

Simultaneous Determination of Cobalt, Copper and Zinc by Energy Dispersive X-ray Fluorescence Spectrometry after Preconcentration on PAR-loaded Ion-Exchange Resin

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A sensitive method for the preconcentration and determination of trace amounts of Co, Cu and Zn by energy-dispersive X-ray fluorescence spectrometry (EDXRF) has been developed. The method is based on the fact that 4-(2-pyridylazo)-resorcinol (PAR) loaded Dowex anion-exchange resin (PAR-resin) can effectively adsorb Co, Cu and Zn at pH 9.0 to form PAR-metal complexes. The detection limits for Co, Cu and Zn were 1.53, 0.31 and 0.21 ppb, respectively. The precisions for five replicate measurements of the three metals were 3.4, 2.7 and 2.1% RSD, and the calibration curves were linear up to 75 µg with correlation coefficients of 0.9975, 0.9980 and 0.9985, respectively. The method was successfully applied for the simultaneous determination of Co, Cu and Zn in seawater samples at ppb levels.

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Introduction

Energy dispersive X-ray fluorescence spectrometry (EDXRF) is a very useful non-destructive technique for the simultaneous determination of elements. Recently, there has been great interest in this technique, and its several applications in element analyses have been reported.¹ Two of the latest papers^{2,3} reviewed the advances in this technique. The major advantage of this technique is its capability for the direct analysis of solid and liquid samples, avoiding, or at least reducing to a minimum, sample handling. Unfortunately, it has relatively poor instrumental detection limits, which are usually at higher than the µg/l level.⁴ A few efforts have been carried out for improving the detection limits of this technique. Generally, these include the precipitation/coprecipitation method using different precipitation agents^{5,6} and the complexing method using different chelating agents-loaded supports.⁷⁻¹¹

Cobalt, copper and zinc are necessary microelements, which people ingest from their daily diet. The World Health Organization (WHO) recommends that 5 - 10 µg of cobalt, 2 mg of copper and 12 - 15 mg of zinc might be the daily optimal intake for adults. These elements are cofactors in many enzymes existing in human bodies. Their deficiencies or excessive intakes may cause serious health problems, and result in Graves' disease,¹² hemolytic anemia,^{13,14} and fatigue.¹⁵ The ocean is an important resource of these elements because many mineral salts are dissolved in seawater. The oceans provide great health benefits to humans, ranging from food and nutritional resources to recreational opportunities, and to new treatments for disease. Recently, the marine food chain has been highlighted. Concerning the simultaneous determination

of cobalt, copper and zinc, classical spectrophotometry is unsuitable for determining the total amount of each element. The simultaneous determination of several individual species is very troublesome due to the unavailability of selective chromogenic reactants. Though inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) should be more efficient among modern instrumental techniques, more expensive instruments were needed. For these reasons, the development of a low-cost methodology for Co, Cu, and Zn determination is very important. The concentrations of Co, Cu, and Zn found in many seawater samples are usually in the ppb range. For most analytical techniques, for instance, X-ray fluorescence, this concentration range is below the quantitative limits, and therefore, some preconcentration methods are necessary.

In the present study, 4-(2-pyridylazo)resorcinol (PAR) was immobilized on a Dowex-1-chloride anion exchange resin to form PAR-resin, and was used as a solid adsorbent for the preconcentration of Co, Cu and Zn ions. Then, the metal ions were directly determined by EDXRF. Moreover, the coexisting metal ions showed no interference. It seems to be a useful method for the determination of these metal ions.

Experimental

Apparatus

A Jordan Valley EX-6600 energy dispersive X-ray fluorescence spectrometer, equipped with a Rh X-ray source and a Si(Li) semiconductor detector (Jordan Valley AR Inc., Austin, TX, USA), was used in this study. The instrumental parameters are listed in Table 1. The samples were placed on a cylindrical plastic device of about 2 cm in height and 3 cm in internal diameter, using an X-ray prolene film (Chemplex

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Table 1 Parameters of the XRF equipment for the determination of metal ions

Parameter		Parameter	
X-ray source	Rhodium tube	Detector	Silicon
Target number	5-Ge-0.000 m	Preset time/s	200
High voltage/kV	20	Atmosphere	Air
Emission current/ μ A	4000	Energy range/keV	10

Industries Inc., Stuart, FL, USA) as the sample support. An Orion 290A digital pH-meter with a combined glass-saturated electrode (Orion Research Inc., Boston, MA, USA) was used to measure the pH values of all solutions. An Alitea U4-Midi sampling pump (Litea AB, Stockholm, Sweden) was used to preconcentrate metal ions from seawater samples.

