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Application Note 77

Elimination of Iron and Aluminum as Matrix Interferences for Determination of Transition Metals Using Chelation Ion Chromatography

INTRODUCTION

The naturally occurring high concentrations of certain metals, such as iron, aluminum, alkali metals and alkaline earth elements, usually interfere with the determination of the trace transition metals. For atomic spectroscopy techniques, several common methods are used in order to minimize the sample matrix interferences. These include spectral background corrections, standard additions, sample dilution, and matrix matching. For chromatographic separation with postcolumn derivitization, the high concentrations of iron and aluminum not only interfere with the separation, but also the detection of other elements of interest. Sample dilution and standard addition are commonly used to reduce matrix effects. These methods help to minimize matrix interferences; however, these methods often result in degraded detection limits and accuracy.

The chelation concentration technique offers the analyst a solution to the detection limit and interference problems commonly experienced when analyzing complex matrices. Selective ion exchange materials such as chelating resins can concentrate transition metals while eliminating alkali and alkaline earth elements. This selective elimination process has been extended to the elements iron and aluminum. By using a matrix selective complexing agent, 95% to 99% of the iron and aluminum are removed, while the other transition metals are quantitatively retained.

In this application note, the determination of trace transition metals in samples that contain high levels of iron and aluminum will be discussed.

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INSTRUMENT REQUIREMENTS

The chelation IC system is comprised of the components listed below. For complete details, please refer to the system configuration for Chelation IC System 2 in Dionex Technical Note 25.

Gradient Pump Module (GPM-2, P/N 37098) or Advanced Gradient Pump (AGP, P/N 43116)

Sample Concentration Module (SCM, P/N 42134)

Reagent Delivery Module (RDM, P/N 37030)

Variable Wavelength Detector Module (VDM-2, P/N 39646, internal or remote cell)

Eluent Degas Module (EDM-2, P/N 39550)

Eluent Container Set, Glass (P/N 38752)

Valve, 4-Way Slider Double Stack, 2000 psi/13.7 MPa (P/N 35914), three (3) required

IonPac® Membrane Reactor (P/N 35354, optional)

Dionex Corporation, P.O. Box 3603, Sunnyvale, CA 94088-3603, (408) 737-0700
Sunnyvale, CA (408) 737-8522; Westmont, IL (708) 789-3660; Houston, TX (713) 847-5652; Smyrna, GA (404) 432-8100; Marlton, NJ (609) 596-0600
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Dionex S.r.l., Rome, Italy, (06) 371-5454
Dionex S.A., Boulogne, France, (1) 4621-6666
Dionex B.V., The Netherlands, (076) 714-800
Nippon Dionex K.K., Yodogawa ku, Osaka, (06) 885-1213

LPN 034660 2.5M 3/92

Knitted Reaction Coil (P/N 39349)
MetPac™ CC-1 Column (P/N 42156)
TMC-1 Column (P/N 42155)
IonPac CG2 (2, P/N 35370)
IonPac CG5 (P/N 37029)
AI-450 or other data acquisition system

SOLUTIONS AND REAGENTS

Ultrapure 2.0 M ammonium acetate, pH 5.5
(1 L, P/N 33440; 6 L, P/N 33441)
Ultrapure 2.0 M nitric acid (1 L, P/N 33442; 6 L, P/N 33443)
Ultrapure 0.1 M ammonium nitrate (1L, P/N 33445)
20 mM Pyrophosphoric acid / 2.0 M ammonium acetate
20% Ultrapure ammonium hydroxide
Ultrapure glacial acetic acid
Chelex-100™, 50–100 mesh (Bio-Rad Laboratories)

The first three reagents used for chelation concentration are available from Dionex in a ready-to-use form. If you wish to prepare your own reagent solutions, please refer to "Preparation of Solutions and Reagents". The other ultrapure reagents are manufactured by Seastar Chemical and Ultrex Reagents. Seastar reagents are available internationally through Fisher Scientific; in North America, Fisher Scientific sells these reagents under the OPTIMA® label. Ultrex reagents are available internationally through J.T. Baker. Chelex-100 is used for eluent purification.

