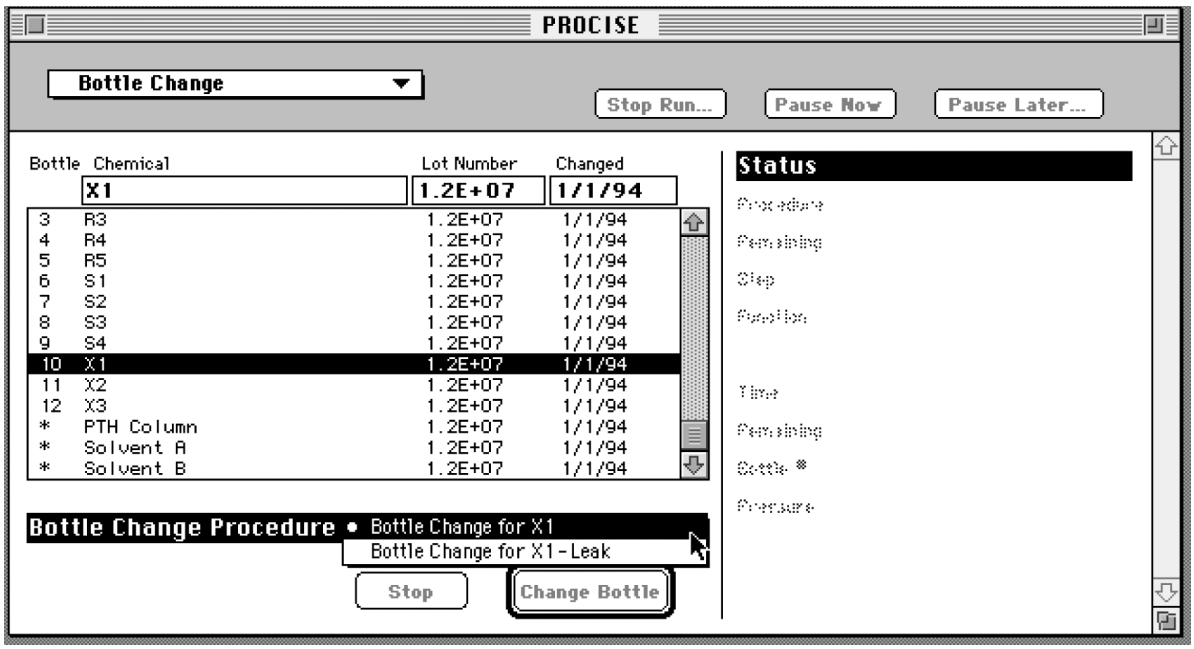

Figure 7-25. Bottle Change dialog box

Creating Tests and Procedures

Overview

To create your own tests and procedures, you can:

- Save a standard test or procedure under a new, unique file name, then edit the test or procedure.
- Select *User Defined Cycle 1* from the Test pop-up menu shown in Figure 7-28, then select and insert steps into the test or procedure.

Procedure

1. Select the Cycles & Procedures dialog box from the dialog box pop-up menu.
2. Select the type of procedure you wish to create from the cycle and procedure category pop-up menu (Figure 7-27 on page 7-37).
3. Select the test or procedure you wish to use as a template from the test and procedure pop-up menu (Figures 7-27 and 7-28 on page 7-37).
4. Pull down the File menu from the main menu bar, and select Save Cycle/Procedures As… (Figure 7-26 on page 7-36).
5. Enter a unique name for the new test/procedure, and click OK.
6. Edit the steps in the procedure as follows:

   To delete a row, highlight the row, and click Delete Row.

   To insert a row, select the function to be inserted from the function list. Highlight the row immediately before the insertion point, and click Insert Row.

IMPORTANT *The maximum number of steps allowed per cycle is 100.*

Every cycle must include a Begin step and an End step.

---

7. Deselect the box labeled Global if the global time not is used.
8. Enter the function time in seconds in the Value box.
9. Save the procedure by pulling down the File menu from the main menu bar, and selecting Save Cycle/Procedure.

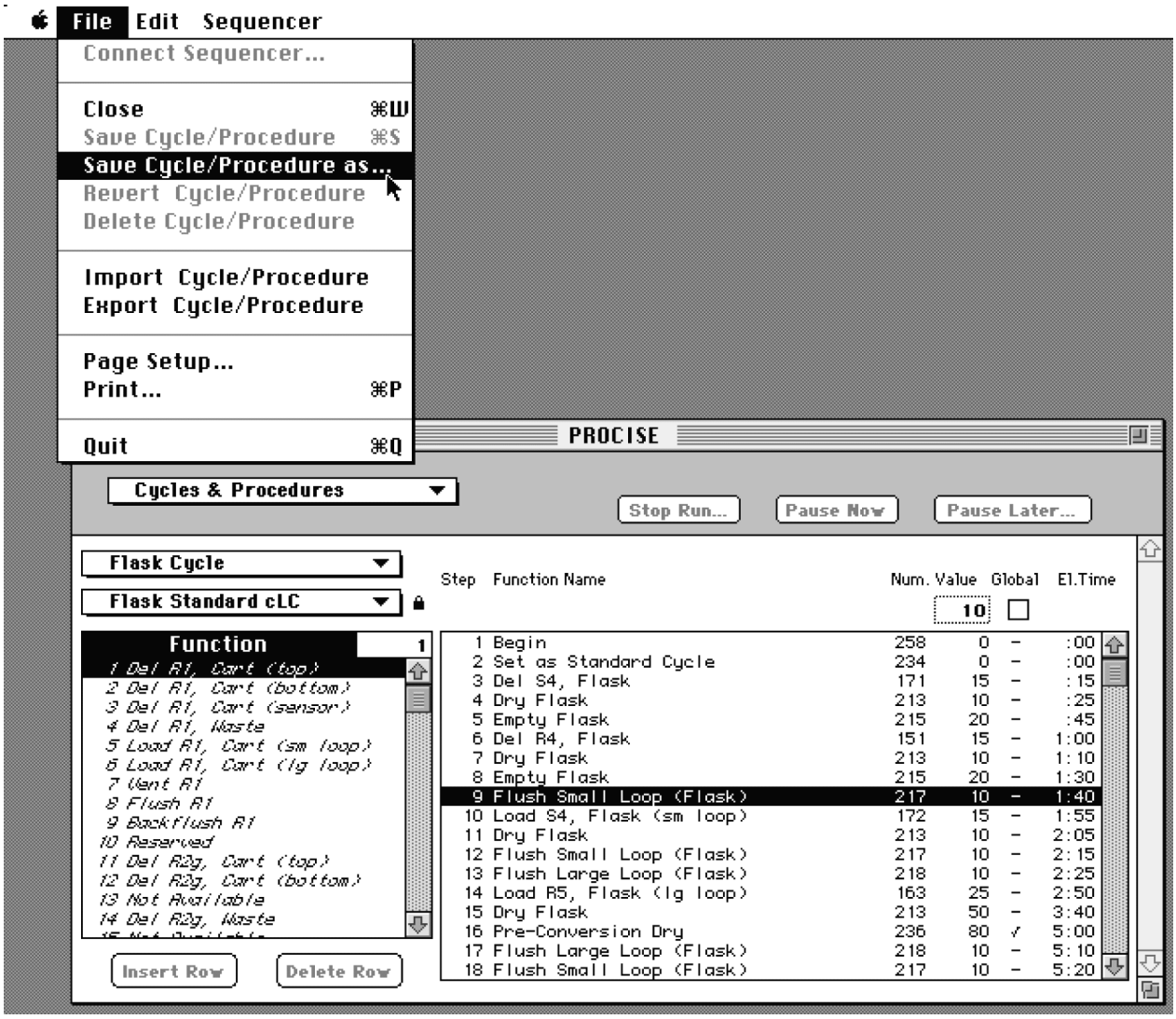

Figure 7-26. Renaming a cycle or procedure

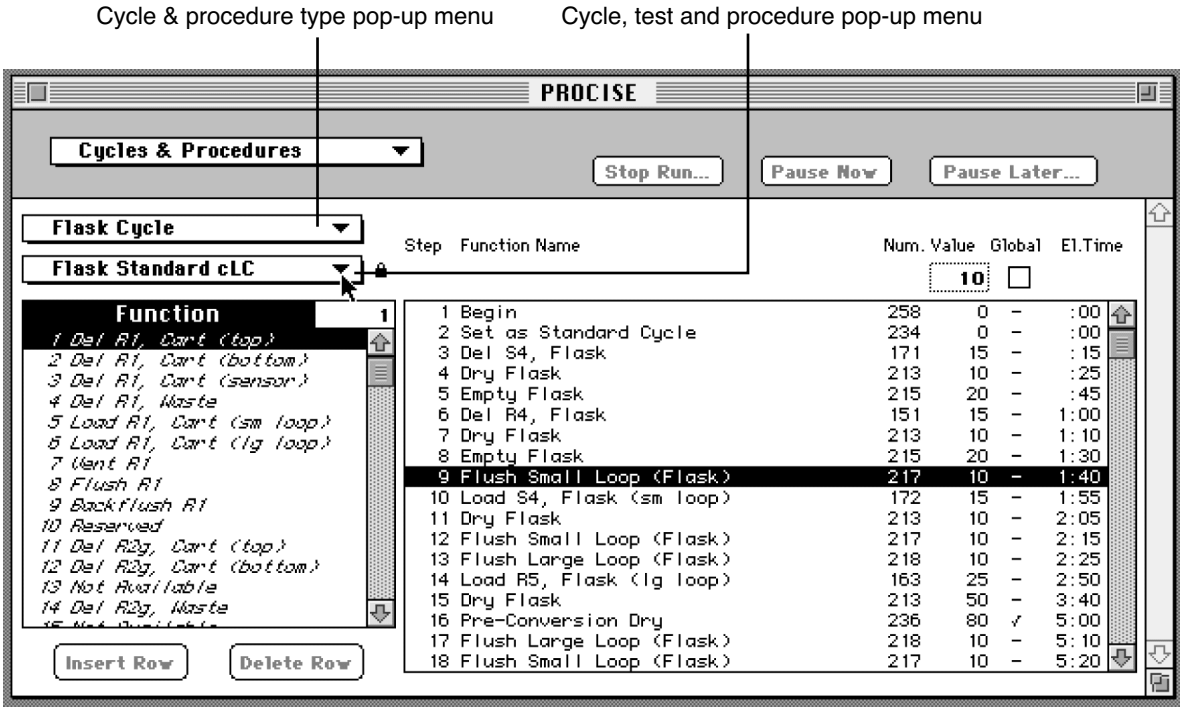

Figure 7-27. Cycles and Procedures dialog box

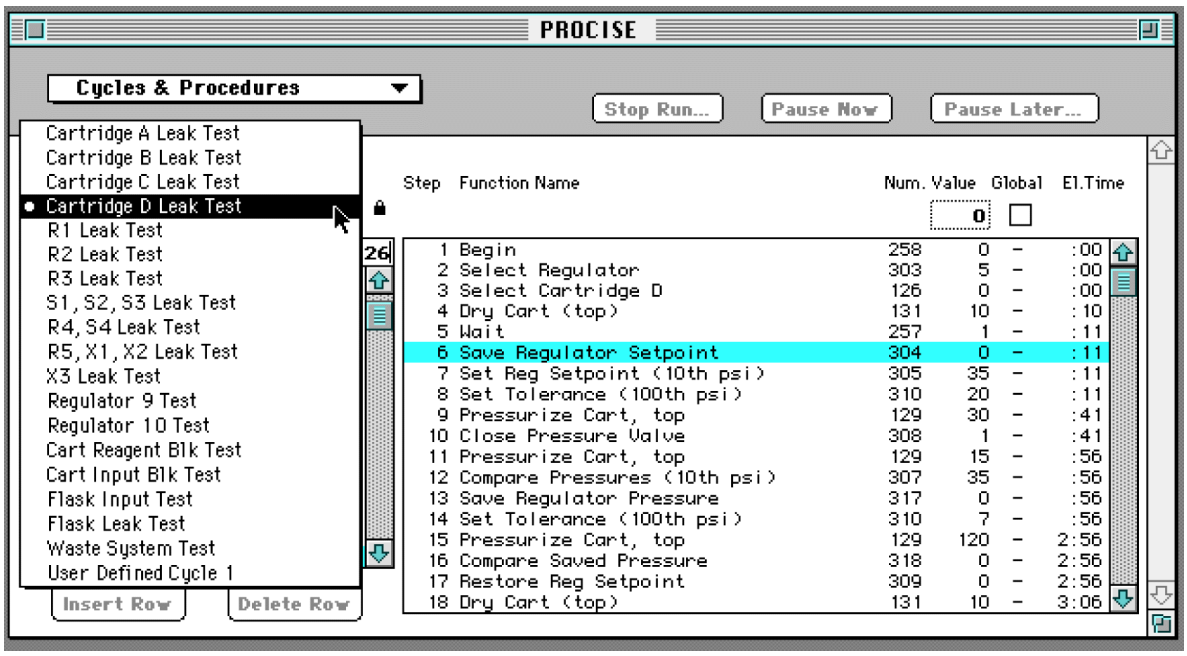

Figure 7-28. The cycle, test and procedure pop-up menu when Leak Procedures is selected from the cycle & procedure type pop-up menu

Macintosh Lock-up Procedures

Gathering Information During a Lock-up

Record the answers to the following general, Macintosh, and sequencer questions.

General

1. Note the circumstances under which the lock-up occurs. This information is critical for determining the cause of the problem.
2. What time did the lock-up occur (morning, over night etc.)?
3. Did any other instrumentation experience a problem?
4. Note the sequence of events that preceded the lock-up?
5. If a system lock-up occurred in the past, did it occur under the same circumstances? Can it be reproduced?

Macintosh

1. Does the cursor move?
2. Are any screen functions active?
3. Is a 610A “Collecting” window open?
4. Is the step time counting down on the Monitor Run screen?
5. Which steps are displayed?
6. What is the most current information in the Event log?

Sequencer

1. Is the door panel COMM LED lit?
2. Is the door panel SEQ LED lit?
3. Is the sequencer is still running? If it is, you will hear the valves clicking.
4. Are any of the red Error LEDs on the inner panel lit?
5. Are any of the green Status LEDs on the inner panel lit?

Recovering from a Lock up

Overview

Try the following suggestions, one at a time, in sequence until normal operation is restored. Resetting (cold booting) the sequencer as described below is a last resort because it will erase the sequencer memory including the current run conditions, chromatogram data and the Event buffer. The Event buffer may contain valuable information which has not yet been transferred to the Macintosh Event Log file on the hard disk.

Procedures

Reboot the Macintosh:

1. Reboot the Macintosh computer. (Re-launch the Procise application if it does not automatically launch as part of the start-up routine).
2. Power-down and power-up the sequencer.
3. Reboot the Macintosh computer.

IMPORTANT	*If communication is re-established after step 3, and you wish to abort the run, wait 5 min before you click Stop. This will ensure that all of the data is transferred from the sequencer to the Macintosh.*

Reset (cold boot) the sequencer:

1. Power-down the sequencer.
2. Unplug the Mel card (left-hand side, upper, rear corner).
3. Power-up the sequencer.
4. Power-down the sequencer.
5. Plug in the Mel card.
6. Power-up the sequencer.
7. Reboot the Macintosh. The message “Execution of Cold start (all RAM has been initialized)” should appear in the Event log. If it does not, make sure that jumper, W6, has been removed from the CPU printed circuit board.

Procedure for Gathering Information after a Lock-up

Record the following information:

1. What version of software or firmware is installed for the following:
   - Mel card
   - Procise operating software
   - 610A software
2. What operating system is running on the Macintosh?
3. Print relevant sections of the Event Log.

Print the 610A status log complete with service information:

1. While in the 610A application, hold down the key while selecting the menu.
2. Select Show Service Info. After a short time, the 610A status log will open. The Macintosh service information will be appended to the end of the 610A status log.
3. Select Print from the File menu.

Recovering from a Power Failure

Overview

Should a power failure occur while the sequencer is running, an error will be generated in the Event buffer, and the run will pause at the end of the cycle once the power returns. If you wish to continue the run, follow the procedure below.

Procedure

1. Check that all the instruments are powered-up, and that the Procise application has automatically launched.
2. Scroll to the Pressures & Temperatures dialog box, and click Execute to turn the heaters back on. The respective heater LEDs on the front panel should illuminate.
3. Scroll to the Start Run dialog box, and click Resume.
4. Relaunch the 610A application.
5. The message “File already exists” may be generated now or later during the run. If this occurs, you will be prompted to rename the 610A data file. Rename the file at this time. The new file you create will contain all the data from the original file.
6. Delete the original 610A file.

Event Log Procedures

Deleting the Event Log

1. Open the Event Log.
2. Select Delete from the pull-down File menu.
3. In response to the dialog box, enter the date of the oldest event log message you wish to keep. This means that all messages prior to this date will be deleted.
4. Click OK.
5. Click Delete in the dialog box that opens.

Using Microsoft Word to Archive and Print the Event log

Overview

If the Microsoft Word application is loaded onto the Macintosh, you can conveniently convert the Event Log file into a Word file, and:

- Print the document in a different format.
- Archive Event Logs on the computer once they exceed 100 kB.

Procedure

Open the Event Log as a Microsoft Word document:

1. Launch the Microsoft Word application.
2. From the pull-down File menu, select Open.
3. Select All Files from the *List files of type* scroll menu on the subsequent dialog box.
4. Locate PROCISE Eventlog from the *Select document* scroll menu. It is in the PROCISE folder which, in turn, is in the Preferences folder.
5. Double-click PROCISE Eventlog to open this file.

Save the event log in an archive folder:

1. Select Save from the pull-down File menu.
2. Click Desktop.
3. Click *New folder,* and name it “Event log archive”.
4. Name the file “Event log (date)”, and click Save.
5. The file, PROCISE Eventlog, may now be deleted.

Choosing a Suitable 610A Reference Peak

Purpose

A fluctuating laboratory temperature can cause all of the peaks in a chromatogram to shift similarly in the same direction. Selecting a reference peak enables the 610A software to compensate for this shift.

Guidelines

The reference peak must be:

- Present in all residue (sequencing) cycles and the PTH-Standard cycle.
- Far from amino acid peaks (± 0.25 min).
- The largest peak if part of a group of non-amino acid peaks.

The PTH-Amino Acid Standard mixture currently includes four peaks that are not amino acids: DMPTU, DPTU, DPU and PMTC. The suitability of these and other reference peaks is as follows:

DMPTU is not suitable as a reference peak because it is not produced as a by-product of the N-methylpiperidine chemistry.

DPTU is only useful as a reference peak if it is larger than the PMTC peak in residue cycles.

PMTC is normally a larger peak than DPTU in residue cycles. As such, it is ideal for use as a reference peak.

Note	*More than the quoted amount of PMTC should be added to the PTH-Amino Acid Standard working solution, since it tends to sublime during the flask dry-downs.*

DPU is the oxidation product of DPTU. It can be used as a reference peak if an adequate amount is generated in each cycle.

A suitable Amino Acid can be used as a reference peak if background is significant in each cycle. In this case, the peak type code is *rc*.

None. If laboratory temperatures are stable and the PTH-column has settled down, you may not need a reference peak.

Fluid Sensor Data Files

Overview

- A separate data file can be generated for each fluid sensor.
- The information in these files is similar to that in the Event log; however, it is reported every time the sensor is used—not just when an error occurs.
- The information in these files can be used to help determine the cause of intermittent delivery problems.
- Sensor data files are stored in the PROCISE folder. The PROCISE folder is located within this hierarchy:

  System Folder → Preferences folder → PROCISE folder

Note	*Fluid sensor data files can grow to 1Mb each (11Mb in total) if the "Always report sensor data" and "Report valve status" options are selected (turned on) all the time. Therefore, use this feature only if you suspect a delivery problem. Delete the files once the problem is resolved.*

Generating Fluid Sensor Data Files

To generate fluid sensor data files:

1. Select the "Always report sensor data" box in the Preferences window. When selected, an X appears in the box.

To stop the generation of fluid sensor data:

1. Deselect the "Always report sensor data" box in the Preferences window.
2. Delete the fluid sensor data files once the delivery problem is resolved.

Note	*Quitting the Procise application automatically deselects the "Always report sensor data" box.*

Opening Fluid Sensor Data Files in Excel 5

Although Excel 5 is not pre-loaded onto the Macintosh, it is the most suitable application for opening fluid sensor data files.

1. Launch the Excel 5 application.
2. Select Open from the File menu.
3. Highlight the desired sensor data file, and select Open.
4. Choose the desired formatting.

Fit all the horizontal information on the screen:

1. Highlight the complete document by clicking the box at the top left-hand corner in the window.
2. From the Format menu, select Columns.
3. Then select Autofit Columns from the pop-up menu.

Display the column title bar while scrolling vertically:

1. Move the cursor over the black box just below the right-hand corner of the window bar. It will change from an arrow into two parallel lines.
2. Click and hold the mouse button while moving the cursor downward until immediately below the column title row. Release the mouse button to split the screen.

Opening Fluid Sensor Data Files in Simpletext

As long as the sensor data files are below a certain size, they can be opened with Simpletext, which is pre-loaded onto the Macintosh.

1. Double-click the Simpletext icon to launch the application.
2. Select Open from the File menu.
3. Highlight the desired sensor data file and select Open. If the file is too large for Simpletext to open, use an alternative text editor.

The format in which Simpletext displays the sensor information is not ideal. You can adjust the size and the font of the title bar. Reducing the type size and/or orientation will allow each step of information to be reported on the same line.

Manual Injection Procedure

Purpose

Use this procedure to manually inject the PTH-Amino Acid Standard if you suspect that the flask chemistry is having a detrimental effect on sequencing results.

Items Required

- Syringe with luer fitting
- 1/4 inch male to luer adaptor (P/N 0382-0007 in Spare parts kit 2)
- 1/4 inch female to 5/16 female union (P/N 0403-0280 in Spare parts kit 2)

Procedure for Manually Injecting the PTH-Standard

Prepare a 0.1pmol / µL solution of PTH-Standard (5 pmol injected onto column):

1. Fill a clean measuring cylinder with approximately 5 mL of HPLC-grade water.
2. Add 10 µL of each PTH-Standard stock solution, including PMTC, to the measuring cylinder.
3. Bring to a total volume of 10 mL using HPLC-grade water.
4. Mix well.

Create a Manual Injection cycle:

1. Scroll to the Cycles & Procedures dialog box, and select Flask Cycle.
2. Select Run Gradient cLC from the list of Flask cycle options.
3. Open the File from the top menu bar, and select Save Cycle/Procedure as.
4. Name the new cycle Manual Injection.
5. Click OK.

Modify the Manual Injection cycle:

1. Select step 1, Begin (Figure 7-29).
2. Select function 226, *Load Position,* from the function list.
3. Click Insert Row. The Global time will be assigned automatically.
4. Select what is now step 6 (*Wait*).
5. Select function 223, *Inject Position,* and click Insert Row.
6. Open the File menu from the top menu bar, and select Save Cycle/Procedure to save the cycle shown in Table 7-2 on page 7-48.

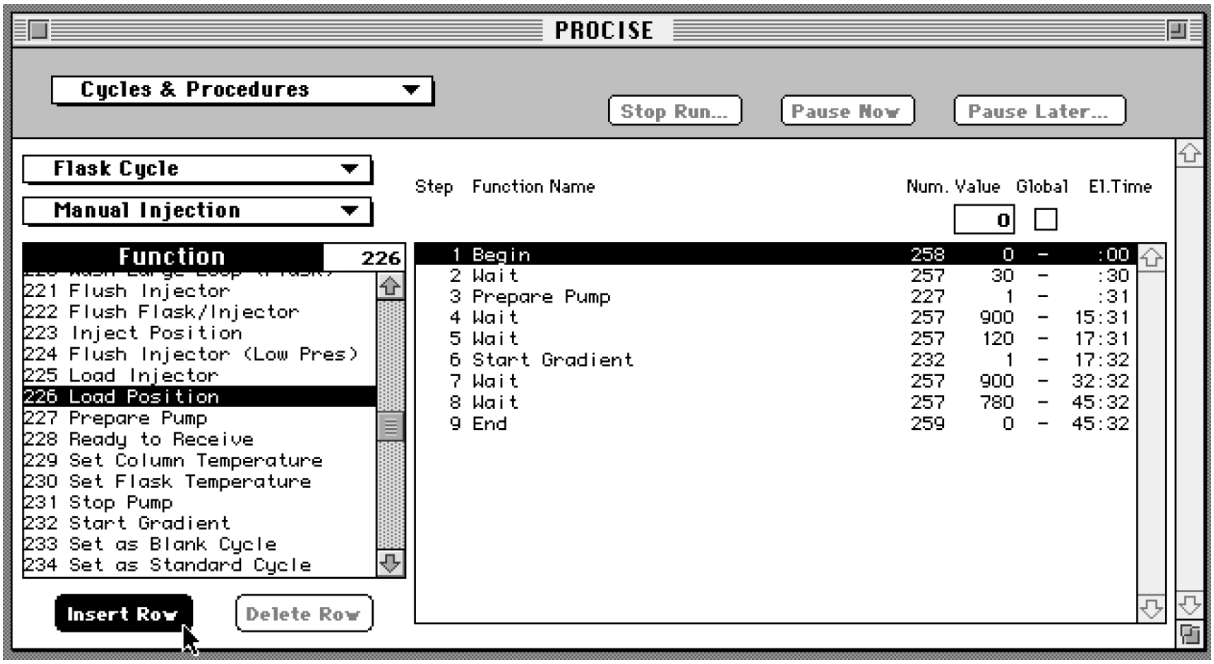

Figure 7-29. Creating a manual injection cycle

Incorporate the Manual Injection cycle into a method:

1. Scroll to the Sequence Methods dialog box, and select the User Defined Method (Figure 7-30).
2. For the Default line, choose *None* for the Cartridge Cycle, *Manual Injection* for the Flask Cycle, and *Normal 1* cLC for the Gradient.
3. Change the cartridge starting temperature to 35 ˚C.
4. Open the File menu, and select Save Method as.
5. Name the method *Manual Injection*.

Table 7-2. Manual injection flask cycle

Step	Function Name	Function Number	Time in sec	Global Time	Elasped Time
1	Begin	258	0	—	:00
2	Load Position	226	0	—	:00
3	Wait	257	30	—	:30
4	Prepare Pump	227	1	√	:31
5	Wait	257	900	—	15:31
6	Wait	257	120	—	17:31
7	Inject Position	223	0	√	17:31
8	Start Gradient	232	1	—	17:32
9	Wait	257	900	—	32:32
10	Wait	257	780	—	45:32
11	End	259	0	—	45:32

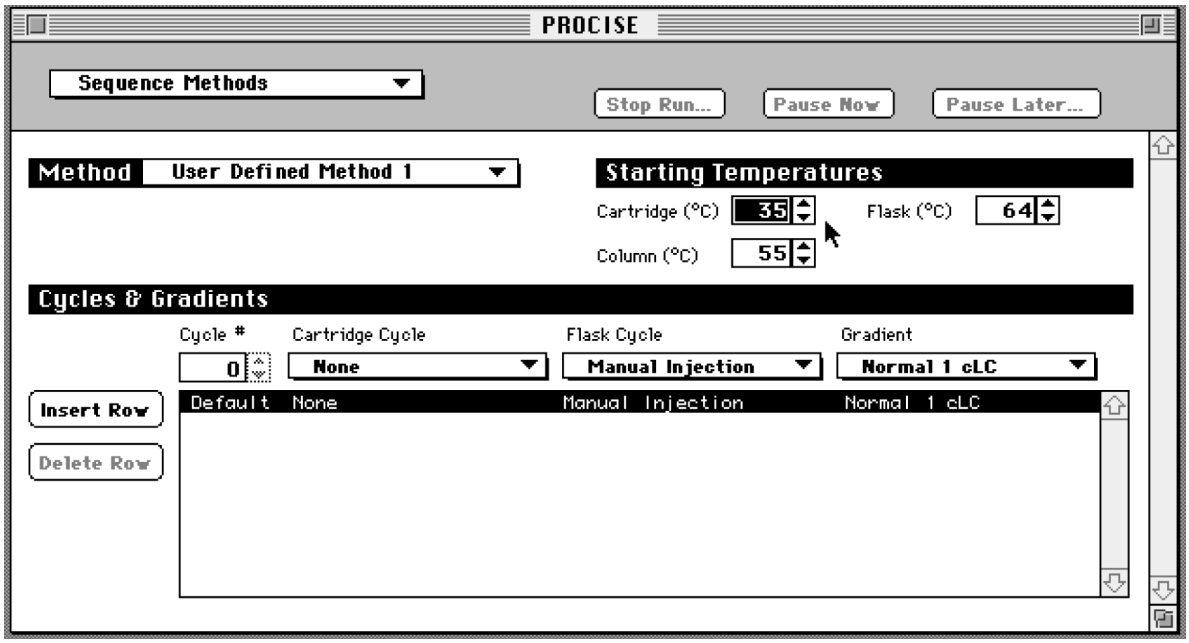

Figure 7-30. Creating a manual injection method

Perform the manual injection:

1. Purge the 140D.
2. Remove the 5/16 -inch fitting from port 42, and connect it to the coupler (white Teflon 5/16-inch to 1/4-inch union).
3. Install the 1/4-inch luer adaptor.
4. From the Start Run dialog box, set up a run using Manual Injection as the Method. Set the number of cycles to 1, and click Start Run.
5. When the Init Sensor procedure starts, you can jump to the last step of the Init Sensor procedure.
6. If the column has reached the correct temperature (LED is cycling), click Next Step if the Flask cycle is *Waiting for Temperatures*.
7. As soon as the *Inject Position* LED on the front panel display is OFF, connect the empty syringe to the luer fitting and flush out the sample loop.
8. Load the PTH-Amino Acid Standard solution into the syringe, and inject it manually into the sample loop.

8 Custom Functions, Cycles, Methods and Gradients

Contents

Introduction

This section describes how to modify and create functions, cycles, methods and gradients. Refer to Appendixes A, B, C and D for a complete listing of the standard functions, cycles, methods and gradients supplied with this system.

Creating and Modifying Functions

The following guidelines and procedure will enable you to create or modify functions.

Guidelines

- Function numbers 401 through 450 are allocated for user-defined functions.
- The standard functions included with the Procise 49X cLC Protein Sequencing System cannot be directly modified, or saved under a different name and then modified. You must create a new function using one of the function numbers reserved for user-defined functions (401–450).
- Only functions created by users can be directly modified.
- The number of valves that can be activated per function is limited.

  For valves 1–23, 34–40, 45, 46, and 63, a maximum of 8 valves can be activated per function.

  For valves 24–33, 41–44, and 47–62, a maximum of 6 valves can be activated per function.
- A maximum of 8 valves total can be activated simultaneously.
- Functions cannot be created or modified while the sequencer is in use.

