

Determination of Transition Metals in Serum and Whole Blood by Ion Chromatography

INTRODUCTION

The determination of transition metals in physiological fluids is of considerable interest in clinical chemistry. In recent years several studies have linked the concentrations of specific transition metals to various diseases. Low serum copper level is used as a marker for Wilson's disease. Serum copper levels are elevated in a large number of chronic and acute illnesses such as Hodgkin's disease, leukemia, and many other malignancies.¹ Zinc is an important nutritive factor as well as a cofactor for many metalloenzymes. Zinc is necessary for the growth and division of cells, especially during the stages of life when growth rates are high. Zinc deficiency is associated with syndromes that cause short stature and dwarfism.² There is also interest in the biochemical relationship of copper and zinc.³ Studies have linked an increase in plasma copper level with decreasing plasma zinc concentration in childhood lymphatic leukemia. Determination of iron in whole blood is used to monitor anemia.

Traditionally, atomic absorption spectrophotometric (AAS) techniques have been used by most clinical chemistry laboratories to determine transition metals in physiological fluids. These techniques have their limitations. Flame AAS has limited sensitivity for copper, and graphite atomic absorption spectrophotometry is susceptible to solute vaporization interferences such as depression of element signal, especially in physiological samples.⁴⁻⁶ The protein content of the physiological fluid samples can cause absorption abnormalities. High sodium chloride content can hamper sensitivity, linearity, and cause burner clogging.

This application note describes an attractive alternative to traditional spectroscopic methods by using the principles of ion exchange. As a sample moves through the ion exchange column, bands of transition metals migrate through at differential rates determined largely by the relative affinities of the different metal-ligand complexes for

the stationary ion exchange sites. A strong metal complexing colorimetric reagent is supplied pneumatically and mixed with the column effluent. The bands of transition metals are then determined at a visible wavelength using an absorbance detector.⁷ Separation between individual metals can be enhanced or altered simply by changing eluents. Figure 1 illustrates the selectivity differences observed on an IonPac® CS5A column when using A) a pyridine-2,6-dicarboxylic acid eluent, and B) an oxalic acid eluent. This method is precise, sensitive, and requires minimum sample preparation.

RECOMMENDED EQUIPMENT

Dionex DX-500 system consisting of:

- GP40 Gradient Pump
- AD20 Absorbance Detector
- LC20 Chromatography Module

PC10 Postcolumn Pneumatic Controller

PC10 Automation Kit (optional)

PeakNet® Chromatography Workstation

REAGENTS AND STANDARDS

Reagents

Deionized water, 17.8 MΩ-cm resistance or higher
MetPac™ PDCA Eluent Concentrate and/or MetPac
Oxalic Acid Eluent Concentrate

MetPac Postcolumn Diluent

4-(2-Pyridylazo)resorcinol, monosodium, monohydrate
(P/N 039672)

Nitric acid, trace-metal grade (Fisher Scientific)

Sulfuric acid (Fisher Scientific)

Hydrogen peroxide, 30% (Fisher Scientific)

Trichloroacetic acid (Fluka Chemika-BioChemika)

Standards

Transition metal standards of 1000 mg/L are available from chemical supply companies (e.g. Aldrich, Sigma) for use with atomic absorption spectrometry. These are always dissolved in dilute acid solutions and can be used as IC standards.

CONDITIONS

Either of two analytical systems may be used for the chromatographic determination of transition metals. The most current method employs the IonPac CS5A, a highly efficient, solvent-compatible, mixed anion/cation exchange column. An older column, the IonPac CS5, may be used for these analyses, but efficiencies are superior and cadmium is better resolved on the CS5A. Refer to Table 1 for a complete listing of experimental conditions for both columns.

PREPARATION OF SOLUTIONS AND REAGENTS

Two eluent systems can be used for transition metal separations with the IonPac CS5A or CS5 column. The PDCA eluent is used for iron, copper, nickel, zinc, cobalt, cadmium, and manganese. The oxalic acid eluent is used for lead, copper, cobalt, zinc, and nickel. Cadmium and manganese coelute using the oxalic acid eluent.