Reagents

All chemicals used were of analytical reagent grade or better. Milli-Q water (Millipore Company, Bedford, Mass, USA) was used throughout the experiment.

A cobalt standard solution (1010 μ g/ml) was obtained from Aldrich (Aldrich Chemical Company Inc., Milwaukee, WI, USA). A copper standard solution (1000 μ g/ml) was obtained from Spex (Spex Certiprep Inc., Metuchen, NJ, USA) and a zinc standard solution (1000 μ g/ml) was obtained from High-Purity (High-Purity, Charleston, SC, USA). Working standard solutions containing 100 μ g/ml cobalt, copper or zinc were prepared from the standard solutions by appropriate dilution. Synthetic standard samples containing 10 μ g/ml each of cobalt(II), copper(II) and zinc(II) were prepared by mixing appropriate volumes of the above-mentioned metal standard solutions and diluting with Milli-Q water.

The PAR solution was prepared by dissolving an appropriate amount of PAR (Shanghai Chemicals Company, Shanghai, China) in minimum quantitative 95% ethanol, then diluting it with Milli-Q water to give a 0.01% PAR solution.

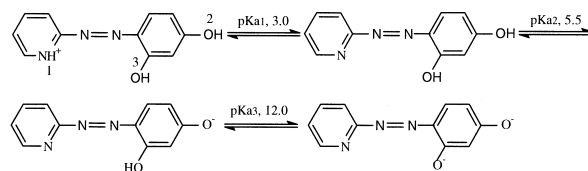
Buffer solutions used for pH values in the range of 2.0 - 12.0 were prepared by dissolving the related buffer powders, obtained from Micro (Micro Essential Laboratory Inc., Brooklyn, NY, USA) in an appropriate proportion of Milli-Q water.

Anion-exchange resin (Dowex-1-chloride, strongly basic anion 8% cross-linking, 200 - 400 mesh) was obtained from Aldrich (Aldrich Chemical Company Inc., Milwaukee, WI, USA). Before use, the resin was pretreated by the following procedure: 50 g of the resin were first washed with acetone, 95% of ethanol and then Milli-Q water. In order to transfer the resin from chloric to hydroxyl type, the resin was then soaked with 20% sodium hydroxide overnight; after that the resin was washed with Milli-Q water several times. Finally, the resin was dried under air at 70°C and kept in a desiccator at room temperature (20°C) until used.

Seawater samples were collected from Tolo Harbor in Shatin, New Territories, Hong Kong. The depth of sampling was about 0.5 m. The distance of the sampling site was about 10 m away from the seaboard.

Procedure

Preparation of 4-(2-pyridylazo)resorcinol (PAR) loaded on the anion-exchange resin (PAR-resin). First, 25 g of the anion-exchange resin, 10 ml of a buffer solution (pH 9.0) and 150 ml of a 0.01% PAR solution were added to a beaker. The mixture was stirred at room temperature (20°C) for 1 min. Under such



Scheme 1 PAR species exists in aqueous solutions.

conditions, PAR in the solution was quantitatively exchanged to the surface of the anion-exchange resin. The obtained PAR-resin was filtered off and washed with 50 ml of Milli-Q water three times. After drying under air at 70°C, the PAR-resin was kept out of light in a desiccator at room temperature (20°C).

Preconcentration of cobalt, copper and zinc using PAR-resin. First, 1.0 g of PAR-resin, 10 ml of a buffer solution (pH 9.0), and different volumes of each of the working solutions (100 μ g/ml) of cobalt(II), copper(II) and zinc(II) were added to a 150 ml beaker. After the mixture was made up to 100 ml with Milli-Q water and stirred mechanically at room temperature (20°C) for 1 min, the colored PAR-resin was filtrated off, washed with Milli-Q water and dried at 70°C for 1 h. The colored PAR-resin was placed on a cylindrical plastic device and determined directly by EDXRF.