Procedure

1. Select the Functions dialog box (Figure 8-1) from the dialog box pop-up menu.

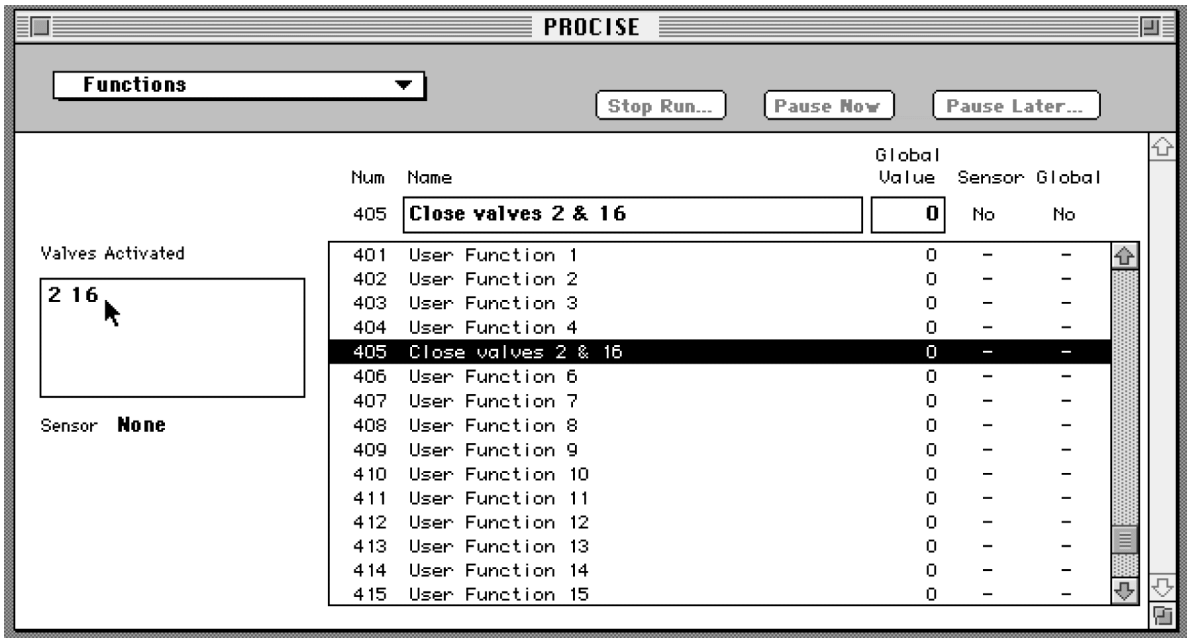

Figure 8-1. Functions dialog box showing user-defined function numbers

2. Select a User Function (401 to 450) from the function list.
3. If you are modifying an existing user-defined function, proceed to step 4.

   If you are creating a new function, highlight the function name field, and enter a unique function name.
4. Move the cursor to the valves activated field.
5. Enter the valve numbers to be activated for the function. Enter a space between each valve number (Figure 8-1).
6. Repeat steps 2 through 5 for all the functions you want to create or modify.
7. Pull down the File menu from the main menu bar. Select Save Function to save the modified or new function.

Setting and Activating a Global Time

Overview

- Each function is activated for a specific period of time. This period can be modified, either locally or globally.
- When the Global Value of a function is changed from the Functions dialog box, the duration of that particular function is automatically changed in every cycle it is used.
- The standard cycles included with this system use Global time values for the *Load X1* cartridge function, and the flask functions *Pre-Conversion Dry, Post-Conversion Dry,* and *Concentrate Sample* only.
- Most functions can be run with a global time setting.
- A check in the Global box for a function used within a cycle or procedure indicates the Global time value set from the Functions dialog box is being used for that function (Figure 8-2).

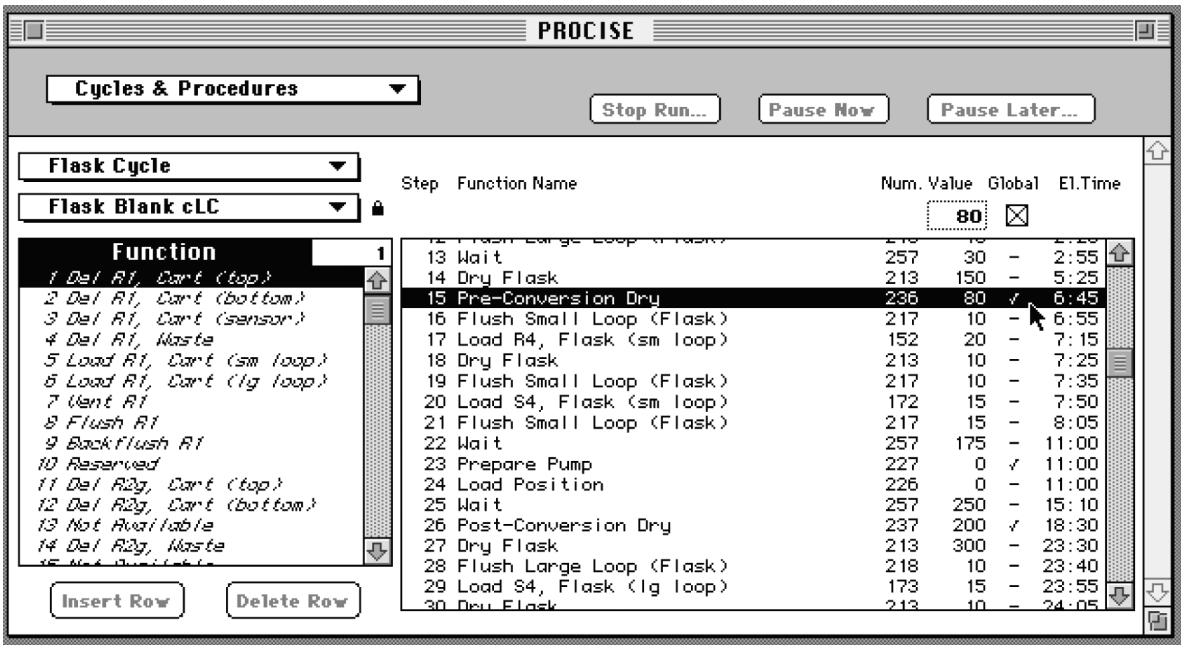

Figure 8-2. Global time values being used for functions 236, 227 and 200

Guidelines

- Global times for a function can be set by modifying the function from the Functions dialog box.
- A global time for a function cannot be directly activated in the standard cycles and procedures provided with this system. You must save a standard cycle or procedure under a new name, or create a new cycle or procedure that includes the modified function.
- The sequencer must be idle to set a global time.
- Global times are set from the Functions dialog box.

Procedure for Setting a Global Time

1. Select the Functions dialog box from the dialog box pop-up menu.
2. Highlight the function from the function list.
3. Enter the desired global time in the Global Value box (Figure 8-3).
4. Open the File menu, and select Save Function.

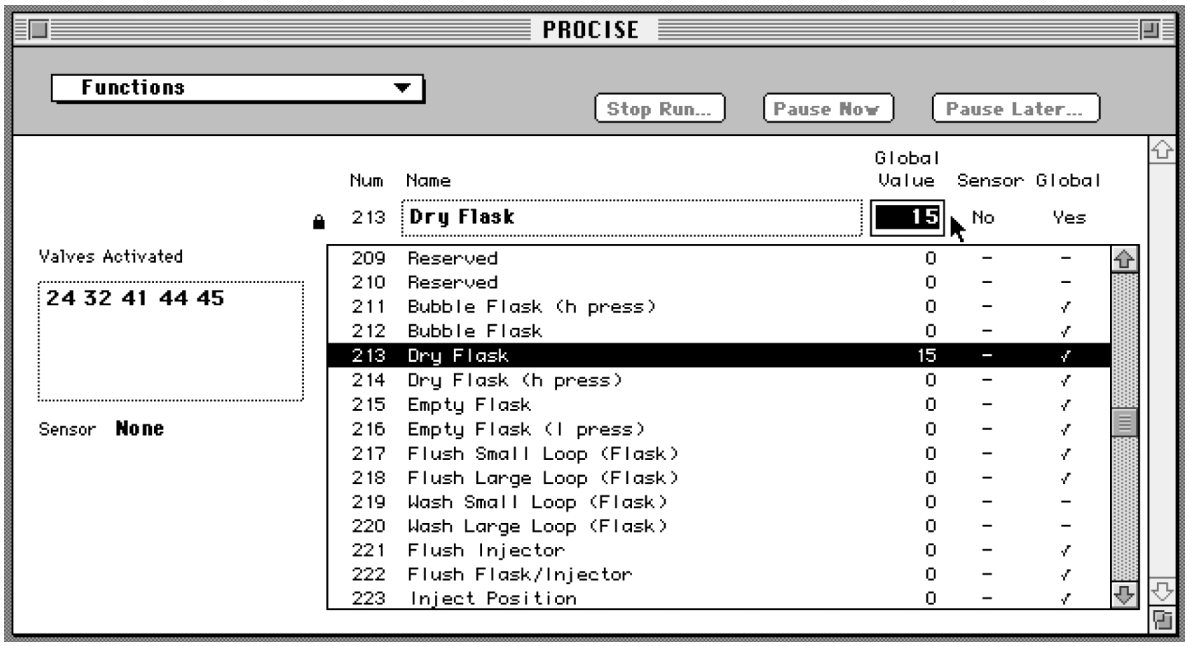

Figure 8-3. Setting a global time value for function 213, Dry Flask

Procedure for Activating a Global Time

Create a custom cycle or procedure:

1. Select the Cycles & Procedures dialog box (Figure 8-4) from the dialog box pop-up menu.

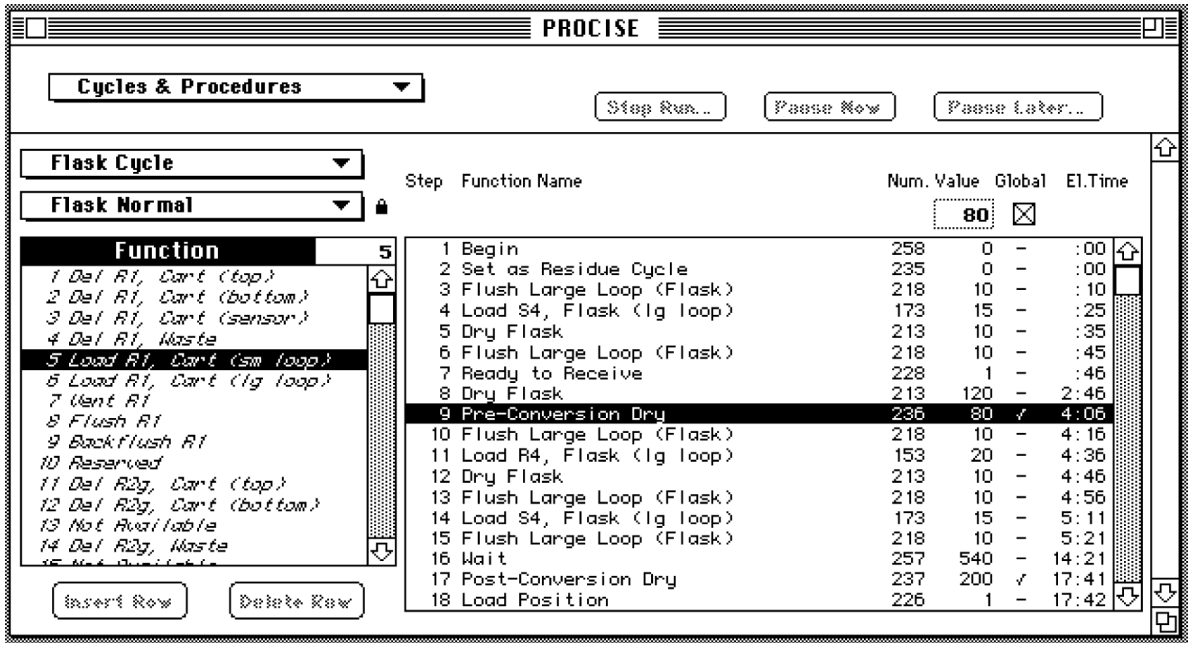

Figure 8-4. Cycles and Procedures dialog box

2. Select the appropriate type of cycle or procedure from the cycle and procedure type pop-up menu.
3. Select the specific cycle or procedure you wish to include the modified function in from the cycle and procedure pop-up menu.
4. Open the File menu, and select Save Cycle/Procedure as.
5. Enter a unique name for the cycle or procedure.

Include the modified function in the custom cycle/procedure:

1. Select the step (function) with the global time to be activated.
2. Click the Global box to activate the global time. An *X* will appear in the box, a check mark in the Global column, and the global value in the Value column (Figure 8-5 on page 8-9).
3. Open the File menu, and select Save Cycle/Procedure.

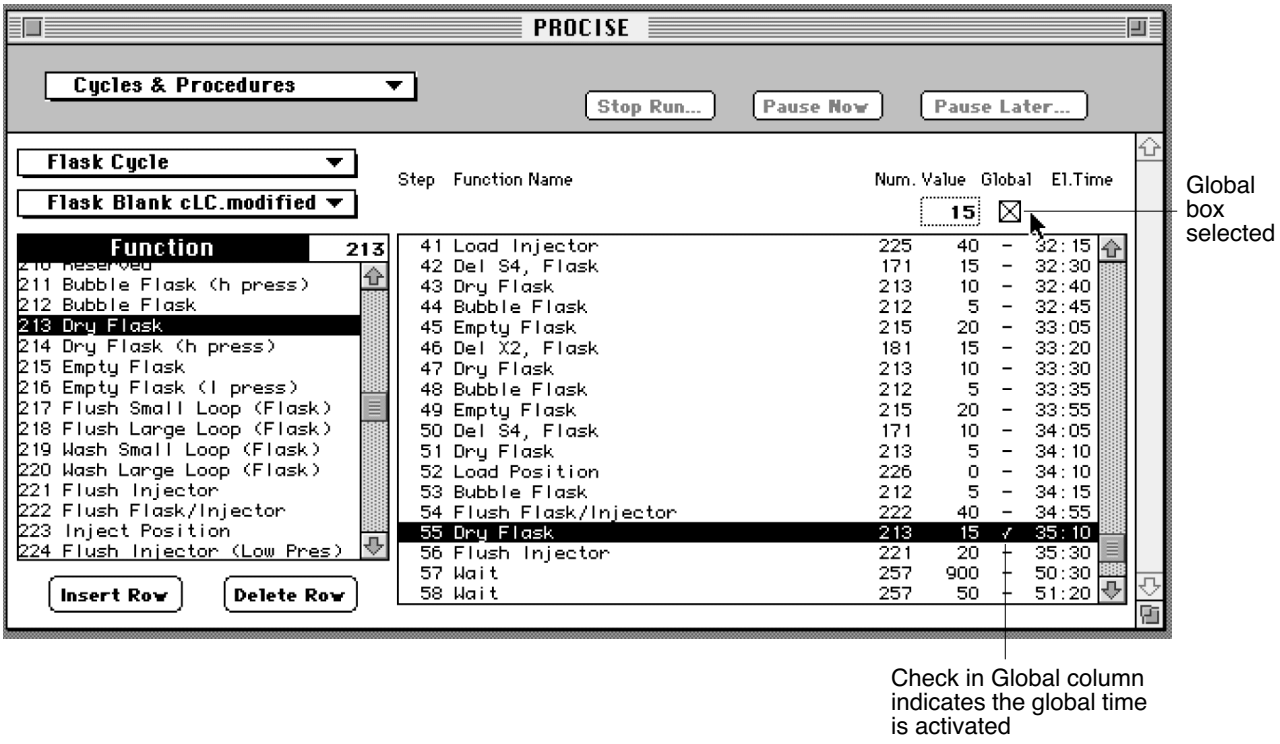

Figure 8-5. Global value for function 213, Dry Flask, is activated in a customized flask blank cycle

Create a custom sequencing method which includes the new cycle/procedure:

1. Open the Sequence Methods dialog box, and select the method you wish to use as a template from the Method pop-up menu.
2. Open the File menu, and select Save Method as.
3. Enter a unique name for the new method.
4. Open the appropriate cycle pop-up menu (Flask Cycle for this example), and select the cycle or gradient you have modified (Figure 8-6 on page 8-10).
5. Open the File menu, and select Save Method.

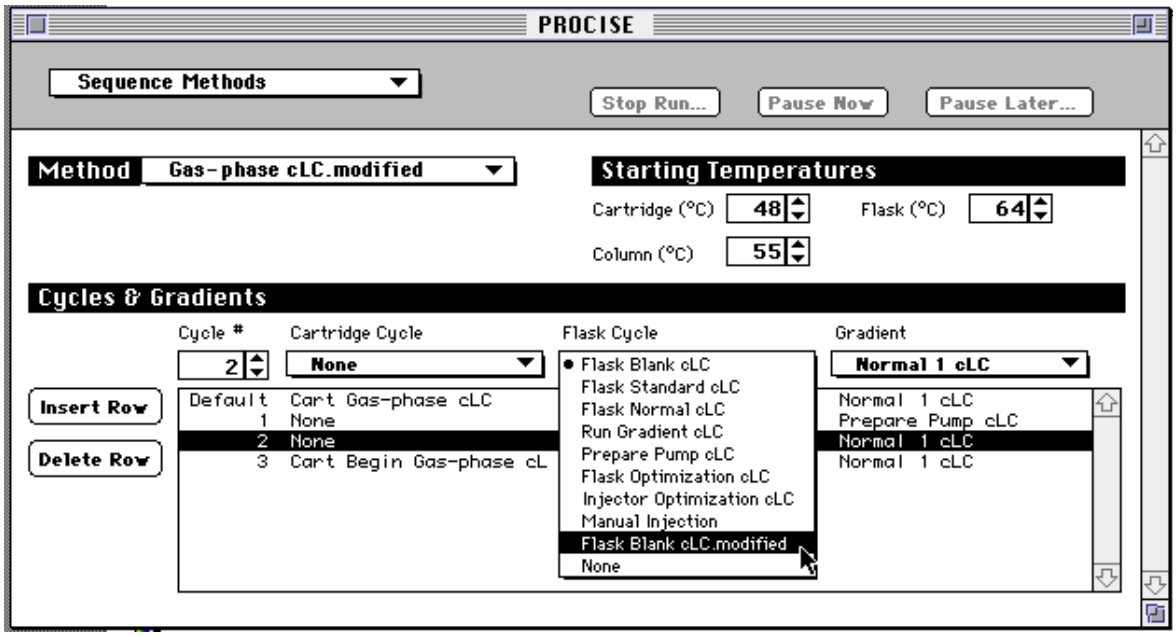

Figure 8-6. Creating a new sequence method to include a customized flask blank cycle

Modifying Cycles

Guidelines

- The standard cartridge and flask cycles included with this system cannot be modified directly. You must create a custom cycle by:
  - Saving an existing cycle under a new name
  - Editing the cycle
  - Saving the changes under the new cycle name
- The maximum number of steps allowed per cycle is 100.
- Every cycle requires a Begin and an End step.
- For Cartridge Cycles:

  The *Ready to Transfer* step in a Cartridge cycle synchronizes with the *Ready to Receive* step in a Flask Cycle. The cartridge cycle must have *Ready to Transfer* and *Transfer Complete* steps to transfer sample from the reaction cartridge to the flask.
- For Flask Cycles:

  The *Ready to Receive* step in a Flask cycle synchronizes with the *Ready to Transfer* step in a Cartridge Cycle. The flask cycle must have a *Ready to Receive* step to receive sample from the cartridge.

  The *Prepare Pump* step starts the 140D and instructs it to equilibrate the column at the initial conditions specified for the start of the gradient. Allow at least 17 min between the *Prepare Pump* and *Load Injector* steps.

Procedure

1. Select the Cycles and Procedures dialog box (Figure 8-4 on page 8-8) from the dialog box pop-up menu.
2. Select the cycle or procedure type from the cycle and procedure type pop-up menu.
3. Select the cycle or procedure from the cycle and procedure pop-up menu.
4. Pull down the File menu from the main menu bar. Select Save Cycle/Procedure As.
5. Type the new cycle name, and click OK.
6. Edit the cycle as follows:

   To delete a row, highlight the row to be deleted, and click Delete Row.

   To insert a row,

   a. Select the function to be inserted from the function list. The function can be selected by using the scroll bar, or by typing the function number at the top, right hand corner of the function list.

   b. Highlight the row immediately before the insertion point, and click Insert.

   To enter the function run time, click the global box to turn the global time off. Type the function time in seconds in the Value box.
7. Pull down the File menu from the main menu bar.
8. Select Save Cycle/Procedures to save your changes.

Creating Cycles

Guidelines

- The maximum number of steps allowed per cycle is 100.
- A cartridge cycle must include a *Ready to Transfer* and *Transfer Complete* step. The *Ready to Transfer* step in a Cartridge cycle synchronizes with the *Ready to Receive* step in a Flask Cycle.
- A flask cycle must include a *Ready to Receive* step to receive sample from the cartridge. The *Ready to Receive* step synchronizes with the *Ready to Transfer* step in a Cartridge Cycle.

  The *Prepare Pump* step starts the 140D, and instructs it to equilibrate the column at the initial conditions specified for the start of the gradient. Allow at least 17 min between the *Prepare Pump* and *Load Injector* steps.
- Every cycle must have a Begin and an End step.

Procedure for Creating Cycles

1. Select the Cycles and Procedures dialog box (Figure 8-4 on page 8-8) from the dialog box pop-up menu.
2. Select the cycle type from the cycle and procedure type pop-up menu.
3. Select User Defined Cycle 1 from the cycle and procedure pop-up menu.
4. Edit the cycle as follows:

   To delete a row, highlight the row to be deleted, and click Delete Row.

   To insert a row,

   a. Select the function to be inserted from the function list. The function can be selected by using the scroll bar, or by typing the function number at the top, right hand corner of the function list.

   b. Highlight the row immediately before the insertion point, and click Insert.

   To enter the function run time, click the global box to turn the global time off. Type the function time in seconds in the Value box.
5. Pull down the File menu from the main menu bar.
6. Select Save Cycle/Procedures As.
7. Type the new cycle name, and click OK.

Modifying Methods

Guidelines

- The standard methods included with this system cannot be modified directly. To modify a standard method, you must:
  - Saving an existing method under a new name
  - Editing that method
  - Save your changes under the new name
- Nine exception cycles are allowed per method.

Procedure

1. Select the Sequence Methods dialog box (Figure 8-7) from the dialog box pop-up menu.
2. Select the method to be copied from the Method pop-up menu.
3. Pull down the File menu from the main menu bar, and select Save Method As.
4. Type the new method name, and click OK.
5. Highlight the default method row.

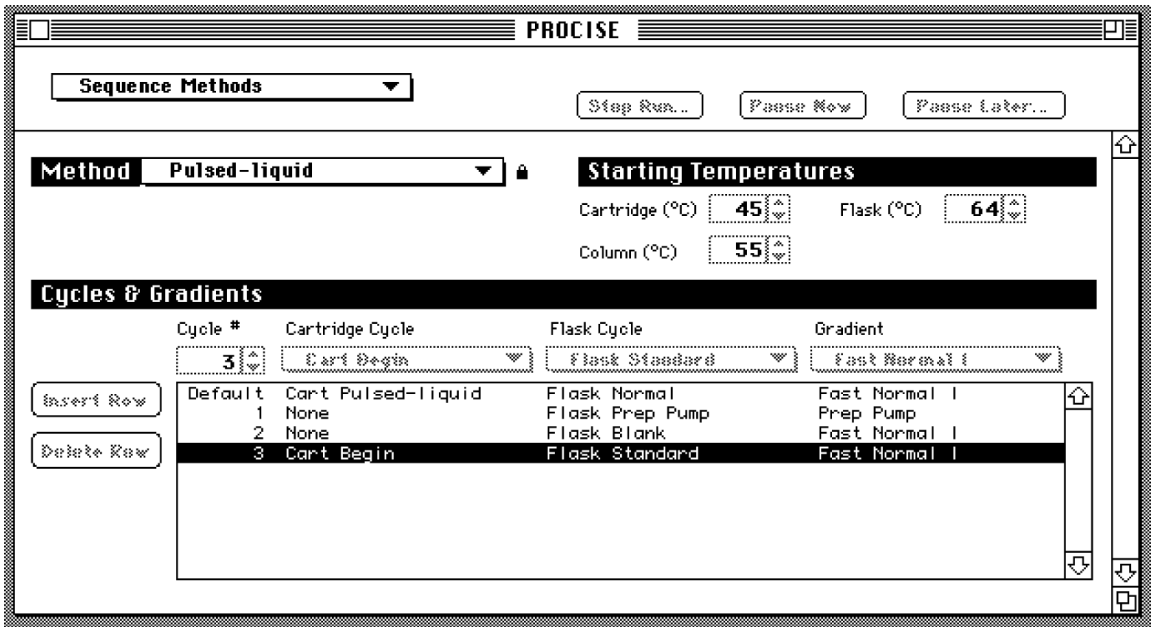

Figure 8-7. Sequence Methods dialog box

6. Select the new cartridge cycle, flask cycle, and/or gradient from each pop-up menu.
7. Edit the method as follows:

   To delete a row, highlight the row to be deleted, and click Delete.

   To add a row,

   a. Highlight the row after which the new row will be inserted.

   b. Click Insert Row.

   c. Move the cursor to the cycle # field, and enter the cycle number to be added as an exception.

   d. Select the new cartridge cycle, flask cycle, and/or gradient from each pop-up menu.
8. If the cartridge, flask, or column temperatures need to be changed, move the cursor to the appropriate temperature field, and enter the desired temperature.
9. Pull down the File menu from the main menu bar, and select Save Method.

Creating Methods

Guidelines

- A method must contain a valid default cycle, the cycle run when there is no exception cycle. The default cycle is not necessarily a canned cycle.
- Nine exception cycles are allowed per method.

Procedure

1. Select the Sequence Methods dialog box (Figure 8-7 on page 8-15) from the dialog box pop-up menu.
2. Select the User Defined method from the Method pop-up menu.
3. Highlight the default method row.
4. Select the new cartridge cycle, flask cycle, and gradient from each pop-up menu.
5. Edit the default method as follows:

   To delete a row, highlight the row to be deleted, and click Delete.

   To add a row,

   a. Highlight the row after which the new row will be inserted, and click Insert Row.

   b. Move the cursor to the cycle # field.

   c. Enter the cycle number to be added as an exception.

   d. Select the proper cartridge cycle, flask cycle, and gradient for the cycle from each pop-up menu.

   e. Enter the desired cartridge, flask and column starting temperatures.
6. Pull down the File menu from the main menu bar, and select Save Method As.
7. Type the new name, and click OK.

Creating and Modifying Gradient Programs

For more detailed information on menus used to control the 140D, refer to the *ABI 140D Microgradient Delivery System User's Manual,* P/N 903586.

Overview of Gradient Programming

- Routine operation of the HPLC components of the system is controlled by Procise control software via the Macintosh.
- Solvent gradient programming changes the retention time of sample species automatically during the course of a single chromatographic run.
- Both gradient programs and changes to the composition (ionic strength) of solvent A3 are used to optimize the retention times of the PTH-amino acids.
- The standard gradient program, *Prepare Pump cLC,* is used in sequencing methods to prepare the 140D, 785A and column for a run.
- One standard, analytical gradient program—*Normal 1 cLC*—is included with this system. The gradient conditions for Normal 1 cLC are listed in Table 8-1.

Table 8-1. HS Normal 1 gradient conditions

Target Pressure:	1500 psi
Target Time:	0.2 min
Pressure Limits:	0 to 3500 psi
Data Collection Time:	28 min

Step #	Time (min)	%B	Flow Rate	Events On	Volume Used	
			(µL/min)		A	B
1	0.0	10	40	12	0	0
2	0.4	12	40	1	14	1
3	4.0	22	40	1	133	25
4	22.0	50	40	1	593	284
5	22.6	90	40	1	600	300
6	23.5	90	40	1	603	332
7	29.0	90	60	0	636	629
8	33.0	50	20	0	660	685

Phases of a Gradient Program

A typical program-controlled gradient run consists of three phases:

- Prepressurization
- Equilibration
- Gradient

Prepressurization Phase

During prepressurization, the 140D rapidly pressurizes to a set of initial conditions specified by the program. Then, the 140D ramps to the conditions desired for equilibration and the first step of the gradient phase—*time-zero* conditions.

Equilibration Phase

During equilibration, the time-zero conditions are held for a specific period of time to allow the system to achieve a steady-state before beginning the gradient.

Gradient Phase

The gradient phase (gradient) is started either by sample injection, or by function 232, *Start Gradient*. Sample separation occurs during the gradient, which consists of a variable number of steps. Each step is characterized by a specific duration, flow rate and mobile phase (solvent) composition. Typically, the composition is gradually changed as a linear function of time from step to step.

External Events

- External events are activated and deactivated by relays located on the back of the 140D (Events 1 through 4 on the 140D terminal block).
- Controlled by programmed runs, events include integrator start, detector autozero, chart recorder start/stop, additional A/D start/stop, or additional data collection start/stop.
- Data collection by the 610A software starts automatically when sample injection occurs.
- Relays are activated (closed) when you select 1, 2, 3, and/or 4 in the Event Column of your gradient program.
- Events remain active until the corresponding numbers are removed from the Event Column, or until the 140D receives an *end of run signal*.
- The *end of run signal* deactivates all external events by opening all the relays.

Gradient Program Parameter Overview

The key parameters of a typical gradient program for the Procise 49X cLC Protein Sequencing System are as follows:

Max Pressure	• Maximum operating pressure for the system. • If the system pressure rises above this value, operation of the 140D is halted. • Choices are 0 through 3500. • The default value is 3500. • Select an upper pressure limit compatible with your column. • As a general rule, set the maximum operating pressure 1000 psi above the expected operating pressure of the system.
Min Pressure	• Minimum operating pressure for the system. • A pressure below the specified value will halt operation of the 140D. • Typical values are 0 to 100 psi. The default value is 0.
Target Pressure	• This is the pressure the 140D is programmed to reach during the first part of the prepressurization phase. • Choices are 0 to 3500 psi. • Typical values are 1500 psi at a flow rate of 40 µL/min. • The default value is 1000 psi. • Generally, the target pressure should be roughly equal to the expected back pressure of the system at the start of the gradient phase.
Target Time	• The amount of time the 140D will take to ramp from the target pressure to the pressure desired for the first step in the gradient. • Choices are 0.1 to 99. • Typical values are 0.2 to 1.0 min for gradients starting with a composition greater than 10% B, and a flow rate greater than 50 µL/min. • Target times as long as 10 min may be required for gradients starting with a 0% B composition and/or a low flow rate such as 10 µL/min. • The default value is 0.1 min.
Equilibrate Time	• A typical equilibration time for the Procise 49X cLC Protein Sequencing System is 18 min.
Data collection time	• The length of time data is collected by the Procise cLC control software. • Sample injection initiates data collection. • The default value is 28 min.

Table 8-2 describes the steps in the gradient phase of a typical programmed run. The gradient program, Normal 1 cLC, is used for this example. Customized programs are created by modifying an existing program, and saving it under a new name.

Table 8-2. Typical steps of the gradient phase of a programmed run

Step	Time	%B	Flow Rate (µL/min)	Events C=closed O=open	Description
1	0.0	12	40	1–C 2–C	The 140D begins pumping at 12% B. Combined flow from pumps A and B is 40 µL/min. Selecting C for event 1 turns the chart recorder on. Selecting C for event 2 autozeroes the detector.
2	0.4	12	40	1–C	This short hold at 12 %B allows for good resolution of peaks S through G. The autozero from step 1 is released by deselecting event 2.
3	4.0	22	40	1–C	From time 0.4 to time 4.0, the 140D linearly increases from 12 %B to 22 %B.
4	22.0	50	40	1–C	From time 4.0 to time 22.0 (18 min), the 140D linearly increases the %B from 22 to 50%.
5	22.6	90	40	1–C	The 140D linearly increases the %B from 50 to 90%. Combined flow from pumps A and B remains constant at 40 µL/min.
6	23.5	90	40	1–C	Flow and composition remain the same for 1.5 min. Contaminants and by-products are removed from the column to clean it for the next sample.
7	29.0	90	60	1–0	Selecting 0 for event 1 turns the chart recorder off.
8	33.0	50	20		The 140D stops flow to the column 33 min after injection, unless it receives another PREPARE PUMP message. The 140D can be programmed to continue running indefinitely after injection until it receives a PREPARE PUMP message. If you choose this option, we recommend you let the 140D run at 20 µL/min, 50 %B.

- The %B values are suggested starting values and may need adjustment to resolve all amino acid peaks. See section 6, “Optimization”, for more information on adjusting the gradient and solvent composition to correct poor resolution of PTH-AAs.
- The smallest time increment for any gradient program step is 0.1 min.