MetPac PDCA Eluent

Dilute 200 mL of the MetPac PDCA Eluent Concentrate to 1.0 L with deionized water.

MetPac Oxalic Acid Eluent

Dilute 100 mL of the MetPac Oxalic Acid Eluent Concentrate to 1.0 L with deionized water.

PAR [4-(2-Pyridylazo)resorcinol] Postcolumn Reagent

Prepare the postcolumn reagent directly in the 1-L plastic reagent reservoir container. Add 0.15 g of 4-(2-pyridylazo)resorcinol, monosodium, monohydrate, to 1.0 L of the MetPac Postcolumn Diluent and ultrasonicate

Table 1 Conditions for Two Analytical Systems Used for the Chromatographic Determination of Transition Metals

Columns	IonPac CS5A analytical and IonPac CG5A guard	IonPac CS5 analytical and IonPac CG5 guard
Eluents	A) MetPac PDCA Eluent (Alternatively, 8.0 mM PDCA, 66 mM potassium hydroxide, 74 mM formic acid, and 5.6 mM potassium sulfate may be used.) B) MetPac Oxalic Acid Eluent (Alternatively, 8 mM oxalic acid, 50 mM potassium hydroxide, and 100 mM tetramethylammonium hydroxide may be used.)	A) 6.0 mM PDCA 40 mM Sodium hydroxide 90 mM Acetic acid B) 50 mM Oxalic acid 95 mM Lithium hydroxide
Flow Rate	1.2 mL/min	1.0 mL/min
Detection	Absorbance, 530 nm	Absorbance, 520 nm
Postcolumn Reagent	0.5 mM PAR, dissolved in MetPac Postcolumn Diluent. (Alternatively, 1.0 M 2-dimethylaminoethanol, 0.50 M ammonium hydroxide, and 0.30 M sodium bicarbonate may be used.)	0.4 mM PAR 1.0 M 2-dimethylaminoethanol 0.50 M Ammonium hydroxide 0.30 M Sodium bicarbonate
Postcolumn Reagent Flow Rate	0.7 mL/min	0.5 mL/min

for five minutes. Add a stir bar and stir for several minutes to ensure that the PAR has completely dissolved. The color of the final solution should be yellow to yellow-orange. Place the reagent container in the reagent reservoir.

SAMPLE PREPARATION

Biological matrices contain higher concentrations of alkali and alkaline earth metals than transition metals. In such instances, a chelation concentration step can be used where the alkali and alkaline earth metals are removed from the matrix and the transition metals are selectively concentrated. For the determination of trace metals in physiological fluids or tissues, the sample must first be

acid-digested to a single phase. In this application, the serum and whole blood samples were digested using the following procedure: to a 250-mL evaporation dish add 10–100 mL of sample. Next, add 5 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2 . Evaporate on a hot plate at medium heat to a volume of 15 to 20 mL. Cover with a watch glass to avoid sample loss by spattering. Transfer the concentrate and any precipitate to a 125 mL conical flask using 5 mL of concentrated HNO_3 . Add 10 mL of concentrated H_2SO_4 and a few boiling chips or glass beads. Evaporate on a hot plate in a hood until dense white fumes of SO_3 appear. If the solution does not clear, add 10 mL of concentrated HNO_3 and repeat evaporation. Remove all HNO_3 before continuing treatment. (All HNO_3 is removed when the solution is clear and no brownish fumes are evident.⁸) Cool and dilute to about 50 mL with eluent.

Alternatively, samples can be digested using nitric acid. Add 40 g of concentrated nitric acid to approximately 75 g of sample. Add a 10-mL aliquot of the digested sample to 20 mL of 2 M ammonium acetate. The final pH of the sample should be 5.5.

