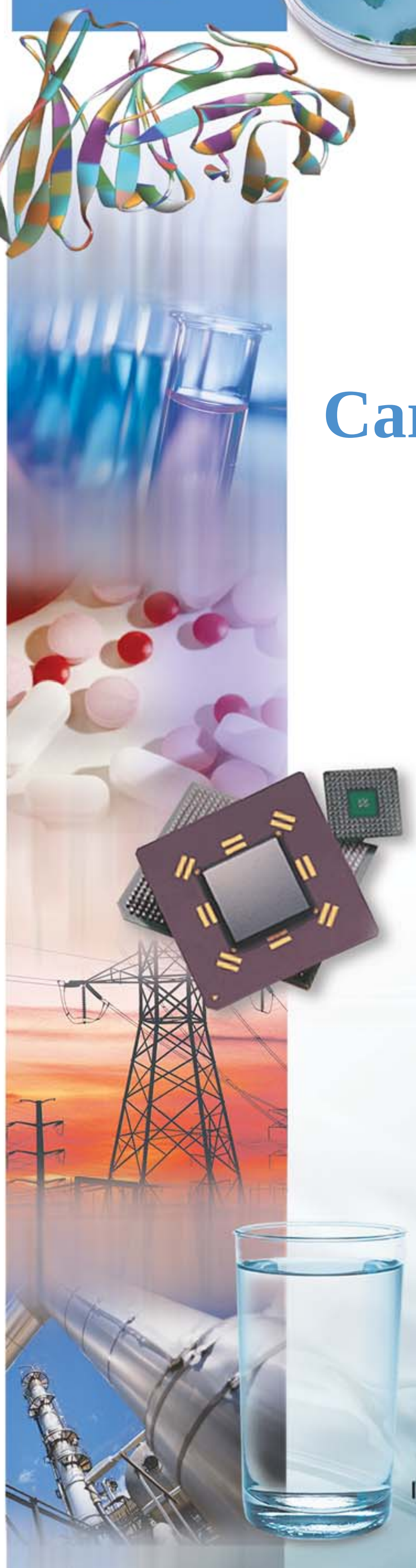
PRODUCT MANUAL

Carbohydrate Removal Accessory



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PRODUCT MANUAL

for the

Carbohydrate Removal Accessory
P/N 063522

Document No. 031923
Revision 03
June 2005

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SECTION 1 - INTRODUCTION

The following instructions are for the installation and use of the Carbohydrate Removal Accessory (CRA), an Accessory Kit for use with Dionex AAA Direct systems. The CRA is used to remove carbohydrate interferences to amino acid analysis.

This fully automated on-line technique is based on retaining amino acids on a SugarTrap column containing cation exchange resin prior to their separation by anion exchange. The carbohydrates are not retained on the SugarTrap and are rinsed out before the transfer of amino acids onto the AminoPac PA10. See P. Jandik, J. Cheng, D. Jensen, S. Manz and N. Avdalovic, *J. Chromatogr. B*., 758 (2001) 189 – 196 for additional details.

The AAA Direct system has to be modified to include the CRA for on-line sugar removal. The CRA consists of a short cation exchange cartridge (the SugarTrap), cartridge holder, a six-port Rheodyne valve, a syringe pump, and a set of PEEK tubing of specified length and internal diameter.

Replacement SugarTrap cartridges are available in sets of 2 (P/N 065321).

Assistance is available for any problem during the shipment or operation of Dionex instrumentation and columns through the Dionex North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or through any of the Dionex Offices listed in "Dionex Worldwide Offices" on the Dionex Reference Library CD-ROM.

For assistance with the Kloehn Transfer Valve (TV) pump, please call Kloehn at 1-800-358-4342.

1.1 Detailed Description of CRA Components

Check these lists carefully. All parts must be available before attempting a CRA installation. Carbohydrate Removal Accessory (P/N 063522) includes the following components.

_ Kloehn VersaPump 6 (Syringe Pump) (Kloehn P/N 54122)

_ 3 Port Valve for the VersaPump 6 (Kloehn P/N 17616, already connected to the pump)

_ Syringe 500 µL (Kloehn P/N 17595, already installed on the pump)

_ Starter Kit for VersaPump 6 (Kloehn P/N 23427)

_ Dionex Reference Library CD-ROM including CRA Product Manual (P/N 053891)

_ Stainless Steel Cartridge Holder (P/N 059456)

_ 2 SugarTrap Cartridges Filled with Cation Exchange Material (P/N 063521)

_ Transfer Valve (TV) Rheodyne Valve (Model Number MX-9900-000) (P/N 063484)

_ 2 Pin Phoenix Connector (already installed on the MX9900-000 leads)

_ 5 Feet of Red Tubing (to make T1, T2, and T3 tubing segments)

_ 5 Feet of Orange PEEK Tubing

_ 5 Feet of Black PEEK Tubing

_ 5 10-32 Couplers

_ 15 Fittings - 10-32

_ 15 Ferules - Double Cone

NOTE: Use the exact lengths specified for T1, T2, and T3. This helps to avoid a time-consuming optimization.

_ T1: Red PEEK Tubing 1 length: 49 cm (Injector port 2 to PEEK union at tubing 2)

_ T2: Red PEEK Tubing 2 length: 10.8 cm (PEEK union at tubing 1 to TV port 2)

_ T3: Red PEEK Tubing 3 length: 18 cm (TV port 1 to outlet of cation exchange column)

_ T4: Red PEEK Tubing 4 (Inlet of cation exchange column to TV port 4)

_ T5: Red PEEK Tubing 5 (TV port 6 to AminoPac PA10 column inlet)

_ T6: Black PEEK Tubing 6 (Gradient mixer to TV port 5)

_ T7: Black PEEK Tubing 7 (from PEEK union at the end of tubing 9 to injector port 3)

_ T8: Orange PEEK Tubing 8 (Transfer Fluid container to syringe pump)

_ T9: Orange PEEK Tubing 9 (Transfer Pump to PEEK union at tubing 7)

_ T10: Orange PEEK Tubing (TV port 3 to waste)

If you do not have the following item, please obtain it from Dionex.