Gradient Programming Guidelines

- The standard gradient programs provided with this system can be modified.
- During a run, the active gradient can be modified. However, the changes you make will effect future runs only, not the current run.
- You can create custom gradient programs by using an existing program as a template, and saving it under a new name.
- Two pump control functions are included with the system:
  - Function 227, *Prepare Pump,* halts the 140D, refills the syringes, pressurizes the pump, and then runs the pump at the time zero conditions specified in the gradient program. Prepare Pump also downloads the gradient program to the 140D. Changes made to a gradient program on the Macintosh will not take effect until the next time the gradient is downloaded from the Macintosh as part of Function 227.
  - Function 232, *Start Gradient,* starts the gradient phase of a program without an injection.
- When programming a cycle, function 227, *Prepare Pump,* must occur **at least 18 min** before the sample is injected on the column. This allows for column equilibration at the time zero conditions defined in the program.
- The smallest time increment for any gradient program step is 0.1 min.

IMPORTANT *Inadequate equilibration will result in variable retention times and resolution.*

- The time of the last step in a program is the *end of run* time. After the last step, the 140D stops flow to the column, and waits for the next Prepare Pump message. When the 140D receives the next Prepare Pump message, it refills, and automatically begins the specified programmed gradient run.

Note *After the last step in the program, the 140D stops flow to the column. In the gradient program, Normal 1 cLC, the 140D stops 33 min after injection unless it receives another Prepare Pump message from the Macintosh. The 140D can also be programmed to continue running after the final step of a program. We* ***strongly recommend*** *you configure the 140D to continue running after the end of a gradient program. Refer to the 140D user's manual for more information.*

- Once the sample is injected onto the column, continue the solvent flow until all sample components elute from the column and pass through the detector. Sample components remaining on the column may elute during a subsequent run, and interfere with peak identification and quantitation.
- A flow rate and %B must be specified in each step of the gradient phase of a program. If a new flow rate is not specified for each step, the value from the previous step is used. If a %B value is not specified for each step, the default value of 0 %B is used.
- The syringes in the 140D have a limited volume; therefore, the time between the *Prepare Pump* and *Load Injector* steps must be limited.
    - Time limit depends upon the time zero flow rate and %B conditions, and the volume of solvents required during the pressurization and analysis cycles.
    - If the limit is exceeded, the 140D continues pumping until one of the syringes empties, or until the start signal arrives.
    - If the start signal is received late (because of a hold or pause in the sequencer cycle), there may not be enough buffer in the syringes to complete the analysis.
    - When the syringes empty, the analysis terminates, and the syringes refill in preparation for the next run before elution is complete.
    - Sample components remaining on the column may elute during a subsequent cycle, and interfere with peak identification and quantitation.

Procedure for Creating or Modifying Gradient Programs

1. Select the Gradient screen. Then select the program you wish to modify from the pull-down list.
2. Highlight the step you wish to change.
3. Modify the value in the Time, % B, Flow Rate, and Events boxes as appropriate.
4. To insert or delete a row, highlight the time line and click Insert Row or Delete Row as appropriate. Alter the time, % B, flow rate, and events as desired.
5. To save your changes, pull down the File menu from the top menu bar, and select either *Save Gradient* or *Save As*.

   When Save Gradient is selected, the original gradient program you selected in step 1 is modified. Changes made to this program will not take effect until the next time the gradient program is used.

   When Save As is selected, you must enter a new, unique name for the modified gradient. The original gradient program you selected in step 1 is not modified.

9 Maintenance

Contents

General System Maintenance

Idle Time Recommendations

Certain procedures should be executed if the Procise 49X cLC Protein Sequencing System is to be idle for any length of time—even one day. The procedures we recommend you follow are based on the length of time the system will be idle.

Length of Idle Time	Recommended Procedures
1 day	*Before leaving the system idle:* • No special treatment is required prior to leaving the system idle. *Before using the system again:* • Run the System Clean-out—X1-X2 Procedure. • Run the Cartridge Line Cleanup Procedure. This procedures washes the valve block, sensor lines, loop, and injector. Refer to Section 7, "Tests and Procedures", page 7-29, for instructions on performing this procedure.
1 to 7 days	*Before leaving the system idle:* • No special treatment is required prior to leaving the system idle. *Before using the system again:* • Run the Start-up Procedure. • Purge the 140D. • Run the Cartridge Line Cleanup Procedure. • Run 1 sequencing cycle on each cartridge before loading samples. Refer to Section 7, "Tests and Procedures", for instructions on performing these procedures.
8 to 14 days	*Before leaving the sequencer idle:* • Configure the Idle Procedure to run every 8 hours while the sequencer is idle. The argon supply must remain connected to the sequencer to run this procedure. *Chose 1 of the following before leaving the HPLC components of the system idle:* • Setup the 140D to free run in manual mode at 5 to 10 μL/min, 50 %B, or • Clean and shut down the 140D and 785A. Refer to Section 7, "Tests and Procedures", and the 140D user's manual for more information on these procedures. *Before using the system again:* • Follow the setup procedures in Section 2, System Setup. • Be sure to load fresh chemicals onto the sequencer, and prepare fresh solvent for the 140D.
	Continued

Length of Idle Time	Recommended Procedures
More than 14 days	***Before leaving the system idle:*** • Run the Short-term Shutdown Procedure. • Empty and rinse the waste bottle on the sequencer. • Setup the 140D to free run in manual mode at 5 to 10 μL/min, 50 %B. Refer to Section 7, "Tests and Procedures", and the 140D user's manual for more information on these procedures. ***To use the system again:*** • Follow the setup procedures in Section 2, System Setup. • Be sure to load fresh chemicals onto the sequencer, and prepare fresh solvent for the 140D.
Complete System Shutdown	***To completely shut the system down:*** • Follow the procedure, "Complete System Shutdown Procedure" on page 9-5. ***To use the system again:*** • Follow the setup procedures in Section 2, System Setup.

Complete System Shutdown Procedure

Overview

We recommend you perform the following procedure for a complete system shutdown. A complete system shutdown means the instruments will be disconnected from the argon and electrical supplies.

Procedures required for a complete system shutdown are:

- Purge the 140D dry
- Run the Delivery Line Backflush procedure (described below)
- Run the System Clean-out X3 procedure (described below)
- Run the System Flush–Argon procedure (described below)
- Remove all reagent and solvent bottles from the sequencer

Caution	***If the reagent and solvent bottles are not removed before a complete system shutdown, the sequencer valve blocks may sustain damage.***

Delivery Line Backflush Procedure Description

- Prepares the system for removal of all reagent bottles.
- Backflushes all reagents and solvents from the delivery lines.
- When the procedure is finished, you will remove all reagent and solvent bottles, and empty them.

System Clean-out – X3 Procedure Description

- Cleans the entire system.
- Requires the heptane from the X3 bottle position be replaced with a bottle of 100% methanol.
- Empty bottles **must** be placed in all the other bottle positions.
- Washes all valve blocks, delivery lines, reaction cartridges, loops, injectors, and reagent bottles with methanol.

System Flush – Argon Procedure Description

- Flushes and dries all sequencer flow paths with argon.
- Resets pressure regulators to the default pressure settings.

Procedure

Purge the 140D dry:

1. Replace the 140D solvent A3 with HPLC-grade or D.I. water.
2. Purge both pumps 3 times at 100%.
3. Run the 140D in manual mode at 50 µL/min, 50 %B for at least 30 min.
4. Place both solvent lines into a bottle of solvent B2. Purge both pumps 3 times at 100%.
5. Disconnect the line at the injector, and place the end of the line in the waste bottle.
6. Freerun the 140D at 200 µL/min, 50% B for 5 min.
7. Place both solvent lines into clean, dry bottles.
8. Purge both pumps 3 times at 100%.

Run the Delivery Line Backflush procedure:

1. From the Test dialog box, click Cleanup.
2. Select the Delivery Line Backflush procedure, and click Start Test.
3. When the test is finished, remove all solvents and reagents from the sequencer.
4. Install a bottle of HPLC-grade methanol in the X3 bottle position.
5. Install empty bottles in all the other bottle positions.

Run the System Clean-out–X3 procedure:

1. Select the System Clean-Out–X3 procedure, and click Start Test.
2. When the procedure is finished, replace the bottle of methanol with an empty bottle.

Run the System Flush–Argon procedure:

1. Select the System Flush–Argon procedure, and click Start Test.
2. When the procedure finishes, remove the waste bottle from the sequencer.
3. Empty, rinse and reinstall the waste bottle.
4. Turn all the instruments off, and disconnect them from the power supply.

Start-up Procedure after a Complete System Shutdown

Recommendation

We recommend you perform the following procedures to start the system up again after a complete shutdown.

For more information on these procedures, refer to Section 7, "Tests and Procedures", and the 140D user's manual.

Procedure

1. Connect the instruments to the power supply, and turn them all on.
2. Load fresh reagents and solvents onto the sequencer using the Start-up Procedure.
3. Prepare and load fresh solvents onto the 140D.
4. Purge the 140D.
5. Run a blank gradient (Run Gradient cLC) from the Start Run dialog box.
6. Check the HPLC components of the system for leaks while the blank gradient is running.

Replacing the Argon Cylinder

Recommendation

Replace the argon cylinder when the tank pressure falls below 100 psi.

Items Required

- Large wrench for removing argon regulator
- Argon cylinder valve key (if necessary)
- Cylinder blanking plug removal tool (if necessary)
- Teflon tape (if necessary)
- Safety goggles

Procedure

WARNING	***Explosion danger. Gas cylinders must be securely fastened upright to a wall, cylinder truck, or cylinder post. Always replace the cylinder cap when the cylinder is not in use, and when it is being moved. Never place cylinders in hallways where they could be hit by a forklift or struck by a falling object. Do not allow grease, oils, or other combustible materials to touch any part of a cylinder.***

Remove the old cylinder:

1. Note the current operating pressure on the regulator.
2. Turn off the argon tank shut-off valve.
3. Open the purge valve on the rear of the instrument to bleed off any residual pressure.
4. Remove the regulator and carefully set it down.
5. Remove the old cylinder.

Install the new cylinder:

1. Fasten the new cylinder securely in place.
2. Wearing safety goggles, briefly open and close the cylinder shut-off valve to remove any debris that may have settled.
3. Screw the regulator onto the new cylinder. Use Teflon tape if appropriate.
4. Open the cylinder shut-off valve. Gas will start to flow out of the instrument purge valve.
5. Close the tank regulator by turning the adjustment knob fully counter-clockwise.

Leak test the new cylinder:

1. Close the tank shut-off valve and wait 30 sec.
2. Using tape, mark where the needle is registering on the tank high-pressure regulator gauge.
3. Wait 1 min and note the reading on the high-pressure gauge.
4. If the pressure has visibly dropped, there is a leak. Determine the cause of the leak, and repair it appropriately.
5. If there are no leaks, turn the tank shut-off valve back on.
6. Adjust the tank regulator to the recommended operating pressure of 65 psi.

Testing the HPLC Components of the System

You can use the Run Gradient cLC sequencing method to test the integrity of the pumping system, and solvent mixing efficiency. This method automatically starts the 140D, equlibrates the column at initial conditions, and starts the gradient. No injection takes place.

Procedure

1. Purge the 140D one time at 100%.
2. In the Start Run dialog box, setup a run as follows:
   - Set the Run Order for Cartridge A to 1st
   - Enter a unique file name for the run
   - Select Run Gradient cLC as the method
   - Set the number of cycles to at least 1
3. Click Start Run.

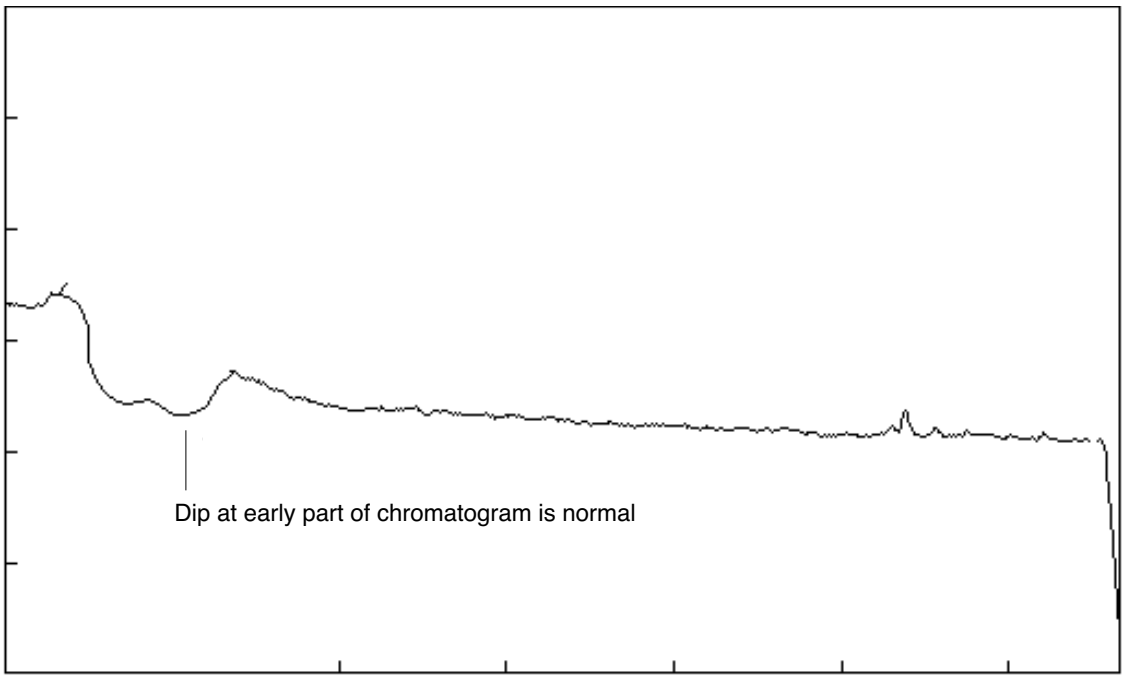

Figure 9-1. Gradient profile with no injection

4. Interpret the results when the run is finished. If the gradient profile is similar to the one shown in Figure 9-1, the HPLC components of the system are functioning properly. If the gradient profile differs significantly from Figure 9-1, troubleshoot the HPLC components of the system to find the source of the problem.

Sequencer Maintenance

User Access to the Internal Components of the Sequencer

As shown in Figure 9-2, you can access the internal components of the Procise 49X cLC Protein Sequencer by:

- Raising the bezel
- Removing the top panel
- Removing the side panels
- Lowering or removing the plumbing plate

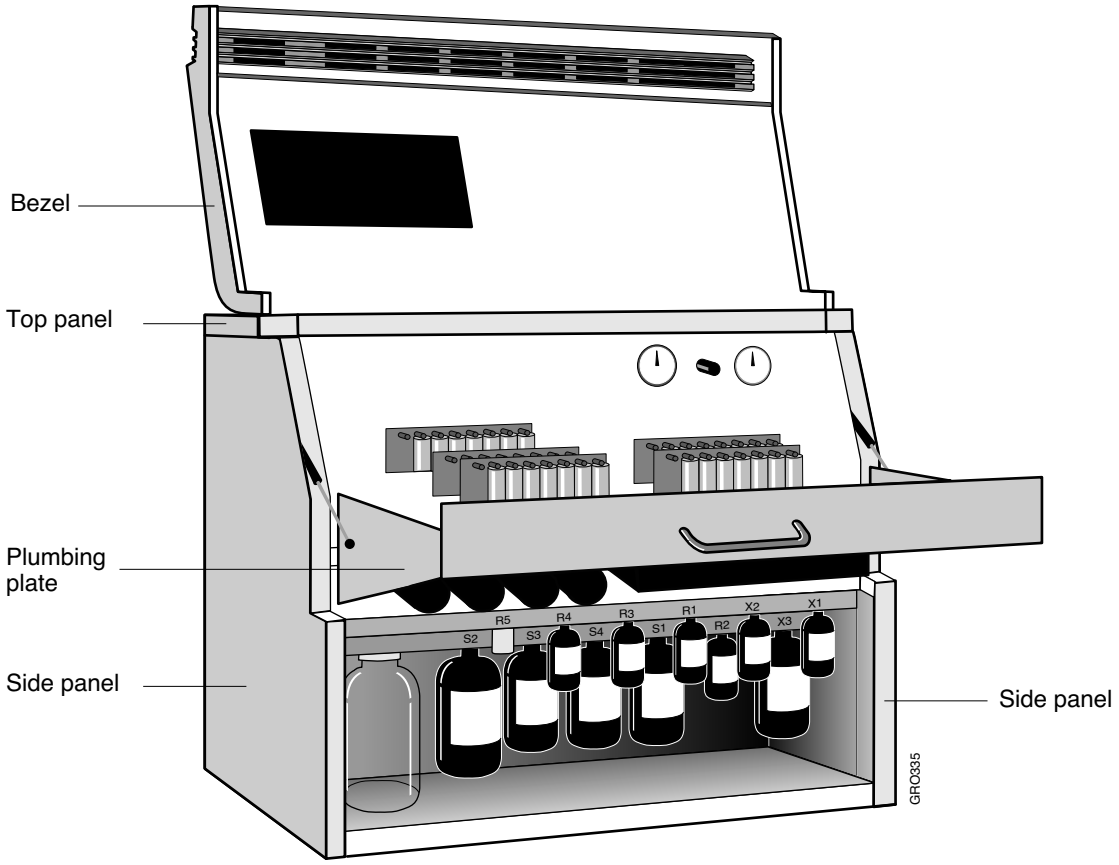

Figure 9-2. User accessibility to the internal components of the sequencer

WARNING ***ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from removal of the rear panel . Therefore, do not remove the rear panel of the sequencer. The panel should be removed by a Applied Biosystems service engineer only.***

Conversion Flask Maintenance Recommendations

- We recommend cleaning the conversion flask vial once a month to prevent residue buildup. Replace the pick-up tube every time the conversion flask vial is cleaned or replaced. Refer to "Cleaning and Replacing the Conversion Flask Vial" on page 9-15 for instructions.

  A dirty conversion flask and pickup line can negatively impact the transfer of sample from the flask to the sample injection loop.

- If white residue builds up on the walls of the conversion flask vial, clean the vial with potassium hydroxide. Replace the pick-up tube every time the conversion flask vial is cleaned or replaced. Refer to "Cleaning and Replacing the Conversion Flask Vial" on page 9-15 for instructions.

- Over time, the conversion flask vial and pick-up tube will need to be replaced. Spare parts are in the flask maintenance kit (P/N 401990). The vial and pick-up tube in this kit are matched, eliminating the need for pick-up tube adjustment. Instructions for removing the conversion flask are included in "Cleaning and Replacing the Conversion Flask Vial" on page 9-15. If the vial is difficult to remove, see "Removing a Stuck Conversion Flask Vial" on page 9-22.

Reaction Cartridge Maintenance Recommendations

- Clean the glass blocks in the reaction cartridges with nitric acid if they become contaminated. See "Cleaning Reaction Cartridge Glass Blocks" on page 9-17 for instructions.

Cartridge Valve Block Maintenance Recommendations

- Cartridge valve blocks can be rinsed with acetone to help reduce non-amino acid background. See "Rinsing the Cartridge Valve Blocks" on page 9-14 for instructions.

- Follow the acetone rinse with a methanol rinse.

Transfer Line Cleaning Recommendations

The Transfer Line Cleanup Procedure cleans the lines between the output block of the cartridges to the flask. Perform this procedure:

- When background becomes excessive.

- On a routine basis (weekly or monthly) as part of regular sequencer maintenance.

This procedure is listed in Section 7, "Tests and Procedures".

Injection System Maintenance Recommendations

- Replace the injector rotor seal once a year. Instructions are on page 9-26.

Rinsing the Cartridge Valve Blocks

- Use the Cartridge Line Cleanup procedure to help reduce non-amino acid background.
- Refer to Section 7, "Tests and Procedures", page 7-29, for information on this procedure.

Cleaning the Cartridge Line

Recommendations

Run the Cartridge Line Cleanup procedure:

- On a routine basis as part of your regular sequencer maintenance (once a month, for example).
- When chemical noise or background becomes too high, and is not due to a dirty sample(s). Verify by running a cartridge with no sample.

Overview

The Cartridge Line Cleanup procedure:

- Cleans the reagent, solvent, input and output valve blocks from the S2 position with methanol.
- Thoroughly washes the cartridge inlet and outlet lines.
- Thoroughly dries the washed areas.

The Cartridge Line Cleanup procedure is listed in Section 7, "Tests and Procedures".

Cleaning and Replacing the Conversion Flask Vial

Recommendations

- Clean the conversion flask vial once a month. This procedure removes the white residue that builds up on the walls of the vial.
- Replace the conversion flask vial whenever residue is visible less than one month after cleaning. The potassium hydroxide used to clean the vial can etch the glass and accelerate the buildup of precipitate.

Items Required

- 2 M solution of potassium hydroxide (KOH)
- Cotton swab or sonicator
- Deionized water
- Replacement pick-up tube (P/N 225053; order by the foot)
- Teflon seal (P/N 004961)
- Tweezers

Cleaning or Replacement Procedure

Remove the flask vial from the sequencer:

1. From the Pressures & Temperatures dialog box, turn off the flask heater, and allow the flask assembly to cool until it is comfortable to touch.
2. Remove the pick-up tube, and discard it appropriately. Do not reuse the old pick-up tube.
3. Unscrew the knurled knob underneath the flask vial. A constant-force spring, the vial, and a Teflon seal should drop out of the housing. If the vial does not slide out, thread a a piece of PEEK tubing through the pick-up line hole and push it out. If the vial is stuck, follow the procedure "Removing a Stuck Conversion Flask Vial" on page 9-22.
4. If the seal remains inside the housing, grip the lip of the seal with tweezers, and pull to remove it.

Caution ***Do not scratch the Kel-F sealing surface.***

5. To clean the vial, proceed to "Clean the flask vial:" on page 9-16. To install a new flask vial, proceed to "Install the cleaned or new flask vial:" on page 9-16.

Clean the flask vial:

1. Choose one of the following:
   - Saturate a cotton swab with potassium hydroxide and clean the inside of the vial.
   - Sonicate the vial in potassium hydroxide for 15 min.
2. Thoroughly rinse the vial with deionized water.

Install the cleaned or new flask vial:

3. Place a new Teflon seal, lip downward, on top of the vial.
4. Insert the vial into the housing.
5. Cut the end of the new pick-up tube at a right-angle to the side of the tube.
6. Install the new pick-up tube. The gap between the tip of the tube and the bottom of the vial should be approximately 1 mm.
7. Run the Flask Leak test from the Test dialog box (Figure 9-3).
8. Optimize the pre- and post-conversion dry-downs by running the Flask Optimization cLC method.
9. From the Sequence Methods dialog box, run the PTH-Standards cLC method 3 times to condition the vial.

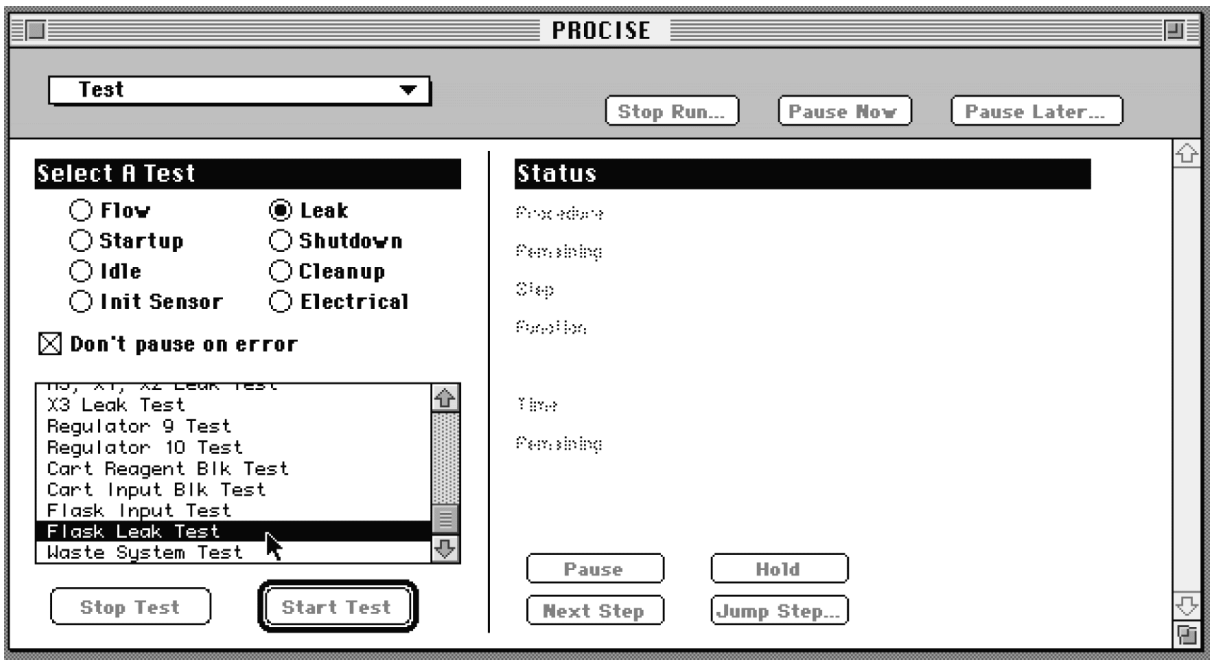

Figure 9-3. Flask leak test

Cleaning Reaction Cartridge Glass Blocks

Recommendations

Perform the following procedure whenever high amino acid background indicates the glass reaction blocks are contaminated with sample.

Items Required

- Concentrated nitric acid
- D.I. water
- Methanol
- Beaker
- Acid-resistant gloves
- Safety goggles
- Sonicator
- Clean, compressed air

Procedure

WARNING	***Concentrated nitric acid is extremely corrosive. Wear safety goggles, a lab coat, and acid-resistant gloves when performing this procedure.***

1. Carefully slide the glass blocks out of the reaction cartridge(s).
2. Remove and discard any cartridge seals and sample supports.
3. Carefully place the blocks into the beaker. To avoid chipping the blocks, do not place on top of each other.
4. Cover the blocks with nitric acid.
5. Place the beaker into the sonicator, and sonicate the blocks for 30 min.
6. Remove the blocks from the beaker, and rinse them 3 times with D.I. water.
7. Rinse the blocks 1 time with methanol.
8. Dry the blocks with clean, compressed air.
9. Discard the nitric acid as appropriate.

The blocks are now ready for use.

Installing New Firmware (Replacing the Mel Card)

Overview

The firmware for the Procise 49X cLC Protein Sequencer resides on the Mel card. The Mel card protrudes from the upper, rear, left-hand side of the sequencer. During the lifetime of the sequencer, Applied Biosystems may release new versions of firmware.

Items Required

- Mel card (version 1.01 is P/N 604256)

Note	*If a different version of the Mel card is installed, the RAM is erased when the instrument is powered-up, resulting in the loss of the fluid sensor dry readings.*

Procedure

1. Turn the main power switch off.
2. Press the button adjacent to the Mel card to eject it. The Mel card protrudes from the upper, rear, left-hand side of the sequencer chassis. The label on the card may or may not face front.
3. Gently push the new Mel card into place. If it does not seat, turn it over and try again.
4. Once the card is properly seated, turn the main power switch on. If the card is functioning correctly, the Ready LED under the front door visor will light in approximately 15 sec.

Replacing a Ratchet Cap Assembly Receptacle

Items Required

- 1/4-in. socket or open-end wrench
- Ratchet cap

Ratchet Cap Type	Part Number
2 oz	003557
8 oz	003558
16 oz	003559

Procedure

Refer to Figure 9-5 on page 9-21 as you perform this procedure.

Remove the old ratchet cap assembly:

1. Backflush the delivery line(s) into the reagent bottle using the appropriate backflush function(s).
2. Power down the sequencer.
3. Remove the appropriate bottle(s).
4. Lower the plumbing plate to expose the valve blocks.
5. Remove the two 1/4-in. stand-off nuts and washers from the housing.
6. Lift the housing over the two studs.
7. Loosen the ratchet cap lid, and remove the ratchet cap receptacle.

Install the new ratchet cap:

1. Screw the lid (P/N 001205) into the new ratchet cap receptacle.
2. Place the assembly (ratchet cap lid, insert and gasket) into the housing.
3. Place the wave spring (P/N 002571) on the underside of the ratchet cap assembly.
4. Reinstall the two washers and 1/4-in. stand-off nuts that hold the ratchet housing in place.
5. Reinstall the bottle(s).
6. Power-up the sequencer.

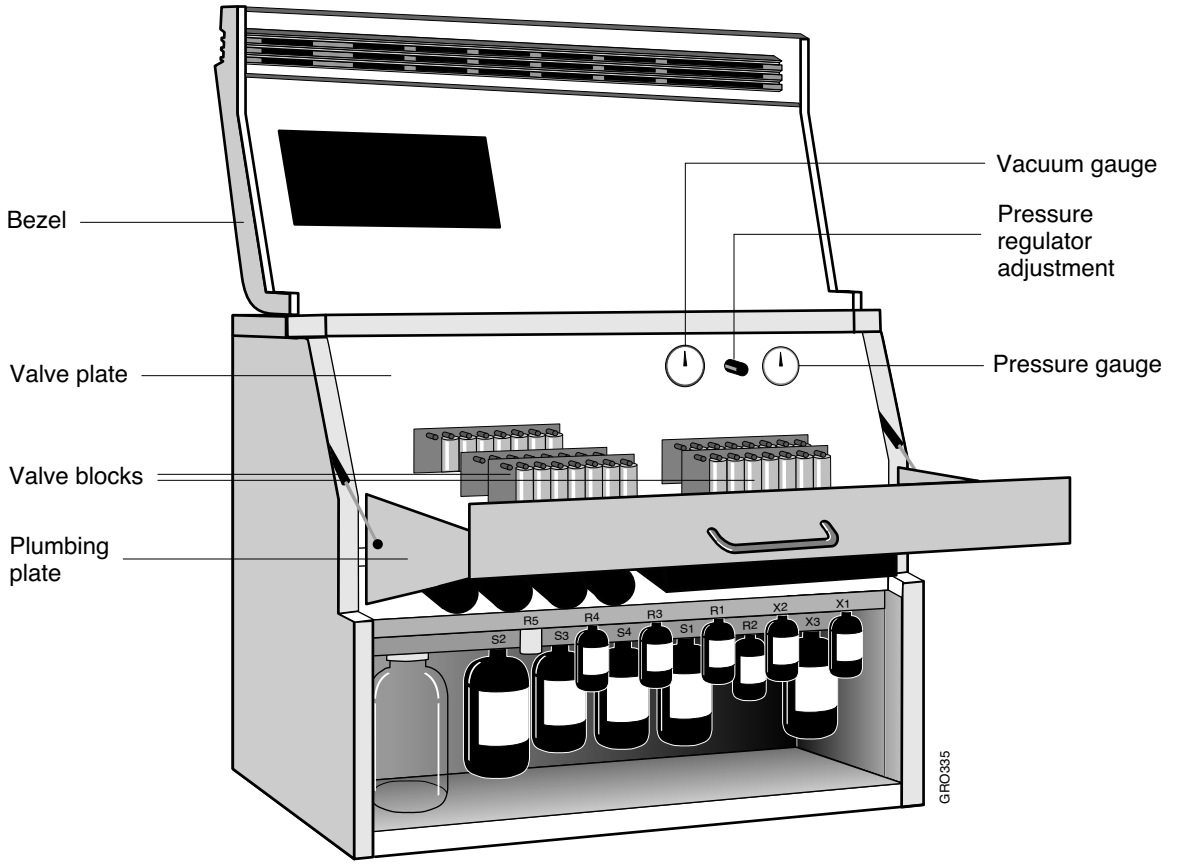

Figure 9-4. Procise 49X cLC Protein Sequencer with front panel open

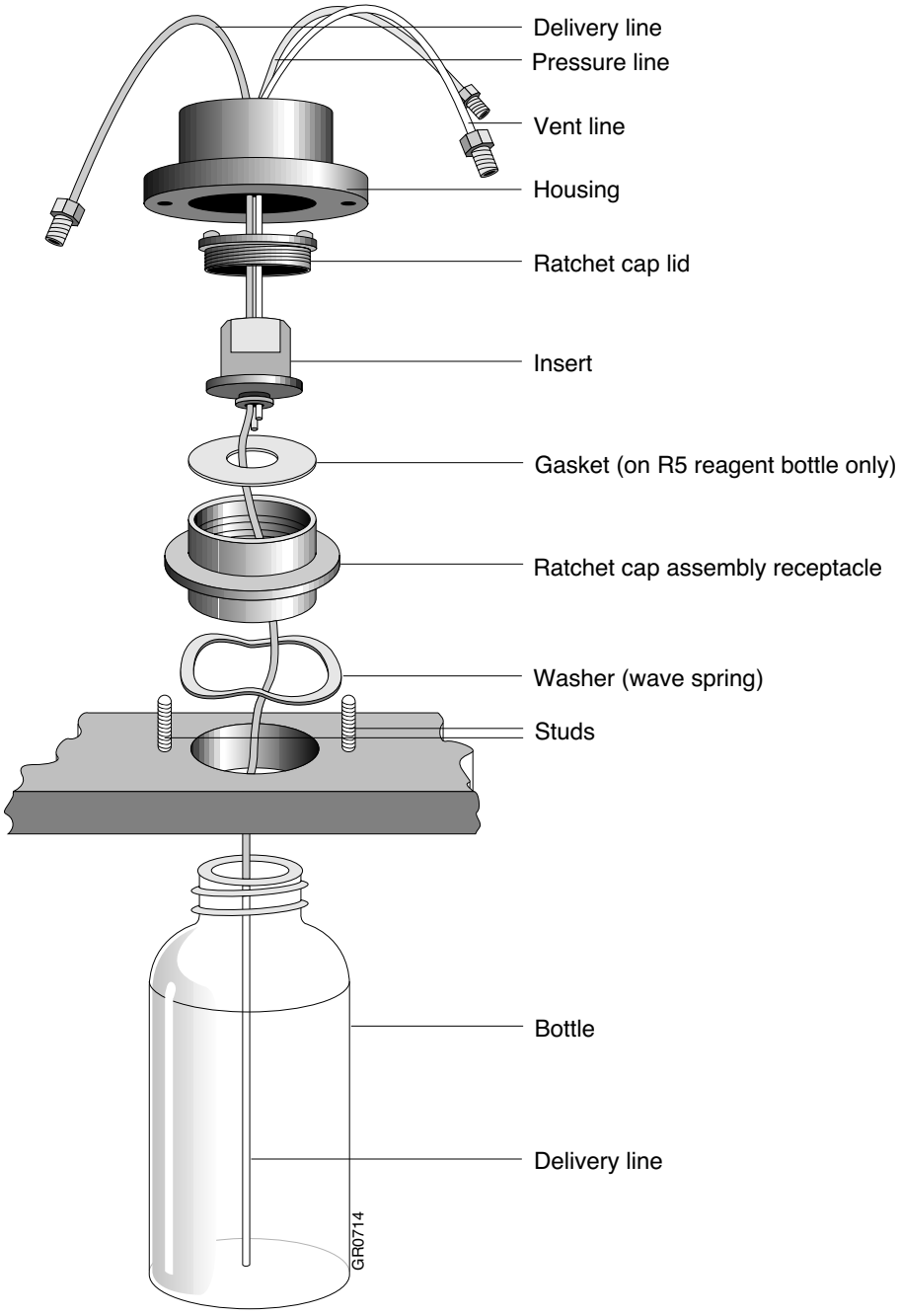

Figure 9-5. R5 reagent bottle racket cap assembly

Removing a Stuck Conversion Flask Vial

Overview

The conversion flask vial can become stuck due to leakage, where salt forms and binds the vial in place. If you cannot push the vial out with PEEK tubing, disassemble the conversion flask and remove the vial as directed below.