CONDITIONS

Chelation Concentration

Columns:	MetPac CC-1, TMC-1
Eluent 1:	20mM Pyrophosphoric acid/ 2.0M ammonium acetate, pH 5.5
Eluent 2:	2.0 M ammonium acetate, pH 5.5±0.1
Eluent 3:	1.0 M Nitric acid
Eluent 4:	0.1 M Ammonium nitrate, pH 3.4±0.3

Analytical Chromatography

Column:	IonPac CS5
Eluent:	0.0060 M Pyridine-2,6-dicarboxylic acid, 0.040 M sodium hydroxide, 0.090 M acetic acid
Flow Rate:	1.0 mL/min

Postcolumn

Derivitization Reagent:	0.5 mM 4-(2-Pyridylazo)resorcinol 1.0 M 2-Dimethylaminoethanol 0.5 M Ammonium Hydroxide 0.3 M Sodium Bicarbonate
Reagent Addition:	Membrane reactor or mixing tee
Reagent Flow Rate:	0.5 mL/min
Reactor:	Packed or knitted reaction coil
Detector:	VDM-2 or UDM, 520 or 530 nm

PREPARATION OF SOLUTIONS AND REAGENTS

Three concentrated reagents are required for eluents in chelation concentration: nitric acid, acetic acid, and ammonium hydroxide. For ultratrace level determinations (sub-ppb), the reagents must be ultrapure grade. For determinations above 5 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.

Preparation of Chelex-100 for Eluent Purification

Suspend approximately 30 g of Chelex-100 (50-100 mesh) resin in 300 mL of 2.0 M nitric acid (trace-metal grade) in a 1-L polyethylene bottle. Using a stir bar, stir the solution for approximately 10 min. Decant the nitric acid and the fine resin particles. Repeat the acid cleaning step twice before rinsing the resin with 500 mL of deionized water. Store the cleaned resin in 200 mL of 2.0 M ammonium acetate, pH 5.5, until use.

20 mM Pyrophosphoric Acid / 2.0 M Ammonium Acetate, pH 5.5 (Eluent 1)

Dissolve 3.7 g of pyrophosphoric acid (97%, Aldrich Chemical Co., Inc.) in 1 L of 2.0 M ammonium acetate, pH 5.5. Since pyrophosphoric acid is not available in ultrapure grade reagent, the trace transition metal contaminants in this solution can be removed by using Chelex-100 resin. Place the cleaned Chelex-100 resin into the pyrophosphoric acid / ammonium acetate solution and stir using a stir bar. After stirring the solution for 1 hr, decant the pyrophosphoric acid / ammonium acetate solution into the glass eluent container.

20 mM Pyrophosphoric Acid / 2.0 M Ammonium Acetate, pH 8.5 (Eluent 1 for Manganese Determination)

Dissolve 3.7 g of pyrophosphoric acid in 200 mL of 2.0 M ammonium acetate, pH 5.5. Add 500 mL of ammonium acetate to this solution. Then, add 60 g (65 mL) of 20% ultrapure ammonium hydroxide. Calibrate a pH meter to pH 7. Pour about 10 mL of the buffer into a small container (e.g., scintillation vial, 10-mL disposable beaker), and measure the pH. If the pH

is below 8.5, add about 5 mL of ammonium hydroxide to the buffer solution. If the pH is above 8.5, add 5 g of acetic acid. Adjust the pH of the ammonium acetate to 8.5 ± 0.1 using ammonium hydroxide. Once the pH is 8.5 ± 0.1, bring to a volume of 1 L. Place the cleaned Chelex-100 resin into the pyrophosphoric acid / ammonium acetate solution and stir using a stir bar. After stirring the solution for 1 hr, decant the pyrophosphoric acid / ammonium acetate solution into the glass eluent container.

2.0 M Ammonium Acetate pH 5.5 ± 0.1 (Eluent 2)

Place 600 mL of deionized or high purity water into a clean 1-L glass eluent container. Tare the bottle. 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, 10-mL disposable beaker, etc.), and measure the pH. If the pH is below 5.4, add about 5 mL of ammonium hydroxide to the buffer solution. If the pH is above 5.6, add 5 g of acetic acid. Adjust the pH of the ammonium acetate to 5.5 ± 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 5.5 ± 0.1, bring to a volume of 1 L.

1.0 M Nitric Acid (Eluent 3)

Place 200 mL of deionized or high purity water in a clean 1-L glass eluent container. Add 89.5 g (63 mL) of ultrapure nitric acid. Add deionized water to bring the final volume to 1 L and mix thoroughly.