_ Clean Glass Container (V =0.2 to 1 L) for Transfer Fluid (water or 0.02% formic acid)

SECTION 2 - INSTALLATION

2.1 Before the Installation

We are assuming that your AAA-Direct is configured and functioning according to the Dionex AAA-Direct Manual.

Before installing the CRA accessory, generate one chromatogram of an 8 µM amino acid standard and a second chromatogram of the 8 µM amino acid standard containing 100 µM glucose. Use the program in Appendix A if you have Chromeleon 6x. If using PeakNet 5x, please create and use the corresponding pump and detector methods in that software.

2.2 Recommended Configuration of CRA Modules

Figure 1
Placement of the CRA Modules

2.3 Installing the Transfer Pump

Do not power up the syringe pump before completing all hardware and software installation steps.

A. Create a folder “Kloehn” (for example in: My Documents). From the Kloehn CD, copy the folders Kcomm and Manuals into that folder. Print the first 30 pages of the VersaPump 6 Manual. Print all 16 pages of the “Kcomm Software…” manual.

B. Initiate Pump Comm installation by double clicking on the Kcom.exe file. Follow the installation steps resulting in a full installation of Pump Comm in a “Kcm” subfolder of the Programs folder of your PC.

C. Verify that the 3-way valve is installed as described on page 14 of the Kloehn V6 Manual and in Figure 1-2 of the same manual. Plug the port B using a blind plug.

D. With the pump outside of the plastic housing verify that the “Card Edge Adapter Board” (Kloehn P/N 23352) is installed according to the instructions on page 15 of the Kloehn V6 Manual.

E. Attach the red connector of the power cable to the four pins marked “Power” on the edge adapter. Follow the instructions in Figure 3-1 (Kloehn V6 Manual). Attach a power cord to the corresponding connector of the Power Supply box. Do not plug in the wall connector yet.

F. Attach the blue connector of the RS232 to the three pins marked "232" on the edge adapter. Follow instruction in Figure 3-1 of the Kloehn V6 Manual. Attach the other end to an available serial port on your PC.

G. The 500 µL syringe should already be installed. Otherwise, remove the syringe screw, screw the syringe into the syringe port. Remember to insert the Teflon washer first (Figure 1-2, Kloehn V6 Manual). Insert the plunger and fasten the syringe screw through the plunger button. Place additional Teflon washers into ports A and C and connect T8 and T9 (Figure 2). Fill the transfer fluid container (0.02% formic acid or water) and insert T8.

H. Plug the power supply into the wall connector. Push the switch on the power cable to power up.

I. Double click on the Kcom icon inside the Kcm subfolder to start the Pump Comm window.

J. Referring to pages 5–7 of the Kcomm manual push the "Setup" button, select Versa 6 as Device Type and select the Comm Port used for RS232 PC to edge adapter connection. Leave the Protocol and Baud Rate settings unchanged. Leave the Device number unchanged as "1".

K. Referring to Appendix C of this CRA manual, load the "Initialize," "Prime," and "Constant Flow 75" strings into the Pump Comm.

L. Load (File, Recall) and run (send) the "Initialize" string.

M. With the T9 disconnected from T7 (**IMPORTANT**: the syringe valve can be damaged otherwise) and T8 immersed in the transfer fluid, load (File, Recall) and run (Send) the "Prime" string of commands. The pump performs five strokes and the syringe is filled with the transfer fluid. If the syringe still contains air bubbles at this point, take it off the pump and prime it manually. Verify that the fluid is coming from the port to which tubing T9 is connected. Verify that the plug (P/N 37628) is installed in the unused port.

N. Load (File/Recall) the Constant Flow 75 string, reconnect T7 and T9. The Transfer Pump installation is now finished.

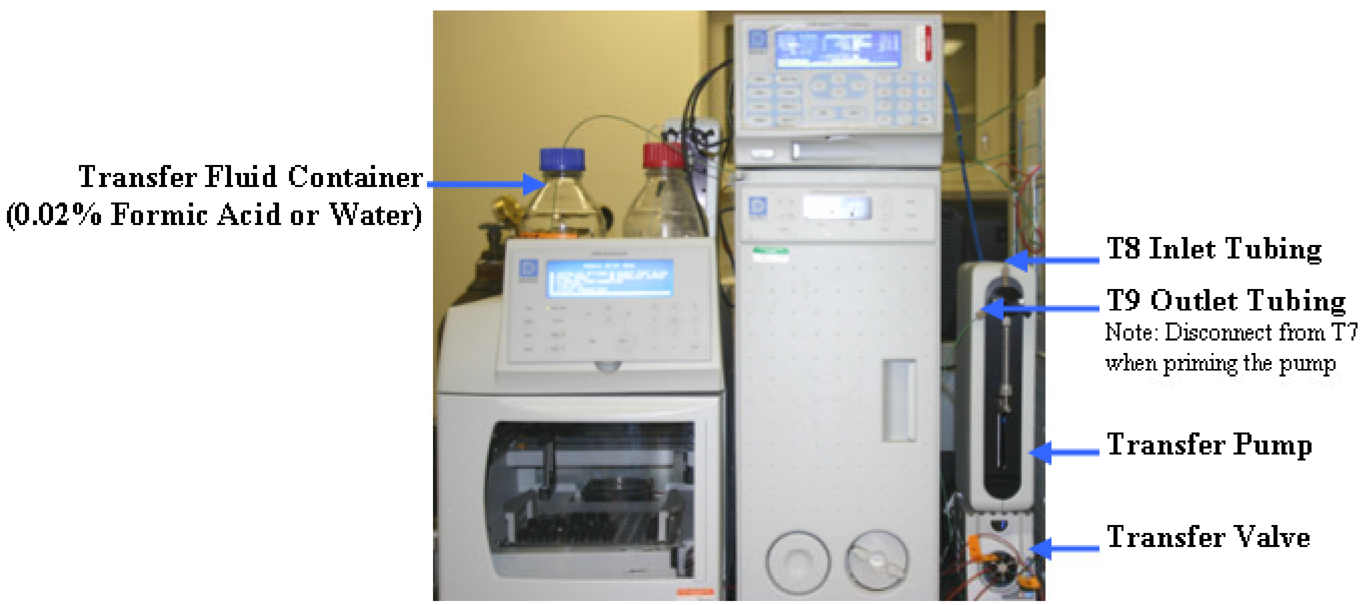

Figure 2
Initial Set Up of Transfer Pump

2.4 Preparing the AAA System

Switch off the cell voltage from the electrochemical detector and stop the pump.