Items Required

- 0.035-in. hex wrench
- Tape
- Hammer
- Water
- Imperial hex driver set
- Pick-up tube (P/N 225053; order by the foot)

Procedure

Remove the conversion flask vial:

1. Power-down the sequencer, and lower the plumbing plate.
2. Remove the pick-up tube.
3. Unplug the flask vent valve and the transfer fluid sensor electrical connections from the distribution board.
4. Using a 0.035 in. hex wrench, loosen the lens set screw at the bottom of the flask assembly, and carefully remove the lens.
5. Loosen the two valve hex screws and the four other hex screws that hold the Kel-F portion of the valve block onto the remaining flask assembly.
6. The valve will now be slightly around of the Kel-F due to the force of the valve spring. Use tape to hold the valve in its current position with respect to the Kel-F.
7. Remove the lines from ports 32 and 38.
8. Lift the Kel-F/valve assembly off the remaining flask assembly, and pull off the vacuum line from the valve. Set these parts aside in a clean environment. If the white Teflon seal has stuck to the bottom of the Kel-F, remove it.
9. Using a hammer and a 5/16-in. bushing as a punch, tap the vial upwards until it becomes dislodged. Remove the vial.

Clean and reinstall the conversion flask vial:

1. Clean the vial and flask holder cavity with water to dissolve any salt.
2. Reconnect the vacuum lines, and screw the vent valve and KEL-F assembly back onto the main flask assembly.
3. Reinstall the tubing and electrical connections.
4. Reinstall the vial and lens.
5. Close the plumbing plate.
6. Power-up the sequencer.

Replacing a Valve Block

Items Required

- Phillips-head screwdriver
- Valve block

Valve Block Type	Part Number
9 port	603454
8 port	603452
4 port with 1 common	603449
4 port	603450

Procedure

Before removing the valve block:

1. Flush the valve block using the appropriate flush function from Functions menu. This will remove as much residue as possible so that an autopsy can be safely conducted afterwards.
2. Backflush the bottles connected to the valve block using the appropriate backflush function from Functions menu.
3. Remove the bottles connected to the valve block.
4. Power-down the sequencer.

Remove the valve block from the sequencer:

1. Remove the bezel from the sequencer.
2. Label the delivery lines connected to the valve block with tape, then disconnect the lines.
3. Loosen the two screws securing the plumbing plate in place and drop the plate to the horizontal position.
4. Pull the vacuum line off the valve block manifold. If the end is discolored, trim or replace the line. Inspect the vacuum assembly and all other vacuum lines for signs of contamination.
5. Unplug the ribbon cable from the valve block printed circuit board (PCB).
6. While holding the valve block, remove the two screws holding the block in place, and remove the valve block.
7. Remove the PCB and bracket from the original valve block.

Install the new valve block:

1. Fit the PCB and bracket onto the new valve block.
2. Screw the new valve block into place.
3. Replace all electrical and plumbing connections.
4. Reinstall the bezel and close the plumbing plate.
5. Reinstall the bottles.
6. Power-up the sequencer.
7. Prime the delivery lines using the appropriate bottle change procedures.

Replacing the Injector Rotor Seal

Recommendations

We recommend the injector rotor seal be changed once a year.

Items Required

- 9/64-in. hex wrench
- 1/4-in. wrench
- Rotor seal (P/N 0173-0015)
- Isolation seal (P/N 0173-0014)

Procedure

Remove the old injector rotor seal:

1. Remove the plumbing plate from the sequencer.
2. Loosen the pre-tee fittings holding the injector full and load sensors in place.
3. Slide the sensors up the tube and out of the way.
4. Do not disconnect the lines from the stator unless you need more room.
5. Loosen the three hex screws and pull the stator from the assembly. The stator face will either come off with the stator or remain with the valve assembly. The 3 dowels in the stator face orient it correctly on the stator.
6. The 2 hex screws holding the retainer ring in place will now be exposed. Loosen and remove these screws.
7. Pull off the retainer ring by screwing 2 of the hex screws a little way into 2 of the threaded bores on the retainer ring.
8. Grasp the screws and pull the ring away from the rest of the assembly.
9. With your finger and thumb, pull the rotor seal off the 4 location pins.

Install the new injector rotor seal:

1. Inspect the white isolation seal behind the rotor seal. Replace the seal if it is worn.
2. Inspect the rear of the original rotor seal, and note the location of the impression made by the shaft bore.
3. Orient the new rotor seal the same way.
4. Reassemble the injector.
5. Inspect the stator face for scratches, and replace it if necessary.
6. Reposition the sensors, and tighten the pre-tee fittings that hold them in place.
7. Reinstall the plumbing plate.

Testing the Conversion Flask Assembly for Leaks

Overview

The following procedure is a more stringent means of leak testing the conversion flask than the flask leak test run from the Test dialog box.

Procedure

1. From the Manual Control dialog box, activate Flask function 171, *Del S4, Flask,* until the liquid just starts to drip into the flask vial.
2. Activate function 213, *Dry Flask,* for 5 sec.
3. Select None for the function fields, and activate valves 41, 44 and 48.
4. Examine the flask. After some initial bubbling, all bubbling should cease in 1 min. If the bubbling continues, a leak is present.
5. Activate function 215, *Empty Flask,* for 10 sec.

Testing the Injector for Blockages

Overview

This procedure checks the HPLC flow paths for blockages.

Procedure

1. Remove the inlet line from the column, and place it in a beaker.
2. On the 140D control panel, press the Manual key to enter manual mode, and free run the instrument.
3. Set the flow rate to 50 µL/min, and the %B to 50.
4. Monitor the pressure for 5 min. The pressure should not rise above 100 psi.
5. If the flow path remains blocked, determine the source of the blockage by breaking fittings in the flow path consecutively from the injector to the dynamic mixer inside the 140D.
6. From the Manual Control dialog box on the Macintosh, toggle the injector position by activating function 223. The pressure should not vary by more than 5 psi. If it does, the sample injection loop may be blocked.
7. Stop the 140D, and reinstall the column inlet line.

Testing Gas Flow Rates

Overview

Gas and vapor flow rates are difficult to measure accurately without the aid of a flow meter. However, the clicking frequency of the pressure control valves (Lee valves) is approximately proportional to the flow rate.

Table 9-1 on page 9-30 lists the clicks per sec for a given function. The pressures listed in the Pressure column differ from the default operating values in order to audibly measure the clicking frequency. If an existing function can be used to measure the flow rate, it is given in the table. If not, the group of valves which must be opened are given instead.

For convenience, the actual flow rate is listed in the table for default operating pressures (V1.0 firmware). Each were measured using a flow meter connected at the 1/4 in. waste outlet line. At high altitudes, flow rates may exceed the range given in the table.

The values in parenthesis are for the actual flush functions which use the manual regulator pressure set at 5.5 psi.

Procedure

WARNING	***This procedure vents the instrument into the laboratory. The waste bottle must be empty. R2 & R3 must be replaced with empty bottles to measure their respective flow rates.***

1. Remove, empty, and reinstall the waste bottle.
2. Remove the vent trap bottle to eliminate fume hood suction.
3. Ensure that the manual regulator is set to 5.5 psi.
4. Flush the respective flow path with argon for 30 sec prior to adjusting pressures and taking flow measurements. For example, activate function 131, Dry cart (top) before measuring the flow rate for “Del R2g cart (top)”.
5. If necessary, replace R2 & R3 with empty bottles.
6. Set the pressure for the particular flow path.
7. Activate the function (or valves) for at least 30 sec before taking a measurement.
8. Calculate the clicks per second, and compare with the values in the table.
9. Restore the default operating pressures when testing is complete.

Table 9-1. Clicks per second

Function or Valves	Pressure (P = pressure regulator)	Clicks per sec	Actual flow rate at Default Operating Pressure (in sccm)
Del R2g cart (top) Function 11	P2 = 0.3 psi	2.5 ± 0.3	23 @ 1.0 psi
Del R3g cart (top) Function 31	P3 = 0.5 psi	1.0 ± 0.2	8.0 @ 1,2 psi
Dry cart (top) Function 131	P5 = 0.2 psi	1.9 ± 0.2	81 @ 3.5 psi
Flush transfer line Function 141	P5 = 0.2 psi	1.7 ± 0.2	78 @ 3.5 psi
Bubble flask Function 212	P9 = 0.2 psi	3.4 ± 0.3	75 @ 1.8 psi
Empty flask Function 215	P8 = 0.2 psi	1.8 ± 0.2	74 @ 3.0 psi
Flush small loop (flask) Function 217	P8 = 0.2 psi	2.5 ± 0.3	100 @ 3.0 psi
Flush large loop (flask) Function 218	P8 = 0.2 psi	2.3 ± 0.3	89 @ 3.0 psi
Flush flask/injector Function 222 (inject position)	P8 = 0.2 psi	0.9 ± 0.3	32 @ 3.0 psi
Flush flask/injector Function 222 (load position)	P8 = 0.2 psi	0.8 ± 0.2	30 @ 3.0 psi
Flush cart reagent block Vlv 1,11,15	P5 = 0.2 psi	2.3 ± 0.2	(Function 135 yields 150) 100 @ 3.5 psi
Flush cart solvent block Vlv 15,16,23	P5 = 0.2 psi	2.3 ± 0.2	(Function 136 yields 150) 100 @ 3.5 psi
Flush input block Vlv 7,11,15,16	P5 = 0.2 psi	2.1 ± 0.2	(Function 137 yields 140) 93 @ 3.5 psi
Flush output block Vlv 10,15,40	P5 = 0.2 psi	2.2 ± 0.2	(Function 138 yields 140) 94 @ 3.5 psi
Flush small loop (cart) Vlv 7,11,15,22	P5 = 0.2 psi	1.4 ± 0.2	(Function 139 yields 95) 57 @ 3.5 psi
Flush large loop (cart) Vlv 7,11,15,21	P5 = 0.2 psi	1.3 ± 0.2	(Function 140 yields 92) 56 @ 3.5 psi
Flush injector Vlv 42,44 (load position)	P9 = 0.2 psi	1.0 ± 0.2	(Function 221 yields 70) 20 @ 1.8 psi

Testing 3-way Valves

Overview

If the 3-way valve fails to switch from the low pressure input to the high pressure (5.5 psi) input during a flush function, the effectiveness of the flush will be compromised. Similarly, if the valve allows high pressure to bleed into the common path, an over-delivery can occur. To determine whether the a 3-way valve is operating correctly, follow the procedure below.

Items Required

- 5/16-in. wrench

Procedure

1. From the Pressures & Temperatures dialog box, set the low pressure input (the *Set* pressure) to the respective 3-way valve to 0.
2. From the Manual Control dialog box, turn on the 3-way valve by entering the valve number (46, 47 or 48) in the *Additional Valves* field.
3. Set both the Cartridge and Flask function numbers to 0 (None).
4. Click Execute.
5. Remove the 3-way valve output line from the valve block, and place the end of the line in a beaker of water. If the valve has switched correctly, a fast stream of bubbles will flow from the tube.
6. From Manual Control, turn the 3-way valve off by clicking All Off. The flow of bubbles should stop.
7. From the Pressures & Temperatures dialog box, reset the input pressure to the correct value. A slower stream of bubbles should flow from the line.
8. Reconnect the line to the valve block.
9. If any of these tests fail, replace the 3-way valve.

Testing Heater Boards

Overview

All the heaters are tested for open and short circuit conditions during the Power On Self Test (POST). An open circuit condition can be caused by a blown thermal fuse, a closed thermal switch, or by a damaged heater element.

Items Required

- Multimeter
- Flat-blade screwdriver

Procedure

1. Power down the instrument.
2. Loosen the two screws securing the plumbing plate at the top, and lower the plate to the horizontal position.
3. Use the test points shown in Table 9-2 to check the element/fuse and thermal switch continuity. The thermal switch should be open during normal operation. Circuit resistance should be greater than 2 kW. All test points are on the Distribution board.

Table 9-2. Heater board test information

Heater Board	Test Points for Fuse/Element Test	Good Resistance	Test Points for Thermal Switch Test (pass if resistance > 2 kW)
Cartridge A	TP9 & TP30	25-30 Ω	TP10 & CR6 anode
Cartridge B	TP9 & TP31	25-30 Ω	TP10 & CR5 anode
Cartridge C	TP9 & TP32	25-30 Ω	TP10 & CR4 anode
Cartridge D	TP9 & TP33	25-30 Ω	TP10 & CR3 anode
Flask	TP9 & TP34	25-30 Ω	TP10 & CR1 anode
Column oven	TP9 & TP35	20-25 Ω	TP10 & CR2 anode

Adjusting the Vacuum Switch Setting

Items Required

- Phillips-head screwdriver
- Flat-blade screwdriver

Procedure

Determine the current vacuum range:

1. Lower the plumbing plate to expose the vacuum and pressure gauges.
2. Remove the top cover from the sequencer. You may need to loosen the two captive shipping screws accessed by removing both side panels.

   The vacuum manifold assembly is located on the right, rear side of the sequencer.
3. Create a leak in the vacuum system using one of the following methods:
   - If your sequencer has a vacuum purge fitting mounted vertically onto the vacuum manifold, loosen the fitting slightly to create a leak.
   - If the manifold does not have a purge fitting, carefully pull a vacuum line off one of the valve blocks to create a leak.
4. Watch the vacuum gauge while you listen for the vacuum assist solenoid valves to open and charge the system.
5. Note the pressure on the vacuum gauge when the valves open (typically 14 to 15 in. Hg).
6. Reseal the leak, and wait for the system to fully recharge.

Adjust the vacuum switch:

1. Turn the adjustment screw in one of the following directions as appropriate:
   - Clockwise to increase the vacuum.
   - Counterclockwise to decrease the vacuum.

   The vacuum system should start recharging when the pressure drops to at least 12 in. Hg.

   The system should stop recharging before the pressure reaches 20 in. Hg.
2. Repeat steps 4 through 6 to verify the adjustment.
3. Replace the top cover, and close the plumbing plate.

Column Maintenance and Replacement

Guidelines

- Replace the guard column once a week. Instructions for replacing the guard column are included in the column replacement procedure, "Replacement Procedure" on page 9-35.
- If metal contamination is suspected, wash the column with phosphate. Follow the procedure on page 9-41, "Washing the 140D and Column with Phosphate".
- Replace the column if the following condition(s) are not improved by adjusting the composition of solvent A3, or by preparing fresh solvents.
  - Chromatography shows consistently broad peaks
  - Tailing peaks
  - Poor separation

  If the separation dramatically improves with the new column, discard the old column.

  Instructions for replacing the column are on page 9-35.

Replacement Procedure

Items Required

- PTH Column (P/N 401882)
- Guard Column (P/N 401883)

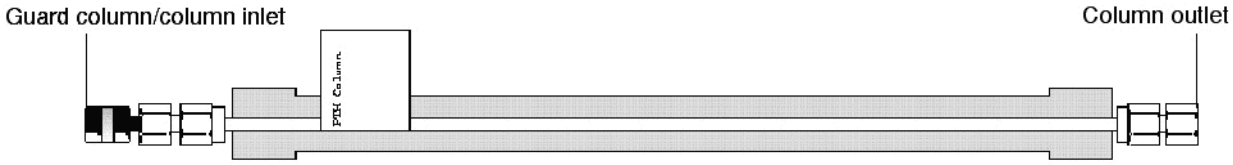

Figure 9-6. Column resting in the lower oven insert

Procedure

Caution	***Handle columns carefully. Damaged columns may leak and must be replaced. Do not scratch or dent the column ends. Dropping or bumping the column can irreversibly damage the consistency of the packed bed, thus impairing separation efficiency.***

Remove the old column:

1. Press Stop on the front panel of the 140D.
2. Press Manual to enter manual mode on the 140D.
3. If the column is to be reused within a short period of time, flush the column with 90 %B for 5 min at a flow rate of 60 µL/min.
4. Change the flow rate to 10 µL/min, and the solvent composition to 70 %B.
5. Remove the top portion of the column oven and oven insert to expose the column.
6. Unscrew the guard column and outlet line from the column.
7. Remove the old column.
8. Unscrew the old guard column from the column inlet line.

Install the new column:

1. Write the date on the label of the new column, and record the column serial number for later use.
2. Connect the new guard column to the inlet line.
3. Wait until liquid starts flowing out the guard column before proceeding to step 4. You want to make sure the guard column is functioning properly before you install the new column.
4. Connect the guard column to the PTH column inlet port. Place the column in the column oven.
5. Wait until the pressure stabilizes and liquid begins coming out of the column before proceeding to step 6.

   If you do not wait until liquid passes through the column, you run the risk of air bubbles later getting trapped in the flowcell.
6. Connect the outlet line to the column outlet port.
7. Cover the column with the oven insert and top cover.
8. Change the flow rate to 40 µL/min, and let the system run for 30 min to stabilize the system pressure.

Record the column change and equilibrate the new column:

1. On the Macintosh, select the Bottle Change dialog box from the dialog box pop-up menu.
2. Click PTH Column in the list of chemicals (Figure 9-7 on page 9-37).
3. Enter the serial number of the new column in the Lot Number box.
4. Open the File menu and select Save Chemical.
5. Select the Start Run dialog box from the dialog box pop-up menu.
6. Setup the sequencer to run the *Run Gradient cLC* method 20 times (Figure 9-8). You can run the β-lactoglobulin standard, blanks, or a combination of both. We recommend running some combination of blanks and the standard.
7. Once a run using the standard is on scale and the baseline is stable (close to flat), column equilibration is complete, and the sequencer is ready for normal use.

Note	*Equilibration of new columns can take up to 24 hours.*

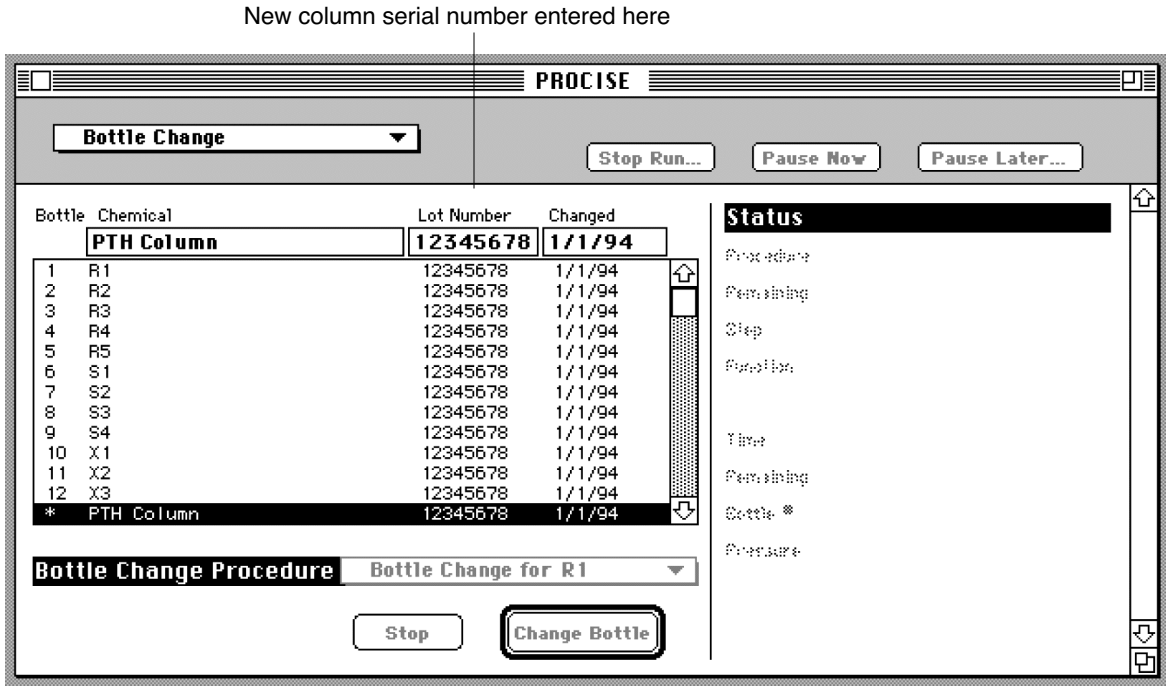

Figure 9-7. Recording a column change

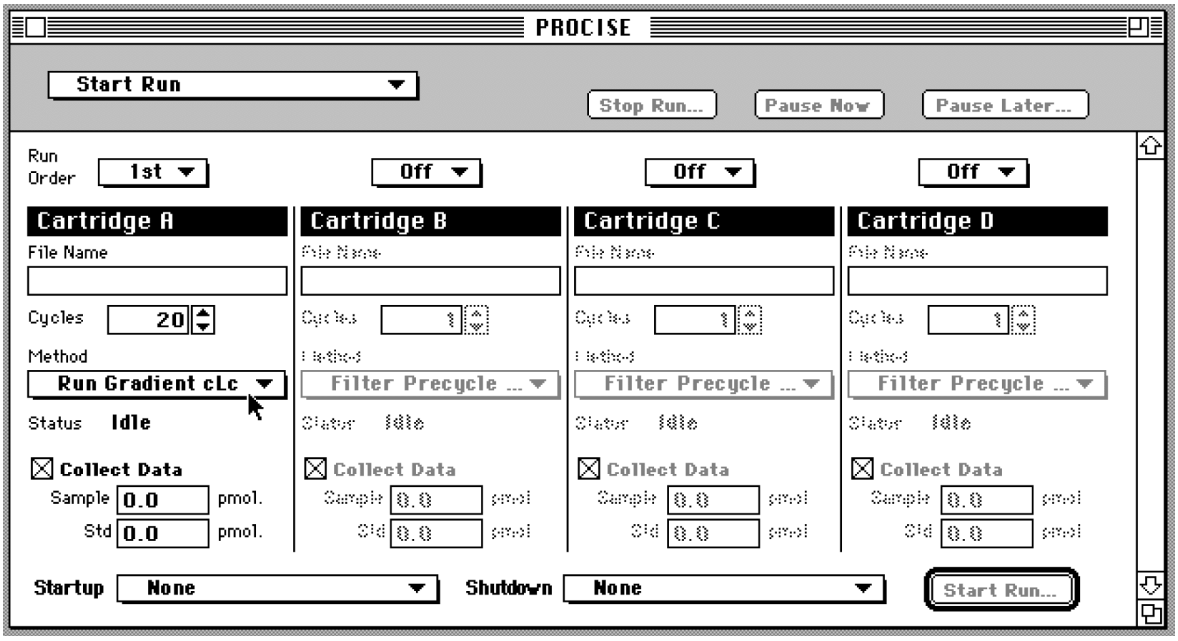

Figure 9-8. Equilibrating the new column using the Normal 1 cLC gradient

Maintaining the 140D

Recommendations

- Replace the piston and head seals every 6 months.
- Clean and inspect each cylinder for damage every time the piston seals are replaced. Once a cylinder is damaged, it must be replaced.
- Replace the rotor seals every 3 to 12 months of continuous use.

Refer to the 140D user's manual for instructions.

If you suspect metal contamination in the HPLC components of the system, we recommend:

- Washing the 140D and column with phosphate. See page 9-41 for this procedure.
- Washing the HPLC components of the system with phosphoric acid. See page 9-43 for this procedure.

Changing Solvents and Purging the 140D

Changing solvents involves:

- Changing the solvent bottles
- Purging the 140D
- Running the 140D to equilibrate the column

Purging the 140D rapidly expels solvents and trapped gases from the pump's syringes. The 140D is equipped with an automatic purge valve to divert the flow of solvent to waste. Every time a solvent is changed, equilibrate the column with the new solvent(s) until the baseline is stable before sequencing or evaluating a separation. Refer to the *ABI 140D Microgradient Delivery System User's Manual* for additional information on changing solvents and purging the pump.

WARNING	***The waste profile in the Safety Summary provides safe handling guidelines, and percent concentration of chemicals in the sequencer waste. Always dispose of all chemicals according to all local, federal and state requirements.***

Procedure

The following procedure for changing solvents A3 and B2 is performed via the 140D control panel. The keys F1, F2, F3, and F4 are referred to as *soft keys*, and are followed by the > symbol (PURGE> for example). The prompts for which you must enter values are shown in all capitals (for example, NUMBER OF PURGES). For more information on this procedure and the 140D control panel, refer to the *ABI 140D Microgradient Delivery System User's Manual*.

Remove the old solvent:

1. Remove the old solvent bottle(s).
2. Check the solvent lines for obstructions or salt deposits. If the lines are not clear, clean or replace them.
3. Check all fittings for salt deposits or indications of leakage. Clean or replace as necessary.
4. From the Ready Screen (main menu, Figure 9-9) on the 140D control panel, press the PURGE> soft key to display the Purge Screen (Figure 9-10).

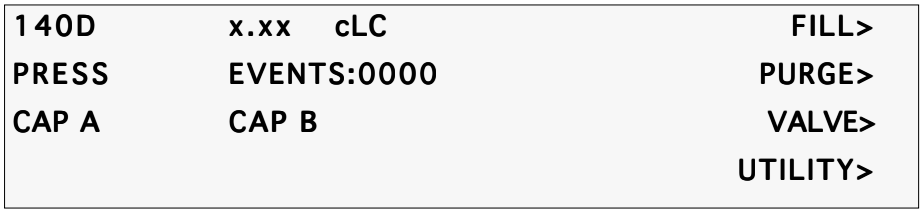

Figure 9-9. Ready Screen

Figure 9-10. Purge Screen

5. Use the arrow keys and numeric keypad to enter 2500 for the PURGE RATE. This is the rate in µL/min at which the cylinders empty.
6. Use the arrow keys to move the cursor to the SYRINGE prompt. Then use the Prev./Next keys to select BOTH.
7. Move the cursor to NUMBER OF PURGES, and enter 7.
8. Move the cursor to PERCENT OF SYRINGE, and enter 20 or more. This is the percent of the syringe to empty, refill and empty again.

Load the fresh solvent by purging the 140D:

1. Place the solvent inlet line into the new bottle, attach the cap, and place the bottle in the bottle holder. Repeat for each new bottle.
2. Press the BEGIN> soft key to start the purge procedure. The 140D and lines are rinsed with fresh solvent. Any air bubbles in the system are removed as well.

   The status of the procedure is displayed along the bottom of the screen on the 140D. To stop the purge procedure, press the Stop key.
3. Press the Manual key to enter manual mode and display the Manual Status screen. The syringes will fill with new solvent.
4. Press the FLOW> soft key. Type 40 to change the flow rate to 40 μL/min. Then press the Enter key.
5. Press the %B> soft key, and type 50 to change the solvent composition to 50 %B. Then press the Enter key.
6. Press the PRESS> soft key, and type 3500 to change the maximum operating pressure to 3500 psi. Then press the Enter key.
7. Allow the 140D to flow at this rate and composition for 10 min to equilibrate the column.
8. Run at least 4 Flask Standard cLC cycles to check PTH-amino acid separation efficiency and reproducibility before sequencing an unknown sample. If the separation is essentially the same as with the old buffers, begin sequencing.

   If the separation changes significantly with the new buffers, you may need to optimize the separation. Compare and evaluate the results of the last two cycles to determine if optimization is required. If so, follow the guidelines listed under “Optimizing the PTH-Amino Acid Separation” in section 6, “Optimization”.

Washing the 140D and Column with Phosphate

Recommendations

Use this procedure to clean the entire pumping system if metal contamination is suspected. This method is preferred over the phosphoric acid method described later because, unlike phosphoric acid, the phosphate can be pumped through the column as well.

Items Required

- Sodium phosphate monobasic or sodium dihydrogen phosphate (NaH_2PO_4). Potassium phosphate monobasic (KH_2PO_4) can be used as a substitute.
- HPLC-grade water
- 500 mL glass beaker

Procedure

Prepare a 0.1 M solution of sodium phosphate (approximately pH 5.0):

Caution	***The pH of the solution must not exceed 7.0. Ensure that sodium phosphate monobasic is used; otherwise, the pH may be too high.***

1. Place 3.45 g of sodium phosphate monobasic (NaH_2PO_4) into a 500 mL beaker.
2. Add 250 mL of HPLC-grade water and mix until thoroughly dissolved.

Wash the 140D and column:

1. Remove the transfer lines from solvents A and B.
2. Purge the 140D once at 100%, using the default flow rate.
3. Place the solvent transfer lines into the phosphate buffer.
4. Perform 3 purges at 100%, using the default flow rate.
5. Press Manual on the 140D control panel, and free run the pump at 50 µL/min, 50% B for 45 to 60 min.
6. Place the solvent transfer lines into HPLC-grade water, and perform 3 purges at 100%.
7. Press Manual on the 140D control panel, and free run the pump at 50 µL/min, 50% B for 45 to 60 min.
8. Place the solvent transfer lines back into the respective solvents.
9. Perform one purge at 100%.

