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Technical Note 28

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Ion Chromatography/Inductively Coupled Argon Plasma (IC/ICAP): A New Technique for Trace Metal Determinations

INTRODUCTION

As the world becomes more environmentally conscious, the need for determining trace elements in diverse and complex matrices increases. Since many elements are regulated at the parts-per-billion (ppb) level, more sensitive and selective analytical methods are required. During the past decade, inductively coupled argon plasma atomic emission spectroscopy (ICAP-AES) has become the predominant technique for trace metal analysis. ICAP instruments offer detection limits in the low to mid ppb range, a range of elements encompassing more than half of the periodic table, rugged instrumentation and rapid analysis times (up to sixty elements in a minute). As with any analytical technique, ICAP does suffer from interferences. In many instances these interferences significantly compromise the detection limits and the accuracy of the determination. Common interferences in ICAP include high concentrations of alkali metals (sodium, potassium), alkaline earth metals (magnesium, calcium), and spectral interferences from elements such as iron and aluminum. As a result, ICAP detection limits are compromised in complex matrices such as brines, seawater, waste water, soils, sludges, and biological fluids and tissues.

Several common methods are used in order to minimize sample matrix effects in ICAP. These include spectral background corrections, inter-element spectral correction, standard additions and matrix matching. While all of these methods help to minimize sample matrix effects, none of these methods completely eliminates the matrix effects.

Ion exchange is a technique which has long been used for the concentration and separation of trace metals. The literature of ion exchange is extensive and the application of ion exchange for sample pretreatment prior to spectroscopic analysis is well known. Ion exchange offers the analyst a solution to the detection limit and interference problems commonly experienced when analyzing complex matrices by ICAP. Ion exchange can concentrate the analytes of interest while at the same time reduce or eliminate common interferences. Using a unique form of ion exchange sample pretreatment called chelation concentration, the analytes of interest are concentrated, while common interferences such as the alkali and alkaline earth metals are eliminated. In this Technical Note we describe a technique based upon the direct coupling of an ion chromatograph (IC) to a simultaneous ICAP for the determination of trace metals in complex matrices. Using chelation concentration, detection limits are lowered 10 to 100 times in complex matrices while eliminating common interferences.

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SUMMARY OF THE TECHNIQUE

A Dionex ion chromatograph comprising the Advanced Gradient Pump (AGP) and Sample Concentration Module (SCM) is interfaced directly to a Thermo Jarrell Ash (TJA) simultaneous ICAP (ICAP 61, or any IBM®-upgraded TJA simultaneous ICAP). The IC performs automated sample pretreatment using chelation concentration with the concentrated sample being pumped directly to the nebulizer. Most cationic transition and lanthanide metals are concentrated while alkali, alkaline earth and anionic species are eliminated.

An acidified sample, containing up to 3% (0.5 M) acid, can be loaded directly to the SCM. The SCM performs on-line buffering on the acidified sample just before the sample effluent enters the concentrator column. In the standard IC/ICAP configuration, a small volume (0.5 to 1.0 mL) of the untreated sample is pumped directly to the nebulizer while the remainder of the acidified sample is buffered with ammonium acetate and then pumped to the chelating concentrator column (MetPac™ CC-1). This configuration allows for "direct nebulization" and "concentration" in a single analysis. The direct nebulization allows for the determination of elements that are not concentrated. When concentrating 5 mL of sample, analysis time is about 8 minutes per sample.

Concentrating 5 mL of sample will lower the standard ICAP detection limits by a factor of 10 to 15. Since chelation concentration also eliminates interferences, the detection limit enhancement is generally much greater in "real world" samples. For example, using the IC to concentrate 5 mL of seawater will improve detection limits 50-fold compared to direct nebulization of seawater.

The IC/ICAP system is controlled, operated and integrated within standard TJA ThermoSpec software. The system can be fully automated with a ThermoSpec supported autosampler.

For more information about the ICAP system and ThermoSpec software, please contact your local Thermo Jarrell Ash representative. In the U.S., please contact the Thermo Jarrell Ash Corporation, 8E Forge Parkway, Franklin, MA 02038; Telephone (508) 520-1880.

INSTRUMENT REQUIREMENTS

The IC/ICAP system consists of a Dionex Chelation Concentration IC and a Thermo Jarrell Ash simultaneous ICAP spectrometer. Figure 1 shows a block schematic of the IC/ICAP system.

You will require the following instrumentation and accessories:

Chelation Concentration System (P/N 42134) comprising:

Advanced Gradient Pump (AGP, P/N 42144/115 V; P/N 42145/220 V)

Sample Concentration Module (SCM, P/N 42134/115V; P/N 42135/230V)

IC/ICAP Installation Kit (P/N 43169; contains eluent containers, air regulator, tubing, power cords and fittings for installation)

MetPac CC-1 Concentrator Column (2 pack; P/N 42156)

In addition to the items listed above, you will also need:

Electrical power

Compressed nitrogen or argon (80–120 psi/550–825 kPa)

Labcart or table for the IC

Standard analytical laboratory equipment such as a balance, pH meter, etc.

If you already own a Dionex Advanced Gradient Pump (AGP), you can perform IC/ICAP with the addition of the Sample Concentration Module (SCM).

Note: The Gradient Pump Module (GPM) may be substituted for the AGP, but only if the GPM firmware is version 3.18 or later (P/N 38546), which permits the GPM to be controlled by TJA ThermoSpec Software. Only the AGP will be referenced throughout the remainder of this technical note.

The SCM contains two single piston-type Dionex QIC Pumps (DQP), four 2000-psi (14-MPa) inert double-stack four-way pneumatically controlled slider valves, and a pulse damper. In addition, a peristaltic pump is used for loading the sample from the autosampler or sample container into the SCM. Use the peristaltic pump supplied with the ICAP. One of the DQPs is used to pump the acidified sample to the mixing tee and the other pump is used for delivering a "carrier" or eluent to the nebulizer. The MetPac CC-1 column used for concentration is also installed in the SCM. All of these components are housed in a single enclosure. The rear panel of the SCM contains bulkhead fittings for connecting wastelines, eluent lines and a sample inlet line. Refer to the *SCM Manual* (P/N 34206) for details pertaining to the operation and maintenance of the individual components of the SCM.