Remove the entire length of black PEEK tubing between the gradient mixer and injector valve from the system.

Remove the entire length of the red PEEK tubing connecting the injection valve to the AminoPac PA10 Guard column.

Remove the Guard column and the red tubing connecting it to the inlet of the AminoPac PA 10 analytical column. Seal the Guard column **by connecting the loose end of the red tubing into the Guard column outlet.** Do not leave the AminoPac PA 10 column inlet or outlet open for longer than ca. 5-10 minutes. Use blind plugs in case you have to interrupt the installation at this point.

2.5 Signal Connections for the Transfer Valve

Place the Rheodyne valve on the bench in front of you.

Connect the green connector (2-pin Phoenix connector) to the RLY-1 OUT of the AAA-Direct pump (behind the front panel).

2.5.1 Dry Test

A. With the Rheodyne to RLY-1 OUT connection in place, conduct a dry test with the Rheodyne valve. The valve can be moved either from the pump front panel (detail screen, select RLY1_0 or RLY1_1) or from the Chromeleon panel screen (control/commands/pump/pump_Relay1/State: open or closed.

B. Verify that the valve moves when switching between RLY1_0 and RLY1_1 or Relay 1 Open and Closed.

2.6 Liquid Connections for the Transfer Pump and Adjusting/Confirming Transfer Flow Rate

We are assuming that an injection valve is located inside the AS50 Autosampler.

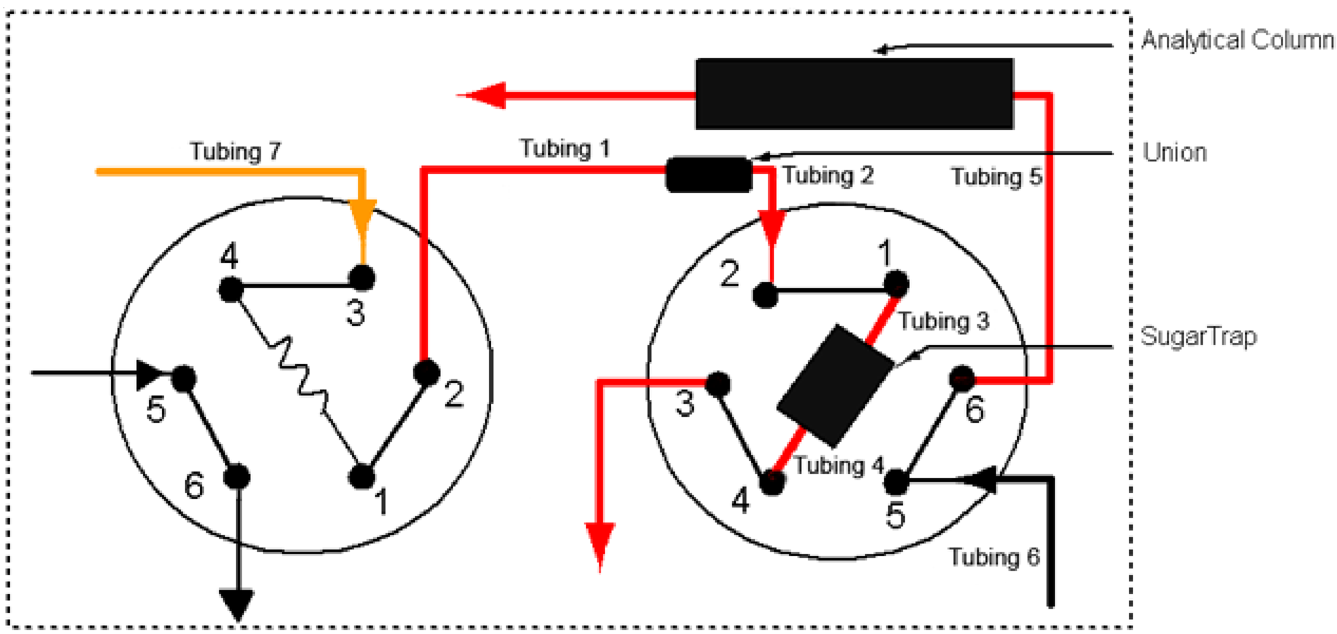

Figure 3
Liquid Connections

Confirm that the tubing connections in the injection valve are as follows (Figure 3): 25 µL sample loop between ports 1 and 4, with the injection port and waste tubing at ports 5 and 6 respectively. Ports 3 and 2 should be open after the removal of red and black tubing as described in Section 2.4.

IMPORTANT
T1, T2, and T3 should have the exact length that is specified in the Introduction.

Connect the tubing T7 from the Transfer Pump outlet to port 3. This segment of tubing should be as short as possible to minimize the delay volume of the Transfer Fluid. Complete all tubing connections shown in Figure 3 but do not install the SugarTrap yet.

Figure 4 shows the two positions of the Transfer Valve. In the Delay position, sample is loaded onto the SugarTrap. In the In-Line portion the sample is being transferred to the analytical column.