Analysis of synthetic standard sample. An aliquot of 1.5 ml of synthetic standard sample was transferred into a 150 ml beaker. Then, 1.0 g of PAR-resin and 10 ml of a buffer solution (pH 9.0) were added successively. The mixture was made up to 100 ml with Milli-Q water and stirred mechanically at room temperature (20°C) for 1 min. The colored PAR-resin was filtered off, washed with Milli-Q water and dried at 70°C for 1 h. The metal ions in the PAR-resin phase were determined by EDXRF, as described above.

Analysis of seawater samples. Each of the seawater samples was pretreated by the following procedure. The pH of a seawater sample was adjusted to 9 using 0.1 mol/l potassium hydroxide. A buffer solution of sodium bicarbonate-sodium carbonate (pH 9, 0.1 mol/l) was added with a ratio of 1 to 50 under stirring. After through mixing, the seawater sample was filtrated through a sintered disc filter funnel with 1.0 - 1.6 μ m pore size. Since a relatively large amount of sample is involved, a column preconcentration method was used. A seawater sample (200 ml) was introduced by the sampling pump through a plastic column of diameter 1.1 cm, which was packed with 1 g of PAR-resin. The sampling pump was used to control the flow rate of the seawater sample at 3.0 ml/min. After all the seawater had flown through the column, PAR-resin packed in the column was taken out and dried at 70°C for 1 h. The metals were determined by EDXRF.

Results and Discussion

Preparation conditions of PAR-resin

In aqueous solutions, PAR exists with four species, including neutral, protonized and two anionic species (shown in Scheme 1). The pH has a great effect on the preparation of PAR-resin. Only anionic PAR can be loaded onto the surface of Dowex-1-chloride resin. However, if PAR with the PAR²⁻ state is exchanged to the surface of Dowex-1-chloride resin, the *o*- and *p*-oxygen atoms of PAR²⁻ will combine with the active sites of Dowex-1-chloride resin. Accordingly, its ability to form metal complexes will decrease sharply. This is because, in general, PAR acts as a tridentate ligand that chelates with metal ions

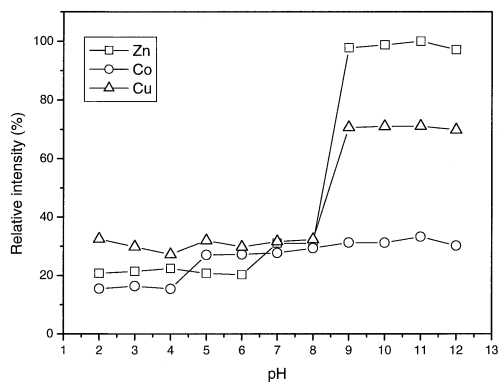


Fig. 1 EDXRF intensity of PAR-metal complexes in different pH. The sample contained 30 μg each of cobalt, copper and zinc. PAR-resin, 1 g; solution volume, 100 ml; buffer solution (pH, from 2 to 12), 10 ml; stirring time, 1 min.

through the pyridine nitrogen atom, the azo-nitrogen atom and the *o*-hydroxyl group. To form a stable PAR-metal complex, the *o*-oxygen atom must be involved.¹⁶ The monovalent PAR anion (PAR) species is the dominant species in pH 5.5–12, which suggests that this pH range is optimum for the preparation of PAR-resin. Under such pH conditions, only the *p*-hydroxyl group ionizes to form PAR, which is then immobilized on the surface of the resin. This is why this pH range (5.5–12) is used to prepare PAR-resin.

Optimum conditions for the preconcentration of cobalt(II), copper(II) and zinc(II)

Effect of pH. The effect of the pH of a sample solution containing 30 μg of each of cobalt(II), copper(II) and zinc(II) in a total volume of 100 ml in the presence of PAR-resin (1 g) was studied over the range of 2 to 12. pH profiles for each metal ion are shown in Fig. 1. It can be seen that the fluorescence intensities reach their maxima for each metal, while in the pH range of 9–11, indicating that this pH range is optimum for the formation of PAR-metal complexes. In addition, in order to prevent the precipitation of metal hydroxides in subsequent steps, pH 9 should be used for preconcentration.