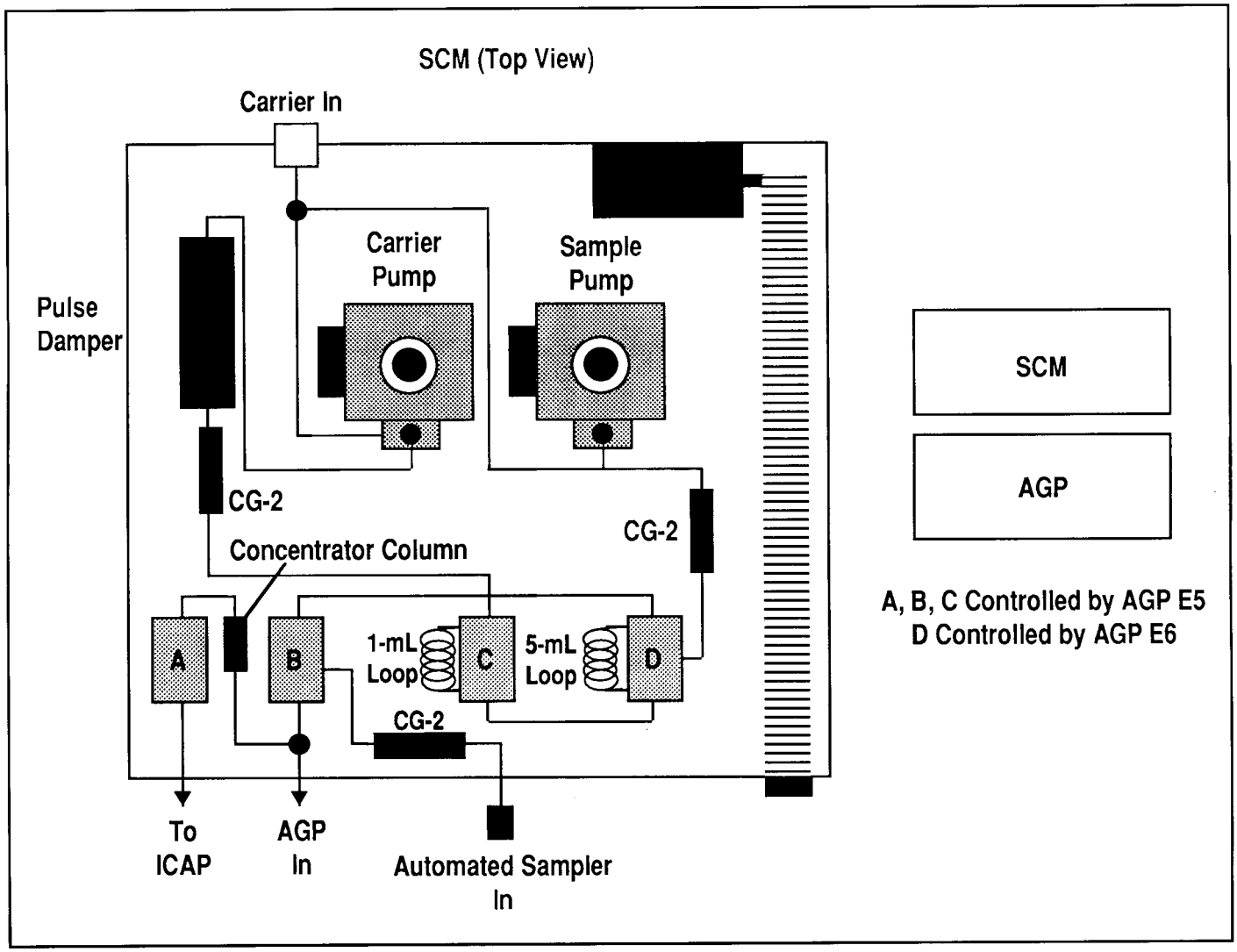

Figure 1 *Top view of the Sample Concentration Module (SCM) configured for ICAP analysis*

The AGP is a microprocessor controlled, high performance quaternary gradient IC pump. The AGP has a metal-free flow path and permits the time dependent selection of up to four eluents, flow rate, and the control of two pairs of air solenoids for external valve control. Controls 5 and 6 (E5 and E6) of the AGP are used to control the four valves present in the SCM. The AGP is programmable from the front panel and can store up to 10 different programs.

The AGP is controlled via an interface cable from the ICAP system controller. Any TJA simultaneous ICAP spectrometer (Models 61, 61E, 1100, 9000) can be interfaced to the Dionex IC as long as the simultaneous spectrometer is used with an IBM® or IBM-compatible computer with TJA ThermoSpec software. For system automation, a ThermoSpec-supported autosampler is required. A TJA type 22 or a TJA 300 autosampler can be used. The autosampler should use the larger sample racks (type 24) to ensure sufficient sample volume.

Questions concerning the compatibility of interfacing a particular TJA simultaneous ICAP instrument to a Dionex IC should be directed to your TJA sales or service representative.

CHEMICALS, REAGENTS AND STANDARDS

A complete list of reagents, preparation, and sources can be found in *Appendix A*. The two reagents used for chelation concentration, 2 M ammonium acetate, pH 5.5 (2 L; P/N 33440), and 2 M nitric acid (1 L; P/N 33442), are available ultrapure and ready to use from Dionex. See *Appendix A* for additional ordering information.

DISCUSSION OF THE METHOD

Concentration Chemistries

The method described in this report was developed to address some of the common analytical problems associated with ICAP. For many elements, the instrument detection limits obtained in ICAP would be sufficient for most analytical needs.

Unfortunately, interferences compromise detection limits. Common interferences in ICAP are the alkali and alkaline earth metals. Specifically, magnesium is a common spectral background interferant for many metals. By using selective ion exchange materials such as chelating resins, analytes may be concentrated while interferences such as the alkali and alkaline earth metals and anions are reduced or eliminated. This form of sample pretreatment is called chelation concentration. Unlike conventional ion exchange concentration methods which are typically not selective for ions of the same valency, chelation concentration is a selective concentration method.

Other common interferences of ICAP include iron and aluminum. These produce spectral interferences that are concentrated with the analytes of interest. A separate concentration method has been developed to selectively eliminate iron and aluminum from the analytes of interest (see Dionex Application Note No. 75). If iron and aluminum are not "chromatographically" removed, interfering element corrections (IECs) should be used for samples which are high in iron or aluminum.

The types of samples for which chelation concentration is applicable include seawater, brines, natural waters, waste waters, sediments, acid digested samples, fusions (KOH or $LiBO_2$), extracts and leaches, concentrated acids or bases as well as biological, botanical and geological materials. Chelation concentration is not intended for use when attempting to determine trace transition metals in the presence of large quantities of other transition metals (e.g., plating baths).

Most samples should be acid digested to ensure that the metals are free in solution and not bound by organic materials such as fulvic or humic acids. Complexing agents in the sample can interfere with concentration efficiency and recoveries. Any digestion, extraction, or fusion can be analyzed by this technique.

The column used for chelation concentration, the MetPac CC-1, contains a macroporous iminodiacetate chelating resin. The column has a capacity of 0.45 milliequivalents. The resin can be used with acid or base concentrations up to 6 M without degradation (*n.b.* do not store the resin in the acid form). The relative selectivity of the resin is:

$$\text{Lanthanides} > Hg >> Cu >> UO_2 > Ni > Pb > Zn > Co > Cd > Fe > Mn > Ba > Ca > Sr > Mg >> Na,K,Li$$

Properties of the MetPac CC-1 chelating resin are shown in Figure 2. The resin has very high affinity for transition and lanthanide metals compared to the alkali and alkaline earth metals. The selectivity of this chelating resin makes it ideal for use with a broad spectrum of sample matrices since most matrices will have high concentrations of alkali and alkaline earth metals relative to the transition metals.

The resin does not concentrate anions such as the halides, nitrate, sulfate, phosphate or organic anions. Unfortunately, anionic species such as arsenic (as arsenate or arsenite) and selenium (as selenate or selenite) are also not concentrated. Other species that are not efficiently concentrated by the MetPac CC-1 column include thallium (Tl^+) and some precious metals. Chromium as chromic ion (Cr^{3+}) is concentrated but not efficiently eluted, while chromate (CrO_4^{2-}) is not concentrated. Table 1 shows elements that are quantitatively concentrated using the MetPac CC-1.

In general, the higher the valency of the metal ion, the more strongly bound the metal ion is to the resin. Since the functional group of the resin is a weak acid (COOH) and a weak base (NH), hydronium ion (H_3O^+) competes strongly with metal ions for the chelating sites. As a result, nitric acid at 0.5 to 2.0 M is an effective eluent. Below a pH of 2.5, the MetPac CC-1 column will not concentrate transition metals. In the pH range of 5 to 6, the resin selectivity is optimized for transition and lanthanide metals relative to alkali and alkaline earth metals. By using an ammonium acetate eluent in this pH range, alkaline earth metals can be eluted while the transition and lanthanide metals remain strongly bound to the resin.

Up to 300 mL of seawater can be concentrated on the MetPac CC-1 column before breakthrough of the transition metals. Brine concentrations of 22% sodium chloride have been concentrated with quantitative recoveries. With a 1.0 M calcium solution, up to 40 mL can be concentrated with quantitative recovery of the transition elements. Flow rates up to 4.0 mL/min can be used with the MetPac CC-1 with quantitative recovery of the transition metals.