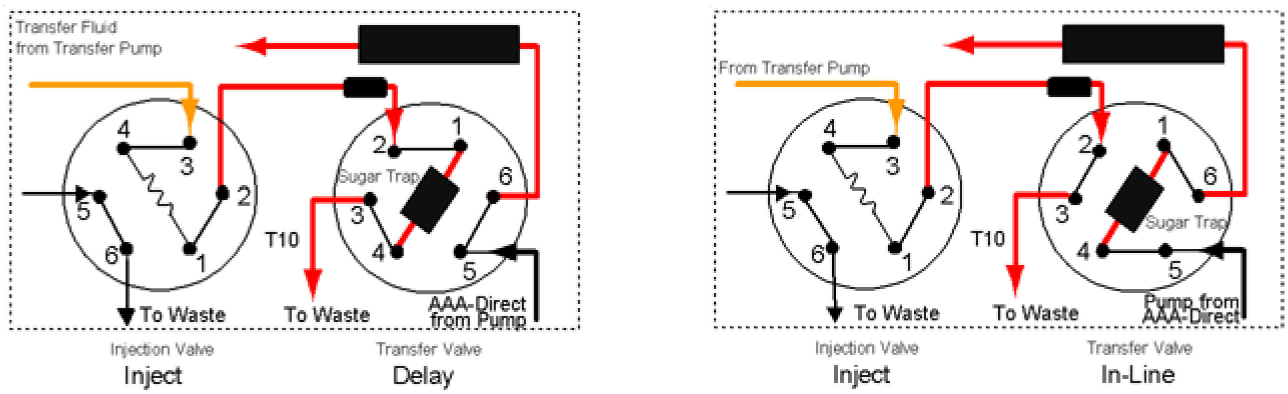

Figure 4
Two Positions of Transfer Valve

NOTE
The SugarTrap column is connected to the Transfer Pump when the Transfer Valve is in the "Delay" position. When the transfer valve is in the "In-Line" position, the transfer fluid flows directly to port 3 and from there to waste (T10 red). At this time, the eluent from the AAA-Direct pump flows through the SugarTrap column. The injection valve remains in the inject position most of the time during system operation. The injection valve is rotated into the load position only when the autosampler is loading the sample loop installed between ports 1 and 4.

2.6.1 Wet Test to Assign the Valve Positions to Chromeleon Commands

The Rheodyne valve can be in one of its two possible positions. We now have to carry out a wet test to verify that the valve-position-to-command assignment is correct (see Figure 5).

A. Start a constant flow (< 100 µL/min) from the Transfer Pump.

IMPORTANT
Do not use the priming flow rate.

B. Carry out several changes between Relay1 open and closed using Chromeleon commands and verify that at Relay Open, the Transfer Fluid is exiting from tubing T3, and at Relay Closed, the same fluid is exiting from tubing T10 (see Figure 5).

C. Correct the command assignment if necessary.

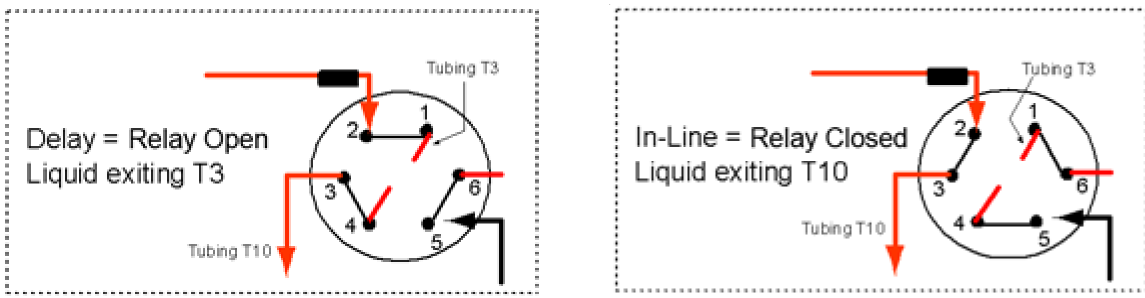

Figure 5
Assigning the Valve Positions to Chromeleon Commands

2.6.2 Pressure Test to Detect Leaks and Obstructions

By conducting this test, users can detect leaks and obstructions in the flow path.

A. Install the SugarTrap cartridge and make sure that all other connections that are shown in Figure 3 are in place.

B. Start the AAA-Direct pump, and the constant flow from the Transfer Pump (<100 µL/min). Turn on the electrochemical cell voltage.

C. Note the pressure reading on the AAA-Direct pump.

D. Verify the set flow rate of the Transfer Fluid by collecting and weighing fractions of liquid captured at tubing T10 (see figure 5).

E. Rotate the Transfer Valve using a Chromeleon command. Verify that the pressure change is < 200 psi and remains the same during several valve rotations. Verify that there are no upsets in the electrochemical detector reading.

F. Check for leaks.

NOTE

At the low flow rates of the Transfer Fluid, the leaks are slow to develop. Allow sufficient time for leaks to develop.

2.7 Testing Recovery of Amino Acids and Efficiency of Carbohydrate Removal

NOTE

In the original version of this carbohydrate method, the SugarTrap was regenerated by injecting 25 µL of 1 M HCl before each injection of a sample (see the literature reference provided in the Introduction). Pure water was used as a Transfer Fluid and each sample run required two independent chromatographic runs in a PeakNet sequence (First run HCl, second run: sample).

We are now recommending the use of 0.02% formic acid as the Transfer Fluid. The injections of 1M HCl are no longer necessary and the programming of sample runs is greatly simplified. It is also possible to use higher Transfer Fluid flow rates (recommended: 75 µL/min) and longer "Delay" times (see Figure 4) to accommodate the removal of higher concentrations of interfering carbohydrates.

A. Make sure that the sample vials (amino acids with and without an added excess of glucose) (Section 1) and an additional vial with water are placed in the autosampler tray. Add another vial containing 100 µM glucose solution. At this point, the evaluation sequence of your browser should already contain the chromatograms of samples 1 and 2. (Figure 6: Lines 1 and 2).

B. Program a sequence of additional injections (samples 3 –6 in Figure 6) for injecting AA standard with and without an added level of glucose and also a pure glucose solution. Inject a water blank first (Figure 6, sample 3) to check the chromatographic background of the system configured for carbohydrate removal.

Should the injection of glucose solution (sample 6) show too high residual level of that compound, run a series of injections with gradually increasing Delay times (Samples 7-12).

If necessary, rerun the amino acid standard at an optimized Delay time.