Caution	***Sodium phosphate will precipitate in acetonitrile. To prevent severe damage to the pumping system, do not allow these two chemicals to mix in the pumping system at any time.***

Recommendation for Preventing Further Metal Contamination

To minimize the possibility of further metal contamination, add sodium phosphate monobasic (NaH_2PO_4) or potassium phosphate (KH_2PO_4) to solvent A, and mix well until completely dissolved. The final concentration of phosphate should be 100 μM. The addition of phosphate may slightly increase the retention time of aspartic and glutamic acid.

Washing the 140D with Phosphoric Acid

Recommendations

- Use this procedure to clean the 140D **only** if metal contamination is suspected.
- An alternative procedure, "Washing the 140D and Column with Phosphate", cleans the entire pumping system including the column with phosphate. This procedure is on page 9-41.

Caution ***Do not run phosphoric acid through the column. Phosphoric acid will severely damage column.***

Items Required

- Phosphoric acid
- Acid-resistant gloves
- Safety goggles
- HPLC-grade water

Procedure

WARNING ***Phosphoric acid is extremely corrosive. Wear safety goggles, a lab coat, and gloves when performing this procedure.***

1. Prepare a 5% solution of phosphoric acid by adding 10 mL of phosphoric acid to 190 mL of HPLC-grade water.
2. Place the solvent A and B transfer lines into the phosphoric acid solution.
3. Perform 5 purges at 100%.
4. Place the solvent transfer lines into a beaker containing 200 mL of HPLC-grade water.
5. Perform 3 purges at 100%.
6. Place the solvent transfer lines back into the respective solvents.
7. Perform 1 purge at 100%.

Dynamic Pressure Monitoring

Overview

Use this procedure to:

- Monitor the pressure of the 140D during a run.
- Test the cylinders in the 140D.

Items Required

- A dual-channel chart recorder

Procedure

1. From the 140D Configuration menu, set the D/A channel to A, and the scale to 3.

Note	*A scale of 3 will ensure that the pressure trace remains on scale throughout the run (0 to 2040 psi). For a more sensitive response, a scale of less than 3 can be used. In this case, the pressure trace will autozero at several points during the gradient.*

2. Connect the red and black input terminals of one of the chart recorder channels to the + and – pressure terminals on the back of the 140D (Figure 9-11 on page 9-45).
3. Set this channel sensitivity to 1 V full scale.
4. Connect the Sec channel to the REC output on the back of the 785A detector.
5. Set this channel sensitivity to 10 mV full scale.
6. Connect the chart recorder external paper feed input to the Event 1 terminals on the back of the 140D.
7. Position both pens using the chart recorder zero controls.
8. Start your run.

The profile of the pressure trace will be gradient specific, but should be consistent from run to run. As shown in Figure 9-12 on page 9-45, Channel 1 will show a trace of the chromatogram. Channel 2 will show the corresponding pressure variation during the gradient.

Peaks that go negative indicate a sudden loss of pressure. This could be due to a scratched cylinder. Such a pressure drop would be consistent with a variation in retention time.

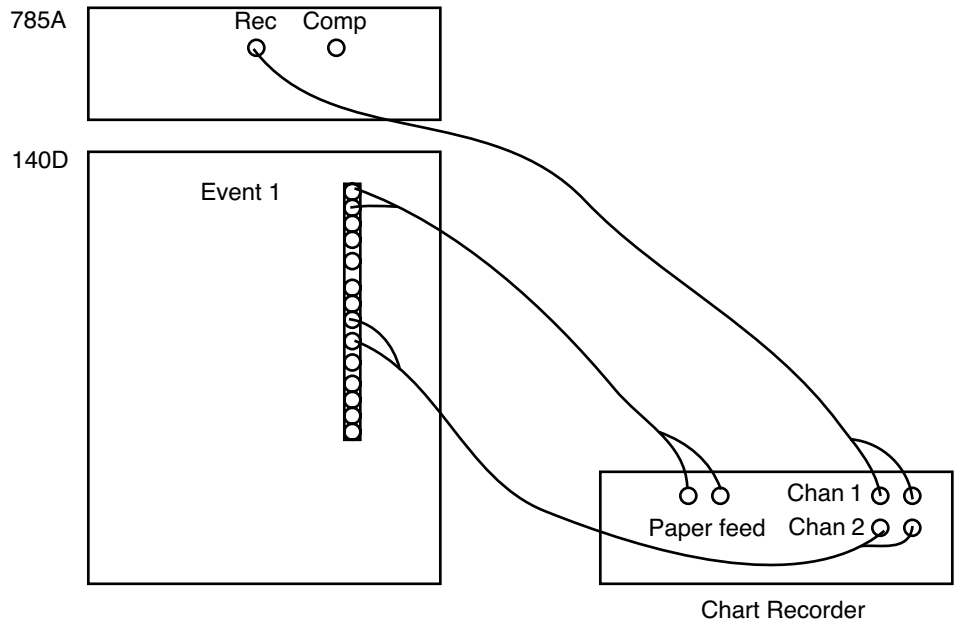

Figure 9-11. Connections for dynamic pressure monitoring

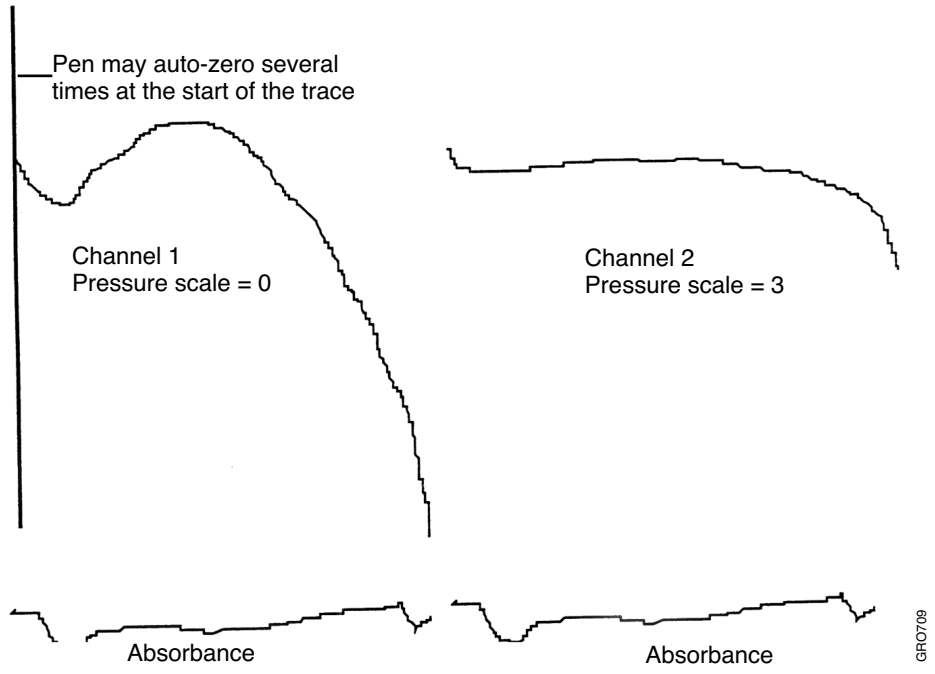

Figure 9-12. Dynamic pressure monitoring

Maintaining the 785A

When to Replace the Lamp

Replace the lamp after every 1500 to 2000 h of normal use. Refer to the 785A user's manual for instructions on how to test and replace the lamp.

WARNING	***ULTRAVIOLET LIGHT HAZARD. Exposure to ultraviolet radiation can cause blindness or permanent eye damage. To prevent eye injury, adjust the detector sensitivity from the ultraviolet to the visible range (500 nm) before beginning any detector maintenance procedures. Always wear protective UV-absorbing glasses when looking into the detector.***

WARNING	***PHYSICAL INJURY HAZARD. The lamp can become very hot while in use. Turn off the power to the lamp and allow it to cool before removing it from the fixture. Always wear heat-resistant gloves when handling the lamp.***

Removing Air Bubbles From the Flowcell

1. Run 90% solvent B through the flowcell at 60 µL/min.
2. If this does not dislodge the bubbles, flush the flowcell with methanol or isopropylalcohol. See "Flushing the Flowcell" on page 9-47 for further instructions.

Cleaning the Flowcell

The flowcell can be cleaned with methanol or isopropylalcohol. Cleaning the flowcell can be helpful if:

- Bubbles are still present after running 90% solvent B through the flowcell at 60 µL/min.
- Severe drift suggests that contamination is leaching from the flowcell windows.

See "Flushing the Flowcell" on page 9-47 for further instructions.

Flushing the Flowcell

Items Required

- Long flat-blade screwdriver
- Two 1/4-in. wrenches
- Methanol or isopropylalcohol
- Protective gloves
- HPLC-grade water
- 5–10 mL disposable syringe

Note	*For most disposable syringes, the luer adaptor can be screwed directly into the flowcell adaptor. If this is not the case, make an adaptor tube.*

Procedure

WARNING	***Wear chemical-resistant gloves when handling methanol. Contact with skin can cause irritation. Absorption through the skin is harmful. Refer to the appropriate material safety data sheet in the Procise 49X cLC Protein Sequencing System Safety Summary for further information.***

Remove the flowcell:

1. Open the front panel of the 785A.
2. Loosen the 3 plastic knurled screws that secure the lid in place.
3. Slide the lid back slightly.
4. Loosen, but do not remove, the clamping screw (located to the right of the flowcell).
5. Use a screwdriver to open the clamp, and remove the flowcell complete with inlet and outlet tubing.
6. Disconnect the flowcell tubing.

IMPORTANT	*When loosening or tightening the flowcell bushings, always use a second wrench to prevent the flowcell adaptor from turning.*

Flush the flowcell:

1. Load the syringe with 5 mL of HPLC-grade water.
2. Flush the flowcell with the water.
3. Load the syringe with 5 mL of methanol or isopropylalcohol.
4. Flush the flowcell with the methanol or isopropylalcohol.
5. If this procedure does not remove the trapped material, disassemble the flowcell, clean it, reassemble it, and test it.

   Instructions for these procedures are included in “Replacing the 785A Detector Flowcell Windows” on page 9-50. Begin with the instructions listed under "Disassemble the flowcell:" on page 9-51.

Re-install the flowcell:

1. Reconnect the inlet and outlet tubing to the flowcell. Do not over-tighten; the walls of the adaptor are thin and easily damaged.

IMPORTANT *When loosening or tightening the flowcell bushings, always use a second wrench to prevent the flowcell adaptor from turning.*

2. Push the flowcell back into the clamp, so the body of the flowcell is flush with the clamp, and the inlet tube is to the right. If necessary, open the clamp with the screwdriver.
3. Keeping the flowcell loose in the clamp, rotate it so that the outlet tubing is approximately 45° to the left of vertical, then clamp the flowcell in place. This orientation prevents the tubing from becoming kinked.
4. Route the inlet and outlet tubing through the slot in the detector head top plate. If the plate is not slotted, route the tubing so it sits in the recesses
5. Tape the tubing in place.

Replacing the Lamp in the 785A UV/VIS Detector

Items Required

- New UV lamp (P/N 2900-0484)
- Flat-blade screwdriver
- UV-protective safety glasses

Procedure

WARNING	***Wear UV-protective glasses when performing this procedure. Allow the lamp to cool before you touch it.***

1. Power-down the 785A, and allow the lamp to cool completely.
2. Release the back panel catch by rotating the knurled knob on the rear of the detector 1/4-turn counter-clockwise.

Note	*The interlock switch in the rear compartment of the detector disconnects the power supply when the back panel is removed.*

3. The lamp is held in place by a spring and catch. The catch is located just above the lamp. Using your fingers or a screwdriver, unhook the catch by pushing it forward and slightly upwards.
4. Unplug the lamp, and pull it horizontally off the locating pins.
5. Install the new lamp over the two locating pins, and secure the retaining spring.
6. Plug in the lamp.
7. Close the back panel, and power up the 785A.

Replacing the 785A Detector Flowcell Windows

Items Required

- Long flat-blade screwdriver
- Small flat-blade screwdriver
- 16 in.-ounce torque screwdriver
- 1/4-in. torque wrench
- Two 1/4-in. wrenches
- Compressed gas for drying
- Methanol or isopropylalcohol
- HPLC-grade water
- One pair of flowcell windows (P/N 7200-0008)
- 5 to 10 mL disposable syringe

Note	*For most disposable syringes, the luer adaptor can be screwed directly into the flowcell adaptor. If this is not the case, make an adaptor tube.*

Procedure

Remove the flowcell:

1. Open the front panel of the 785A.
2. Loosen the 3 plastic knurled screws that secure the lid in place.
3. Slide the lid back slightly.
4. Loosen, but do not remove, the clamping screw located to the right of the flowcell.
5. Use a screwdriver to open the clamp, and remove the flowcell complete with inlet and outlet tubing.
6. Disconnect the flowcell tubing.

IMPORTANT	*When loosening or tightening the flowcell bushings, always use a second wrench to prevent the flowcell adaptor from turning.*

Disassemble the flowcell:

1. While holding the front and rear cell apertures in place with your finger and thumb, loosen and remove the three aperture screws.
2. Remove the apertures. If the windows remain in the flowcell body, pry them out with your fingernail, or blow compressed gas into one of the adaptors.
3. If material is trapped inside the flowcell, remove both flowcell adaptors.
4. Soak or sonicate the adaptors and flowcell body in HPLC-grade water.
5. Dry the flowcell components with compressed gas.

Rebuild and test the flowcell:

1. **Being extremely careful not to touch the face of the window,** drop one of the windows into to the rear cell counter-bore.
2. Place the rear aperture on top so the aperture and flowcell body screw holes line up.
3. While holding the rear aperture in place, drop the other window into the front counter-bore, and position the front aperture on top.
4. While holding both apertures in place with your finger and thumb, loosely tighten the three aperture screws.
5. Tighten each screw in turn slightly to keep the apertures parallel with the flowcell body. Tighten each screw to a final torque of 16 in.-ounces.
6. If the flowcell adaptors were removed, screw them back into place using the 1/4-in. torque wrench.
7. Load the syringe with isopropylalcohol or methanol, and flush the flowcell. Flushing removes any residue from inside the cell, thus minimizing the opportunity for bubbles to become trapped.
8. Reconnect the tubing to the flowcell.

IMPORTANT *While reconnecting the tubing, prevent the flowcell adaptor from turning by holding it in place with a wrench. Do not over-tighten the bushing. The adaptor walls are quite thin and easily damaged.*

9. Check the flowcell for leakage by free running the 140D in Manual mode for 5 min at 90 %B and 60 µL/min.

Reinstall the flowcell:

1. Push the flowcell back into the clamp, so the body of the flowcell is flush with the clamp, and the inlet tube is to the right. If necessary, open the clamp with the screwdriver.
2. Keeping the flowcell loose in the clamp, rotate it so that the outlet tubing is approximately 45° to the left of vertical, then clamp the flowcell in place. This orientation prevents the tubing from becoming kinked.
3. Route the inlet and outlet tubing through the slot in the detector head top plate. If the plate is not slotted, route the tubing so it sits in the recesses
4. Tape the tubing in place.

Testing the Dry Cell

Overview

If noise, spikes, drift or stepping are evident on the baseline, use this procedure to test the integrity of the following system components:

- Line voltage stability
- 785A electronics and lamp
- Signal cable between the 785A and the sequencer
- Procise 24-bit A/D convertor

Items Required

- Strip chart recorder and signal cable (if available)
- Dry cell aperture (normally taped inside front compartment of detector)

Procedure

Setup the instruments for the test:

1. Replace the flowcell with the spare dry cell aperture. Do not disconnect the flowcell from the plumbing.
2. Set the wavelength to 238 nm, the rise time to 1.0 sec, and the range to 0.001.
3. Connect the REC output to the chart recorder. Leave the COMP output connected to the sequencer.
4. Set the chart recorder scale to 10 mV full scale, and the speed to 2 mm/min.
5. To collect data, select the Start Run dialog box.
6. Configure a run as follows:
   - Set the Run Order for Cartridge A to 1st
   - Enter a unique file name for the run
   - Select Run Gradient cLC from the Method pop-up menu

Run the test:

1. Click Start Run.
2. As soon as the Init Sensor procedure starts, jump to the End step.
3. Click Next Step when the Flask cycle begins.
4. When the 140D starts to run, jump to step 5, Start Gradient.
5. If you do not want the 140D to run, press the Stop key on the front of the 140D.
6. If a *Collecting* window is not displayed, ensure that the 610A Data Analysis software is launched, and that *Display New Procise Data* is checked under the Acquisition menu.

Interpret the test results:

- Noise should be no greater than 2 x 10–5 AU.
- 20 μAU peak-to-peak as read from the 610A.
- 0.2 mV peak-to-peak on the chart recorder.
- Drift should be no greater than 1 x 10-4 AU/hour after warm-up.
- The baseline should be free of spikes and steps apart from the initial auto-zero.

If both 610A and chart recorder traces have excessive noise, steps or spikes, suspect the:

- UV lamp
- Detector electronics
- Line voltage

If only the 610A data is affected, suspect the:

- COMP output
- Signal cable from the 785A to the sequencer
- Sequencer I/O PCB
- Sequencer power supply

Visually Testing the 785A Wavelength

Note	*This is not an accurate wavelength test. However, it will reveal gross errors that affect sequencing results.*

WARNING	***To avoid eye injury, always wear UV-protective goggles when performing this procedure.***

1. Press the WAVE> soft key on the 785A front panel.
2. Press 656, then Enter. If the display indicates the wavelength is driving towards 656 nm, proceed to step 8.
3. Press the MORE> soft key.
4. When the next menu appears, press the UTIL> soft key to display the utility menu.
5. Press the MORE> soft key.
6. Move the cursor to the LIMITS field, and press Next to display 190 to 700.
7. Press DONE>, then EXIT> to return to the Main menu.
8. Open the detector head door and observe the light emitted from the sample and reference cells. Both lights should be a bright red color.
9. If the color is not bright red, either the wavelength is incorrect, or the lamp is not lit. Make repairs or adjustments as appropriate.

Macintosh Maintenance

Guidelines

The Macintosh computer is easily maintained by following these guidelines:

- Restart the Macintosh once a day to defragment the RAM.
- Rebuild the desktop once a month. To rebuild the desktop, restart the Macintosh while holding down the OPTION and keys.

Following these guidelines will help prevent computer lock-ups from occurring.

Reformatting the Macintosh Hard Drive

Items Required

- System software

Procedure

Reformat the hard drive:

Caution	***Performing a low level format will delete all files from the hard drive. Ensure that important files are backed-up first.***

1. Make a back-up copy of the Procise Chemistry file, 610A data files and any user-specified files.
2. Load the system software CD-ROM, and restart the Mac while holding down the C key. Once the self-tests have passed (smiley face appears), release the C key and hold down the Shift key until the message *Extensions off* appears.
3. Once the Mac has booted from the CD, open the Utilities folder.
4. Double-click on Drive Set-up, and highlight the hard disk.
5. Open the Functions menu from the upper menu bar, and choose Initialization options.
6. Once the dialog box appears, click the Low level format and Zero data check boxes.
7. Click OK.

Install the operating system:

1. Once the formatting is complete, open the System Software Install folder.
2. Double-click the System installer to install the operating system.

Install the Procise control and 610A software:

1. Insert Procise disk 1, and double-click Installer.
2. Click OK when the splash screen appears.
3. Click Customize, and select all but the Chemistry file.
4. Follow the installer instructions.
5. Install the Chemistry file that was backed-up earlier into the PROCISE folder (inside the Preferences folder).
6. Save an alias of the Procise application in the Startup items folder.
7. Restart the Macintosh.

10 User Bulletins

A User Bulletin is an advisory issued by Applied Biosystems. User bulletins contain new information, advances or procedures that may immediately influence your use of Applied Biosystems instruments.

This section of the user's manual is intended as a storage space for any user bulletins you may receive regarding your Procise 49X cLC Protein Sequencing System.

Appendix A Standard Functions

The following is a complete list of the standard functions provided by Applied Biosystems for the Procise 49X cLC Protein Sequencing System.

Function Number	Reagent	Function Name	Valves
001	R1	Del R1, Cart (top)	6, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40
002	R1	Del R1, Cart (bottom)	6, 11, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16
003	R1	Del R1, Cart (sensor)	6, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40
004	R1	Del R1, Waste	6, 1
005	R1	Load R1, Cart (sm loop)	6, 7, 22
006	R1	Load R1, Cart (lg loop)	6, 7, 21
007	R1	Vent R1	55
008	R1	Flush R1	55
009	R1	Backflush R1	6, 11, 55, 15
010	R1	Reserved	
011	R2g	Del R2g, Cart (top)	3, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40
012	R2g	Del R2g, Cart (bottom)	3, 11, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16
013	R2g	Not Available	
014	R2g	Del R2g, Waste	3, 1
015	R2g	Not Available	
016	R2g	Not Available	
017	R2g	Vent R2g	58
018	R2g	Flush R2g	58
019	R2g	Backflush R2g	3, 11, 58, 15
020	R2g	Reserved	
021	R3	Del R3, Cart (top)	8, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40
022	R3	Del R3, Cart (bottom)	8, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16

Function Number	Reagent	Function Name	Valves
023	R3	Del R3, Cart (sensor)	8, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40
024	R3	Del R3, Waste	8, 23, 16
025	R3	Load R3, Cart (sm loop)	8, 23, 22
026	R3	Load R3, Cart (lg loop)	8, 23, 21
027	R3	Vent R3	53
028	R3	Flush R3	53
029	R3	Backflush R3	8, 53, 15
030	R3	Transfer R3, Cart (gas)	7, 11, 15, 17, 18, 19, 20, 34, 35, 36, 37, 40
031	R3g	Del R3g, Cart (top)	9, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40
032	R3g	Del R3g, Cart (bottom)	9, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16
033	R3g	Not Available	
034	R3g	Del R3g, Waste	9, 23, 16
035	R3g	Not Available	
036	R3g	Not Available	
037	R3g	Vent R3g	53
038	R3g	Flush R3g	53
039	R3g	Backflush R3g	9, 53, 15
040	R3g	Reserved	
041	S1	Del S1, Cart (top)	14, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40
042	S1	Del S1, Cart (bottom)	14, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16
043	S1	Del S1, Cart (sensor)	14, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40
044	S1	Del S1, Waste	14, 23, 16
045	S1	Load S1, Cart (sm loop)	7, 11, 14, 22
046	S1	Load S1, Cart (lg loop)	7, 11, 14, 21
047	S1	Vent S1	56
048	S1	Flush S1	56
049	S1	Backflush S1	14, 56, 15
050	S1	Reserved	

Function Number	Reagent	Function Name	Valves
051	S2	Del S2, Cart (top)	12, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40
052	S2	Del S2, Cart (bottom)	12, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16
053	S2	Del S2, Cart (sensor)	12, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40
054	S2	Del S2, Waste	12, 23, 16
055	S2	Load S2, Cart (sm loop)	7, 11, 12, 22
056	S2	Load S2, Cart (lg loop)	7, 11, 12, 21
057	S2	Vent S2	54
058	S2	Flush S2	54
059	S2	Backflush S2	12, 54, 15
060	S2	Reserved	
061	S3	Del S3, Cart (top)	13, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40
062	S3	Del S3, Cart (bottom)	13, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16
063	S3	Del S3, Cart (sensor)	13, 23, 17, 18, 19, 20, 34, 35, 36, 37, 40
064	S3	Del S3, Waste	13, 23, 16
065	S3	Load S3, Cart (sm loop)	7, 11, 13, 22
066	S3	Load S3, Cart (lg loop)	7, 11, 13, 21
067	S3	Vent S3	52
068	S3	Flush S3	52
069	S3	Backflush S3	13, 52, 15
070	S3	Reserved	
071	X1	Del X1, Cart (top)	5, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40
072	X1	Del X1, Cart (bottom)	5, 11, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16
073	X1	Del X1, Cart (sensor)	5, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40
074	X1	Del X1, Waste	5, 1
075	X1	Load X1, Cart (sm loop)	5, 7, 22
076	X1	Load X1, Cart (lg loop)	5, 7, 21

Function Number	Reagent	Function Name	Valves
077	X1	Vent X1	59
078	X1	Flush X1	59
079	X1	Backflush X1	5, 11, 59, 15
080	X1	Reserved	
081	X1g	Del X1g, Cart (top)	2, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40
082	X1g	Del X1g, Cart (bottom)	2, 11, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16
083	X1g	Not Available	
084	X1g	Del X1g, Waste	2, 1
085	X1g	Not Available	
086	X1g	Not Available	
087	X1g	Vent X1g	59
088	X1g	Flush X1g	59
089	X1g	Backflush X1g	2, 11, 59, 15
090	X1g	Reserved	
091	X3	Del X3, Cart (top)	4, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40
092	X3	Del X3, Cart (bottom)	4, 11, 10, 17, 18, 19, 20, 34, 35, 36, 37, 16
093	X3	Del X3, Cart (sensor)	4, 7, 17, 18, 19, 20, 34, 35, 36, 37, 40
094	X3	Del X3, Waste	4, 1
095	X3	Load X3, Cart (sm loop)	4, 7, 22
096	X3	Load X3, Cart (lg loop)	4, 7, 21
097	X3	Vent X3, Cart	60
098	X3	Flush X3, Cart	60
099	X3	Backflush X3, Cart	4, 11, 60, 15
100	X3	Reserved	
101	S1	Wash Input Block (S1)	7, 11, 14, 16
102	S1	Wash Output Block (S1)	14, 10, 40
103	S1	Transfer to Flask (S1)	14,23,17,18,19,20,34,35,36,37, 38,45
104	S1	Transfer to FC (S1)	14,23,17,18,19,20,34,35,36,37, 39

Function Number	Reagent	Function Name	Valves
105	S1	Reserved	
106	S2	Wash Input Block (S2)	7, 11, 12, 16
107	S2	Wash Output Block (S2)	12, 10, 40
108	S2	Transfer to Flask (S2)	12, 23, 17, 18, 19, 20, 34, 35, 36, 37, 38, 45
109	S2	Transfer to FC (S2)	12, 23, 17, 18, 19, 20, 34, 35, 36, 37, 39
110	X1	Wash Input Blk (X1)	
111	S3	Wash Input Block (S3)	7, 11, 13, 16
112	S3	Wash Output Block (S3)	13, 10, 40
113	S3	Transfer to Flask (S3)	13, 23, 17, 18, 19, 20, 34, 35, 36, 37, 38, 45
114	S3	Transfer to FC (S3)	13, 23, 17, 18, 19, 20, 34, 35, 36, 37, 39
115	X1	Wash Output Blk (X1)	
116	X3	Wash Input Block (X3)	4, 7, 16
117	X3	Wash Output Block (X3)	4, 11, 10, 40
118	X3	Transfer to Flask (X3)	4, 7, 17, 18, 19, 20, 34, 35, 36, 37, 38, 45
119	X3	Transfer to FC (X3)	4, 7, 17, 18, 19, 20, 34, 35, 36, 37, 39
120	X3	Reserved	
121		Transfer to Flask (gas)	15, 23, 17, 18, 19, 20, 34, 35, 36, 37, 38, 45
122		Transfer to FC (gas)	15, 23, 17, 18, 19, 20, 34, 35, 36, 37, 39
123		Select Cartridge A	
124		Select Cartridge B	
125		Select Cartridge C	
126		Select Cartridge D	
127		Ready Transfer to Flask	
128		Transfer Complete	
129		Pressurize Cart, top	23, 17, 18, 19, 20, 15
130		Pressurize Cart, bottom	10, 34, 35, 36, 37, 15

Function Number	Reagent	Function Name	Valves
131		Dry Cart (top)	23, 17, 18, 19, 20, 34, 35, 36, 37, 40, 15
132		Dry Cart (bottom)	10, 17, 18, 19, 20, 34, 35, 36, 37, 16, 15
133		Dry Cart (high, top)	23, 17, 18, 19, 20, 34, 35, 36, 37, 15, 40, 46
134		Dry Cart (high, bottom)	10, 17, 18, 19, 20, 34, 35, 36, 37, 16, 15, 46
135		Flush Cart Reagent Block	1, 11, 15, 46
136		Flush Cart Solvent Block	15, 16, 23, 46
137		Flush Input Block	7, 11, 15, 16, 46
138		Flush Output Block	10, 15, 40, 46
139		Flush Small Loop (Cart)	7, 11, 15, 22, 46
140		Flush Large Loop (Cart)	7, 11, 15, 21, 46
141		Flush Transfer Line	15, 10, 38, 45
142		Set Cart Temperature	
143		Wash Cart Reagent Block	1, 11, 12
144		Wash Cart Solvent Block	12, 16, 23
145		Wash Small Loop (Cart)	7, 11, 12, 22
146		Wash Large Loop (Cart)	7, 11, 12, 21
147		End Cartridge Select	
148		Cartridge Wait	17,18,19,20,34,35,36,37
149	S2	Wash Transfer Line (S2)	10, 12, 38, 45
150	X1	Wash Transfer Line (X1)	
151	R4	Del R4, Flask	28, 32, 45
152	R4	Load R4, Flask (sm loop)	28, 30
153	R4	Load R4, Flask (lg loop)	28, 31
154	R4	Vent R4	51
155	R4	Flush R4	51
156	R4	Backflush R4	28, 51, 24
157	R4	Del R4, Waste	28, 31
158	R4	Reserved	
159	R4	Reserved	

Function Number	Reagent	Function Name	Valves
160	R4	Reserved	
161	R5	Del R5, Flask	29, 32, 45
162	R5	Load R5, Flask (sm loop)	29, 30
163	R5	Load R5, Flask (lg loop)	29, 31
164	R5	Vent R5	49
165	R5	Flush R5	49
166	R5	Backflush R5	29, 49, 24
167	R5	Del R5, Waste	29, 31
168	R5	Reserved	
169	R5	Reserved	
170	R5	Reserved	
171	S4	Del S4, Flask	25, 32, 45
172	S4	Load S4, Flask (sm loop)	25, 30
173	S4	Load S4, Flask (lg loop)	25, 31
174	S4	Vent S4	50
175	S4	Flush S4	50
176	S4	Backflush S4	25, 50, 24
177	S4	Del S4, Waste	25, 31
178	S4	Reserved	
179	S4	Reserved	
180	S4	Reserved	
181	X2	Del X2, Flask	27, 32, 45
182	X2	Load X2, Flask (sm loop)	27, 30
183	X2	Load X2, Flask (lg loop)	27, 31
184	X2	Vent X2	57
185	X2	Flush X2	57
186	X2	Backflush X2	27, 57, 24
187	X2	Del X2, Waste	27, 31
188	X2	Reserved	
189	X2	Reserved	
190	X2	Reserved	
191	X2g	Del X2g, Flask	33, 32, 45

Function Number	Reagent	Function Name	Valves
192	X2g	Not Available	
193	X2g	Not Available	
194	X2g	Vent X2g	57
195	X2g	Flush X2g	57
196	X2g	Backflush X2g	33, 57, 24
197	X2g	Del X2g, Waste	31, 33
198	X2g	Reserved	
199	X2g	Reserved	
200	X2g	Reserved	
201	X3	Del X3, Flask	26, 32, 45
202	X3	Load X3, Flask (sm loop)	26, 30
203	X3	Load X3, Flask (lg loop)	26, 31
204	X3	Vent X3, Flask	60
205	X3	Flush X3, Flask	60
206	X3	Backflush X3, Flask	26, 60, 24
207	X3	Del X3, Waste, Flask	26, 31
208	X3	Reserved	
209	X3	Reserved	
210	X3	Reserved	
211		Bubble Flask (h press)	41, 44, 45, 48
212		Bubble Flask	41, 44, 45
213		Dry Flask	24, 32, 41, 44, 45
214		Dry Flask (h press)	24, 32, 41, 44, 45, 48
215		Empty Flask	24, 32, 41, 43
216		Empty Flask (l press)	24, 32, 41, 43, 47
217		Flush Small Loop (Flask)	24, 30
218		Flush Large Loop (Flask)	24, 31
219		Wash Small Loop (Flask)	25, 30
220		Wash Large Loop (Flask)	25, 31
221		Flush Injector	42, 44, 48
222		Flush Flask/Injector	24, 32, 41, 42
223		Inject Position	