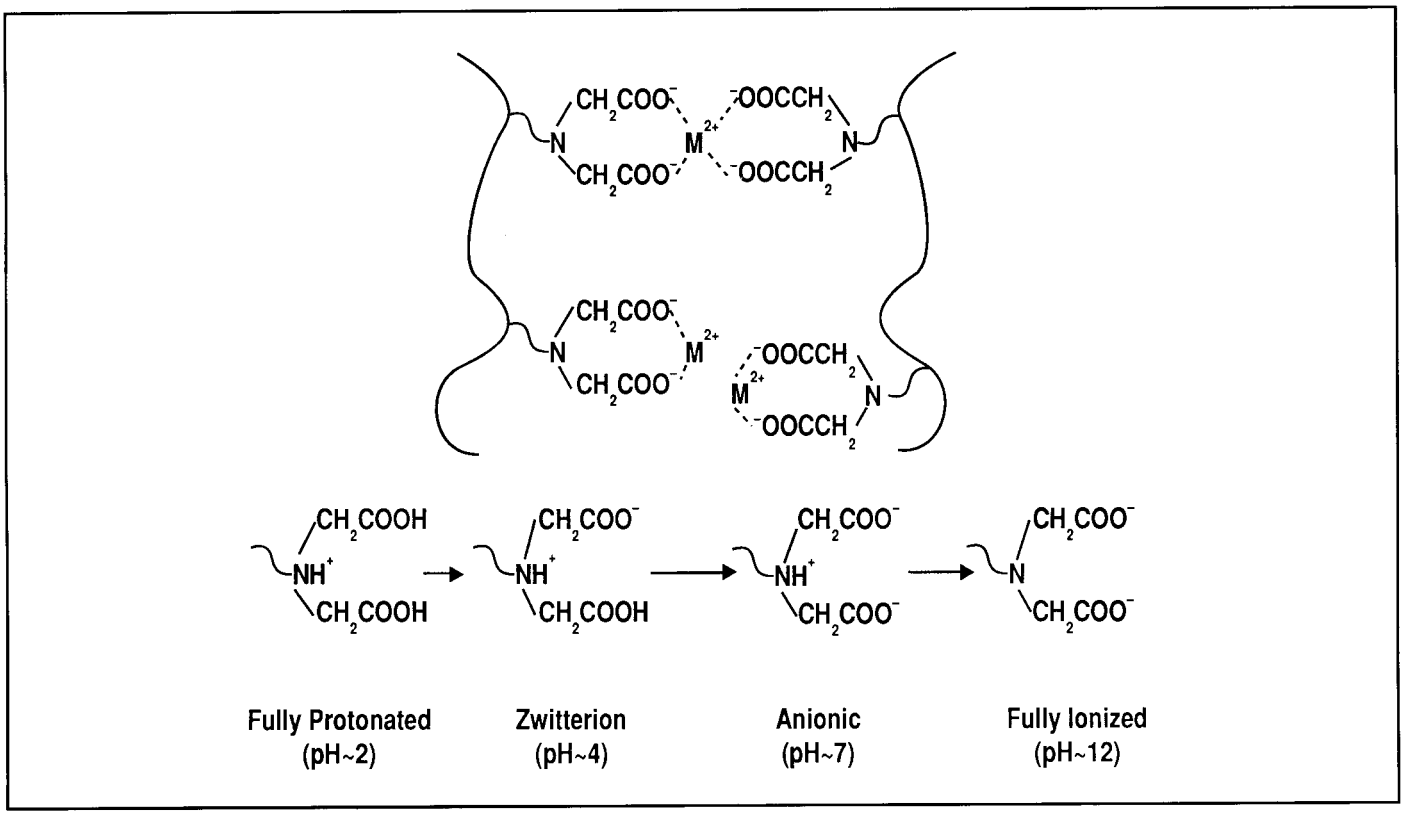

Figure 2 *Properties of MetPac CC-1 Chelating Resin*

Table 1 Retention Characteristics of the MetPac CC-1 Column

Metal Ion	Quantitative	Metal Ion	Quantitative
Ti(IV)	Yes	Cd(II)	Yes
V(IV,V)	Yes	In(III)	Yes
Cr(III)	No	Y(III)	Yes
Mn(II)	Yes	Lanthanides	Yes
Fe(II, III)	Yes	Hg(II)	Yes
Co(II)	Yes	Pb(II)	Yes
Ni(II)	Yes	Al(III)	Yes
Cu(II)	Yes	Tl(I, II)	No
Zn(II)	Yes	As(III< IV)	No
Ag(I)	No	Se(IV, VI)	No

Chelation concentration consists of four major processes.

1. A known volume of the sample is buffered on-line and passed through the MetPac CC-1 column. Most polyvalent cations are quantitatively concentrated from the sample while anions pass through the column. Alkali metals are weakly retained. Metals which are quantitatively concentrated are listed in Table 1.

2. Weakly bound alkaline earth metal ions such as magnesium and calcium are selectively eluted with a 2 M ammonium acetate eluent (pH 5.5, 9 to 12 mL), which is pumped by the AGP. During this elution process, at least 98% of the magnesium and 95% of the calcium on the column will be eliminated. Some manganese (10 to 15%) will be eluted. This does not preclude quantitation of manganese since the percentage of manganese eluted during the ammonium acetate wash is constant.

3. Next, the concentrated transition and lanthanide metals are eluted in a 100 to 200 µL volume using 1 to 2 M nitric or hydrochloric acid (8 to 10 mL) delivered from the AGP.

4. Finally, the MetPac CC-1 is converted back to the ammonium form using 2 M ammonium acetate (5 to 6 mL). This regeneration step prepares the concentrator column for the next sample.

It is important to use reagents and water which have very low metal contamination (less than 1 ppb). Any trace metals in the reagents will be concentrated as a "blank" and subsequently eluted with the sample. The system blank results from contamination in the chelation concentration reagents and the system. Generally, iron and zinc are the most common transition metal contaminants, but a small amount of copper may be observed as well. Care must be taken to minimize reagent and sample contamination during preparation and handling. Reagent purity will usually dictate the detection limits. A description of the necessary reagents are listed in Appendix A.

SEQUENCING OF THE IC AND ICAP FOR AUTOMATION

This section will describe the sequencing and operation of the system components. The IC/ICAP system has been designed for use either manually or in the fully automated mode. Table 2 presents the standard AGP program for the MetPac CC-1 column. Since the IC is controlled by the ICAP computer, the discussion below places the IC functions relative to the ICAP. The following describes the various operations of the IC during the gradient program.

1. **Time 0.0**
   Valves A, B, and C switch ON. The peristaltic pump begins pulling sample from the autosampler through the loops and out to waste. The AGP is pumping ammonium acetate through the column for regeneration.

2. **Time 2.0**
   Valves A, B, and C are OFF and valve D is ON. The carrier pump is pumping through the 1-mL loop and bringing the previously loaded sample to the nebulizer. At the same time, the sample pump has begun pumping the carrier solution (0.1 M nitric acid) at a flow rate of about 2.0 mL/min through the 5-mL loop, which was previously loaded with the sample. The acidified sample from the sample loop is mixed with 2.0 M ammonium acetate buffer from the AGP, and the buffered sample passes through the concentrator column and out to waste.