Calculate the recoveries of all amino acids for the optimized Delay time.

No.	Name	Type	Pos	Inj. Vol.	Program
1	AA Standard plus Glc before installation of CRA	Unknown	2	25.0	76_24Method without CRA
2	AA Standard before installation of CRA	Unknown	3	25.0	76_24Method without CRA
3	Water after installation of CRA	Unknown	1	25.0	1p8 CRA 76_24
4	AA Standard after installation of CRA	Unknown	3	25.0	1p8 CRA 76_24
5	AA Standard plus Glc after installation of CRA	Unknown	2	25.0	1p8 CRA 76_24
6	100uM Glc with 1.8delay	Unknown	4	25.0	1p8 CRA 76_24
7	100uM with 2.0	Unknown	4	25.0	2p0
8	100uM with 2.2	Unknown	4	25.0	2p2
9	100uM with 2.4	Unknown	4	25.0	2p4
10	100uM with 2.6	Unknown	14	25.0	2p6
11	100uM with 2.8	Unknown	14	25.0	2p8
12	100uM with 3.0	Unknown	1	25.0	3p0
13	end	Unknown	1	25.0	End

Evaluation Sequence

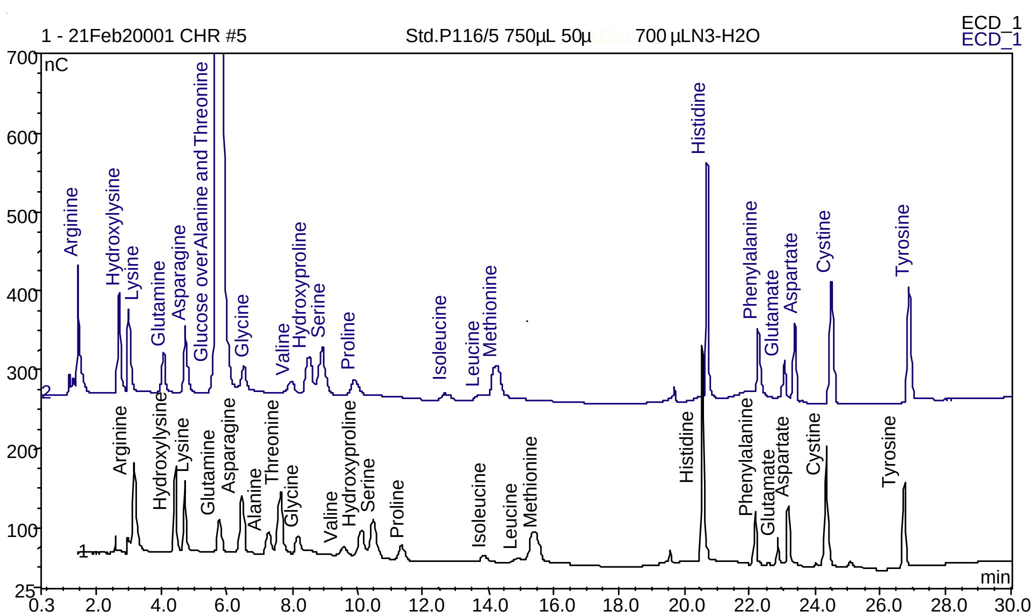

Figure 7
Samples 1 and 5 from Figure 6
Comparison of Chromatograms With and Without the SugarTrap Installed

APPENDIX A - PROGRAM FOR DIRECT INJECTION

	Pressure.LowerLimit =	0
	Pressure.UpperLimit =	4000
	%A.Equate =	“%A Water”
	%B.Equate =	“%B 250 mM NaOH”
	%C.Equate =	“%C 1 M NaAc”
	%D.Equate =	“%D 100 mM HAc”
	Flush	Volume = 250
	Wait	FlushState
	NeedleHeight =	3
	CutSegmentVolume =	0
	SyringeSpeed =	3
	pH.LowerLimit =	10.0
	pH.UpperLimit =	13.0
	Log	pH.Value
	ECD.Data_Collection_Rate =	0.8
	Cycle =	0
	Cell =	On
	Waveform Time = 0.00, Potential = 0.13	
	Waveform Time = 0.04, Potential = 0.13	
	Waveform Time = 0.05, Potential = 0.33	
	Waveform Time = 0.21, Potential = 0.33, Integration = Begin	
	Waveform Time = 0.22, Potential = 0.55	
	Waveform Time = 0.46, Potential = 0.55	
	Waveform Time = 0.47, Potential = 0.33	
	Waveform Time = 0.56, Potential = 0.33, Integration = End	
	Waveform Time = 0.57, Potential = -1.67	
	Waveform Time = 0.58, Potential = -1.67	
	Waveform Time = 0.59, Potential = 0.93	
	Waveform Time = 0.60, Potential = 0.13	
	WaitForTemperature =	False
	Wait	SamplePrep
0.000	Flow =	0.25
	%B =	24.0
	%C =	0.0
	%D =	0.0
	Curve =	5
	Load	
	Inject	
	Wait	InjectState
	Flow =	0.25
	%B =	24.0
	%C =	0.0
	%D =	0.0
	Curve =	5
	ECD_1.AcqOn	

2.000	Flow =	0.25
	%B =	24.0
	%C =	0.0
	%D =	0.0
	Curve =	5
8.000	Flow =	0.25
	%B =	36.0
	%C =	0.0
	%D =	0.0
	Curve =	8
11.000	Flow =	0.25
	%B =	36.0
	%C =	0.0
	%D =	0.0
	Curve =	8
18.000	Flow =	0.25
	%B =	20.0
	%C =	40.0
	%D =	0.0
	Curve =	8
21.000	Flow =	0.25
	%B =	16.0
	%C =	40.0
	%D =	0.0
	Curve =	5
23.000	Flow =	0.25
	%B =	16.0
	%C =	70.0
	%D =	0.0
	Curve =	8
45.000	Flow =	0.25
	%B =	16.0
	%C =	70.0
	%D =	0.0
	Curve =	8
45.100	Flow =	0.25
	%B =	0.0
	%C =	0.0
	%D =	100.0
	Curve =	8
47.100	ECD_1.AcqOff	
	Flow =	0.25
	%B =	0.0
	%C =	0.0
	%D =	100.0
	Curve =	8