Function Number	Reagent	Function Name	Valves
224		Flush Injector (Low Pres)	42, 44
225		Load Injector	24, 32, 41, 42, 47
226		Load Position	
227		Prepare Pump	
228		Ready to Receive	41, 44, 45
229		Set Column Temperature	
230		Set Flask Temperature	
231		Stop Pump	
232		Start Gradient	
233		Set as Blank Cycle	
234		Set as Standard Cycle	
235		Set as Residue Cycle	
236		Pre-Conversion Dry	24, 32, 41, 44, 45
237		Post-Conversion Dry	24, 32, 41, 44, 45
238		Concentrate Sample	24, 32, 41, 44, 45
239		Flask Equilibrate	24,32,45,47
240	S1	Wash Cart Solvent Block S1	14,16,23
241	S1	Wash Cart Reagent Block S1	1,11,14
242	S1	Wash Cart Small Loop S1	7,11,14,22
243	S1	Wash Cart Large Loop S1	7,11,14,21
244		Reserved	
245		Reserved	
246		Reserved	
247		Reserved	
248		Reserved	
249		Inject Pos/Collect Data	
250		Start Grad/No Data Coll	
251		490A Relay 1 Off	
252		490A Relay 1 On	
253		490A Relay 1 Pulse	
254		490A Relay 2 Off	
255		490A Relay 2 On	

Function Number	Reagent	Function Name	Valves
256		490A Relay 2 Pulse	
257		Wait	
258		Begin	
259		End	
260		Pause for Bottle Change	
261		Set for Bottle R1	
262		Set for Bottle R2	
263		Set for Bottle R3	
264		Set for Bottle R4	
265		Set for Bottle R5	
266		Set for Bottle S1	
267		Set for Bottle S2	
268		Set for Bottle S3	
269		Set for Bottle S4	
270		Set for Bottle X1	
271		Set for Bottle X2	
272		Set for Bottle X3	
273		Init Sm Loop Snsr, Cart	
274		Init Lg Loop Snsr, Cart	
275		Init Cart A Snsr	
276		Init Cart B Snsr	
277		Init Cart C Snsr	
278		Init Cart D Snsr	
279		Init Transfer Snsr	
280		Init Sm Loop Snsr, Flask	
281		Init Lg Loop Snsr, Flask	
282		Init Injector Load Snsr	
283		Init Injector Full Snsr	
284		Open Valves 11,15	11, 15
285		Injector Sim Load	24, 32, 41, 42, 47
286		X3 to R2	3, 4, 58
287		X3 to R3g	4, 9, 11, 53

Function Number	Reagent	Function Name	Valves
288		X3 to X1g	2, 4, 59
289		X3 to X2g	26, 33, 57
290		Vent 16 Test	7, 11, 15, 16
291		Vent 30 Test	24, 30, 47
292		Vent 43 Test	43, 44
293		Open Valves 15,23	15, 23
294		Open Valve 24	24
295		Open Valves 24,32	24, 32
296		Open Valves 24,32,45	24,32,45
297		Flask Out Test	44
298		Flask Reag Blk, Hi Test	24, 47
299		Open Valves 49,57,59	49, 57, 59
300		Waste Test	43, 44
301		Pause	
302		Use Valves of Function	
303		Select Regulator	
304		Save Regulator Setpoint	
305		Set Reg Setpoint (10th psi)	
306		Wait With Valves On	
307		Compare Pressures (10th psi)	
308		Close Pressure Valve	
309		Restore Reg Setpoint	
310		Set Tolerance (100th psi)	
311		Test Valves	
312		Test Heaters	
313		Test Pressure Board	
314		Test 12-Bit A/D	
315		Test 24-Bit A/D	
316		Test Rheodyne	
317		Save Regulator Pressure	
318		Compare Saved Pressure	
319		Compare HP Inlet (10th psi)	

Function Number	Reagent	Function Name	Valves
320		Select Heater	
321		Save Heater Setpoint	
322		Restore Heater Setpoint	
323		Inc. Heater Setpoint (°C)	
324		Dec. Heater Setpoint (°C)	
325		Set Heater Tolerance (100th°C)	
326		Compare Temperatures	
327		Reset Vacuum On Count	
328		Log Vacuum On Count	
329		Set Flow Meter Tolerance(SC-CM)	
330		Compare Flow Meter (SCCM)	
331		Tare Sartorius	
332		Log Weight	
333		X3 to R1	4, 6, 55
334		X3 to R3	4, 8, 11, 53
335		X3 to R4	26, 28, 51
336		X3 to R5	26, 29, 49
337		X3 to S1	4, 11, 14, 56
338		X3 to S2	4, 11, 12, 54
339		X3 to S3	4, 11, 13, 52
340		X3 to S4	25, 26, 50
341		X3 to X1	4, 5, 59
342		X3 to X2	26, 27, 57
343		X3 to Cart A (bottom)	4, 10, 11, 16, 17, 34
344		Open Valves 7,11,15,16	7, 11, 15, 16
345		Open Valves 1,11,15	1, 11, 15
346		Open Valves 1,11	1, 11
347		Open Valves 1,15,16,40	1, 15, 16, 40
348		Open Valves 11,15,16	11, 15, 16
349		Open Valves 15,23	15, 23
350		Open Valves 10,15,45	10, 15, 45

Function Number	Reagent	Function Name	Valves
351		Open Valve 30	30
352		Flask Reag Blk Test	24
353		Open Valves 24,45	24, 45
354		Open Valve 43	43
355		Open Valves 44,45	44, 45
356		Reserved	
357		Reserved	
358		Reserved	
359		Reserved	
360		Reserved	
361		Reserved	
362		Reserved	
363		Reserved	
364		Reserved	
365		Reserved	
366		Reserved	
367		Reserved	
368		Reserved	
369		Reserved	
370		Reserved	
371		Reserved	
372		Reserved	
373		Reserved	
374		Reserved	
375		Reserved	
376		Reserved	
377		Reserved	
378		Reserved	
379		Reserved	
380		Reserved	
381		Reserved	
382		Reserved	

Function Number	Reagent	Function Name	Valves
383		Reserved	
384		Reserved	
385		Reserved	
386		Reserved	
387		Reserved	
388		Reserved	
389		Reserved	
390		Reserved	
391		Reserved	
392		Reserved	
393		Reserved	
394		Reserved	
395		Reserved	
396		Reserved	
397		Reserved	
398		Reserved	
399		Reserved	
400		Reserved	
401		User Function 1	
402		User Function 2	
403		User Function 3	
404		User Function 4	
405		User Function 5	
406		User Function 6	
407		User Function 7	
408		User Function 8	
409		User Function 9	
410		User Function 10	
411		User Function 11	
412		User Function 12	
413		User Function 13	
414		User Function 14	

Function Number	Reagent	Function Name	Valves
415		User Function 15	
416		User Function 16	
417		User Function 17	
418		User Function 18	
419		User Function 19	
420		User Function 20	
421		User Function 21	
422		User Function 22	
423		User Function 23	
424		User Function 24	
425		User Function 25	
426		User Function 26	
427		User Function 27	
428		User Function 28	
429		User Function 29	
430		User Function 30	
431		User Function 31	
432		User Function 32	
433		User Function 33	
434		User Function 34	
435		User Function 35	
436		User Function 36	
437		User Function 37	
438		User Function 38	
439		User Function 39	
440		User Function 40	
441		User Function 41	
442		User Function 42	
443		User Function 43	
444		User Function 44	
445		User Function 45	
446		User Function 46	

Function Number	Reagent	Function Name	Valves
447		User Function 47	
448		User Function 48	
449		User Function 49	
450		User Function 50	

Appendix B Standard Cycles

The following is a complete list of the standard cycles provided by Applied Biosystems for the Procise 49X cLC Protein Sequencing System.

Flask Cycles

Table B-1. Flask Blank cLC

Step Number	Function Number	Function Name
1	258	Begin
2	233	Set as Blank Cycle
3	171	Del S4, Flask
4	213	Dry Flask
5	215	Empty Flask
6	151	Del R4, Flask
7	213	Dry Flask
8	215	Empty Flask
9	218	Flush Large Loop (Flask)
10	173	Load S4, Flask (lg loop)
11	213	Dry Flask
12	218	Flush Large Loop (Flask)
13	257	Wait
14	213	Dry Flask
15	236	Pre-Conversion Dry
16	217	Flush Small Loop (Flask)
17	152	Load R4, Flask (sm loop)
18	213	Dry Flask
19	217	Flush Small Loop (Flask)
20	172	Load S4, Flask (sm loop)
21	217	Flush Small Loop (Flask)
22	257	Wait
23	227	Prepare Pump
24	226	Load Position
25	257	Wait

Step Number	Function Number	Function Name
26	237	Post-Conversion Dry
27	213	Dry Flask
28	218	Flush Large Loop (Flask)
29	173	Load S4, Flask (lg loop)
30	213	Dry Flask
31	218	Flush Large Loop (Flask)
32	173	Load S4, Flask (lg loop)
33	213	Dry Flask
34	218	Flush Large Loop (Flask)
35	257	Wait
36	238	Concentrate Sample
37	221	Flush Injector
38	224	Flush Injector (Low Pres)
39	238	Concentrate Sample
40	239	Flask Equilibrate
41	225	Load Injector
42	171	Del S4, Flask
43	213	Dry Flask
44	212	Bubble Flask
45	215	Empty Flask
46	181	Del X2, Flask
47	213	Dry Flask
48	212	Bubble Flask
49	215	Empty Flask
50	171	Del S4, Flask
51	213	Dry Flask
52	226	Load Position
53	212	Bubble Flask
54	222	Flush Flask/Injector
55	213	Dry Flask
56	221	Flush Injector
57	257	Wait
58	257	Wait

Step Number	Function Number	Function Name
59	259	End

Table B-2. Flask Standard cLC

Step Number	Function Number	Function Name
1	258	Begin
2	234	Set as Standard Cycle
3	171	Del S4, Flask
4	213	Dry Flask
5	215	Empty Flask
6	151	Del R4, Flask
7	213	Dry Flask
8	215	Empty Flask
9	217	Flush Small Loop (Flask)
10	172	Load S4, Flask (sm loop)
11	213	Dry Flask
12	217	Flush Small Loop (Flask)
13	218	Flush Large Loop (Flask)
14	163	Load R5, Flask (lg loop)
15	213	Dry Flask
16	236	Pre-Conversion Dry
17	218	Flush Large Loop (Flask)
18	217	Flush Small Loop (Flask)
19	152	Load R4, Flask (sm loop)
20	213	Dry Flask
21	217	Flush Small Loop (Flask)
22	172	Load S4, Flask (sm loop)
23	217	Flush Small Loop (Flask)
24	257	Wait
25	212	Bubble Flask
26	257	Wait
27	212	Bubble Flask
28	257	Wait
29	212	Bubble Flask

Step Number	Function Number	Function Name
30	227	Prepare Pump
31	226	Load Position
32	257	Wait
33	212	Bubble Flask
34	257	Wait
35	212	Bubble Flask
36	257	Wait
37	237	Post-Conversion Dry
38	213	Dry Flask
39	257	Wait
40	218	Flush Large Loop (Flask)
41	173	Load S4, Flask (lg loop)
42	213	Dry Flask
43	218	Flush Large Loop (Flask)
44	173	Load S4, Flask (lg loop)
45	213	Dry Flask
46	218	Flush Large Loop (Flask)
47	257	Wait
48	238	Concentrate Sample
49	221	Flush Injector
50	224	Flush Injector (Low Pres)
51	238	Concentrate Sample
52	239	Flask Equilibrate
53	225	Load Injector
54	171	Del S4, Flask
55	213	Dry Flask
56	212	Bubble Flask
57	215	Empty Flask
58	181	Del X2, Flask
59	213	Dry Flask
60	212	Bubble Flask
61	215	Empty Flask
62	171	Del S4, Flask

Step Number	Function Number	Function Name
63	213	Dry Flask
64	212	Bubble Flask
65	226	Load Position
66	222	Flush Flask/Injector
67	221	Flush Injector
68	257	Wait
69	257	Wait
70	259	End

Table B-3. Flask Normal cLC

Step Number	Function Numer	Function Name
1	258	Begin
2	235	Set as Residue Cycle
3	218	Flush Large Loop (Flask)
4	173	Load S4, Flask (lg loop)
5	213	Dry Flask
6	218	Flush Large Loop (Flask)
7	228	Ready to Receive
8	213	Dry Flask
9	236	Pre-Conversion Dry
10	217	Flush Small Loop (Flask)
11	152	Load R4, Flask (sm loop)
12	213	Dry Flask
13	217	Flush Small Loop (Flask)
14	172	Load S4, Flask (sm loop)
15	217	Flush Small Loop (Flask)
16	257	Wait
17	212	Bubble Flask
18	257	Wait
19	212	Bubble Flask
20	257	Wait
21	212	Bubble Flask
22	227	Prepare Pump
23	226	Load Position
24	257	Wait
25	212	Bubble Flask
26	257	Wait
27	212	Bubble Flask
28	257	Wait
29	237	Post-Conversion Dry
30	213	Dry Flask
31	218	Flush Large Loop (Flask)
32	173	Load S4, Flask (lg loop)

Step Number	Function Numer	Function Name
33	213	Dry Flask
34	218	Flush Large Loop (Flask)
35	173	Load S4, Flask (lg loop)
36	213	Dry Flask
37	218	Flush Large Loop (Flask)
38	257	Wait
39	238	Concentrate Sample
40	221	Flush Injector
41	224	Flush Injector (Low Pres)
42	238	Concentrate Sample
43	239	Flask Equilibrate
44	225	Load Injector
45	171	Del S4, Flask
46	213	Dry Flask
47	212	Bubble Flask
48	215	Empty Flask
49	181	Del X2, Flask
50	213	Dry Flask
51	212	Bubble Flask
52	215	Empty Flask
53	171	Del S4, Flask
54	213	Dry Flask
55	212	Bubble Flask
56	226	Load Position
57	222	Flush Flask/Injector
58	221	Flush Injector
59	257	Wait
60	259	End

Table B-4. Run Gradient cLC

Step Number	Function Number	Function Name
1	258	Begin
2	257	Wait
3	227	Prepare Pump
4	257	Wait
5	257	Wait
6	232	Start Gradient
7	257	Wait
8	257	Wait
9	259	End

Table B-5. Prepare Pump cLC

Step Number	Function Number	Function Name
1	258	Begin
2	257	Wait
3	227	Prepare Pump
4	257	Wait
5	259	End

Table B-6. Flask Optimization cLC

Step Number	Function Number	Function Name
1	258	Begin
2	235	Set as Residue Cycle
3	218	Flush Large Loop (Flask)
4	215	Empty Flask
5	173	Load S4, Flask (lg loop)
6	213	Dry Flask
7	218	Flush Large Loop (Flask)
8	228	Ready to Receive
9	213	Dry Flask
10	301	Pause
11	236	Pre-Conversion Dry
12	301	Pause
13	218	Flush Large Loop (Flask)
14	152	Load R4, Flask (sm loop)
15	213	Dry Flask
16	218	Flush Large Loop (Flask)
17	173	Load S4, Flask (lg loop)
18	218	Flush Large Loop (Flask)
19	301	Pause
20	213	Dry Flask
21	237	Post-Conversion Dry
22	259	End

Table B-7. Injector Optimization cLC

Step Number	Function Number	Function Name
1	258	Begin
2	222	Flush Flask/Injector
3	221	Flush Injector
4	215	Empty Flask
5	226	Load Position
6	218	Flush Large Loop (Flask)
7	173	Load S4, Flask (lg loop)
8	213	Dry Flask
9	218	Flush Large Loop (Flask)
10	173	Load S4, Flask (lg loop)
11	213	Dry Flask
12	218	Flush Large Loop (Flask)
13	257	Wait
14	238	Concentrate Sample
15	221	Flush Injector
16	224	Flush Injector (Low Pres)
17	238	Concentrate Sample
18	239	Flask Equilibrate
19	225	Load Injector
20	259	End

Flow Cycles

Table B-8. Sensor and Delivery Test

Step Number	Function Number	Function Name
1	258	Begin
2	226	Load Position
3	139	Flush Small Loop (Cart)
4	5	Load R1, Cart (sm loop)
5	139	Flush Small Loop (Cart)
6	145	Wash Small Loop (Cart)
7	139	Flush Small Loop (Cart)
8	137	Flush Input Block
9	140	Flush Large Loop (Cart)
10	76	Load X1, Cart (lg loop)
11	140	Flush Large Loop (Cart)
12	303	Select Regulator
13	304	Save Regulator Setpoint
14	305	Set Reg Setpoint (10th psi)
15	137	Flush Input Block
16	26	Load R3, Cart (lg loop)
17	309	Restore Reg Setpoint
18	34	Del R3g, Waste
19	136	Flush Cart Solvent Block
20	144	Wash Cart Solvent Block
21	136	Flush Cart Solvent Block
22	143	Wash Cart Reagent Block
23	135	Flush Cart Reagent Block
24	111	Wash Input Block (S3)
25	137	Flush Input Block
26	107	Wash Output Block (S2)
27	138	Flush Output Block
28	140	Flush Large Loop (Cart)
29	146	Wash Large Loop (Cart)
30	140	Flush Large Loop (Cart)

Step Number	Function Number	Function Name
31	136	Flush Cart Solvent Block
32	123	Select Cartridge A
33	43	Del S1, Cart (sensor)
34	131	Dry Cart (top)
35	53	Del S2, Cart (sensor)
36	131	Dry Cart (top)
37	63	Del S3, Cart (sensor)
38	131	Dry Cart (top)
39	118	Transfer to Flask (X3)
40	121	Transfer to Flask (gas)
41	124	Select Cartridge B
42	53	Del S2, Cart (sensor)
43	131	Dry Cart (top)
44	125	Select Cartridge C
45	63	Del S3, Cart (sensor)
46	131	Dry Cart (top)
47	126	Select Cartridge D
48	63	Del S3, Cart (sensor)
49	131	Dry Cart (top)
50	147	End Cartridge Select
51	136	Flush Cart Solvent Block
52	212	Bubble Flask
53	215	Empty Flask
54	221	Flush Injector
55	152	Load R4, Flask (sm loop)
56	213	Dry Flask
57	217	Flush Small Loop (Flask)
58	162	Load R5, Flask (sm loop)
59	213	Dry Flask
60	217	Flush Small Loop (Flask)
61	202	Load X3, Flask (sm loop)
62	213	Dry Flask
63	217	Flush Small Loop (Flask)

Step Number	Function Number	Function Name
64	182	Load X2, Flask (sm loop)
65	213	Dry Flask
66	217	Flush Small Loop (Flask)
67	215	Empty Flask
68	218	Flush Large Loop (Flask)
69	173	Load S4, Flask (lg loop)
70	213	Dry Flask
71	218	Flush Large Loop (Flask)
72	173	Load S4, Flask (lg loop)
73	213	Dry Flask
74	218	Flush Large Loop (Flask)
75	221	Flush Injector
76	257	Wait
77	225	Load Injector
78	222	Flush Flask/Injector
79	259	End

Table B-9. R5 Large Loop Cal cLC

Step Number	Function Number	Function Name
1	258	Begin
2	218	Flush Large Loop (Flask)
3	163	Load R5, Flask (lg loop)
4	213	Dry Flask
5	218	Flush Large Loop (Flask)
6	163	Load R5, Flask (lg loop)
7	213	Dry Flask
8	218	Flush Large Loop (Flask)
9	163	Load R5, Flask (lg loop)
10	213	Dry Flask
11	218	Flush Large Loop (Flask)
12	163	Load R5, Flask (lg loop)
13	213	Dry Flask
14	218	Flush Large Loop (Flask)
15	163	Load R5, Flask (lg loop)
16	213	Dry Flask
17	218	Flush Large Loop (Flask)
18	285	Injector Sim Load
19	222	Flush Flask/Injector
20	253	490A Relay 1 Pulse
21	259	End

Idle Cycles

Table B-10. Idle Procedure

Step Number	Function Number	Function Name
1	258	Begin
2	8	Flush R1
3	18	Flush R2g
4	28	Flush R3
5	48	Flush S1
6	58	Flush S2
7	68	Flush S3
8	78	Flush X1
9	98	Flush X3, Cart
10	155	Flush R4
11	165	Flush R5
12	175	Flush S4
13	185	Flush X2
14	259	End

Leak Cycles

Table B-11. Cartridge A Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	123	Select Cartridge A
4	131	Dry Cart (top)
5	257	Wait
6	304	Save Regulator Setpoint
7	305	Set Reg Setpoint (10th psi)
8	310	Set Tolerance (100th psi)
9	129	Pressurize Cart, top
10	308	Close Pressure Valve
11	129	Pressurize Cart, top
12	307	Compare Pressures (10th psi)
13	317	Save Regulator Pressure
14	310	Set Tolerance (100th psi)
15	129	Pressurize Cart, top
16	318	Compare Saved Pressure
17	309	Restore Reg Setpoint
18	131	Dry Cart (top)
19	147	End Cartridge Select
20	259	End

Table B-12. Cartridge B Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	124	Select Cartridge B
4	131	Dry Cart (top)
5	257	Wait
6	304	Save Regulator Setpoint
7	305	Set Reg Setpoint (10th psi)
8	310	Set Tolerance (100th psi)
9	129	Pressurize Cart, top
10	308	Close Pressure Valve
11	129	Pressurize Cart, top
12	307	Compare Pressures (10th psi)
13	317	Save Regulator Pressure
14	310	Set Tolerance (100th psi)
15	129	Pressurize Cart, top
16	318	Compare Saved Pressure
17	309	Restore Reg Setpoint
18	131	Dry Cart (top)
19	147	End Cartridge Select
20	259	End

Table B-13. Cartridge C Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	125	Select Cartridge C
4	131	Dry Cart (top)
5	257	Wait
6	304	Save Regulator Setpoint
7	305	Set Reg Setpoint (10th psi)
8	310	Set Tolerance (100th psi)
9	129	Pressurize Cart, top
10	308	Close Pressure Valve
11	129	Pressurize Cart, top
12	307	Compare Pressures (10th psi)
13	317	Save Regulator Pressure
14	310	Set Tolerance (100th psi)
15	129	Pressurize Cart, top
16	318	Compare Saved Pressure
17	309	Restore Reg Setpoint
18	131	Dry Cart (top)
19	147	End Cartridge Select
20	259	End

Table B-14. Cartridge D Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	126	Select Cartridge D
4	131	Dry Cart (top)
5	257	Wait
6	304	Save Regulator Setpoint
7	305	Set Reg Setpoint (10th psi)
8	310	Set Tolerance (100th psi)
9	129	Pressurize Cart, top
10	308	Close Pressure Valve
11	129	Pressurize Cart, top
12	307	Compare Pressures (10th psi)
13	317	Save Regulator Pressure
14	310	Set Tolerance (100th psi)
15	129	Pressurize Cart, top
16	318	Compare Saved Pressure
17	309	Restore Reg Setpoint
18	131	Dry Cart (top)
19	147	End Cartridge Select
020	259	End

Table B-15. R1 Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	7	Vent R1
4	9	Backflush R1
5	304	Save Regulator Setpoint
6	305	Set Reg Setpoint (10th psi)
7	310	Set Tolerance (100th psi)
8	257	Wait
9	308	Close Pressure Valve
10	257	Wait
11	307	Compare Pressures (10th psi)
12	317	Save Regulator Pressure
13	310	Set Tolerance (100th psi)
14	257	Wait
15	318	Compare Saved Pressure
16	7	Vent R1
17	310	Set Tolerance (100th psi)
18	307	Compare Pressures (10th psi)
019	309	Restore Reg Setpoint
20	8	Flush R1
21	4	Del R1, Waste
22	135	Flush Cart Reagent Block
23	143	Wash Cart Reagent Block
24	135	Flush Cart Reagent Block
25	259	End

Table B-16. R2 Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	304	Save Regulator Setpoint
4	305	Set Reg Setpoint (10th psi)
5	310	Set Tolerance (100th psi)
6	257	Wait
7	308	Close Pressure Valve
8	257	Wait
9	307	Compare Pressures (10th psi)
10	317	Save Regulator Pressure
11	310	Set Tolerance (100th psi)
12	257	Wait
013	318	Compare Saved Pressure
14	17	Vent R2g
15	310	Set Tolerance (100th psi)
16	307	Compare Pressures (10th psi)
17	309	Restore Reg Setpoint
18	18	Flush R2g
19	259	End

Table B-17. R3 Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	27	Vent R3
4	29	Backflush R3
5	304	Save Regulator Setpoint
6	305	Set Reg Setpoint (10th psi)
7	310	Set Tolerance (100th psi)
8	257	Wait
9	308	Close Pressure Valve
10	257	Wait
11	307	Compare Pressures (10th psi)
12	317	Save Regulator Pressure
13	310	Set Tolerance (100th psi)
14	257	Wait
15	318	Compare Saved Pressure
16	27	Vent R3
17	310	Set Tolerance (100th psi)
18	307	Compare Pressures (10th psi)
19	309	Restore Reg Setpoint
20	28	Flush R3
21	24	Del R3, Waste
22	136	Flush Cart Solvent Block
23	144	Wash Cart Solvent Block
24	136	Flush Cart Solvent Block
25	259	End

Table B-18. S1, S2, S3 Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	47	Vent S1
4	49	Backflush S1
5	59	Backflush S2
6	69	Backflush S3
7	304	Save Regulator Setpoint
8	305	Set Reg Setpoint (10th psi)
9	310	Set Tolerance (100th psi)
10	257	Wait
11	308	Close Pressure Valve
12	257	Wait
13	307	Compare Pressures (10th psi)
14	317	Save Regulator Pressure
15	310	Set Tolerance (100th psi)
16	257	Wait
17	318	Compare Saved Pressure
18	47	Vent S1
19	310	Set Tolerance (100th psi)
20	307	Compare Pressures (10th psi)
21	305	Set Reg Setpoint (10th psi)
22	257	Wait
23	308	Close Pressure Valve
24	57	Vent S2
25	307	Compare Pressures (10th psi)
26	305	Set Reg Setpoint (10th psi)
27	257	Wait
28	308	Close Pressure Valve
29	67	Vent S3
30	307	Compare Pressures (10th psi)
31	309	Restore Reg Setpoint
32	48	Flush S1

Step Number	Function Number	Function Name
33	44	Del S1, Waste
34	136	Flush Cart Solvent Block
35	64	Del S3, Waste
36	136	Flush Cart Solvent Block
37	54	Del S2, Waste
38	136	Flush Cart Solvent Block
39	259	End

Table B-19. R4, S4 Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	174	Vent S4
4	156	Backflush R4
5	176	Backflush S4
6	304	Save Regulator Setpoint
7	305	Set Reg Setpoint (10th psi)
8	310	Set Tolerance (100th psi)
9	257	Wait
10	308	Close Pressure Valve
11	257	Wait
12	307	Compare Pressures (10th psi)
13	317	Save Regulator Pressure
14	310	Set Tolerance (100th psi)
15	257	Wait
16	318	Compare Saved Pressure
17	154	Vent R4
18	310	Set Tolerance (100th psi)
19	307	Compare Pressures (10th psi)
20	305	Set Reg Setpoint (10th psi)
21	257	Wait
22	308	Close Pressure Valve
23	174	Vent S4
24	307	Compare Pressures (10th psi)
25	309	Restore Reg Setpoint
26	157	Del R4, Waste
27	218	Flush Large Loop (Flask)
28	220	Wash Large Loop (Flask)
29	218	Flush Large Loop (Flask)
30	259	End

Table B-20. R5, X1, X2 Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	77	Vent X1
4	79	Backflush X1
5	186	Backflush X2
6	166	Backflush R5
7	304	Save Regulator Setpoint
8	305	Set Reg Setpoint (10th psi)
9	310	Set Tolerance (100th psi)
10	257	Wait
11	308	Close Pressure Valve
12	257	Wait
13	307	Compare Pressures (10th psi)
14	317	Save Regulator Pressure
15	310	Set Tolerance (100th psi)
16	257	Wait
17	318	Compare Saved Pressure
18	164	Vent R5
19	310	Set Tolerance (100th psi)
20	307	Compare Pressures (10th psi)
21	305	Set Reg Setpoint (10th psi)
22	257	Wait
23	308	Close Pressure Valve
24	77	Vent X1
25	307	Compare Pressures (10th psi)
26	305	Set Reg Setpoint (10th psi)
27	257	Wait
28	308	Close Pressure Valve
29	184	Vent X2
30	307	Compare Pressures (10th psi)
31	309	Restore Reg Setpoint
32	185	Flush X2

Step Number	Function Number	Function Name
33	74	Del X1, Waste
34	135	Flush Cart Reagent Block
35	143	Wash Cart Reagent Block
36	135	Flush Cart Reagent Block
37	187	Del X2, Waste
38	218	Flush Large Loop (Flask)
39	167	Del R5, Waste
40	218	Flush Large Loop (Flask)
41	220	Wash Large Loop (Flask)
42	218	Flush Large Loop (Flask)
43	259	End

Table B-21. X3 Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	97	Vent X3, Cart
4	99	Backflush X3, Cart
5	206	Backflush X3, Flask
6	304	Save Regulator Setpoint
7	305	Set Reg Setpoint (10th psi)
8	310	Set Tolerance (100th psi)
9	257	Wait
10	308	Close Pressure Valve
11	257	Wait
12	307	Compare Pressures (10th psi)
13	317	Save Regulator Pressure
14	310	Set Tolerance (100th psi)
15	257	Wait
16	318	Compare Saved Pressure
17	97	Vent X3, Cart
18	310	Set Tolerance (100th psi)
19	307	Compare Pressures (10th psi)
20	309	Restore Reg Setpoint
21	98	Flush X3, Cart
22	94	Del X3, Waste
23	135	Flush Cart Reagent Block
24	207	Del X3, Waste, Flask
25	218	Flush Large Loop (Flask)
26	259	End

Table B-22. Regulator 9 Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	212	Bubble Flask
4	304	Save Regulator Setpoint
5	305	Set Reg Setpoint (10th psi)
6	310	Set Tolerance (100th psi)
7	257	Wait
8	308	Close Pressure Valve
9	257	Wait
10	307	Compare Pressures (10th psi)
11	317	Save Regulator Pressure
12	310	Set Tolerance (100th psi)
13	257	Wait
14	318	Compare Saved Pressure
15	212	Bubble Flask
16	310	Set Tolerance (100th psi)
17	307	Compare Pressures (10th psi)
18	309	Restore Reg Setpoint
19	212	Bubble Flask
20	259	End

Table B-23. Regulator 10 Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	223	Inject Position
4	257	Wait
5	221	Flush Injector
6	304	Save Regulator Setpoint
7	305	Set Reg Setpoint (10th psi)
8	310	Set Tolerance (100th psi)
9	257	Wait
10	308	Close Pressure Valve
11	257	Wait
12	307	Compare Pressures (10th psi)
13	317	Save Regulator Pressure
14	310	Set Tolerance (100th psi)
15	257	Wait
16	318	Compare Saved Pressure
17	285	Injector Sim Load
18	310	Set Tolerance (100th psi)
19	307	Compare Pressures (10th psi)
20	309	Restore Reg Setpoint
21	285	Injector Sim Load
22	259	End

Table B-24. Cartridge Reagent Block Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	131	Dry Cart (top)
4	304	Save Regulator Setpoint
5	305	Set Reg Setpoint (10th psi)
6	310	Set Tolerance (100th psi)
7	284	Open Valves 11,15
8	308	Close Pressure Valve
9	284	Open Valves 11,15
10	307	Compare Pressures (10th psi)
11	317	Save Regulator Pressure
12	310	Set Tolerance (100th psi)
13	284	Open Valves 11,15
14	318	Compare Saved Pressure
15	345	Open Valves 1,11,15
16	310	Set Tolerance (100th psi)
17	307	Compare Pressures (10th psi)
18	309	Restore Reg Setpoint
19	344	Open Valves 7,11,15,16
20	259	End