0.10 M Ammonium Nitrate, pH 3.4 ± 0.3 (Eluent 4)

Place 200 mL of deionized water into a clean 1-L glass eluent container. Add 8.9 g (6.3 mL) of ultrapure nitric acid. Next, add 7.6 g (8.5 mL) of ultrapure 20% ammonium hydroxide. Add sufficient water deionized water to give a final volume of 1 L and mix thoroughly. Calibrate pH meter to pH 4.0. Take a 10-mL aliquot of the solution and measure the pH. Add either 0.10 M ammonium hydroxide or 0.10 M nitric acid in 3 to 5-mL aliquots to the bulk solution to adjust the pH. Continue taking aliquots and adjusting the pH to 3.4 ± 0.3.

PDCA Stock Solution

0.060 M PDCA
0.40 M Sodium Hydroxide
Place 200 mL of deionized water into a clean 1-L polyethylene bottle. Add 32 g (21 mL) of 50% sodium hydroxide and stir with a stir bar. While stirring, add 10.0 g of pyridine-2,6-dicarboxylic acid. Continue to stir for about 10 min or until all the PDCA has dissolved. Dilute to 1 L and stir thoroughly. Label the solution "0.060 M PDCA, 0.40 M NaOH".

Acetic Acid Stock Solution

0.90 M Acetic Acid
Place 200 mL of deionized water into a clean 1-L polyethylene bottle. Add 54 g (52 mL) of trace-metal grade acetic acid and dilute to 1 L. Label the solution "0.90 M Acetic Acid".

PDCA Eluent

0.0060 M PDCA
0.040 M Sodium Hydroxide
0.090 M Acetic Acid
Add 100 g (100 mL) of the PDCA and acetic acid solutions to a 1-L glass eluent container. Dilute to 1 L with deionized water. Label the container "0.0060 M PDCA, 0.040 M NaOH, 0.090 M Acetic Acid". The eluent should have a final pH of 4.6.

PAR Postcolumn Reagent

0.5 mM 4-(2-Pyridylazo)resorcinol
1.0 M 2-Dimethylaminoethanol
0.5 M Ammonium Hydroxide
0.3 M Sodium Bicarbonate
Prepare PAR directly in 1-L plastic reagent reservoir container (P/N 37054). To 200 g (200 mL) of deionized water, add 31 g (35 mL) of trace-metal grade ammonium hydroxide. Next, add 0.12 g of 4-(2-pyridylazo)resorcinol, monosodium, monohydrate, and ultrasonicate for 5 min. Stir the solution for several minutes with a stir bar to ensure that PAR has completely dissolved. Add 500 g (500 mL) of deionized water and then 89 g of 2-dimethylaminoethanol (DMAEOH). The solution should turn from red to orange yellow. Add 25.4 g of sodium bicarbonate and stir thoroughly until dissolved. Fill the reagent container with deionized water up to the threads on the neck, and stir. The color of the final solution should be yellow to yellow orange. Place the reagent container in the reagent reservoir.

PREPARATION OF STANDARDS

Standards should be prepared daily. Certain metals, especially iron (III), are not stable at pH 5.5 for more than a day. The standards described below are intended for the determination of metals in the low-ppb (ng/mL) range. If quantification at high levels is required, standards can be prepared at concentrations five times greater than those listed. The standards listed below are intended for use with the PDCA eluent.

Transition Metal Stock Solution

Using a variable volume micropipet, add the following volumes of 1000-ppm atomic absorption standards to a 100-mL volumetric flask.

Metal Ion	Volume (µL)	Final Concentration (µg/mL)
Fe^{3+}	200	2.00
Cu^{2+}	200	2.00
Ni^{2+}	400	4.00
Zn^{2+}	400	4.00
Co^{2+}	400	4.00
Mn^{2+}	400	4.00

Next, add about 1 mL of concentrated nitric acid (ultrapure) and dilute to volume. This stock solution will be used to prepare the calibration standards. The calibration standards can be prepared in 100-mL volumetric flasks or LDPE bottles. Be sure that the flasks or bottles have been thoroughly cleaned.

Single Level Calibration Standard

If you are using a 100-mL volumetric flask to prepare the standard, add 200 µL of stock solution and 15 mL of the 2 M ammonium acetate buffer and bring to volume.

If you are using a polyethylene or Teflon bottle, tare the empty bottle (without the cap) on a top loading balance.

Using a micropipet, add 200 µL of stock solution and 15 g of the 2 M ammonium acetate buffer. Next, add water to give a total mass of 100 g (±0.1 g). This single level calibration standard will have the following concentrations.