47.200	Flow =	0.25
	%B =	80.0
	%C =	0.0
	%D =	0.0
	Curve =	8
49.200	Flow =	0.25
	%B =	80.0
	%C =	0.0
	%D =	0.0
	Curve =	5
49.300	Flow =	0.25
	%B =	24.0
	%C =	0.0
	%D =	0.0
	Curve =	5
74.000		
	Flow =	0.25
	%B =	24.0
	%C =	0.0
	%D =	0.0
	Curve =	5
	End	

APPENDIX B - PROGRAM FOR CARBOHYDRATE ELIMINATION AND SUBSEQUENT SEPARATION OF AMINO ACIDS

	Pressure.LowerLimit =	0
	Pressure.UpperLimit =	4000
	%A.Equate =	“%A Water”
	%B.Equate =	“%B 250 mM NaOH”
	%C.Equate =	“%C 1 M NaAc”
	%D.Equate =	“%D 100 mM HAc”
	Flush	Volume = 250
	Wait	FlushState
	NeedleHeight =	3
	CutSegmentVolume =	0
	SyringeSpeed =	3
	pH.LowerLimit =	10.0
	pH.UpperLimit =	13.0
	Log	pH.Value
	ECD.Data_Collection_Rate =	0.8
	Cycle =	0
	Cell =	On
	Waveform Time = 0.00, Potential = 0.13	
	Waveform Time = 0.04, Potential = 0.13	
	Waveform Time = 0.05, Potential = 0.33	
	Waveform Time = 0.21, Potential = 0.33, Integration = Begin	
	Waveform Time = 0.22, Potential = 0.55	
	Waveform Time = 0.46, Potential = 0.55	
	Waveform Time = 0.47, Potential = 0.33	
	Waveform Time = 0.56, Potential = 0.33, Integration = End	
	Waveform Time = 0.57, Potential = -1.67	
	Waveform Time = 0.58, Potential = -1.67	
	Waveform Time = 0.59, Potential = 0.93	
	Waveform Time = 0.60, Potential = 0.13	
	WaitForTemperature =	False
	Wait	SamplePrep
0.000	Flow =	0.25
	%B =	24.0
	%C =	0.0
	%D =	0.0
	Curve =	5
	Load	
	Inject	
	Wait	InjectState
	Flow =	0.25
	%B =	24.0
	%C =	0.0
	%D =	0.0
	Curve =	5

1.800	Pump_Relay_1 closed	
	ECD_1.AcqOn	
2.000	Flow =	0.25
	%B =	24.0
	%C =	0.0
	%D =	0.0
	Curve =	5
2.30	Pump_Relay_1 open	
8.000	Flow =	0.25
	%B =	36.0
	%C =	0.0
	%D =	0.0
	Curve =	8
11.000	Flow =	0.25
	%B =	36.0
	%C =	0.0
	%D =	0.0
	Curve =	8
18.000	Flow =	0.25
	%B =	20.0
	%C =	40.0
	%D =	0.0
	Curve =	8
21.000	Flow =	0.25
	%B =	16.0
	%C =	40.0
	%D =	0.0
	Curve =	5
23.000	Flow =	0.25
	%B =	16.0
	%C =	70.0
	%D =	0.0
	Curve =	8
45.000	Flow =	0.25
	%B =	16.0
	%C =	70.0
	%D =	0.0
	Curve =	8
45.100	Flow =	0.25
	%B =	0.0
	%C =	0.0
	%D =	100.0
	Curve =	8

47.100	ECD_1.AcqOff	
	Flow =	0.25
	%B =	0.0
	%C =	0.0
	%D =	100.0
	Curve =	8
47.200	Flow =	0.25
	%B =	80.0
	%C =	0.0
	%D =	0.0
	Curve =	8
49.200	Flow =	0.25
	%B =	80.0
	%C =	0.0
	%D =	0.0
	Curve =	5
49.300	Flow =	0.25
	%B =	24.0
	%C =	0.0
	%D =	0.0
	Curve =	5
74.000		
	Flow =	0.25
	%B =	24.0
	%C =	0.0
	%D =	0.0
	Curve =	5
	End	

APPENDIX C - WRITING, LOADING AND USING SHORT PROGRAMS (STRINGS) CONTROLLING THE SYRINGE PUMP

NOTE: all instructions given here are for a 24,000 step version of Versa6 pump (Kloehn) with a 500 µL syringe connected to its syringe port.

Before reading the following text you should complete the installation of the syringe pump as described in Section 2.3 of this manual. In completing the installation, you will have printed out the first 30 pages of the VersaPump 6 Manual and 16 pages of the “Kcomm Software…” manual.

Here are several examples of strings for use with the V6pump:

Prime

Carries out five pump strokes at ca. 4 sec per stroke. Useful for priming the syringe or changing the fluids in the syringe. Do not use without T8 being immersed in a fluid and T9 disconnected from T7.

o1A0gV6000A24000o-3V6000A0o1G5R

Constant Flow 25

Delivers a constant flow of transfer fluid at ca. 25 µL/min. See V6 Manual page 70 for instructions how to program other flow rates.

W4A0:Ao1A24000o-3gD1M28G23000JAR

Constant Flow 75

Delivers a constant flow of transfer fluid at ca. 75 µL/min. See V6 Manual page 28 for instructions how to program other flow rates. G value is set for maximum possible number of repeats. See V6 Manual page 38.

o1A0gV6000A24000o-3V60A0o-1G32768R

Initialize

W4R

All the way down

Moves the plunger to the lowest possible position

A24000R

All the way up

Moves the plunger to the highest possible position

A0R

Call Kloehn at 1-800-358-4342 if you need assistance.