AGP Program for Chelation Concentration IC/ICAP Program 1 (for 5 mL sample loop)

E1: 2 M Ammonium acetate, pH 5.5 E2: 2 M Nitric acid E3: Deionized water

t (min)	%E1	%E2	%E3	V5	V6	Flow Rate (mL/min)	Event
0.0	100	0	0	1	0	2.0	buffer column, load loop
2.0	100	0	0	0	1	2.0	dir. neb., load column
5.0	100	0	0	0	0	4.0	selective elution
6.2	100	0	0	0	0	4.0	
6.3	0	75	25	0	0	4.0	start elution of metals
6.7	0	75	25	0	0	2.0	
6.9	0	75	25	1	1	2.0	conc. metals to neb.
8.3	0	75	25	1	1	2.0	
8.4	100	0	0	0	0	0.0	buffer column, stop EXP.

3. **Time 5.0**
   Valves A, B, C, and D are OFF. The contents of the 1-mL loop have been pumped to the nebulizer and the carrier pump is still delivering carrier to the nebulizer. The 5-mL sample has been loaded on the concentrator column and the selective elution of calcium and magnesium from the column is in progress.

4. **Time 6.3**
   Valves A, B, C, and D are OFF. The carrier pump is still delivering carrier to the nebulizer. The AGP now switches to nitric acid and begins eluting the concentrated metals. The sample pump is still on and is pumping carrier solution to waste.

5. **Time 6.9**
   Valves A, B, C, and D are ON. The AGP flow rate drops to 2.0 mL/min as the concentrated band is being eluted of the column. The 5-mL sample loop is being rinsed with the carrier (0.1 M nitric acid).

6. **Time 8.4**
   Valves A, B, C, and D are OFF. The carrier pump is now switched in line with the nebulizer. The exposure will be completed by this time. In the automated mode, a new sample cycle will begin with the autosampler proceeding to the rinse station and then to the next sample.

The IC is controlled by a relay from the ICAP system controller. When an ICAP run is initiated, the computer sends a signal to the intelligent controller. The controller either begins in a flush mode or in an exposure mode. At the initiation of an exposure, a signal is sent to the AGP. If the AGP is in the "start" mode, the signal from the ICAP controller to the AGP will reset the AGP program to time 0.0 and the program will begin to run. If the ICAP method is written with a "flush" time, the system controller begins the flush cycle but no signal is sent to the AGP. As a result, the AGP continues its program uninterrupted.

Sample loading starts at the beginning of the AGP program (t = 0.0). The flush mode is not required at the beginning of the ICAP sequence. Two steps occur at the beginning of the AGP program. At 0.0 minute, the AGP starts conditioning the chelating concentrator column by pumping the buffer solution through the column and to the nebulizer. The IC also starts loading sample to the 5-mL loop and then to the 1-mL loop, and out to waste. Typically, a minimum of 6 mL of sample is required. However, in order to prevent carryover and to completely flush the sample loops, it is recommended to pump at least 9 mL of sample. Since the peristaltic pump is operating at approximately 5 mL/min, the sample loading time of 1.8 to 2.0 min is required. The subsequent steps following the sample loading and column conditioning include on-line buffering of the acidified sample, selective elution of interfering species and acid wash.

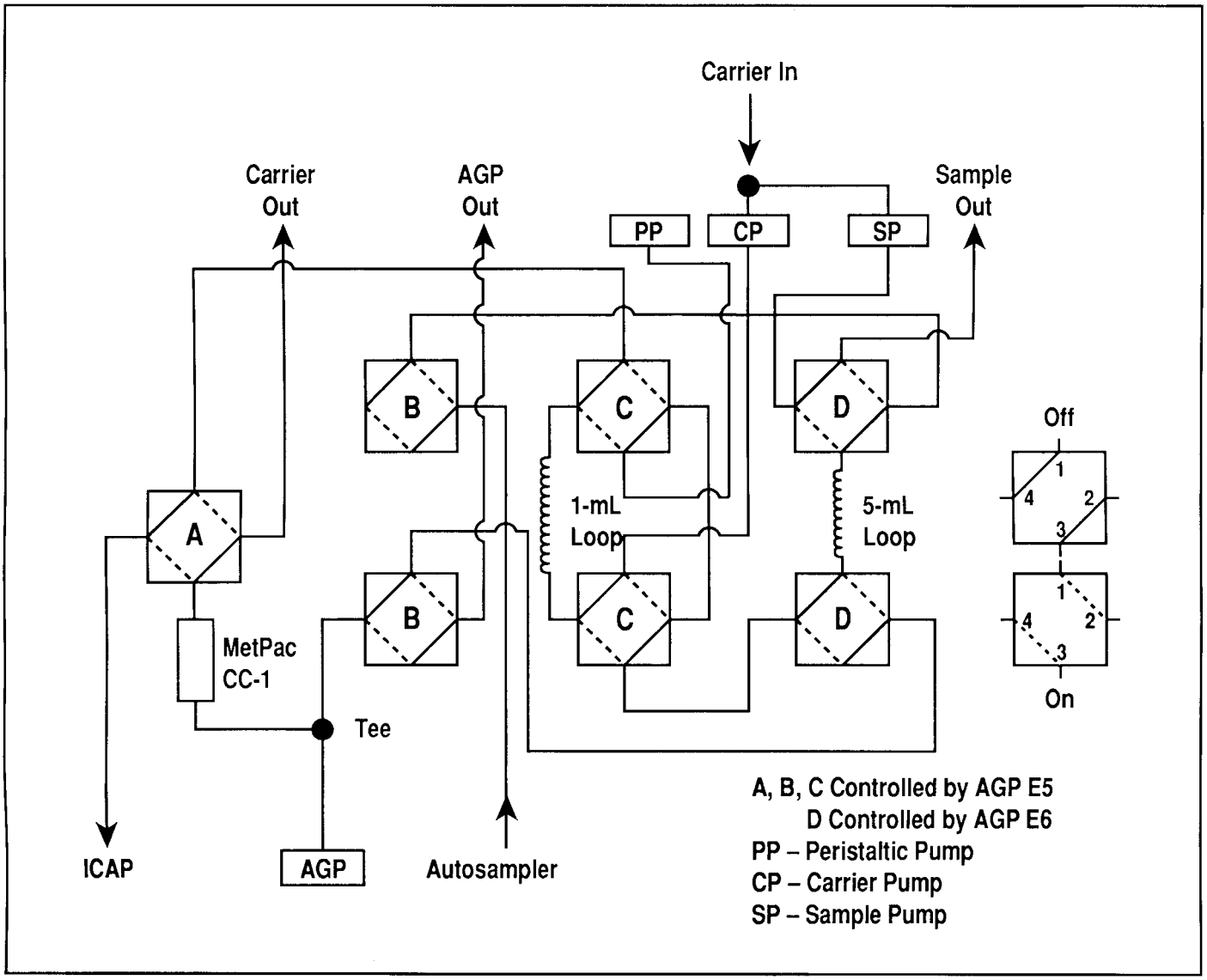

Figure 3 *Schematic for Sample Concentration Module (SCM) automated on-line buffering*

SYSTEM CONFIGURATION AND SET-UP

Figure 3 shows a detailed pneumatic and hydraulic schematic of the IC system. The SCM is factory-configured with a 1-mL sample loop for direct nebulization and a 5 mL loop for concentration. The following set-up procedure is designed for the standard (factory) SCM configuration.

Pneumatic Connections

Locate the four colored air tubings at the rear panel of the AGP and SCM. Using the small barbed couplers (P/N 42241), couple the air tubing together by matching the colors (pink-pink, yellow-yellow, green-green and blue-blue). Next, connect about 2 ft (60 cm) of air tubing (P/N 30091) to the small barbed fitting on the back of the AGP. Insert a barbed tee (P/N 30538) into the end of this tubing. One arm of the tee will go to the nitrogen or argon source (regulator) and the other arm will go to the inlet of the eluent bottle regulator (P/N 38201). Using the required lengths of tubing, connect the tee to the gas source and to the eluent pressure regulator. Use the 1/4-in. to 10/32 brass reducer (P/N 30087) and the 10/32 x 1/16-in. barbed fitting (P/N 30071) to connect the air tubing to the source regulator.