Metal Ion	Concentration (ng/mL)
Fe^{3+}	4.0
Cu^{2+}	4.0
Ni^{2+}	8.0
Zn^{2+}	8.0
Co^{2+}	8.0
Mn^{2+}	8.0

For calibration, concentrate at least 5 mL of this solution.

Multilevel Calibration Standards

The multilevel calibration method recommended uses standards at four concentrations. Using the procedure given in "Single Level Calibration Standard," prepare standards as given below.

Level	Volume, Stock Solution (µL)
L1	100
L2	200
L3	500
L4	1000

The multilevel calibration standards will have the following concentrations of metal ions.

Metal Ion	L1	L2	L3	L4
	Concentration (mg/mL)			
Fe^{3+}	2.0	4.0	10.0	20.0
Cu^{2+}	2.0	4.0	10.0	20.0
Ni^{2+}	4.0	8.0	20.0	40.0
Zn^{2+}	4.0	8.0	20.0	40.0
Co^{2+}	4.0	8.0	20.0	40.0
Mn^{2+}	4.0	8.0	20.0	40.0

Depending on the concentration of metal ions in the sample, the volume of the standards to be concentrated can be varied.

SAMPLE PREPARATION

To avoid hydrolysis of transition metals during long storage, it is recommended that the pH of the sample be maintained at 1–2. If the sample is digested with concentrated acid, it must be neutralized with ammonium hydroxide to pH 1–2 prior to analysis. Avoid using pipets and glassware, which usually contaminate the samples.

If the sample contains more than 400-ppm iron and more than 600-ppm aluminum in the final dilution, the sample should be further diluted. Note that the mass ratio of iron to aluminum and transition metals should not exceed 20,000 to 1. For example, if the sample contains 2% iron and 1-ppm copper, the sample must be diluted at least 50-fold to give less than 400-ppm iron and 2.5-ppb copper.

Caution: Samples should not contain high (%) levels of silica. Silicate may be precipitated in the column at pH 5.5. Digestion of such sample with concentrated hydrofluoric acid prior to chromatography is strongly recommended.

DISCUSSION OF THE METHOD

The method described in this application note was developed for determining trace transition metals in complex matrices containing high levels of alkali metals, alkaline earth elements, iron, and aluminum. The removal of iron and aluminum is based upon the electroselectivity difference between iron and aluminum with pyrophosphate eluate and iron and aluminum with iminodiacetate functionality on the stationary phase (MetPac CC-1). The other transition metals are not eluted by pyrophosphate and are quantitatively retained by the MetPac CC-1 chelating resin. The alkali and alklaline earth metals in the MetPac CC-1 column are removed with ammonium acetate eluent.

The selective elimination of iron and aluminum using chelation sample pretreatment is possible only with an on-line buffering system. Off-line buffering of the samples that contain the high levels of iron and aluminum (<100 ppm) would result in precipitation of iron and aluminum. The SCM is equipped with a high pressure valve system that allows the acidified sample to be neutralized on-line with a known quantity of buffer solution. The neutralization by on-line buffering is instantaneous and the sample preconcentration processes occur in a few seconds. As a result, hydrolysis of aquated metal ions and adsorption of metal–hydroxide complexes on the surface of inert polymeric tubing is prevented.

The chelation concentration with selective elimination of iron and aluminum can be described as shown in Figure 1. A complexing agent is used which will selectively bind iron and aluminum, thus preventing uptake by the chelating resin during concentration. This approach not only prevents the precipitation of iron and aluminum at high concentration, it also allows an effective removal of iron and aluminum from the MetPac CC-1 column. The relatively stable metal–pyrophosphate (PP) complexes formed during on-line neutralization step (in-situ) do not interact with iminodiacetate and are not retained in the column. Figure 1 shows the scheme of the selective concentration using MetPac CC-1 with complexing agents.

The high levels of iron, aluminum, and manganese that are normally found in rock, sediment, and soil samples can be separated from trace transition metals by using 20 mM pyrophosphate, pH 5.5. Moreover, the rare earth, lanthanide, and transuranium elements, which normally are present in rock samples and may interfere with the analysis, are eliminated from the MetPac CC-1. The analytes of interest, such as copper, cadmium, cobalt, nickel, and zinc, are quantitatively retained by the MetPac CC-1 chelating resin. The IC/ICAP data indicated in Figure 2 shows recovery of metals using the MetPac CC-1 at various pyrophosphate concentrations. If manganese is one of the analytes of interest, it can be determined by using 10 mM pyrophosphate at pH 8.5 as noted in Table 1.