Table B-25. Cartridge Input Block Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	131	Dry Cart (top)
4	304	Save Regulator Setpoint
5	305	Set Reg Setpoint (10th psi)
6	310	Set Tolerance (100th psi)
7	293	Open Valves 15,23
8	308	Close Pressure Valve
9	293	Open Valves 15,23
10	307	Compare Pressures (10th psi)
11	317	Save Regulator Pressure
12	310	Set Tolerance (100th psi)
13	293	Open Valves 15,23
14	318	Compare Saved Pressure
15	344	Open Valves 7,11,15,16
16	310	Set Tolerance (100th psi)
17	307	Compare Pressures (10th psi)
18	309	Restore Reg Setpoint
19	344	Open Valves 7,11,15,16
20	259	End

Table B-26. Flask Input Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	304	Save Regulator Setpoint
4	305	Set Reg Setpoint (10th psi)
5	310	Set Tolerance (100th psi)
6	294	Open Valve 24
7	308	Close Pressure Valve
8	294	Open Valve 24
9	307	Compare Pressures (10th psi)
10	317	Save Regulator Pressure
11	294	Open Valve 24
12	310	Set Tolerance (100th psi)
13	318	Compare Saved Pressure
14	218	Flush Large Loop (Flask)
15	310	Set Tolerance (100th psi)
16	307	Compare Pressures (10th psi)
17	309	Restore Reg Setpoint
18	218	Flush Large Loop (Flask)
19	259	End

Table B-27. Flask Leak Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	304	Save Regulator Setpoint
4	305	Set Reg Setpoint (10th psi)
5	310	Set Tolerance (100th psi)
6	295	Open Valves 24,32
7	308	Close Pressure Valve
8	295	Open Valves 24,32
9	307	Compare Pressures (10th psi)
10	317	Save Regulator Pressure
11	295	Open Valves 24,32
12	310	Set Tolerance (100th psi)
13	318	Compare Saved Pressure
14	296	Open Valves 24,32,45
15	310	Set Tolerance (100th psi)
16	307	Compare Pressures (10th psi)
17	309	Restore Reg Setpoint
18	296	Open Valves 24,32,45
19	259	End

Table B-28. Waste System Test

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	304	Save Regulator Setpoint
4	299	Open Valves 49,57,59
5	310	Set Tolerance (100th psi)
6	308	Close Pressure Valve
7	77	Vent X1
8	307	Compare Pressures (10th psi)
9	317	Save Regulator Pressure
10	77	Vent X1
11	310	Set Tolerance (100th psi)
12	318	Compare Saved Pressure
13	309	Restore Reg Setpoint
14	259	End

Sensor Cycles

Table B-29. Init Sensor cLC

StepNumber	Function Number	Function Name
1	258	Begin
2	145	Wash Small Loop (Cart)
3	139	Flush Small Loop (Cart)
4	273	Init Sm Loop Snsr, Cart
5	146	Wash Large Loop (Cart)
6	140	Flush Large Loop (Cart)
7	274	Init Lg Loop Snsr, Cart
8	107	Wash Output Block (S2)
9	112	Wash Output Block (S3)
10	138	Flush Output Block
11	123	Select Cartridge A
12	132	Dry Cart (bottom)
13	275	Init Cart A Snsr
14	147	End Cartridge Select
15	124	Select Cartridge B
16	132	Dry Cart (bottom)
17	276	Init Cart B Snsr
18	147	End Cartridge Select
19	125	Select Cartridge C
20	132	Dry Cart (bottom)
21	277	Init Cart C Snsr
22	147	End Cartridge Select
23	126	Select Cartridge D
24	132	Dry Cart (bottom)
25	278	Init Cart D Snsr
26	147	End Cartridge Select
27	149	Wash Transfer Line (S2)
28	141	Flush Transfer Line
29	279	Init Transfer Snsr
30	215	Empty Flask

StepNumber	Function Number	Function Name
31	171	Del S4, Flask
32	213	Dry Flask
33	215	Empty Flask
34	219	Wash Small Loop (Flask)
35	217	Flush Small Loop (Flask)
36	280	Init Sm Loop Snsr, Flask
37	220	Wash Large Loop (Flask)
38	218	Flush Large Loop (Flask)
39	281	Init Lg Loop Snsr, Flask
40	226	Load Position
41	221	Flush Injector
42	257	Wait
43	221	Flush Injector
44	282	Init Injector Load Snsr
45	283	Init Injector Full Snsr
46	259	End

Shutdown Cycles

Table B-30. Short-Term Shutdown cLC

Step Number	Function Number	Function Name
1	258	Begin
2	145	Wash Small Loop (Cart)
3	242	Wash Cart Small Loop S1
4	139	Flush Small Loop (Cart)
5	146	Wash Large Loop (Cart)
6	243	Wash Cart Large Loop S1
7	140	Flush Large Loop (Cart)
8	143	Wash Cart Reagent Block
9	241	Wash Cart Reagent Block S1
10	135	Flush Cart Reagent Block
11	144	Wash Cart Solvent Block
12	240	Wash Cart Solvent Block S1
13	136	Flush Cart Solvent Block
14	106	Wash Input Block (S2)
15	111	Wash Input Block (S3)
16	110	Wash Input Blk (X1)
17	137	Flush Input Block
18	107	Wash Output Block (S2)
19	112	Wash Output Block (S3)
20	115	Wash Output Blk (X1)
21	150	Wash Transfer Line (X1)
22	141	Flush Transfer Line
23	138	Flush Output Block
24	135	Flush Cart Reagent Block
25	215	Empty Flask
26	9	Backflush R1
27	19	Backflush R2g
28	29	Backflush R3
29	39	Backflush R3g
30	49	Backflush S1

Step Number	Function Number	Function Name
31	59	Backflush S2
32	69	Backflush S3
33	79	Backflush X1
34	89	Backflush X1g
35	99	Backflush X3, Cart
36	219	Wash Small Loop (Flask)
37	217	Flush Small Loop (Flask)
38	220	Wash Large Loop (Flask)
39	218	Flush Large Loop (Flask)
40	171	Del S4, Flask
41	213	Dry Flask
42	215	Empty Flask
43	171	Del S4, Flask
44	213	Dry Flask
45	222	Flush Flask/Injector
46	156	Backflush R4
47	166	Backflush R5
48	176	Backflush S4
49	186	Backflush X2
50	196	Backflush X2g
51	206	Backflush X3, Flask
52	259	End

Table B-31. Post-Run Valve Blk Wash X1-X2

Step Number	Function Number	Function Name
1	258	Begin
2	215	Empty Flask
3	181	Del X2, Flask
4	213	Dry Flask
5	74	Del X1, Waste
6	110	Wash Input Blk (X1)
7	115	Wash Output Blk (X1)
8	150	Wash Transfer Line (X1)
9	257	Wait
10	115	Wash Output Blk (X1)
11	136	Flush Cart Solvent Block
12	115	Wash Output Blk (X1)
13	136	Flush Cart Solvent Block
14	115	Wash Output Blk (X1)
15	136	Flush Cart Solvent Block
16	115	Wash Output Blk (X1)
17	136	Flush Cart Solvent Block
18	141	Flush Transfer Line
19	136	Flush Cart Solvent Block
20	140	Flush Large Loop (Cart)
21	135	Flush Cart Reagent Block
22	137	Flush Input Block
23	138	Flush Output Block
24	141	Flush Transfer Line
25	212	Bubble Flask
26	213	Dry Flask
27	215	Empty Flask
28	181	Del X2, Flask
29	217	Flush Small Loop (Flask)
30	218	Flush Large Loop (Flask)
31	213	Dry Flask
32	226	Load Position

33	285	Injector Sim Load
34	222	Flush Flask/Injector
35	171	Del S4, Flask
36	213	Dry Flask
37	215	Empty Flask
38	259	End

Startup Cycles

Table B-32. Startup Procedure cLC

Step Number	Function Number	Function Name
1	258	Begin
2	8	Flush R1
3	4	Del R1, Waste
4	135	Flush Cart Reagent Block
5	98	Flush X3, Cart
6	116	Wash Input Block (X3)
7	137	Flush Input Block
8	78	Flush X1
9	74	Del X1, Waste
10	150	Wash Transfer Line (X1)
11	141	Flush Transfer Line
12	135	Flush Cart Reagent Block
13	18	Flush R2g
14	14	Del R2g, Waste
15	58	Flush S2
16	54	Del S2, Waste
17	136	Flush Cart Solvent Block
18	143	Wash Cart Reagent Block
19	135	Flush Cart Reagent Block
20	303	Select Regulator
21	305	Set Reg Setpoint (10th psi)
22	28	Flush R3
23	24	Del R3, Waste
24	34	Del R3g, Waste
25	136	Flush Cart Solvent Block
26	144	Wash Cart Solvent Block
27	136	Flush Cart Solvent Block
28	68	Flush S3
29	64	Del S3, Waste
30	136	Flush Cart Solvent Block

Step Number	Function Number	Function Name
31	48	Flush S1
32	44	Del S1, Waste
33	136	Flush Cart Solvent Block
34	144	Wash Cart Solvent Block
35	136	Flush Cart Solvent Block
36	106	Wash Input Block (S2)
37	111	Wash Input Block (S3)
38	137	Flush Input Block
39	107	Wash Output Block (S2)
40	112	Wash Output Block (S3)
41	138	Flush Output Block
42	207	Del X3, Waste, Flask
43	218	Flush Large Loop (Flask)
44	155	Flush R4
45	157	Del R4, Waste
46	218	Flush Large Loop (Flask)
47	165	Flush R5
48	167	Del R5, Waste
49	218	Flush Large Loop (Flask)
50	185	Flush X2
51	187	Del X2, Waste
52	218	Flush Large Loop (Flask)
53	175	Flush S4
54	177	Del S4, Waste
55	218	Flush Large Loop (Flask)
56	171	Del S4, Flask
57	213	Dry Flask
58	215	Empty Flask
59	171	Del S4, Flask
60	213	Dry Flask
61	215	Empty Flask
62	259	End

Electrical Cycles

Table B-33. Electrical Test Procedure

Step Number	Function Number	Function Name
1	258	Begin
2	311	Test Valves
3	312	Test Heaters
4	313	Test Pressure Board
5	314	Test 12-Bit A/D
6	315	Test 24-Bit A/D
7	316	Test Rheodyne
8	259	End

Cleanup Cycles

Table B-34. Delivery Line Backflush

Step Number	Function Number	Function Name
1	258	Begin
2	7	Vent R1
3	9	Backflush R1
4	17	Vent R2g
5	19	Backflush R2g
6	27	Vent R3
7	29	Backflush R3
8	39	Backflush R3g
9	47	Vent S1
10	49	Backflush S1
11	59	Backflush S2
12	69	Backflush S3
13	77	Vent X1
14	79	Backflush X1
15	89	Backflush X1g
16	97	Vent X3, Cart
17	99	Backflush X3, Cart
18	206	Backflush X3, Flask
19	154	Vent R4
20	156	Backflush R4
21	164	Vent R5
22	166	Backflush R5
23	174	Vent S4
24	176	Backflush S4
25	184	Vent X2
26	186	Backflush X2
27	196	Backflush X2g
28	259	End

Table B-35. System Cleanout–X3

Step Number	Function Number	Function Name
1	258	Begin
2	223	Inject Position
3	303	Select Regulator
4	305	Set Reg Setpoint (10th psi)
5	303	Select Regulator
6	305	Set Reg Setpoint (10th psi)
7	303	Select Regulator
8	305	Set Reg Setpoint (10th psi)
9	303	Select Regulator
10	305	Set Reg Setpoint (10th psi)
11	303	Select Regulator
12	305	Set Reg Setpoint (10th psi)
13	303	Select Regulator
14	305	Set Reg Setpoint (10th psi)
15	303	Select Regulator
16	305	Set Reg Setpoint (10th psi)
17	303	Select Regulator
18	305	Set Reg Setpoint (10th psi)
19	94	Del X3, Waste
20	135	Flush Cart Reagent Block
21	341	X3 to X1
22	135	Flush Cart Reagent Block
23	333	X3 to R1
24	135	Flush Cart Reagent Block
25	286	X3 to R2
26	19	Backflush R2g
27	287	X3 to R3g
28	39	Backflush R3g
29	342	X3 to X2
30	288	X3 to X1g
31	89	Backflush X1g
32	334	X3 to R3

33	337	X3 to S1
34	338	X3 to S2
35	339	X3 to S3
36	96	Load X3, Cart (lg loop)
37	140	Flush Large Loop (Cart)
38	95	Load X3, Cart (sm loop)
39	139	Flush Small Loop (Cart)
40	140	Flush Large Loop (Cart)
41	123	Select Cartridge A
42	257	Wait
43	343	X3 to Cart A (bottom)
44	132	Dry Cart (bottom)
45	124	Select Cartridge B
46	257	Wait
47	118	Transfer to Flask (X3)
48	121	Transfer to Flask (gas)
49	215	Empty Flask
50	125	Select Cartridge C
51	257	Wait
52	118	Transfer to Flask (X3)
53	131	Dry Cart (top)
54	126	Select Cartridge D
55	257	Wait
56	118	Transfer to Flask (X3)
57	121	Transfer to Flask (gas)
58	147	End Cartridge Select
59	136	Flush Cart Solvent Block
60	135	Flush Cart Reagent Block
61	137	Flush Input Block
62	135	Flush Cart Reagent Block
63	138	Flush Output Block
64	336	X3 to R5
65	218	Flush Large Loop (Flask)
66	335	X3 to R4

67	218	Flush Large Loop (Flask)
68	202	Load X3, Flask (sm loop)
69	217	Flush Small Loop (Flask)
70	289	X3 to X2g
71	196	Backflush X2g
72	203	Load X3, Flask (lg loop)
73	218	Flush Large Loop (Flask)
74	340	X3 to S4
75	213	Dry Flask
76	215	Empty Flask
77	118	Transfer to Flask (X3)
78	121	Transfer to Flask (gas)
79	222	Flush Flask/Injector
80	226	Load Position
81	201	Del X3, Flask
82	213	Dry Flask
83	222	Flush Flask/Injector
84	9	Backflush R1
85	19	Backflush R2g
86	29	Backflush R3
87	49	Backflush S1
88	59	Backflush S2
89	69	Backflush S3
90	79	Backflush X1
91	97	Vent X3, Cart
92	99	Backflush X3, Cart
93	206	Backflush X3, Flask
94	156	Backflush R4
95	166	Backflush R5
96	176	Backflush S4
97	186	Backflush X2
98	196	Backflush X2g
99	259	End

Table B-36. System Flush - Argon

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	305	Set Reg Setpoint (10th psi)
4	303	Select Regulator
5	305	Set Reg Setpoint (10th psi)
6	303	Select Regulator
7	305	Set Reg Setpoint (10th psi)
8	303	Select Regulator
9	305	Set Reg Setpoint (10th psi)
10	303	Select Regulator
11	305	Set Reg Setpoint (10th psi)
12	303	Select Regulator
13	305	Set Reg Setpoint (10th psi)
14	303	Select Regulator
15	305	Set Reg Setpoint (10th psi)
16	303	Select Regulator
17	305	Set Reg Setpoint (10th psi)
18	226	Load Position
19	9	Backflush R1
20	19	Backflush R2g
21	29	Backflush R3
22	39	Backflush R3g
23	49	Backflush S1
24	59	Backflush S2
25	69	Backflush S3
26	79	Backflush X1
27	89	Backflush X1g
28	99	Backflush X3, Cart
29	206	Backflush X3, Flask
30	156	Backflush R4
31	166	Backflush R5
32	176	Backflush S4

33	186	Backflush X2
34	196	Backflush X2g
35	135	Flush Cart Reagent Block
36	136	Flush Cart Solvent Block
37	123	Select Cartridge A
38	121	Transfer to Flask (gas)
39	132	Dry Cart (bottom)
40	124	Select Cartridge B
41	121	Transfer to Flask (gas)
42	125	Select Cartridge C
43	131	Dry Cart (top)
44	126	Select Cartridge D
45	131	Dry Cart (top)
46	147	End Cartridge Select
47	137	Flush Input Block
48	138	Flush Output Block
49	139	Flush Small Loop (Cart)
50	140	Flush Large Loop (Cart)
51	214	Dry Flask (h press)
52	217	Flush Small Loop (Flask)
53	218	Flush Large Loop (Flask)
54	222	Flush Flask/Injector
55	223	Inject Position
56	222	Flush Flask/Injector
57	215	Empty Flask
58	303	Select Regulator
59	305	Set Reg Setpoint (10th psi)
60	303	Select Regulator
61	305	Set Reg Setpoint (10th psi)
62	259	End

Table B-37. Cartridge Line Cleanup cLC

Step Number	Function Number	Function Name
1	258	Begin
2	143	Wash Cart Reagent Block
3	144	Wash Cart Solvent Block
4	106	Wash Input Block (S2)
5	107	Wash Output Block (S2)
6	145	Wash Small Loop (Cart)
7	146	Wash Large Loop (Cart)
8	123	Select Cartridge A
9	142	Set Cart Temperature
10	51	Del S2, Cart (top)
11	52	Del S2, Cart (bottom)
12	51	Del S2, Cart (top)
13	52	Del S2, Cart (bottom)
14	51	Del S2, Cart (top)
15	52	Del S2, Cart (bottom)
16	51	Del S2, Cart (top)
17	52	Del S2, Cart (bottom)
18	51	Del S2, Cart (top)
19	148	Cartridge Wait
20	51	Del S2, Cart (top)
21	148	Cartridge Wait
22	52	Del S2, Cart (bottom)
23	148	Cartridge Wait
24	147	End Cartridge Select
25	124	Select Cartridge B
26	142	Set Cart Temperature
27	51	Del S2, Cart (top)
28	52	Del S2, Cart (bottom)
29	51	Del S2, Cart (top)
30	52	Del S2, Cart (bottom)
31	51	Del S2, Cart (top)
32	52	Del S2, Cart (bottom)

33	51	Del S2, Cart (top)
34	52	Del S2, Cart (bottom)
35	51	Del S2, Cart (top)
36	148	Cartridge Wait
37	51	Del S2, Cart (top)
38	148	Cartridge Wait
39	52	Del S2, Cart (bottom)
40	148	Cartridge Wait
41	147	End Cartridge Select
42	125	Select Cartridge C
43	142	Set Cart Temperature
44	51	Del S2, Cart (top)
45	52	Del S2, Cart (bottom)
46	51	Del S2, Cart (top)
47	52	Del S2, Cart (bottom)
48	51	Del S2, Cart (top)
49	52	Del S2, Cart (bottom)
50	51	Del S2, Cart (top)
51	52	Del S2, Cart (bottom)
52	51	Del S2, Cart (top)
53	148	Cartridge Wait
54	51	Del S2, Cart (top)
55	148	Cartridge Wait
56	52	Del S2, Cart (bottom)
57	148	Cartridge Wait
58	147	End Cartridge Select
59	126	Select Cartridge D
60	142	Set Cart Temperature
61	51	Del S2, Cart (top)
62	52	Del S2, Cart (bottom)
63	51	Del S2, Cart (top)
64	52	Del S2, Cart (bottom)
65	51	Del S2, Cart (top)
66	52	Del S2, Cart (bottom)

67	51	Del S2, Cart (top)
68	52	Del S2, Cart (bottom)
69	51	Del S2, Cart (top)
70	148	Cartridge Wait
71	51	Del S2, Cart (top)
72	148	Cartridge Wait
73	52	Del S2, Cart (bottom)
74	148	Cartridge Wait
75	147	End Cartridge Select
76	135	Flush Cart Reagent Block
77	136	Flush Cart Solvent Block
78	137	Flush Input Block
79	138	Flush Output Block
80	139	Flush Small Loop (Cart)
81	140	Flush Large Loop (Cart)
82	123	Select Cartridge A
83	131	Dry Cart (top)
84	147	End Cartridge Select
85	124	Select Cartridge B
86	131	Dry Cart (top)
87	147	End Cartridge Select
88	125	Select Cartridge C
89	131	Dry Cart (top)
90	147	End Cartridge Select
91	126	Select Cartridge D
92	131	Dry Cart (top)
93	147	End Cartridge Select
94	259	End

Table B-38. Clean Transfer Line with X1

Step Number	Function Number	Function Name
1	258	Begin
2	141	Flush Transfer Line
3	150	Wash Transfer Line (X1)
4	141	Flush Transfer Line
5	215	Empty Flask
6	150	Wash Transfer Line (X1)
7	141	Flush Transfer Line
8	215	Empty Flask
9	150	Wash Transfer Line (X1)
10	141	Flush Transfer Line
11	215	Empty Flask
12	135	Flush Cart Reagent Block
13	259	End

Bottle Cycles

Table B-39. Bottle Change for R1

Step	Function #	Function Name
1	258	Begin
2	261	Set for Bottle R1
3	303	Select Regulator
4	7	Vent R1
5	9	Backflush R1
6	260	Pause for Bottle Change
7	8	Flush R1
8	4	Del R1, Waste
9	135	Flush Cart Reagent Block
10	143	Wash Cart Reagent Block
11	135	Flush Cart Reagent Block
12	259	End

Table B-40. Bottle Change for R1–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	261	Set for Bottle R1
3	303	Select Regulator
4	7	Vent R1
5	9	Backflush R1
6	260	Pause for Bottle Change
7	304	Save Regulator Setpoint
8	305	Set Reg Setpoint (10th psi)
9	310	Set Tolerance (100th psi)
10	257	Wait
11	308	Close Pressure Valve
12	257	Wait
13	307	Compare Pressures (10th psi)
14	317	Save Regulator Pressure
15	310	Set Tolerance (100th psi)

16	257	Wait
17	318	Compare Saved Pressure
18	309	Restore Reg Setpoint
19	8	Flush R1
20	4	Del R1, Waste
21	135	Flush Cart Reagent Block
22	143	Wash Cart Reagent Block
23	135	Flush Cart Reagent Block
24	259	End

Table B-41. Bottle Change for R2

Step Number	Function Number	Function Name
1	258	Begin
2	262	Set for Bottle R2
3	303	Select Regulator
4	17	Vent R2g
5	19	Backflush R2g
6	260	Pause for Bottle Change
7	18	Flush R2g
8	143	Wash Cart Reagent Block
9	135	Flush Cart Reagent Block
10	259	End

Table B-42. Bottle Change for R2–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	262	Set for Bottle R2
3	303	Select Regulator
4	17	Vent R2g
5	19	Backflush R2g
6	260	Pause for Bottle Change
7	304	Save Regulator Setpoint
8	305	Set Reg Setpoint (10th psi)
9	310	Set Tolerance (100th psi)
10	257	Wait
11	308	Close Pressure Valve
12	257	Wait
13	307	Compare Pressures (10th psi)
14	317	Save Regulator Pressure
15	310	Set Tolerance (100th psi)
16	257	Wait
17	318	Compare Saved Pressure
18	309	Restore Reg Setpoint
19	18	Flush R2g

20	143	Wash Cart Reagent Block
21	135	Flush Cart Reagent Block
22	259	End

Table B-43. Bottle Change for R3

Step Number	Function Number	Function Name
1	258	Begin
2	263	Set for Bottle R3
3	303	Select Regulator
4	304	Save Regulator Setpoint
5	27	Vent R3
6	29	Backflush R3
7	39	Backflush R3g
8	260	Pause for Bottle Change
9	28	Flush R3
10	305	Set Reg Setpoint (10th psi)
11	24	Del R3, Waste
12	309	Restore Reg Setpoint
13	34	Del R3g, Waste
14	144	Wash Cart Solvent Block
15	136	Flush Cart Solvent Block
16	259	End

Table B-44. Bottle Change for R3–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	263	Set for Bottle R3
3	303	Select Regulator
4	27	Vent R3
5	29	Backflush R3
6	39	Backflush R3g
7	260	Pause for Bottle Change
8	304	Save Regulator Setpoint
9	305	Set Reg Setpoint (10th psi)
10	310	Set Tolerance (100th psi)
11	257	Wait
12	308	Close Pressure Valve
13	257	Wait

14	307	Compare Pressures (10th psi)
15	317	Save Regulator Pressure
16	310	Set Tolerance (100th psi)
17	257	Wait
18	318	Compare Saved Pressure
19	309	Restore Reg Setpoint
20	28	Flush R3
21	305	Set Reg Setpoint (10th psi)
22	24	Del R3, Waste
23	309	Restore Reg Setpoint
24	34	Del R3g, Waste
25	144	Wash Cart Solvent Block
26	136	Flush Cart Solvent Block
27	259	End

Table B-45. Bottle Change for R4

Step Number	Function Number	Function Name
1	258	Begin
2	264	Set for Bottle R4
3	303	Select Regulator
4	154	Vent R4
5	156	Backflush R4
6	260	Pause for Bottle Change
7	155	Flush R4
8	157	Del R4, Waste
9	218	Flush Large Loop (Flask)
10	220	Wash Large Loop (Flask)
11	218	Flush Large Loop (Flask)
12	259	End

Table B-46. Bottle Change for R4–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	264	Set for Bottle R4
3	303	Select Regulator
4	154	Vent R4
5	156	Backflush R4
6	260	Pause for Bottle Change
7	304	Save Regulator Setpoint
8	305	Set Reg Setpoint (10th psi)
9	310	Set Tolerance (100th psi)
10	257	Wait
11	308	Close Pressure Valve
12	257	Wait
13	307	Compare Pressures (10th psi)
14	317	Save Regulator Pressure
15	310	Set Tolerance (100th psi)
16	257	Wait
17	318	Compare Saved Pressure

18	309	Restore Reg Setpoint
19	155	Flush R4
20	157	Del R4, Waste
21	218	Flush Large Loop (Flask)
22	220	Wash Large Loop (Flask)
23	218	Flush Large Loop (Flask)
24	259	End

Table B-47. Bottle Change for R5

Step Number	Function Number	Function Name
1	258	Begin
2	265	Set for Bottle R5
3	303	Select Regulator
4	164	Vent R5
5	166	Backflush R5
6	260	Pause for Bottle Change
7	165	Flush R5
8	167	Del R5, Waste
9	218	Flush Large Loop (Flask)
10	220	Wash Large Loop (Flask)
11	218	Flush Large Loop (Flask)
12	259	End

Table B-48. Bottle Change for R5–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	265	Set for Bottle R5
3	303	Select Regulator
4	164	Vent R5
5	166	Backflush R5
6	260	Pause for Bottle Change
7	304	Save Regulator Setpoint
8	305	Set Reg Setpoint (10th psi)
9	310	Set Tolerance (100th psi)
10	257	Wait
11	308	Close Pressure Valve
12	257	Wait
13	307	Compare Pressures (10th psi)
14	317	Save Regulator Pressure
15	310	Set Tolerance (100th psi)
16	257	Wait
17	318	Compare Saved Pressure

18	309	Restore Reg Setpoint
19	165	Flush R5
20	167	Del R5, Waste
21	218	Flush Large Loop (Flask)
22	220	Wash Large Loop (Flask)
23	218	Flush Large Loop (Flask)
24	259	End

Table B-49. Bottle Change for S1

Step Number	Function Number	Function Name
1	258	Begin
2	266	Set for Bottle S1
3	303	Select Regulator
4	47	Vent S1
5	49	Backflush S1
6	260	Pause for Bottle Change
7	48	Flush S1
8	44	Del S1, Waste
9	136	Flush Cart Solvent Block
10	144	Wash Cart Solvent Block
11	136	Flush Cart Solvent Block
12	259	End

Table B-50. Bottle Change for S1–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	266	Set for Bottle S1
3	303	Select Regulator
4	47	Vent S1
5	49	Backflush S1
6	260	Pause for Bottle Change
7	304	Save Regulator Setpoint
8	305	Set Reg Setpoint (10th psi)
9	310	Set Tolerance (100th psi)
10	257	Wait
11	308	Close Pressure Valve
12	257	Wait
13	307	Compare Pressures (10th psi)
14	317	Save Regulator Pressure
15	310	Set Tolerance (100th psi)
16	257	Wait
17	318	Compare Saved Pressure

18	309	Restore Reg Setpoint
19	48	Flush S1
20	44	Del S1, Waste
21	136	Flush Cart Solvent Block
22	144	Wash Cart Solvent Block
23	136	Flush Cart Solvent Block
24	259	End

Table B-51. Bottle Change for S2

Step Number	Function Number	Function Name
1	258	Begin
2	267	Set for Bottle S2
3	303	Select Regulator
4	57	Vent S2
5	59	Backflush S2
6	260	Pause for Bottle Change
7	58	Flush S2
8	54	Del S2, Waste
9	136	Flush Cart Solvent Block
10	259	End

Table B-52. Bottle Change for S2–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	267	Set for Bottle S2
3	303	Select Regulator
4	57	Vent S2
5	59	Backflush S2
6	260	Pause for Bottle Change
7	304	Save Regulator Setpoint
8	305	Set Reg Setpoint (10th psi)
9	310	Set Tolerance (100th psi)
10	257	Wait
11	308	Close Pressure Valve
12	257	Wait
13	307	Compare Pressures (10th psi)
14	317	Save Regulator Pressure
15	310	Set Tolerance (100th psi)
16	257	Wait
17	318	Compare Saved Pressure
18	309	Restore Reg Setpoint
19	58	Flush S2

20	54	Del S2, Waste
21	136	Flush Cart Solvent Block
22	259	End

Table B-53. Bottle Change for S3

Step Number	Function Number	Function Name
1	258	Begin
2	268	Set for Bottle S3
3	303	Select Regulator
4	67	Vent S3
5	69	Backflush S3
6	260	Pause for Bottle Change
7	68	Flush S3
8	64	Del S3, Waste
9	136	Flush Cart Solvent Block
10	259	End

Table B-54. Bottle Change for S3–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	268	Set for Bottle S3
3	303	Select Regulator
4	67	Vent S3
5	69	Backflush S3
6	260	Pause for Bottle Change
7	304	Save Regulator Setpoint
8	305	Set Reg Setpoint (10th psi)
9	310	Set Tolerance (100th psi)
10	257	Wait
11	308	Close Pressure Valve
12	257	Wait
13	307	Compare Pressures (10th psi)
14	317	Save Regulator Pressure
15	310	Set Tolerance (100th psi)
16	257	Wait
17	318	Compare Saved Pressure
18	309	Restore Reg Setpoint
19	68	Flush S3

20	64	Del S3, Waste
21	136	Flush Cart Solvent Block
22	259	End

Table B-55. Bottle Change for S4

Step Number	Function Number	Function Name
1	258	Begin
2	269	Set for Bottle S4
3	303	Select Regulator
4	174	Vent S4
5	176	Backflush S4
6	260	Pause for Bottle Change
7	175	Flush S4
8	177	Del S4, Waste
9	218	Flush Large Loop (Flask)
10	259	End

Table B-56. Bottle Change for S4–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	269	Set for Bottle S4
3	303	Select Regulator
4	174	Vent S4
5	176	Backflush S4
6	260	Pause for Bottle Change
7	304	Save Regulator Setpoint
8	305	Set Reg Setpoint (10th psi)
9	310	Set Tolerance (100th psi)
10	257	Wait
11	308	Close Pressure Valve
12	257	Wait
13	307	Compare Pressures (10th psi)
14	317	Save Regulator Pressure
15	310	Set Tolerance (100th psi)
16	257	Wait
17	318	Compare Saved Pressure
18	309	Restore Reg Setpoint
19	175	Flush S4

20	177	Del S4, Waste
21	218	Flush Large Loop (Flask)
22	259	End

Table B-57. Bottle Change for X1

Step Number	Function Number	Function Name
1	258	Begin
2	270	Set for Bottle X1
3	303	Select Regulator
4	77	Vent X1
5	79	Backflush X1
6	89	Backflush X1g
7	260	Pause for Bottle Change
8	78	Flush X1
9	74	Del X1, Waste
10	84	Del X1g, Waste
11	143	Wash Cart Reagent Block
12	135	Flush Cart Reagent Block
13	259	End