The Pump Comm window you opened during installation step 2.3 I is shown below:

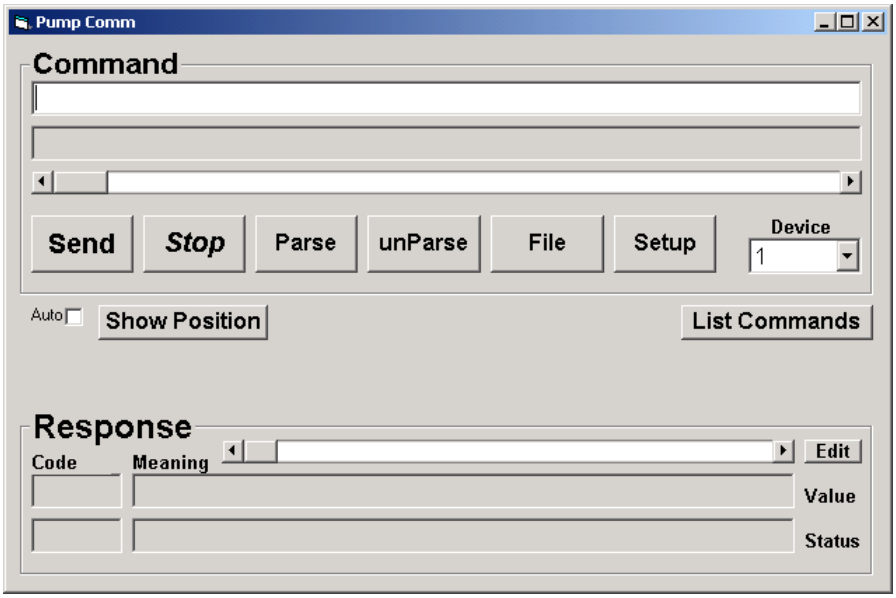

Figure 8

After completing the step 2.3 J, start loading the string from this Appendix into the Pump Comm as described in step 2.3 K. Highlight the string entitled Prime (only the string, not the title or description in italics). Copy the highlighted string and paste it into Command window using Ctrl V.

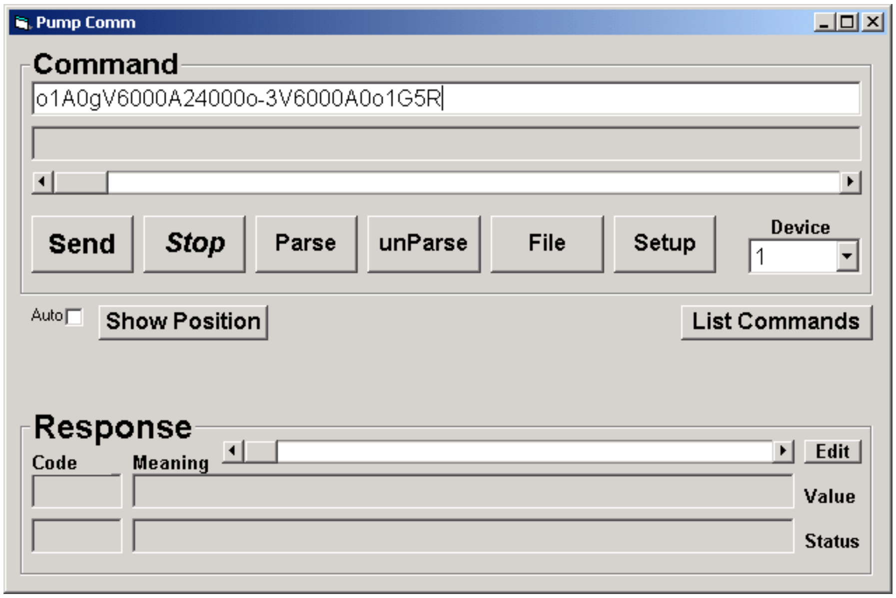

Figure 9

Click on File button to open the Program sub window.

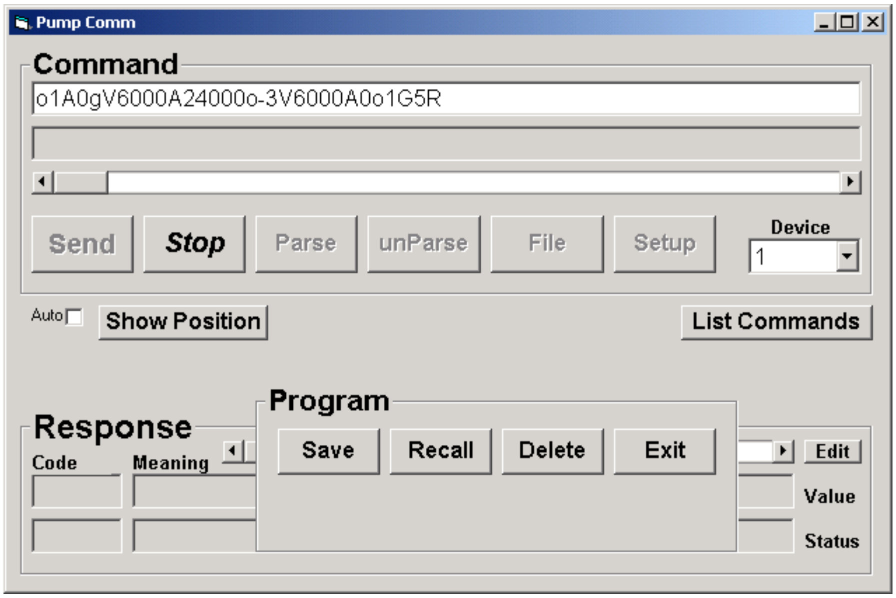

Figure 10

Click on Save to open an input field for string names. Type Prime and push the Save button again. The Prime string is now stored. Store the Constant Flow 75 string by repeating the above steps.