The recommended eluent for this application is pyridine-2,6-dicarboxylic acid (PDCA), which is a strong complexing agent that separates metal ion complexes by anion exchange. The 0.006M PDCA eluent is best suited for iron, copper, nickel, zinc, cobalt, cadmium, and manganese (see Technical Note 25 for complete details). Lead, on the other hand, can be determined using the oxalic acid eluent (again, see Technical Note 25 for more details).

The elimination of iron and aluminum by chelation ion chromatography has been applied to the determination of transition metals in geological materials. The matrix components that potentially interfere with the chromatographic separation and the postcolumn derivatization are eliminated during chelation concentration. An example of matrix components eliminated by chelation concentration is shown in Table 2. This table summarizes the interfering species present in the USGS Basalt rock sample. The results of analysis and the chromatogram are shown in Figure 2.

Example - Iron, Aluminum with Pyrophosphate (M^{3+} – Al^{3+}, Fe^{3+})

In-Situ -

$$M^{3+} + PP^{4-} \overset{K_{MPP}}{\rightleftharpoons} MPP^{-}$$

$$M^{3+} + 2PP^{4-} \overset{K_{MPP_2}}{\rightleftharpoons} M(PP)_2^{5-}$$

$$2M^{3+} + PP^{4-} \overset{K_{M_2PP}}{\rightleftharpoons} M_2PP^{2+}$$

Resin -

$$M^{3+} + R - N(CH_2COO^-)_2 \overset{K_R}{\rightleftharpoons} R - N(CH_2COO)M^{+}$$

$$M^{3+} + 2R - N(CH_2COO^-)_2 \overset{K_{R_2}}{\rightleftharpoons} 2R - N(CH_2COO)M^{-}$$

For Selective Concentration: $K_{MPP} >> K_R$ and $K_{MPP_2} >> K_{R_2}$

Figure 1 Chelation concentration using MetPac CC-1 with complexing agents

Table 1 Metal recovery from high calcium–iron–aluminum matrix using MetPac CC-1 with pyrophosphate for complexation

Element	Amount Added (ng/g)	Found (pH 5.5) ng/g (%RSD)*	Found (pH 8.5) ng/g (%RSD)*
Ca	50,000	15	15
Al	50,000	5	5
Fe	100,000	10	10
Pb	100	98.4 (3.08%)	99.3 (0.93%)
Cd	100	98.1 (1.20%)	99.5 (0.61%)
Cu	100	104 (0.80%)	135 (0.31%)
Co	100	100 (0.96%)	102 (0.25%)
Mn	100	70.2 (2.46%)	135 (0.49%)
Ni	100	98.3 (1.09%)	102 (1.07%)
Zn	100	98.2 (1.69%)	106 (1.17%)

*n = 4

Table 2 Matrix composition of USGS Basalt (BHVO-1) rock sample	
Element	**Concentration**
SiO_2	49.94%*
Al_2O_3	13.80%
Fe_2O_3	12.23%
MnO	0.168%
MgO	7.23%
CaO	11.40%
TiO_2	2.71%

* If sample contains high concentration of SiO_2, it must be treated with hydrofluoric acid prior to chelation concentration.

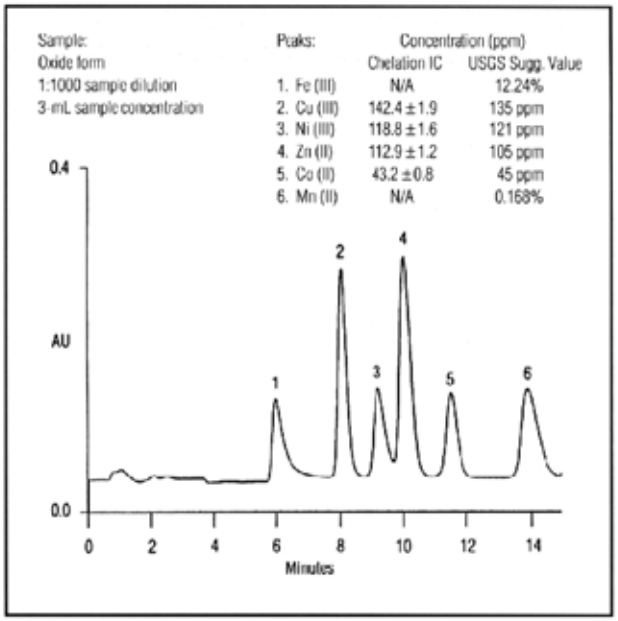

Figure 2 Determination of transition metals in USGS Basalt (BHVO-1) by chelation IC

SYSTEM PREPARATION, SET-UP, AND TEST

For complete details in system preparation and setup, operation and automation; refer to Technical Note 25.