A trichloroacetic acid deproteinization procedure is sometimes used for serum and plasma samples. Add 0.2 mL of 50% trichloroacetic acid to 0.4 mL of serum or plasma sample. Centrifuge the mixture for 5 minutes at 1500 x g. Inject an appropriate volume of the supernatant (e.g. 25–50 μL).

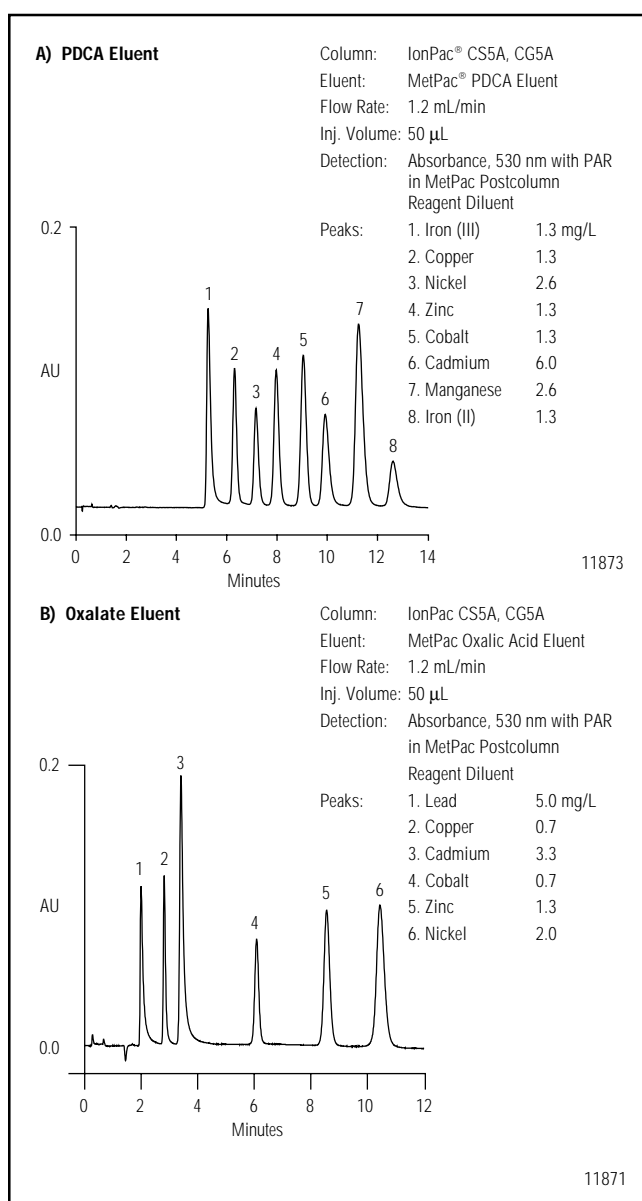


Figure 1. Separation of transition metals using A) PDCA Eluent and B) Oxalate Eluent (IonPac CS5A).

DISCUSSION AND RESULTS

The method outlined in this application note permits rapid separation of various transition metals. The separations are based on one of two different eluent systems. The first is a pyridine-2,6-dicarboxylic acid (PDCA) eluent, which is a strong complexing agent that separates the metal ion complexes by anion exchange. PDCA is best suited for iron(II) and iron(III), copper, nickel, zinc, cobalt, cadmium, and manganese (see Figure 2). This method allows one to speciate the oxidation states of iron, Fe(II) and Fe(III). However, since ferrous ion is easily oxidized to ferric, oxygen must be removed from the eluent by degassing. Oxygen should also be purged from the analytical column by pumping 0.1 M sodium sulfite (12.6 g/L Na_2SO_3) through the column for 2 hours.