Next, connect the air tubing to the eluent container caps (P/N 41004). Start by cutting one of the two 1/8-in. Teflon® lines flush with the bottom of the cap. Repeat this for all three eluent container caps. Next, cut the same tubing about 2 in. above the eluent container cap. This line will be used to connect the argon or nitrogen for pressurizing the eluent bottles. (The eluent bottle caps should contain a white TFE O-ring (P/N 41078) and not a black rubber O-ring. If black O-rings are present, replace with the TFE O-ring.) Insert a barbed coupler (P/N 42241) into the trimmed Teflon line of cap E1. Insert a barbed tee (P/N 30538) into the trimmed Teflon lines of caps E2 and E3. Connect the eluent caps using the air tubing (P/N 30091 or equivalent). This completes the pneumatic set-up.

Hydraulic Connections

Refer to the *AGP* and *SCM Operator's Manuals* for details pertaining to the installation and operation of the respective modules. Begin the hydraulic connections by connecting the three eluent lines from the three eluent container caps to the front panel eluent ports of the AGP. Notice that the eluent lines are labeled 1, 2 and 3. Be sure that the eluent lines are connected to the appropriate eluent port of the AGP.

Connect the eluent line from the four liter plastic eluent container (P/N 39164) to the CARRIER IN port of the SCM rear panel. Next, connect the three blue waste lines (P/N 39341) to the ports of the SCM rear panel labeled CARRIER OUT, AGP OUT, and SAMPLE OUT and place them in a waste container. For the sample inlet line, connect the 0.037-in. I.D. x 36-in. (92-cm) length of pink tubing to valve B, port 2, in the SCM. Locate THE SAMPLE IN port of the rear panel, and use the 1/8-in. I.D. tubing (white tubing) to connect this port to the peristaltic pump inlet. Finally, remove the end fitting from the 0.020-in. I.D. x 36-in. (92-cm) tubing connected to valve A, port 4 of the SCM. Using a pair of pliers, stretch the end of this tubing as to taper the tubing to about two-thirds of its original outside diameter. Using about 3/8 in. (1 cm) of 0.030-in. I.D. Tygon® tubing as a coupler, connect the tapered tubing to 6 in. (15 cm) of the nebulizer tubing. This is the actual liquid interface between the IC and the ICAP. Place the tubing in a waste container for the system test. This completes the hydraulic connections.

Electrical Connections

Verify that the front PUMP 1 and PUMP 2 power switches of the SCM are off. Using the power cords provided (P/N 96078), connect the AC receptacles on the rear panels of the SCM and AGP to the white outlets of the power strip located at the upper rear section of the system enclosure. Next, connect the AC receptacle of the power strip enclosure to an AC power outlet.

Next, install the interface cable (P/N 43044). One end of the interface cable connects to the rear panel of the AGP (via the telephone plug) and the other end connects to a connector in the ICAP main power board. **Start by turning off the line voltage to the ICAP intelligent controller. The circuit breaker to be turned off is located on the rear power panel of the ICAP instrument.** Next, loosen (or remove) the screws on the rear power panel and carefully open the hinged power panel to reveal the electronics. On the left side of the electronics panel is a printed circuit board containing three 12-pin Molex nylon connectors in a row. Carefully remove the center Molex connector (J3-N) by gripping the sides of the connector and pulling straight up. Note the positions of the wires in the connector. Using the Molex pin extractor tool, remove the wire(s) at 1 and 10 or 4 and 7 position in the Molex connector attached to the interface cable (see Fig. 4) and insert the wires of the interface cable into the same position. Finally, plug the interface cable Molex connector into the circuit board where

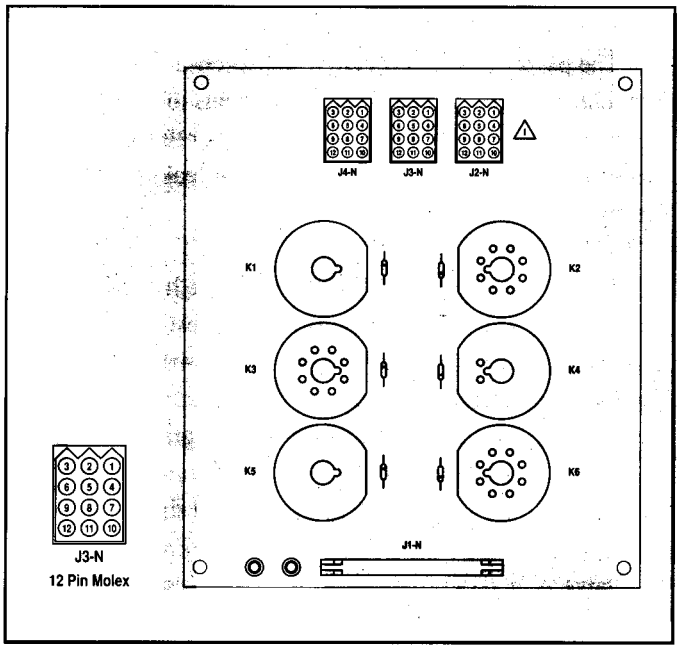

Figure 4 *Diagram of Molex connectors on the ICAP main power circuit board*

the original Molex connector was located. Carefully close the power panel being careful not to pinch or sever the interface cable.

To the right of the Molex connectors are six relay sockets. Check to see that there is a relay (labeled SOURCE or K1) installed in the relay socket labeled K1 as shown in Figure 4. This relay will be used to control the IC.

Finally, connect the interface cable to the relay connector on the rear panel of the AGP. Check to see that the AGP relay dip switches located on the right top cover of the AGP are in the OFF (forward) position. When the ICAP initiates an exposure, the AGP will be reset and will begin to execute the AGP program. This completes the electrical connections.

SYSTEM PREPARATION

1. Confirm that the SCM is configured as shown in Figure 3. Be sure that an IonPac® CG2 column is installed between the AGP and valve B (before the mixing tee) of the SCM. Confirm that the other two CG-2 columns are also installed on the two DQP outlet check valves (see Fig. 1). Install a MetPac CC-1 column between the coupled lines connecting valve A and the mixing tee.

2. Remove the mixer (GM-2 or GM-3) from the low pressure side of the AGP. This mixer is located between the valve manifold and the priming block of the AGP. Connect the two lines using a coupler (P/N 39056). Do not install a mixer on the high pressure side of the AGP (i.e., between the AGP and the SCM). Refer to the *AGP Operator's Manual* for details of the AGP.

3. Prepare 1 L of 0.20 M oxalic acid by dissolving 25.2 g of reagent grade oxalic acid dihydrate in 1 L of deionized water. This eluent will be used to clean the AGP eluent flow path.

4. Connect the 0.20 M oxalic acid to Port 1 (E1) of the AGP. Pump the oxalic acid through the AGP and to waste at 2.0 mL/min for 10 minutes. Repeat this procedure for ports 2, 3, and 4. This helps to remove any trace metals from the AGP flow path.

5. Place the sample inlet tube into the 0.20 M oxalic acid solution. Fill each of the three 1-L eluent glass bottles and the 4-L carrier bottle with 500 mL of 0.20 M oxalic acid. Enter the following program for system preparation. This program is entered from the front panel of the AGP. Refer to the *AGP Operator's Manual* for details on programming the AGP.

Time	E1	E2	E3	V5	V6	Flow
0.0	100	0	0	1	0	2.0
5.0	0	100	0	1	1	2.0
10.0	0	0	100	0	1	2.0
15.0	100	0	0	0	0	2.0
20.0	100	0	0	0	0	0.0

Before turning the pump switches on, confirm that the sample pump and the carrier pump are primed and their flow rates are set at 2.0 mL/min. Turn the peristaltic pump switch on and adjust its flow rate to at least 5.0 mL/min. Start the AGP and run the above program for 2 to 3 times.