1. A schematic diagram of the SCM configured for operation in Chelation IC System 2 is provided in Figure 4 in Technical Note 25. Confirm that the system is configured and plumbed as shown.

2. Ensure that there are no metal components in the flow path, including tubing end fittings (stainless steel washers, omni-fittings), columns, and valves, which contain stainless steel. Replace all omni-grippers with Thermo-Flare™ washers.

3. Follow the step-by-step "System Preparation" instructions given in Technical Note 25.

4. Four eluents are required for this application:

   E1: 20 mM pyrophosphoric acid / 2.0 M ammonium acetate
   E2: 2.0 M ammonium acetate
   E3: 1.0 M nitric acid
   E4: 0.1 M ammonium nitrate

5. If a 5-mL sample loop is used, enter the program provided in Table 3 into the gradient pump. For other sample loops, refer to the Appendix for instructions on how to create an appropriate gradient pump program.

6. Follow the step-by-step "System Test" instructions given in Technical Note 25.

SYSTEM OPERATION

The sequencing and operation of the system components are described below. This system configuration has been designed for use in the fully automated mode, except for the sample introduction step. If the required sample volume is not more than 3 mL, the Dionex ASM autosampler may be used. The sample must be introduced using a syringe or peristaltic pump unless the autosampler used is capable of delivering more than 5 mL of sample.

1. Confirm that the system is configured as given in Technical Note 25. Check to see that the system has a 80–120 psi (550–830 kPa) inert gas supply.

2. Turn the absorbance detector on. If the VDM-2 is being used, set the wavelength to 530 nm. If a filter based detector is being used, be sure the filter is 520 or 530 nm. Turn on the visible lamp and set the sensitivity to 0.2 AUFS. Be sure that the detector output is connected to a data collection system (integrator or ACI/AI-450).

3. Enter the program listed in Table 3. Check the program carefully by listing each step of the program.

4. Reset the gradient program at time 0.0 min. Turn on the carrier pump (Pump 1, 1.0 mL/min) and the RDM. Confirm that the PAR reagent is flowing through the detector by measuring the flow rate out of the waste line.

Table 3 Gradient program for chelation concentration

E1: 20 mM Pyrophosphoric Acid / 2.0 M Ammonium Acetate
E2: 2.0 M Ammonium Acetate
E3: 1.0 M Nitric Acid
E4: 0.1 M Ammonium Nitrate

t	%E1	%E2	%E3	%E4	V5	V6	Flow Rate (mL/min)
0.0	100	0	0	0	1	0	3.0
2.0	100	0	0	0	0	1	2.0
6.0	100	0	0	0	1	0	3.0
6.1	0	100	0	0	1	0	3.0
7.0	0	100	0	0	1	0	1.2
7.1	0	0	100	0	1	1	1.2
12.0	0	0	100	0	1	1	1.2
12.1	0	0	0	100	1	1	2.0
13.0	0	0	0	100	0	0	3.0
15.0	0	0	0	100	0	0	3.0
15.1*	0	0	100	0	1	0	4.0
16.0	0	0	100	0	1	0	4.0
17.0	0	0	100	0	1	0	4.0
18.0	0	100	0	0	1	0	0.0

* Begin sample analysis

5. Turn on the integrator or monitor and begin to monitor the baseline. At 0.2 AUFS, an essentially noise-free and drift-free baseline should be observed.

6. Step 1: Once the baseline is stable, start the gradient pump and press RUN. Confirm that valve 5 is ON and valve 6 is OFF. The sample or the standard can be loaded via the autosampler. If the autosampler is not used, use a syringe to load the sample by drawing the sample through the sample inlet. The sample pH should be 1–2. While the sample introduction step is in progress, the gradient pump is pumping 20 mM pyrophosphate to regenerate / equilibrate the MetPac CC-1 column. Note that the next step occurs at 2.0 min. If the sample introduction takes more than 2.0 min, adjust the gradient pump program accordingly (see the Appendix).