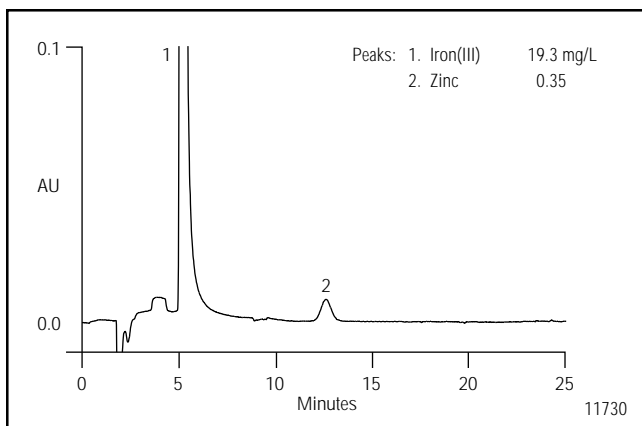


Figure 2. Determination of iron(III) and zinc in whole blood (IonPac CS5, PDCA eluent).

An alternative eluent system uses an oxalic acid-based eluent, which is a moderate strength complexing agent that separates the metals by a mixed mode mechanism. The oxalate eluent separates lead, copper, cobalt, zinc, and nickel (see Figure 3). Cadmium and manganese coelute with this eluent.

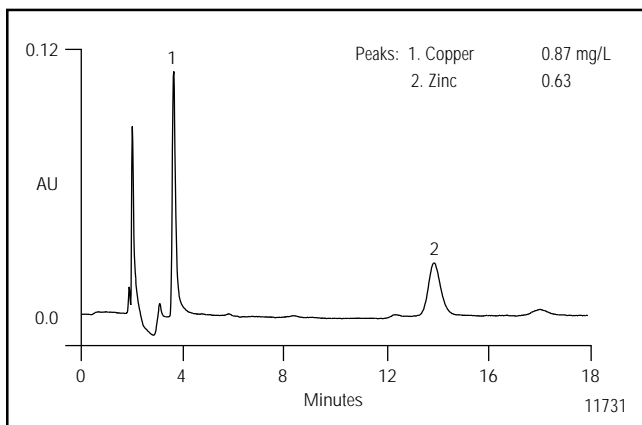


Figure 3. Determination of copper and zinc in serum (IonPac CS5, Oxalic Acid Eluent).

The separated metals from the analytical column enter a postcolumn reaction system where they are derivatized with 4-(2-pyridylazo)resorcinol and then detected at 520–530 nm using a UV/visible absorbance detector. This method is ideal for complex matrices such as physiological fluids. This method is highly sensitive, specific, and precise. Analyte recoveries for various physiological fluid matrices are listed in Tables 2 and 3.

Table 2 Precision and Recovery Data for Transition Metals in Whole Blood^a

Analyte	Amount Found (mg/L)	Amount Spiked (mg/L)	Mean Recovery (%)	RSD (%)
Iron(III) ^b	420	—	—	2.7
Zinc	1.2	3.0	95	1.3

^an = 7 replicates, 50 μ L injected.

^bIron(III) was not spiked.

Table 3 Precision and Accuracy Data for Transition Metals in Serum^a

Analyte	Amount Found (mg/L)	Amount Spiked (mg/L)	Mean Recovery (%)	RSD (%)	MDL ^b μ g/L
Copper	0.57	1.0	98	3.8	45
Zinc	0.85	2.0	95	2.4	70

^an = 7 replicates, 50 μ L injected.

^bMDL = SD \times (t)_{99%}.

PRECAUTIONS

The analytical flow path must have no metal components. This includes tubing end fittings, stainless steel washers, omni-fittings, etc., as well as columns and valves that contain stainless steel. Use caution in preparing and transferring reagents to minimize contamination. The prepared PAR is easily oxidized. If at anytime the PAR takes on a red color, it has been contaminated and should be discarded. Prepared reagents should be stored under an inert gas, such as nitrogen or helium, and used within two weeks of preparation. Be sure that the eluent is being pumped through the columns when the postcolumn pneumatic controller is turned on. Failure to do so may cause the PAR reagent to back up through the analytical column.

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