Table B-58. Bottle Change for X1–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	270	Set for Bottle X1
3	303	Select Regulator
4	77	Vent X1
5	79	Backflush X1
6	89	Backflush X1g
7	260	Pause for Bottle Change
8	304	Save Regulator Setpoint
9	305	Set Reg Setpoint (10th psi)
10	310	Set Tolerance (100th psi)
11	257	Wait
12	308	Close Pressure Valve
13	257	Wait
14	307	Compare Pressures (10th psi)
15	317	Save Regulator Pressure
16	310	Set Tolerance (100th psi)

17	257	Wait
18	318	Compare Saved Pressure
19	309	Restore Reg Setpoint
20	78	Flush X1
21	74	Del X1, Waste
22	84	Del X1g, Waste
23	143	Wash Cart Reagent Block
24	135	Flush Cart Reagent Block
25	259	End

Table B-59. Bottle Change for X2

Step Number	Function Number	Function Name
1	258	Begin
2	271	Set for Bottle X2
3	303	Select Regulator
4	184	Vent X2
5	186	Backflush X2
6	196	Backflush X2g
7	260	Pause for Bottle Change
8	185	Flush X2
9	187	Del X2, Waste
10	197	Del X2g, Waste
11	220	Wash Large Loop (Flask)
12	218	Flush Large Loop (Flask)
13	259	End

Table B-60. Bottle Change for X2–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	271	Set for Bottle X2
3	303	Select Regulator
4	184	Vent X2
5	186	Backflush X2
6	196	Backflush X2g
7	260	Pause for Bottle Change
8	304	Save Regulator Setpoint
9	305	Set Reg Setpoint (10th psi)
10	310	Set Tolerance (100th psi)
11	257	Wait
12	308	Close Pressure Valve
13	257	Wait
14	307	Compare Pressures (10th psi)
15	317	Save Regulator Pressure
16	310	Set Tolerance (100th psi)

17	257	Wait
18	318	Compare Saved Pressure
19	309	Restore Reg Setpoint
20	185	Flush X2
21	187	Del X2, Waste
22	197	Del X2g, Waste
23	220	Wash Large Loop (Flask)
24	218	Flush Large Loop (Flask)
25	259	End

Table B-61. Bottle Change for X3 Both

Step Number	Function Number	Function Name
1	258	Begin
2	272	Set for Bottle X3
3	303	Select Regulator
4	97	Vent X3, Cart
5	99	Backflush X3, Cart
6	206	Backflush X3, Flask
7	260	Pause for Bottle Change
8	98	Flush X3, Cart
9	94	Del X3, Waste
10	135	Flush Cart Reagent Block
11	143	Wash Cart Reagent Block
12	135	Flush Cart Reagent Block
13	207	Del X3, Waste, Flask
14	218	Flush Large Loop (Flask)
15	220	Wash Large Loop (Flask)
16	218	Flush Large Loop (Flask)
17	259	End

Table B-62. Bottle Change for X3 Both–Leak

Step Number	Function Number	Function Name
1	258	Begin
2	272	Set for Bottle X3
3	303	Select Regulator
4	97	Vent X3, Cart
5	99	Backflush X3, Cart
6	206	Backflush X3, Flask
7	260	Pause for Bottle Change
8	304	Save Regulator Setpoint
9	305	Set Reg Setpoint (10th psi)
10	310	Set Tolerance (100th psi)
11	257	Wait
12	308	Close Pressure Valve

13	257	Wait
14	307	Compare Pressures (10th psi)
15	317	Save Regulator Pressure
16	310	Set Tolerance (100th psi)
17	257	Wait
18	318	Compare Saved Pressure
19	309	Restore Reg Setpoint
20	98	Flush X3, Cart
21	94	Del X3, Waste
22	135	Flush Cart Reagent Block
23	143	Wash Cart Reagent Block
24	135	Flush Cart Reagent Block
25	207	Del X3, Waste, Flask
26	218	Flush Large Loop (Flask)
27	220	Wash Large Loop (Flask)
28	218	Flush Large Loop (Flask)
29	259	End

Cartridge Cycles

Table B-63. Cartridge Begin cLC

Step	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	305	Set Reg Setpoint (10th psi)
4	145	Wash Small Loop (Cart)
5	139	Flush Small Loop (Cart)
6	242	Wash Cart Small Loop S1
7	139	Flush Small Loop (Cart)
8	144	Wash Cart Solvent Block
9	136	Flush Cart Solvent Block
10	240	Wash Cart Solvent Block S1
11	136	Flush Cart Solvent Block
12	106	Wash Input Block (S2)
13	137	Flush Input Block
14	101	Wash Input Block (S1)
15	137	Flush Input Block
16	107	Wash Output Block (S2)
17	138	Flush Output Block
18	102	Wash Output Block (S1)
19	138	Flush Output Block
20	131	Dry Cart (top)
21	139	Flush Small Loop (Cart)
22	25	Load R3, Cart (sm loop)
23	30	Transfer R3, Cart (gas)
24	139	Flush Small Loop (Cart)
25	145	Wash Small Loop (Cart)
26	139	Flush Small Loop (Cart)
27	136	Flush Cart Solvent Block
28	240	Wash Cart Solvent Block S1
29	136	Flush Cart Solvent Block
30	241	Wash Cart Reagent Block S1

Step	Function Number	Function Name
31	135	Flush Cart Reagent Block
32	101	Wash Input Block (S1)
33	137	Flush Input Block
34	102	Wash Output Block (S1)
35	138	Flush Output Block
36	136	Flush Cart Solvent Block
37	257	Wait
38	131	Dry Cart (top)
39	63	Del S3, Cart (sensor)
40	148	Cartridge Wait
41	61	Del S3, Cart (top)
42	148	Cartridge Wait
43	131	Dry Cart (top)
44	53	Del S2, Cart (sensor)
45	148	Cartridge Wait
46	51	Del S2, Cart (top)
47	148	Cartridge Wait
48	51	Del S2, Cart (top)
49	148	Cartridge Wait
50	51	Del S2, Cart (top)
51	148	Cartridge Wait
52	61	Del S3, Cart (top)
53	148	Cartridge Wait
54	61	Del S3, Cart (top)
55	148	Cartridge Wait
56	61	Del S3, Cart (top)
57	131	Dry Cart (top)
58	137	Flush Input Block
59	11	Del R2g, Cart (top)
60	140	Flush Large Loop (Cart)
61	6	Load R1, Cart (lg loop)
62	131	Dry Cart (top)
63	140	Flush Large Loop (Cart)

Step	Function Number	Function Name
64	135	Flush Cart Reagent Block
65	11	Del R2g, Cart (top)
66	131	Dry Cart (top)
67	140	Flush Large Loop (Cart)
68	6	Load R1, Cart (lg loop)
69	131	Dry Cart (top)
70	140	Flush Large Loop (Cart)
71	135	Flush Cart Reagent Block
72	11	Del R2g, Cart (top)
73	116	Wash Input Block (X3)
74	241	Wash Cart Reagent Block S1
75	137	Flush Input Block
76	135	Flush Cart Reagent Block
77	131	Dry Cart (top)
78	63	Del S3, Cart (sensor)
79	148	Cartridge Wait
80	61	Del S3, Cart (top)
81	148	Cartridge Wait
82	51	Del S2, Cart (top)
83	148	Cartridge Wait
84	51	Del S2, Cart (top)
85	148	Cartridge Wait
86	131	Dry Cart (top)
87	63	Del S3, Cart (sensor)
88	148	Cartridge Wait
89	61	Del S3, Cart (top)
90	148	Cartridge Wait
91	61	Del S3, Cart (top)
92	148	Cartridge Wait
93	61	Del S3, Cart (top)
94	148	Cartridge Wait
95	61	Del S3, Cart (top)
96	131	Dry Cart (top)

Step	Function Number	Function Name
97	259	End

Table B-64. Cartridge Begin Gas-phase cLC

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	305	Set Reg Setpoint (10th psi)
4	145	Wash Small Loop (Cart)
5	139	Flush Small Loop (Cart)
6	242	Wash Cart Small Loop S1
7	139	Flush Small Loop (Cart)
8	144	Wash Cart Solvent Block
9	136	Flush Cart Solvent Block
10	240	Wash Cart Solvent Block S1
11	136	Flush Cart Solvent Block
12	106	Wash Input Block (S2)
13	137	Flush Input Block
14	101	Wash Input Block (S1)
15	137	Flush Input Block
16	107	Wash Output Block (S2)
17	138	Flush Output Block
18	102	Wash Output Block (S1)
19	138	Flush Output Block
20	131	Dry Cart (top)
21	39	Backflush R3g
22	34	Del R3g, Waste
23	31	Del R3g, Cart (top)
24	136	Flush Cart Solvent Block
25	240	Wash Cart Solvent Block S1
26	136	Flush Cart Solvent Block
27	241	Wash Cart Reagent Block S1
28	135	Flush Cart Reagent Block
29	101	Wash Input Block (S1)

30	137	Flush Input Block
31	102	Wash Output Block (S1)
32	138	Flush Output Block
33	136	Flush Cart Solvent Block
34	257	Wait
35	131	Dry Cart (top)
36	63	Del S3, Cart (sensor)
37	148	Cartridge Wait
38	61	Del S3, Cart (top)
39	148	Cartridge Wait
40	131	Dry Cart (top)
41	53	Del S2, Cart (sensor)
42	148	Cartridge Wait
43	51	Del S2, Cart (top)
44	148	Cartridge Wait
45	51	Del S2, Cart (top)
46	148	Cartridge Wait
47	51	Del S2, Cart (top)
48	148	Cartridge Wait
49	61	Del S3, Cart (top)
50	148	Cartridge Wait
51	61	Del S3, Cart (top)
52	148	Cartridge Wait
53	61	Del S3, Cart (top)
54	131	Dry Cart (top)
55	137	Flush Input Block
56	11	Del R2g, Cart (top)
57	140	Flush Large Loop (Cart)
58	6	Load R1, Cart (lg loop)
59	131	Dry Cart (top)
60	140	Flush Large Loop (Cart)
61	135	Flush Cart Reagent Block
62	11	Del R2g, Cart (top)
63	131	Dry Cart (top)

64	140	Flush Large Loop (Cart)
65	6	Load R1, Cart (lg loop)
66	131	Dry Cart (top)
67	140	Flush Large Loop (Cart)
68	135	Flush Cart Reagent Block
69	11	Del R2g, Cart (top)
70	116	Wash Input Block (X3)
71	241	Wash Cart Reagent Block S1
72	137	Flush Input Block
73	135	Flush Cart Reagent Block
74	131	Dry Cart (top)
75	63	Del S3, Cart (sensor)
76	148	Cartridge Wait
77	61	Del S3, Cart (top)
78	148	Cartridge Wait
79	51	Del S2, Cart (top)
80	148	Cartridge Wait
81	51	Del S2, Cart (top)
82	148	Cartridge Wait
83	131	Dry Cart (top)
84	63	Del S3, Cart (sensor)
85	148	Cartridge Wait
86	61	Del S3, Cart (top)
87	148	Cartridge Wait
88	61	Del S3, Cart (top)
89	148	Cartridge Wait
90	61	Del S3, Cart (top)
91	148	Cartridge Wait
92	61	Del S3, Cart (top)
93	131	Dry Cart (top)
94	259	End

Table B-65. Cartridge Precycle cLC

Step Number	Function Number	Function Name
1	258	Begin
2	303	Select Regulator
3	305	Set Reg Setpoint (10th psi)
4	143	Wash Cart Reagent Block
5	135	Flush Cart Reagent Block
6	11	Del R2g, Cart (top)
7	140	Flush Large Loop (Cart)
8	6	Load R1, Cart (lg loop)
9	131	Dry Cart (top)
10	140	Flush Large Loop (Cart)
11	135	Flush Cart Reagent Block
12	11	Del R2g, Cart (top)
13	131	Dry Cart (top)
14	140	Flush Large Loop (Cart)
15	6	Load R1, Cart (lg loop)
16	131	Dry Cart (top)
17	140	Flush Large Loop (Cart)
18	135	Flush Cart Reagent Block
19	11	Del R2g, Cart (top)
20	146	Wash Large Loop (Cart)
21	140	Flush Large Loop (Cart)
22	143	Wash Cart Reagent Block
23	135	Flush Cart Reagent Block
24	131	Dry Cart (top)
25	63	Del S3, Cart (sensor)
26	61	Del S3, Cart (top)
27	148	Cartridge Wait
28	51	Del S2, Cart (top)
29	148	Cartridge Wait
30	51	Del S2, Cart (top)
31	148	Cartridge Wait
32	51	Del S2, Cart (top)

33	148	Cartridge Wait
34	131	Dry Cart (top)
35	61	Del S3, Cart (top)
36	148	Cartridge Wait
37	61	Del S3, Cart (top)
38	131	Dry Cart (top)
39	61	Del S3, Cart (top)
40	131	Dry Cart (top)
41	61	Del S3, Cart (top)
42	131	Dry Cart (top)
43	111	Wash Input Block (S3)
44	137	Flush Input Block
45	139	Flush Small Loop (Cart)
46	25	Load R3, Cart (sm loop)
47	30	Transfer R3, Cart (gas)
48	136	Flush Cart Solvent Block
49	131	Dry Cart (top)
50	53	Del S2, Cart (sensor)
51	51	Del S2, Cart (top)
52	148	Cartridge Wait
53	51	Del S2, Cart (top)
54	148	Cartridge Wait
55	131	Dry Cart (top)
56	139	Flush Small Loop (Cart)
57	25	Load R3, Cart (sm loop)
58	30	Transfer R3, Cart (gas)
59	136	Flush Cart Solvent Block
60	131	Dry Cart (top)
61	53	Del S2, Cart (sensor)
62	51	Del S2, Cart (top)
63	148	Cartridge Wait
64	51	Del S2, Cart (top)
65	148	Cartridge Wait
66	131	Dry Cart (top)

67	139	Flush Small Loop (Cart)
68	25	Load R3, Cart (sm loop)
69	30	Transfer R3, Cart (gas)
70	136	Flush Cart Solvent Block
71	131	Dry Cart (top)
72	53	Del S2, Cart (sensor)
73	51	Del S2, Cart (top)
74	148	Cartridge Wait
75	51	Del S2, Cart (top)
76	148	Cartridge Wait
77	131	Dry Cart (top)
78	139	Flush Small Loop (Cart)
79	25	Load R3, Cart (sm loop)
80	30	Transfer R3, Cart (gas)
81	136	Flush Cart Solvent Block
82	144	Wash Cart Solvent Block
83	136	Flush Cart Solvent Block
84	145	Wash Small Loop (Cart)
85	139	Flush Small Loop (Cart)
86	131	Dry Cart (top)
87	53	Del S2, Cart (sensor)
88	51	Del S2, Cart (top)
89	148	Cartridge Wait
90	51	Del S2, Cart (top)
91	148	Cartridge Wait
92	131	Dry Cart (top)
93	61	Del S3, Cart (top)
94	148	Cartridge Wait
95	131	Dry Cart (top)
96	259	End

Table B-66. Cartridge-pulsed Liquid 6 mm Glass Fiber Filter cLC

Step Number	Function Number	Function Name
1	258	Begin
2	139	Flush Small Loop (Cart)
3	135	Flush Cart Reagent Block
4	137	Flush Input Block
5	11	Del R2g, Cart (top)
6	140	Flush Large Loop (Cart)
7	6	Load R1, Cart (lg loop)
8	131	Dry Cart (top)
9	140	Flush Large Loop (Cart)
10	135	Flush Cart Reagent Block
11	11	Del R2g, Cart (top)
12	131	Dry Cart (top)
13	140	Flush Large Loop (Cart)
14	6	Load R1, Cart (lg loop)
15	131	Dry Cart (top)
16	140	Flush Large Loop (Cart)
17	135	Flush Cart Reagent Block
18	11	Del R2g, Cart (top)
19	131	Dry Cart (top)
20	140	Flush Large Loop (Cart)
21	6	Load R1, Cart (lg loop)
22	131	Dry Cart (top)
23	140	Flush Large Loop (Cart)
24	135	Flush Cart Reagent Block
25	11	Del R2g, Cart (top)
26	116	Wash Input Block (X3)
27	137	Flush Input Block
28	241	Wash Cart Reagent Block S1
29	135	Flush Cart Reagent Block
30	131	Dry Cart (top)
31	142	Set Cart Temperature
32	63	Del S3, Cart (sensor)

33	131	Dry Cart (top)
34	63	Del S3, Cart (sensor)
35	131	Dry Cart (top)
36	53	Del S2, Cart (sensor)
37	148	Cartridge Wait
38	51	Del S2, Cart (top)
39	148	Cartridge Wait
40	131	Dry Cart (top)
41	53	Del S2, Cart (sensor)
42	148	Cartridge Wait
43	51	Del S2, Cart (top)
44	148	Cartridge Wait
45	51	Del S2, Cart (top)
46	148	Cartridge Wait
47	131	Dry Cart (top)
48	63	Del S3, Cart (sensor)
49	131	Dry Cart (top)
50	63	Del S3, Cart (sensor)
51	131	Dry Cart (top)
52	63	Del S3, Cart (sensor)
53	131	Dry Cart (top)
54	63	Del S3, Cart (sensor)
55	131	Dry Cart (top)
56	106	Wash Input Block (S2)
57	137	Flush Input Block
58	101	Wash Input Block (S1)
59	137	Flush Input Block
60	138	Flush Output Block
61	107	Wash Output Block (S2)
62	138	Flush Output Block
63	102	Wash Output Block (S1)
64	138	Flush Output Block
65	139	Flush Small Loop (Cart)
66	25	Load R3, Cart (sm loop)

67	30	Transfer R3, Cart (gas)
68	139	Flush Small Loop (Cart)
69	145	Wash Small Loop (Cart)
70	136	Flush Cart Solvent Block
71	240	Wash Cart Solvent Block S1
72	241	Wash Cart Reagent Block S1
73	101	Wash Input Block (S1)
74	102	Wash Output Block (S1)
75	242	Wash Cart Small Loop S1
76	136	Flush Cart Solvent Block
77	135	Flush Cart Reagent Block
78	137	Flush Input Block
79	139	Flush Small Loop (Cart)
80	136	Flush Cart Solvent Block
81	138	Flush Output Block
82	131	Dry Cart (top)
83	142	Set Cart Temperature
84	127	Ready Transfer to Flask
85	141	Flush Transfer Line
86	63	Del S3, Cart (sensor)
87	148	Cartridge Wait
88	121	Transfer to Flask (gas)
89	141	Flush Transfer Line
90	53	Del S2, Cart (sensor)
91	148	Cartridge Wait
92	121	Transfer to Flask (gas)
93	128	Transfer Complete
94	11	Del R2g, Cart (top)
95	131	Dry Cart (top)
96	61	Del S3, Cart (top)
97	131	Dry Cart (top)
98	259	End

Table B-67. Cartridge-pulsed Liquid Prosorb cLC

Step Number	Function Number	Function Name
1	258	Begin
2	139	Flush Small Loop (Cart)
3	135	Flush Cart Reagent Block
4	137	Flush Input Block
5	11	Del R2g, Cart (top)
6	140	Flush Large Loop (Cart)
7	6	Load R1, Cart (lg loop)
8	131	Dry Cart (top)
9	140	Flush Large Loop (Cart)
10	135	Flush Cart Reagent Block
11	11	Del R2g, Cart (top)
12	131	Dry Cart (top)
13	140	Flush Large Loop (Cart)
14	6	Load R1, Cart (lg loop)
15	131	Dry Cart (top)
16	140	Flush Large Loop (Cart)
17	135	Flush Cart Reagent Block
18	11	Del R2g, Cart (top)
19	131	Dry Cart (top)
20	140	Flush Large Loop (Cart)
21	6	Load R1, Cart (lg loop)
22	131	Dry Cart (top)
23	140	Flush Large Loop (Cart)
24	135	Flush Cart Reagent Block
25	11	Del R2g, Cart (top)
26	116	Wash Input Block (X3)
27	137	Flush Input Block
28	241	Wash Cart Reagent Block S1
29	135	Flush Cart Reagent Block
30	131	Dry Cart (top)
31	142	Set Cart Temperature
32	63	Del S3, Cart (sensor)

33	131	Dry Cart (top)
34	63	Del S3, Cart (sensor)
35	131	Dry Cart (top)
36	53	Del S2, Cart (sensor)
37	148	Cartridge Wait
38	51	Del S2, Cart (top)
39	148	Cartridge Wait
40	131	Dry Cart (top)
41	53	Del S2, Cart (sensor)
42	148	Cartridge Wait
43	51	Del S2, Cart (top)
44	148	Cartridge Wait
45	51	Del S2, Cart (top)
46	148	Cartridge Wait
47	131	Dry Cart (top)
48	63	Del S3, Cart (sensor)
49	131	Dry Cart (top)
50	63	Del S3, Cart (sensor)
51	131	Dry Cart (top)
52	63	Del S3, Cart (sensor)
53	131	Dry Cart (top)
54	63	Del S3, Cart (sensor)
55	131	Dry Cart (top)
56	106	Wash Input Block (S2)
57	137	Flush Input Block
58	101	Wash Input Block (S1)
59	137	Flush Input Block
60	138	Flush Output Block
61	107	Wash Output Block (S2)
62	138	Flush Output Block
63	102	Wash Output Block (S1)
64	138	Flush Output Block
65	140	Flush Large Loop (Cart)
66	26	Load R3, Cart (lg loop)

67	30	Transfer R3, Cart (gas)
68	140	Flush Large Loop (Cart)
69	146	Wash Large Loop (Cart)
70	136	Flush Cart Solvent Block
71	240	Wash Cart Solvent Block S1
72	241	Wash Cart Reagent Block S1
73	101	Wash Input Block (S1)
74	102	Wash Output Block (S1)
75	243	Wash Cart Large Loop S1
76	136	Flush Cart Solvent Block
77	135	Flush Cart Reagent Block
78	137	Flush Input Block
79	140	Flush Large Loop (Cart)
80	136	Flush Cart Solvent Block
81	138	Flush Output Block
82	131	Dry Cart (top)
83	142	Set Cart Temperature
84	127	Ready Transfer to Flask
85	141	Flush Transfer Line
86	63	Del S3, Cart (sensor)
87	148	Cartridge Wait
88	121	Transfer to Flask (gas)
89	141	Flush Transfer Line
90	53	Del S2, Cart (sensor)
91	148	Cartridge Wait
92	121	Transfer to Flask (gas)
93	128	Transfer Complete
94	11	Del R2g, Cart (top)
95	131	Dry Cart (top)
96	61	Del S3, Cart (top)
97	131	Dry Cart (top)
98	259	End

Table B-68. Cartridge Gas-phase cLC

Step Number	Function Number	Function Name
1	258	Begin
2	139	Flush Small Loop (Cart)
3	135	Flush Cart Reagent Block
4	137	Flush Input Block
5	11	Del R2g, Cart (top)
6	140	Flush Large Loop (Cart)
7	6	Load R1, Cart (lg loop)
8	131	Dry Cart (top)
9	140	Flush Large Loop (Cart)
10	135	Flush Cart Reagent Block
11	11	Del R2g, Cart (top)
12	131	Dry Cart (top)
13	140	Flush Large Loop (Cart)
14	6	Load R1, Cart (lg loop)
15	131	Dry Cart (top)
16	140	Flush Large Loop (Cart)
17	135	Flush Cart Reagent Block
18	11	Del R2g, Cart (top)
19	131	Dry Cart (top)
20	140	Flush Large Loop (Cart)
21	6	Load R1, Cart (lg loop)
22	131	Dry Cart (top)
23	140	Flush Large Loop (Cart)
24	135	Flush Cart Reagent Block
25	11	Del R2g, Cart (top)
26	116	Wash Input Block (X3)
27	137	Flush Input Block
28	241	Wash Cart Reagent Block S1
29	135	Flush Cart Reagent Block
30	131	Dry Cart (top)
31	142	Set Cart Temperature
32	63	Del S3, Cart (sensor)

33	131	Dry Cart (top)
34	63	Del S3, Cart (sensor)
35	131	Dry Cart (top)
36	53	Del S2, Cart (sensor)
37	148	Cartridge Wait
38	51	Del S2, Cart (top)
39	148	Cartridge Wait
40	131	Dry Cart (top)
41	53	Del S2, Cart (sensor)
42	148	Cartridge Wait
43	51	Del S2, Cart (top)
44	148	Cartridge Wait
45	51	Del S2, Cart (top)
46	148	Cartridge Wait
47	131	Dry Cart (top)
48	63	Del S3, Cart (sensor)
49	131	Dry Cart (top)
50	63	Del S3, Cart (sensor)
51	131	Dry Cart (top)
52	63	Del S3, Cart (sensor)
53	131	Dry Cart (top)
54	63	Del S3, Cart (sensor)
55	131	Dry Cart (top)
56	106	Wash Input Block (S2)
57	137	Flush Input Block
58	101	Wash Input Block (S1)
59	137	Flush Input Block
60	138	Flush Output Block
61	107	Wash Output Block (S2)
62	138	Flush Output Block
63	102	Wash Output Block (S1)
64	138	Flush Output Block
65	34	Del R3g, Waste
66	31	Del R3g, Cart (top)

67	136	Flush Cart Solvent Block
68	240	Wash Cart Solvent Block S1
69	241	Wash Cart Reagent Block S1
70	101	Wash Input Block (S1)
71	102	Wash Output Block (S1)
72	136	Flush Cart Solvent Block
73	135	Flush Cart Reagent Block
74	137	Flush Input Block
75	136	Flush Cart Solvent Block
76	138	Flush Output Block
77	257	Wait
78	131	Dry Cart (top)
79	142	Set Cart Temperature
80	127	Ready Transfer to Flask
81	141	Flush Transfer Line
82	63	Del S3, Cart (sensor)
83	148	Cartridge Wait
84	121	Transfer to Flask (gas)
85	141	Flush Transfer Line
86	53	Del S2, Cart (sensor)
87	148	Cartridge Wait
88	121	Transfer to Flask (gas)
89	128	Transfer Complete
90	11	Del R2g, Cart (top)
91	131	Dry Cart (top)
92	61	Del S3, Cart (top)
93	131	Dry Cart (top)
94	259	End

Table B-69. Flask Optimization cLC

Step Number	Function Number	Function Name
1	258	Begin
2	131	Dry Cart (top)
3	142	Set Cart Temperature
4	127	Ready Transfer to Flask
5	141	Flush Transfer Line
6	63	Del S3, Cart (sensor)
7	148	Cartridge Wait
8	121	Transfer to Flask (gas)
9	141	Flush Transfer Line
10	53	Del S2, Cart (sensor)
11	148	Cartridge Wait
12	121	Transfer to Flask (gas)
13	128	Transfer Complete
14	131	Dry Cart (top)
15	259	End

Appendix C Standard Sequencing Methods

The following is a complete list of the standard methods provided by Applied Biosystems for the Procise 49X cLC Protein Sequencing System.

Table C-1. Filter Precycle cLC

Method Name	Temperatures	Cartridge Cycles	Flask Cycles	Pump Cycles
Filter Precycle cLC	Cartridge Temp: 48 ˚C	Cart-PL 6mmGFF cLC	Flask Normal cLC	Normal 1 cLC
	Flask Temp: 64 ˚C	None	Prepare Pump cLC	Prepare Pump cLC
	Column Temp: 55 ˚C	Cart Precycle cLC	Flask Blank cLC	Normal 1 cLC
		Cart Precycle cLC	Flask Standard cLC	Normal 1 cLC
Pulsed-liquid Prosorb cLC	Cartridge Temp: 48 ˚C	Cart-PL Prosorb cLC	Flask Normal cLC	Normal 1 cLC
	Flask Temp: 64 ˚C	None	Prepare Pump cLC	Prepare Pump cLC
	Column Temp: 55 ˚C	None	Flask Blank cLC	Normal 1 cLC
		Cart Begin cLC	Flask Standard cLC	Normal 1 cLC
Pulsed-Liquid cLC	Cartridge Temp: 48 ˚C	Cart-PL 6mm GFF cLC	Flask Normal cLC	Normal 1 cLC
	Flask Temp: 64 ˚C	None	Prepare Pump cLC	Prepare Pump cLC
	Column Temp: 55 ˚C	None	Flask Blank cLC	Normal 1 cLC
		Cart Begin cLC	Flask Standard cLC	Normal 1 cLC
Gas-phase cLC	Cartridge Temp: 48 ˚C	Cart Gas-phase cLC	Flask Normal cLC	Normal 1 cLC
	Flask Temp: 64 ˚C	None	Prepare Pump cLC	Prepare Pump cLC
	Column Temp: 55 ˚C	None	Flask Blank cLC	Normal 1 cLC
		Cart Begin Gas-phase cLC	Flask Standard cLC	Normal 1 cLC
PTH-Standards cLC	Cartridge Temp: 35 ˚C	None	Flask Standard cLC	Normal 1 cLC
	Flask Temp: 64 ˚C	None	Prepare Pump cLC	Prepare Pump cLC
	Column Temp: 55 ˚C			
Run Gradient cLC	Cartridge Temp: 35 ˚C	None	Run Gradient cLC	Normal 1 cLC
	Flask Temp: 64 ˚C			
	Column Temp: 55 ˚C			
Flask Optimization cLC	Cartridge Temp: 35 ˚C	Flask Optimization cLC	Flask Optimization cLC	None
	Flask Temp: 64 ˚C			
	Column Temp: 55 ˚C			

Method Name	Temperatures	Cartridge Cycles	Flask Cycles	Pump Cycles
Injector Optimization cLC	Cartridge Temp: 35 ˚C	None	Injector Optimization cLC	None
	Flask Temp: 64 ˚C			
	Column Temp: 55 ˚C			

Appendix D Standard Gradient Programs

The following is a complete list of the standard gradient programs provided by Applied Biosystems for the Procise 49X cLC Protein Sequencing System.

Table D-1. Normal 1 cLC

Gradient Name	Time	%B	µL/min	Event
Normal 1 cLC	0.0	10	40	12
	0.4	12	40	1
	4.0	22	40	1
	22.0	48	40	1
	22.6	90	40	1
	23.5	90	40	1
	29.0	90	60	0
	33.0	50	20	0
Max Pressure: 4000 psi Min Pressure: 0 psi Target Pressure: 1500 psi		Target Time: 0.2 min Data Collection Time: 28 min		

Table D-2. Prepare Pump cLC

Gradient Name	Time	%B	µL/min	Event
Prepare Pump cLC	0.0	50	45	0
	30.0	50	45	0
Max Pressure: 3000 psi Min Pressure: 0 psi Target Pressure: 2000 psi		Target Time: 2.0 min Data Collection Time: 28 min		

Appendix E Warranty

Applied Biosystems warrants to the customer that, for a period ending on the earlier of one year(s) from the completion of install ation or fifteen (15) month(s) from the date of shipment to the customer (the "Warranty Period"), the PROCISE 49X cLC Protein Sequencer purchased by the customer (the "Instrument") will be free from defects in material and workmanship, and will perform in accordance with the specifications set forth in the Product Specification Sheet (the "Specifications").

During the Warranty Period, if the Instrument's hardware becomes damaged or contaminated or if the Instrument otherwise fails to meet the Specifications, Applied Biosystems will repair or replace the Instrument so that it meets the Specifications, at Applied Biosystems expense. However, if the Instrument's hardware becomes damaged or contaminated, or if the chemical performance of that Instrument otherwise deteriorates, due to solvents and/or reagents other than those supplied or expressly recommended by Applied Biosystems, Applied Biosystems will return the Instrument to Specification at the customer's request and at the customer's expense. After this service is performed, coverage of the parts repaired or replaced will be restored thereafter for the remainder of the original Warranty Period.

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Appendix F Amino Acid Abbreviations & Symbols

Amino Acid	Abbreviation	Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

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