6. Replace the 0.20 M oxalic acid in the 4-L carrier bottle with 0.1 M nitric acid. Be sure the cap has an O-ring for proper sealing. Refer to Appendix A for details on preparing the carrier solution.

7. Clean three 1-L glass eluent bottles by filling them with 0.20 M oxalic acid. Allow the acid to remain in the eluent bottle for at least 4 hours. Prepare eluents as described in Appendix A. Use caution in preparing and transferring these reagents in order to minimize contamination. Connect the filled eluent bottles to the appropriate eluent cap connected to the AGP. Be sure that the eluents are plumbed to the proper ports of the AGP (E1: 2 M ammonium acetate; E2: 2 M nitric acid; E3: deionized water). Adjust the eluent bottle regulator to 4 to 6 psi and check for any gas leaks.

8. Prime the AGP with each eluent using the following procedure. Enter Program 2 into the AGP. Set the flow rate to 3.0 mL/min and enter 100% of E1. Start the AGP and loosen the needle valve located on the pressure transducer housing. This will flush any air out of the eluent lines and the pump components and which will prime the pump. Repeat this procedure for E2 and E3. Be sure to tighten the needle valve upon completing the priming.

9. Enter the following AGP program for chelation concentration. This program is entered from the front panel of the AGP. Refer to the *AGP Operator's Manual* for details on programming the AGP. Check the program carefully for accuracy by listing the program. (This program is also shown in Table 1.)

Time	E1	E2	E3	V5	V6	Flow
0.0	100	0	0	1	0	2.0
2.0	100	0	0	0	1	2.0
5.0	100	0	0	0	0	4.0
6.2	100	0	0	0	0	4.0
6.3	0	75	25	0	0	4.0
6.7	0	75	25	0	0	1.5
6.9	0	75	25	1	1	1.5
8.3	0	75	25	1	1	1.5
8.4	100	0	0	0	0	0.0

SYSTEM TEST

The purpose of this system test is to ensure that all chromatographic and chemical components of the system are operating properly. Refer to Figure 3 for the system schematic. Be sure to check all fittings for leaks during the system test.

1. The system test begins with a test of the hydraulic system. If the system fails the hydraulics test at any point, determine the source of the plumbing error. Begin by using Program 1. With the AGP in the stop-hold position, press RESET. This will set the program to time 0.0.

2. Press START on the AGP. Eluent (E1) should begin to flow to the mixing tee, through the MetPac CC-1 and valve A, and out to the nebulizer. Check the interface tubing on valve A, port 4, of the SCM to confirm that eluent is flowing to the ICAP.

3. Next, prime the carrier pump by loosening the tubing fitting which is screwed into the outlet check valve. Since the carrier reservoir is pressurized (5 psi or 35 kPa), the 0.1 M nitric acid solution should begin to flow out of the check valve. As the carrier solution begins to flow, turn on the carrier pump by pressing the PUMP 1 power switch on the SCM front panel. After about 5 seconds, replace the outlet check valve tubing fitting. It is generally only necessary to finger tighten the fittings. If the fitting leaks, tighten it another 1/8 of a turn using a 5/16-in. open-end wrench.

4. Set the stroke dial of the carrier pump to about 8.00. (Refer to the *SCM Operator's Manual* for details on adjusting the flow rate.) After about 90 seconds, check that the carrier is flowing out of the CARRIER OUT tubing.

5. Calibrate the carrier pump flow rate by mass or volume to 1.8 to 2.0 mL/min. Be sure that the carrier reservoir is pressurized to 4 to 6 psi. Turn off the carrier pump (PUMP 1).

6. Repeat step 3 on the sample pump. Press PUMP 2 power switch on the SCM front panel. Check that the carrier is flowing out of the SAMPLE OUT tubing. Calibrate the sample pump flow rate by mass or volume to 1.8 to 2.0 mL/min. Turn off the sample pump (PUMP 2).

7. Place the sample inlet tube in a container of deionized water and start the peristaltic pump. If the sample loop is not filled, it may take about 120 seconds before DI water begins to exit the waste line of the peristaltic pump. Adjust the peristaltic pump flow rate to at least 5 mL/min.

8. List the AGP program to 2.0 minutes and press run. This will forward the program to 2.0 minutes. Check to see that the eluent is flowing out of the CARRIER OUT tubing. Stop the AGP and confirm that the eluent flow stops from the CARRIER OUT tubing. Turn on the sample pump (Pump 2). Check that the carrier is flowing out of the CARRIER OUT tubing. Turn on the carrier pump (PUMP 1) and confirm that the carrier flows out of the interface tubing.

9. List the AGP program to 5.0 minutes and press start and run. Stop and start the pump to confirm that the carrier from sample pump is flowing out of the SAMPLE OUT tubing.

   If the sample pump looses prime, prime the sample pump by loosening the tubing fitting on the outlet check valve. If there is no liquid in the check valve, use a squirt bottle and squirt some deionized water into the check valve. This will aid in priming the pump. Replace the outlet check valve fitting. If you are going to use an autosampler, connect the autosampler probe to the sample inlet line.

10. With the AGP in the start-run mode, start an ICAP exposure. As soon as the ICAP controller starts the exposure (red EXP light is on), the AGP will reset to time 0.0 and the program will be executed. If the run is aborted, the AGP will continue to run. Allow the AGP to run to the end of the program. Reset and run the program one more time to be sure that the MetPac CC-1 is thoroughly regenerated.

11. Connect the interface tubing to the nebulizer. Turn on the carrier pump and the argon to the nebulizer and torch. Confirm nebulization of the carrier by looking for mist in the spray chamber. At this point, the ICAP torch can be ignited. This completes the system test.

IC/ICAP OPERATION

This section describes the integrated operation of the IC/ICAP system. For details on ThermoSpec software, refer to the appropriate TJA manual.

Methods Development

1. Begin by writing a ThermoSpec method entitled IC/ICAP. Table 1 lists the elements which can be concentrated using the MetPac CC-1 column. Under METHODS DEVELOPMENT/SET-UP, select the elements of interest (F1) and also select the duplicate (F3) function for each element. Select the elements and the duplicate of each element in the order in which you want them printed in the report. In addition, select sodium, potassium, magnesium, and calcium, if available. Do not duplicate these elements. Press F9 to save the element selection.

   Note: The order in which the elements are printed in the analysis report can be selected by the user under F8, OPTIONS from the main menu under METHODS DEVELOPMENT.

2. Next, press F3 for output. Change the NUMBER OF REPEATS to 1 and the CONCENTRATION to ppm or ppb. Select print limits as desired. Press F9 to save.

3. For F5, ELEMENT INFO, the element heading will be changed and the standard(s) concentrations entered. Start by changing the element heading. For the elements in timing group 1 (direct nebulization), enter the element symbol followed by "-DIR". For example, Cu-DIR, Fe-DIR, Na-DIR, etc. For the elements in the timing group 2 (concentrated), enter the element symbol followed by "-CON". For example, Cu-CON, Fe-CON, etc. Next, check to see that elements labeled "-DIR" are in timing group 1 and those elements labeled "-CON" are in timing group 2.

   Enter the appropriate concentration for each element. Refer to Appendix B for recommended standard concentrations. Generally a two-point calibration routine is used, a blank and a high standard, HIGH STD. Be sure that the correct concentration is entered for each element and that only a high standard and a blank appear in the method. Press F9 to save. Be sure to save the entire method.

4. At this point it is necessary to run a time scan. Press F6 for SCAN and F7 for TIME SCAN. The TIME SCAN feature is used to determine the retention times of the direct nebulization and the concentrated bands. Place the sample inlet tube into the high standard and turn on the peristaltic pump.