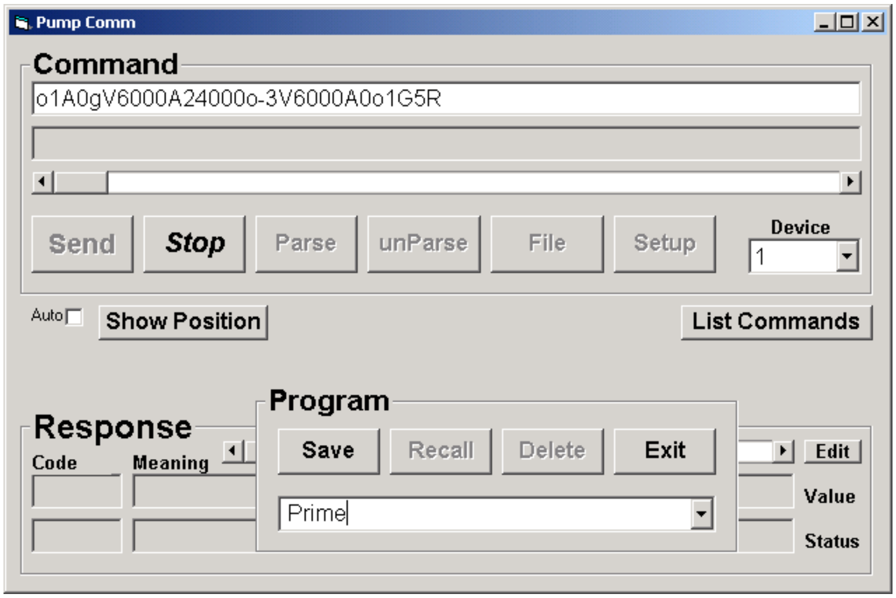

Figure 11

To activate a string push File/Recall then scroll to the string you need to work with and push Enter.

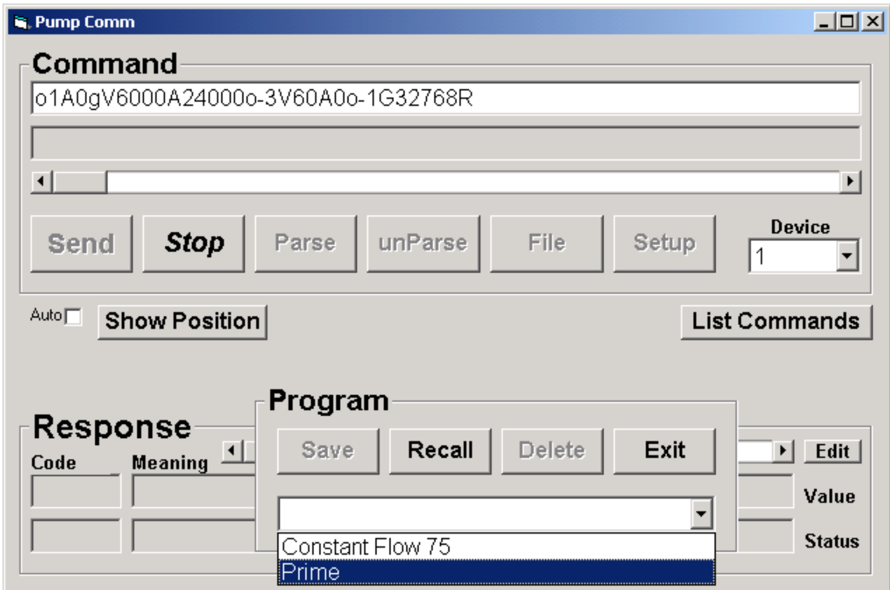

Figure 12

The string then appears in the Command field as shown below. After pushing the Send button the syringe pump begins executing the commands defined in the string.

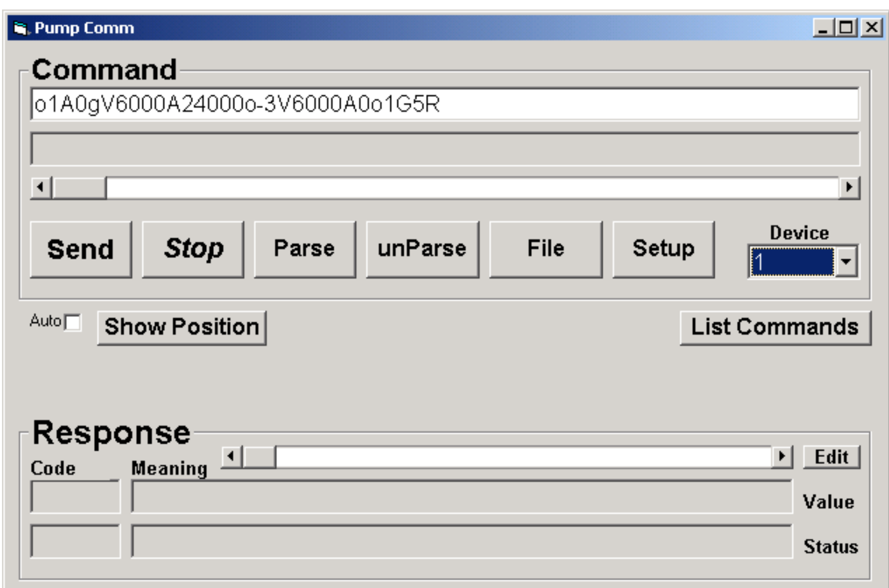

Figure 13

Prime: Explanation of commands

o1	set the valve to port A (clockwise)
A0	send the syringe to zero (all the way up)
g	begin a repeat loop
V6000	plunger speed 6000 steps/second (4 seconds/stroke)
A24000	fill the syringe (move plunger all the way down)
o-3	set the valve to port C (counterclockwise)
V6000	plunger speed 6000 steps/second (4 seconds/stroke)
A0	send the syringe to zero
o1	set the valve to port A (clockwise)
G5	repeat all commands between g and G five times

The “R” at the end means run this program now.

Constant Flow 75: Explanation of commands

W4	initialize the syringe
A0	send the syringe to zero
:A	Label A
o1	set the valve to port A (clockwise)
A24000	fill the syringe
o-3	set the valve to port C (counterclockwise)
g	begin a repeat loop
D1	dispense 1 step
M28	pause 28 milliseconds
G23000	dispense 23000 steps
JA	do the fill-dispense cycle again forever

The “R” at the end means run this program now.