Effect of time. The effect of the stirring time on extracting metals was studied for the three metal ions at pH 9. It was found that the reactions of PAR-resin with the metals were completed in 1 min under the experimental conditions, and an increase in the stirring time did not have any effect on the formation of PAR-metal complexes on the surfaces of PAR-resin. Fast kinetics indicates that the equilibria between metal ions and PAR-resin are easily achieved. This may be related to the large surface area and pore size of the resin. This property is particularly useful in the determination of metal ions at extremely low concentrations. The fast kinetics and high capacity also suggest that PAR-resin can also be used as a column packing for the extraction of trace amounts of metal ions.

Molar ratio of PAR to metal ion in PAR-resin

The molar ratio of PAR to metal ion in the resin was determined according to the molar ratio method.¹⁷ The molar ratio of PAR to each of three metal ions was found to be 2:1. The results were identical with those in the solution, which the molar ratio of PAR to metal ion in the PAR-metal complexes $[\text{M}(\text{PAR})_2]$ was 2:1.¹⁸ In all reactions of PAR with metal ions, Co(II) is very special.¹⁹ When it reacts with PAR, two steps are involved in aqueous solutions. At the initial stage, Co(II) reacts

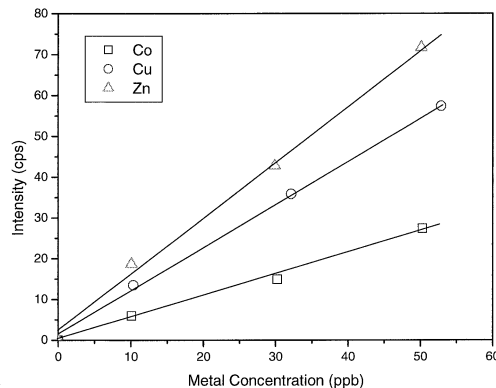


Fig. 2 Multielement calibration curves for cobalt, copper, and zinc.

with PAR to form a red complex, $[\text{Co}^{\text{II}}(\text{PAR})_2]^0$. Then, the complex is oxidized by oxygen to form a more stable red complex, $[\text{Co}^{\text{III}}(\text{PAR})_2]^+$. The PAR-Co(III), once formed, is very stable and is not decomposed in the presence of EDTA, even in a strongly acidic solution.^{18,19}

Calibrations, precisions and detection limits

Multielement calibration curves were obtained for cobalt, copper and zinc in the range of 0–75 μg under the optimum conditions. Calibration curves with good linearity are expressed as follows:

$$\text{Intensity (Co)} = 0.0023 + 0.5328C_{\text{Co}} \times V/W, R = 0.9975;$$

$$\text{Intensity (Cu)} = 1.1429 + 1.0719C_{\text{Cu}} \times V/W, R = 0.9992;$$

$$\text{Intensity (Zn)} = 1.8874 + 1.3945C_{\text{Zn}} \times V/W, R = 0.9980.$$

Here, the intensity is units of counts per second (cps); V denotes the volume of the sample solution (ml); W is the weight of PAR-resin (mg) and C_{Co} , C_{Cu} and C_{Zn} represent the concentrations (ppb) of Co(II), Cu(II) and Zn(II) in the sample, respectively.

The precisions of the developed method for cobalt, copper and zinc for 5 replicate determinations at 10 μg of each of Co(II), Cu(II) and Zn(II) levels were 3.4, 2.7 and 2.1% of the relative standard deviation (RSD), respectively, according to the IUPAC method.²⁰

The detection limits of the method for three metal ions were calculated as the concentration corresponding to a three-fold standard deviation (3σ method)²⁰ obtained by ten subsequently repeated measurements of a blank sample. The detection limits for Co, Cu and Zn were 1.53, 0.31 and 0.21 ppb, respectively.

Analysis of a synthetic standard sample

In order to demonstrate the accuracy of the proposed method, it was checked by the simultaneous determination of the three metal ions in a synthetic standard sample containing 15 μg of each metal in 100 ml. The results are given in Table 2. It can be seen that the results obtained by EDXRF are in good agreement with the added standards.