5. In the TIME SCAN mode, the spectrophotometer monitors the plasma for a user-specified period of time (much like a chromatographic detector). Enter an integration "slice" of 5.0 and press ENTER. Enter 100 for the number of time slice. This will result in a time scan of 500 seconds. If the eluents are prepared properly, the concentrated sample band should elute between 425 and 475 seconds.

6. Press F1, RUN. The controller goes to an exposure mode (the EXP LED will light), a signal is sent to the AGP that resets the program to time 0.0, and the AGP program begins running. The exposure will end in 500 seconds.

7. The results of the time scan displayed on the screen should look like Figure 5. For the concentrated elements, two discrete regions are present in the time scan. The first region between 140 seconds and 160 seconds is the result of direct nebulization. This represents the 1-mL loop of raw sample that was delivered to the nebulizer by the carrier pump. The second region is typically between 425 and 475 seconds and represents the 5-mL loop of sample that was concentrated and subsequently eluted off the MetPac CC-1 column. Press F1, EXPAND, and move the cursor to determine the time at which the peak begins to elute. Note this time for comparison to the next run. Using the cursor, determine the width of the peak at the base. Typical base width is 25 to 35 seconds.

   Use the cursor to determine the time at which a steady state of (PURE SAMPLE) sample is present in the direct nebulization. Steady state occurs at the elevated, flat portion of the direct nebulization region. This usually occurs between 140 and 160 seconds. Note the time of the steady state.

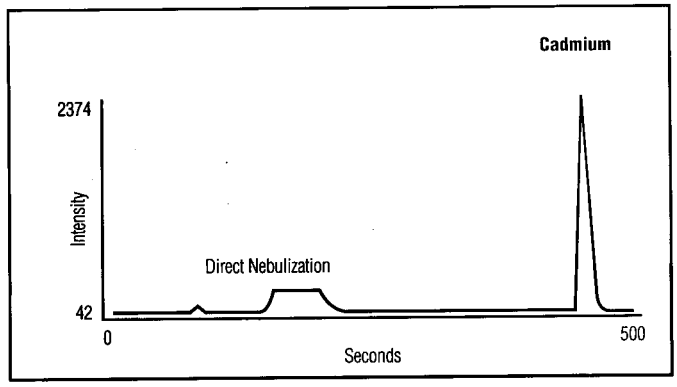

Figure 5 *Time study using chelation concentration for cadmium*

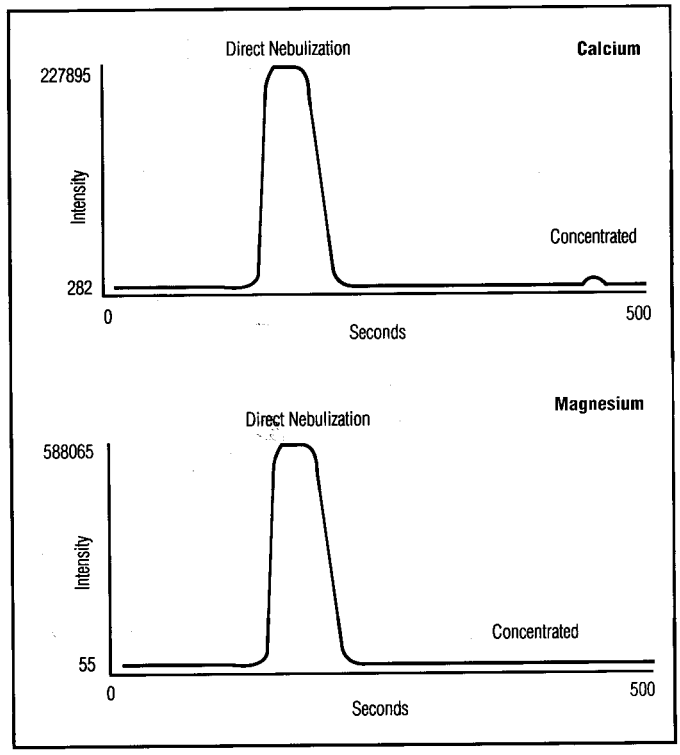

Figure 6 *Time studies using chelation concentration for calcium and magnesium*

Elements that are not present (or greatly reduced) in the concentrated band include sodium, potassium, magnesium, and calcium. Figure 6 shows the time scans for calcium and magnesium. For magnesium, notice a large signal in the direct nebulization, but virtually no signal in the concentrated band. This time scan shows the benefit of the matrix elimination capability of the method.

8. Use the F5, OVERLAY function to overlay several of the time scans. Notice that all of the concentrated metals elute with nearly the same retention times. The time scan can be stored if required.

9. Repeat the time scan. The retention time of the peak should not vary by more than 3 seconds. Continue to repeat the time scan until the retention time variation is within 3 seconds. Return to the main menu for methods development.

10. Use the F2, INTERNAL STANDARDS function to enter the timing groups. For timing group 1, enter a preintegration time (in seconds) determined from the direct nebulization region of the time scan. This preintegration time is the time from the beginning of the exposure to steady state. This is typically about 140 seconds. For the integration time, enter 10 to 20 seconds, depending on the duration of the steady state signal.

    For timing group 2, enter a preintegration time determined from the concentration region of the time scan. The preintegration time is the time from the beginning of the exposure to when the peak(s) begin to elute. It is advisable to enter a preintegration time about 3 to 5 seconds less than the measured times to allow for minor shifts in retention times. For the integration time, add about 5 seconds to the measured base width and enter this value (typically 35 to 45 seconds).

11. Press F9, SAVE, to complete the methods development. Be sure to save the entire method.

Standardization

Standardization of the ICAP system with the IC is performed in the same manner as normal ICAP standardization. Standardization can be performed manually or with the autosampler.

1. In the ANALYSIS section of ThermoSpec, press F3 for STANDARDIZATION. For the IC/ICAP method, two entries should appear: BLANK and HIGH STD. Prepare a high standard as directed in Appendix B. The blank contains 1% nitric acid (trace metal grade).

2. Run the blank. After standardization with the blank, compare the intensity values obtained for the direct ("DIR") and concentrated ("CON") timing groups for the appropriate elements. The intensity values should be approximately the same and should correspond to blank intensities obtained without the IC. The CON values for elements such as zinc, iron, and copper may be significantly higher (2 to 4 times) than the DIR values. This results from trace metals in the ammonium acetate or nitric acid solutions being concentrated on the column and subsequently eluted. In general, if the reagent solutions are relatively pure, the CON values for iron and zinc should not be greater than three times the DIR value. No appreciable copper blank should be present. If the blank values appear within range, press F9, SAVE, and continue.

3. Next, run the high standard. Compare the intensity values obtained for the direct and concentrated values. For all elements concentrated, the CON value should be greater than the DIR value. If the values are in range press, F9, SAVE, and complete the standardization (press F9 again). If the CON values are not at least two times greater than the DIR values, then either an insufficient amount of the high standard was loaded or the standard was not prepared properly.

4. Finally, run the high standard as a sample. The reported values should be within 1 to 3% of the calibrated values. If the values are out of range, run the standard again. If the result is still out of range, run another time scan and repeat the standardization procedure. This completes standardization. The system is now ready for sample analysis.

Sample Preparation

It is beyond the scope of this technical note to describe in detail all of the techniques of ultratrace analysis in terms of sample collection, storage, and handling. However, several points are discussed below that are applicable to sample preparation before analyzing samples by the method described in this technical note.

Samples should be collected in **clean** polyethylene containers. In order to stabilize the sample for storage, the sample should be acidified to a pH 1.5 to 2. Be sure to use ultrapure nitric acid to adjust the pH.