7. Step 2: Valve 5 is OFF and valve 6 is ON. The sample pump delivers the deionized water through the sample loop that was previously loaded with the sample. The sample stream is now mixing with the 20 mM pyrophosphate from the gradient pump, and the buffered sample passes through the MetPac CC-1 column.

8. Step 3: Valve 5 is ON and valve 6 is ON. Valve E now is switched to the LOAD position in which the TMC-1 column is placed in-line with the gradient pump. The gradient pump delivers 1.0 M nitric acid to the MetPac CC-1 column. The concentrated metal ions are eluted off the column, and the 1.0 M acid stream is diluted on-line to approximately 0.37 M with deionized water from the sample pump before it passes through the TMC-1 column. This step maximizes the removal of concentrated metal ions from the MetPac CC-1 and places them on the TMC-1 column in a tight band.

9. Step 4: Valve 5 is OFF and valve 6 is OFF. The gradient pump delivers 0.1 M ammonium nitrate to the TMC-1. This step is required to convert the TMC-1 from the hydronium form to the ammonium form.

10. Step 5: Valve 5 is ON and valve 6 is OFF. Valve E is now switched to the INJECT position in which the TMC-1 column is placed in-line with the IonPac CS5 column. The gradient pump delivers 1.0 M nitric acid to the MetPac CC-1 for 2 min (4.0 mL/min), followed by 2.0 M ammonium acetate for 1.0 min (4.0 mL/min) before the end of the chelation concentration process.

If you wish to start the chelation concentration on the next sample, you may start at this time. Remember that the analysis of the previous sample must be completed before proceeding to step 3 in which the TMC-1 column is switched in-line with the MetPac CC-1. The time to complete the analysis of transition metals is normally within 15 min. The gradient pump may be adjusted so that step 3 can proceed at 15.0 min.

11. Reset the gradient pump and data collection device. Start the overall cycle *without* injecting the sample. This run will represent the system blank. Repeat this cycle at least three times or until the blank is reproducible.

12. At this point, the system is ready for calibration and sample analysis. Refer to the appropriate section of Technical Note 25 for details on calibration and quantification.

APPENDIX: SAMPLE LOOP

The 1-mL and 5-mL sample loops are available and supplied with the SCM. If you wish to prepare a sample loop larger than 5.0 mL, use an appropriate length of 1/8-in. I.D. Tefzel® tubing.

To create a proper gradient program for a new sample loop, use the worksheet shown in Table 3 and follow the steps below:

1. Determine the loop loading time (*L*) starting from the sample source to the sample loop. If an autosampler is used, determine how much time the autosampler completes the loading step. If a syringe is used, 2.0 min is appropriate. Be sure that the sample loop is completely filled. A minimum of 2.0 min is required for the first step.

2. Determine the sample loading time (*C*) from sample loop to the MetPac CC-1 column. This value can be obtained by divided the sample loop size (mL) by the sample pump flow rate (mL/min). For proper sample loading, an additional 1.0 min is normally included.

   Example: Sample loop size = 7 mL
   Sample pump flow rate = 2.0 mL/min
   Sample loading time = C =
   (7 mL/2.0 mL/min) + 1.0 min = 4.5 min

3. Enter the *L* and *C* values in the work sheet shown in Table 3. Calculate and enter the new time. Enter the new program into the gradient pump.

Table 3 Gradient Program Work Sheet

t (min)	Enter New Time	E1	E2	E3	E4	V5	V6	Flow (mL/min)
0.0		100	0	0	0	1	0	3.0
L		100	0	0	0	0	1	2.0
L + C		100	0	0	0	1	0	3.0
L + C + 1.1		0	100	0	0	1	0	3.0
L + C + 2.0		0	100	0	0	1	0	1.2
L + C + 2.1		0	0	100	0	1	1	1.2
L + C + 7.0		0	0	100	0	1	1	1.2
L + C + 7.1		0	0	0	100	1	1	2.0
L + C + 8.0		0	0	0	100	0	0	3.0
L + C + 10		0	0	0	100	0	0	3.0
L + C + 10.1*		0	0	100	0	1	0	4.0
L + C + 11		0	0	100	0	1	0	4.0
L + C + 12		0	0	100	0	1	0	4.0
L + C + 13		0	100	0	0	1	0	0.0

*Begin sample analysis