Determination of seawater

Seawater samples were determined, and the result is given in Table 2. The seawater collected in Tolo Harbor contained 5.0 \pm 0.2 ppb Cu and 55.7 \pm 1.3 ppb Zn (mean \pm standard deviation for three measurements). The concentration of Co in this study was below the detection limit of 1.53 ppb.

Table 2 Concentrations of cobalt, copper and zinc in a seawater sample

Sample	Co, ppb		Cu, ppb		Zn, ppb	
	Added	Found	Added	Found	Added	Found
Seawater						
This method		< 1.5		5.0 ± 0.2		55.7 ± 1.3
ICP-AES		1.0 ± 0.0		4.8 ± 0.1		53.1 ± 2.2
Synthetic sample	150.0	148.6 ± 2.7	150.0	152.3 ± 5.2	150.0	151.0 ± 4.1

Conclusions

Three metal ions including cobalt(II), copper(II) and zinc(II) in seawater samples can be quantified by EDXRF after preconcentration on a PAR-loaded resin. This simple method is accurate and reproducible. It has the potential for preconcentrating other metal ions from environmental samples.

References

1. E. Menendez-Alonso, S. J. Hill, M. E. Foulkes, and J. S. Crighton, *J. Anal. At. Spectrom.*, **1999**, *14*, 187.
2. P. J. Potts, A. T. Eills, M. Holmes, P. Kregsamer, C. Strelis, M. West, and P. Wobrauschek, *J. Anal. At. Spectrom.*, **2000**, *15*, 1417.
3. P. J. Otts, A. T. Ellis, P. Kregsamer, J. Arshall, C. Trelis, M. Est, and P. Wobrauschek, *J. Anal. At. Spectrom.*, **2002**, *17*, 1439.
4. N. Ekinci, R. Ekinci, and Y. Sahin, *J. Quant. Spectrosc. RA.*, **2002**, *74*, 783.
5. Y. Iwata, Y. Isihibasi, N. Gunji, and H. Yoshikawa, *Bunseki Kagaku*, **1991**, *40*, 361.
6. O. W. Lau and S. Y. Ho, *Anal. Chim. Acta*, **1993**, *280*, 269.
7. V. Bhagavathy, M. L. P. Reddy, T. P. Rao, and A. D. Damodaran, *J. Radioanal. Nucl. Chem.*, **1991**, *149*, 35.
8. R. Panayappan, D. L. Venezky, J. V. Gilfrich, and L. S. Birks, *Anal. Chem.*, **1978**, *50*, 1125.
9. I. E. De Vito, A. N. Masi, and R. A. Olsina, *Talanta*, **1999**, *49*, 929.
10. K. N. Belikov, A. B. Blank, N. I. Shevtsov, O. Y. Nadzhafova, and M. M. Tananaiko, *Anal. Chim. Acta*, **1999**, *383*, 277.
11. M. Foerster and K. H. Lieser, *Fresenius' Z. Anal. Chem.*, **1981**, *309*, 355.
12. S. Cornelis, S. Uttenweiler-Joseph, V. Panneels, G. Vassart, and S. Costagliola, *Biochemistry*, **2001**, *40*, 9860.
13. J. E. Kiss, D. Berman, and D. Van Thiel, *Transfusion*, **1998**, *38*, 327.
14. D. Mosha, J. Wangabo, and G. Mhinzi, *Food Chem.*, **1996**, *57*, 205.
15. M. B. Salzman, E. M. Smith, and C. Koo, *J. Pediat. Hematol. Onc.*, **2002**, *24*, 582.
16. A. E. Visser, S. T. Griffin, D. H. Hartman, and R. D. Rogers, *J. Chromatogr., B*, **2000**, *743*, 107.
17. N. Menek, S. Topcu, and M. Ucar, *Anal. Lett.*, **2001**, *34*, 1733.
18. R. G. Anderson and G. Nickless, *Analyst*, **1967**, *92*, 1093.
19. K. Mochizuki, T. Imamura, T. Ito, and M. Fujimoto, *Bull. Chem. Soc. Jpn.*, **1978**, *51*, 1743.
20. L. A. Currie, *Anal. Chim. Acta*, **1999**, *391*, 105.