To ensure complete recovery of metals using chelation concentration, metal ions should not be bound by any strong complexing agents or be present as hydroxy complexes. Acid digestion is a general technique used to destroy complexing agents or to minimize their complexation ability. If you are analyzing samples that may contain large amounts of organic materials (e.g., humic acids), it is advisable to digest the sample. If there is solid material in the sample, it should be digested. In general, if you have used digestion for sample pretreatment prior to metal analysis, those same digestion procedures can be used in this method. **Never attempt to concentrate a sample that contains solids or suspended materials.**

Because sample preparation plays a critical role in the accuracy and the precision of analytical measurements, any contamination from sample handling or addition of reagents to the sample must be avoided or minimized. Since the pH of any digested or undigested sample is normally maintained in the acidic range, a direct introduction of such acid samples into an analytical system would minimize the sample contamination. The acidified sample can be neutralized by on-line addition of a buffer solution to the sample stream before it enters the chelator column. Because the sample pH is maintained in the acidic range, the adsorption, hydrolysis, and precipitation of metal ions that can occur during sample preparation will be avoided.

An acidified sample, containing up to 0.5 M acid, can be loaded directly. The SCM performs on-line buffering on the acidified sample just before the sample effluent enters the MetPac CC-1 column. If the sample contains more than 0.5 M acid (e.g. digested samples), it is recommended that 4 to 6 M ammonium acetate be used to neutralize the acidified sample. Use port 4 of the AGP for a 4.0 M ammonium acetate (pH 5.5) solution, and correct the percent eluent on the AGP to E4 at time 2.0 min and 5.0 min.

Sample Analysis

Samples can be run either manually or automated using the IC/ICAP method. To run samples manually, place the sample probe (inlet) in the sample solution before starting the ICAP sequence.

In the automated mode, set up the autosampler with type 24 racks (14 samples per rack). Each sample vial must contain at least 12 mL of buffered sample. Make the first sample in the first rack a blank; this will allow for proper sequencing of the IC, ICAP and autosampler. Be sure to enter the appropriate dilution factor for each sample in the autosampler table.

APPENDIX A: PREPARATION OF REAGENTS

The following reagents are required. The two eluents used for chelation concentration are available from Dionex in a ready-to-use form. If you wish to prepare your own reagent solutions, information for ordering ultrapure acids and ammonium hydroxide is also provided. Three concentrated reagents are required for eluents in this method: Nitric acid, acetic acid, and ammonium hydroxide. For ultratrace level determinations (sub-ppb) the reagents must be ultrapure grade. For determinations above 1 ppb, high quality trace metal grade reagents can be used. Any metal impurity in these reagents will be concentrated with your sample constituting a system blank.

Chelation Concentration Reagents:
Dionex Ultrapure Eluents

2 M Ammonium acetate, pH 5.5 (1 L, P/N 33440; 6 L, P/N 33441)

2 M Nitric acid (1 L, P/N 33442; 6 L, P/N 33443) or acetic acid, ultrapure (Seastar or Ultrex, 1 L)

Ammonium hydroxide, ultrapure (Seastar or Ultrex, 1 L)

Nitric acid, ultrapure (Seastar or Ultrex, 1 L)

Oxalic acid dihydrate (100 g), trace metal grade

Metal standards, 1000 or 10,000 ppm, 100 mL each

In North America, Seastar Ultrapure Reagents can be obtained through Fisher Scientific under the Optima label. Ultrex reagents can be obtained through Van Waters and Rogers (VWR) Scientific. If you cannot obtain these reagents through these sources, please contact:

Seastar Chemical
318 Second Ave. South
Seattle, WA 98104
U.S.A.
Tel: (206) 623-2855

Ultrex Reagents
J.T. Baker
222 Red School Lane
Phillipsburg, NJ 08865
U.S.A.
Tel: (201) 859-9357

Eluents and Standard Preparation

Before preparing eluents and standards, thoroughly clean the eluent containers as directed in "System Preparation", earlier in this Technical Note. Be sure that the eluent bottle caps have a white TFE seal and **not** a black rubber seal. Prepare all eluents directly in the 1-L glass eluent containers. Transfer reagents directly from their container. Avoid using pipets or graduated cylinders unless they have been thoroughly cleaned.

Chelation Concentration Eluents: Transition Metals

Use only ultrapure chemicals and deionized water (less than 0.5 ppb for each metal) for preparation of these reagents. Caution must be used in preparing this reagent in order to minimize metal contamination. Do not place anything in the eluent container (including stir bars). When adjusting the pH of the ammonium acetate and ammonium nitrate, do not place the pH electrode in the bulk solution. Instead, take aliquots of the solutions to check the pH.

Eluent 1: 2 M Ammonium Acetate, pH 5.4 ±0.1

Place 600 mL of deionized water into a clean 1-L glass eluent container. Add 121 g (115 mL) of ultrapure glacial acetic acid and mix thoroughly. In a fume hood, **slowly** add 120 g (130 mL) of 20% ultrapure ammonium hydroxide and mix thoroughly. Agitate the bottle to thoroughly mix the solution. Calibrate a pH meter to pH 7. Pour about 10 mL of the buffer into a small container (e.g., scintillation vial or 10 mL disposable beaker) and measure the pH. If the pH is below 5.3, add about 5 mL of ammonium hydroxide to the buffer solution. If above pH 5.5, add 5 g of acetic acid. Adjust the pH of the ammonium acetate to 5.4 ±0.1 using acetic acid if the pH is greater than 5.5, or ammonium hydroxide if the pH is less than 5.5. Once the pH is 5.4 ±0.1, bring up to a volume of 1 L.

Eluent 2: 2.0 M Nitric Acid

Place 200 mL of deionized water into a clean 1-L glass eluent container. Add 179 g (126 mL) of ultrapure nitric acid. Add deionized water to bring the final volume to 1 L and mix thoroughly.

Eluent 3: Deionized Water—HPLC Grade

Carrier Solution: 0.1 M Nitric Acid

Place about 1 L of deionized water into the 4 L plastic eluent container. Add 89 g (63 mL) of concentrated nitric acid and an additional 3 L of deionized water.

Concentrated Sample Buffer: 6 M Ammonium Acetate, pH 5.5

Place 200 mL of deionized water into a clean 1-L glass eluent container. Add 363 g (345 mL) of ultrapure glacial acetic acid and mix thoroughly. In a fume hood, **slowly** add 360 g (390 mL) of 20% ultrapure ammonium hydroxide and mix thoroughly. Agitate the bottle to thoroughly mix the solution. Calibrate a pH meter to pH 7. Pour about 10 mL of the buffer into a small container (e.g., scintillation vial or 10 mL disposable beaker) and measure the pH. If the pH is

below 5.3, add about 15 mL of ammonium hydroxide to the buffer solution. If above pH 5.5, add 15 g of acetic acid. Adjust the pH of the ammonium acetate to 5.4 ±0.1 using acetic acid if the pH is greater than 5.5, or ammonium hydroxide if the pH is less than 5.5. Once the pH is 5.4 ±0.1, bring up to a volume of 1 L.

APPENDIX B: PREPARATION OF STANDARDS

Listed below is a typical standard Dionex uses for a variety of sample matrices. Since the analytes of interest are concentrated, the concentration of the high standard used should not exceed 1 ppm. It is convenient to prepare a 10X concentrate or stock solution of the standard and prepare the high standard by dilution of the stock solution. Prepare the stock solution given below.

Metals	Concentration (ppm)
Alkali	200
Alkaline earth	100
Transition metals	1.0
Al, Fe, Pb	5.0

To prepare 100 mL of the standard listed below, place 10 mL of the stock solution in a 100 mL volumetric flask. Add 1 mL of concentrated nitric acid, trace metal grade. Bring the solution to a final volume of 100 mL.

Metals	Concentration (ppm)
Alkali	20
Alkaline earth	10
Transition metals	0.1
Al, Fe, Pb	0.5

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