

Flow Injection Photometric Determination of Zinc and Copper With Zincon Based on the Variation of the Stability of the Complexes With pH

Pablo Richter^{*a}, M. Inés Toral^a, A. Eugenia Tapia^b and Emely Fuenzalida^a

^a Department of Chemistry, Faculty of Sciences, University of Chile, P.O. Box 653, Santiago, Chile

^b Department of Technology, Technologic Metropolitan University, P.O. Box 9845, Santiago, Chile

A flow injection photometric method for the sequential determination of zinc and copper in mixtures was developed based on the variation of the stability of the chromogenic complexes between the analytes and the reagent zincon with pH. At pH 5.0 only the Cu–zincon complex exists, whereas at pH 9.0 the copper and zinc chelates co-exist. A three-channel manifold was implemented containing two alternating buffer streams (pH 5 and 9) which permit the colored reaction products to be formed sequentially at both pH values, and consequently the mixtures can be resolved. A continuous preconcentration unit (Chelex-100) was used in order to increase the sensitivity of the method, thus allowing the analysis of water samples in which the analytes are present at the ng ml^{-1} level. On the other hand, preconcentration was not required when the analytes were determined in brass. Under the optimum conditions and using a preconcentration time of 2 min, the detection limits (3σ) were found to be 0.35 and 0.80 ng ml^{-1} for zinc and copper, respectively. The repeatability of the method, expressed as the RSD, was in all instances less than 3.1%. Considering the sequential determination of both species, a sampling rate of 70 h^{-1} was obtained if preconcentration of the samples was not required.

Keywords: Flow injection; sequential determination; copper; zinc; zincon; water; brass

In analytical chemistry, multi-elemental determinations are in increasing demand. At present, the use of an ICP with either MS and AES detection is probably the best selection when the interest is in multi-elemental determinations at trace levels. However, when the costs involved in instrumental acquisition and maintenance are considered, normally mostly laboratories opt for alternative techniques.

Flow injection analysis (FIA) has been applied in many fields of natural sciences. The basic aim of FIA was initially devoted to the rapid and precise determination of a single species in a large number of samples.¹ However, the versatility of this technique permits the easy design of devices for the determination of several species in a sample, which commonly implies some decrease in the sampling rate. In order to resolve mixtures of analytes by FIA, diverse alternatives have been proposed which are based on different approaches,^{2–11} including differential kinetics, the use of several reagents and reaction media, coupling of FIA and chromatography, computational methods and coupled techniques.

Copper and zinc are often found together in a great number of samples of different nature. Consequently, the simultaneous determination of both species at different concentration levels is in great demand.

From an analytical point of view, when a distinction can be established between the chemical reactivity of two or more species with a common reagent, this can be very useful in

developing methods for the simultaneous determination of analytes in mixtures. In this context, the different rate of the reactions between copper and zinc with a common reagent, zincon, has served as the basis for the resolution of their binary mixtures by using an FIA differential kinetic method.⁴ In addition to the different kinetic reactivities, the Cu– and Zn–zincon complexes also show variations in stability with the pH of the medium. In this work, using the Cu–Zn–zincon system, copper and zinc could be sequentially determined in a continuous flow process based on the variation in the stability of the complexes with pH.

Analytical reactions involving zincon as a chromogenic reagent have been used previously for spectrophotometric determinations of copper and zinc by conventional manual procedures.^{12,13} Liu *et al.*¹¹ reported an FIA procedure for determining both species sequentially using zincon. This approach, which involves the selective masking of copper using the merging zone technique, permits the determination of both analytes in serum at the $\mu\text{g ml}^{-1}$ level.

The FIA method reported here is based on the fact that at pH < 5.5 only the Cu^{II}–Zincon complex exists, whereas at pH 9.0 the Zn^{II} and Cu^{II} chelates co-exist. A three-channel manifold with two alternating buffer streams (pH 5.0 and 9.0) was used to implement the method. Determinations below 0.3 $\mu\text{g ml}^{-1}$ required the use of a preconcentration unit containing Chelex-100 chelating resin. Sodium citrate was included as a masking agent in both buffer streams in order to avoid interferences from iron, aluminum and manganese. The method was applied to the determination of both elements in tap water and brass.

Experimental

Instruments and Apparatus

Absorbances were measured at 612 nm with a Shimadzu (Kyoto, Japan) UV-160 spectrophotometer equipped with a Hellma (Jamaica, NY, USA) Model 178.010-OS flow cell. An Orion (Cambridge, MA, USA) Model 701 digital ion analyzer with glass and saturated calomel electrodes were used for pH measurements. Two four-channel Ismatec fixed-speed peristaltic pumps fitted with Tygon tubes, Teflon flow injection tubes of 0.56 mm id, two Rheodyne (Cotati, CA, USA) Model 5041 injection valves, two Teflon PTFE three-way connectors, a Teflon PTFE three-way selecting valve and a microcolumn made of Tygon tubing (1.5 cm long, 2.5 mm i.d.) were also used.

Reagents

All chemicals were of analytical-reagent grade. De-ionized water (NANOpure ultrapure water system; Barnstead, Dubuque, IA, USA) was used throughout. Working standard solutions of copper and zinc were prepared by dilution of aqueous 1000 mg l^{-1} stock standard solutions. A 1.40×10^{-4} M solution of 2-carboxy-2'-hydroxy-5'-sulfoformacylbenzol (zincon) was prepared in 0.02 M sodium hydroxide. Sodium



acetate–acetic acid buffer solution (pH 5) was prepared in 30% ethanol and a pH of 5 was reached by adding acetic acid to 0.2 M sodium acetate solution. Citrate (0.1 M) was added to this solution as a masking agent. A Clark and Lubs buffer (pH 9) was prepared adding 21.3 ml of 0.5 M sodium hydroxide to 50 ml of 0.5 M boric acid in 0.2 M potassium chloride and diluting to 200 ml. Citrate (0.2 M) was added to this buffer solution.

An iminodiacetic acid chelating resin (Chelex-100) was used for pre-concentration of the analytes from water samples and also 0.1 M nitric acid was used as eluting solution.

Manifold and Procedure

A schematic diagram of the proposed FIA system is depicted in Fig. 1. The manifold contained two injection valves in series. A Chelex-100 microcolumn was located in the loop of one of the valves (IV_1), in which the analytes were pre-concentrated by passing the sample solution through the loop for a pre-set interval (T_p) at a flow rate of 3.0 ml min^{-1} . The loop ($50 \mu\text{l}$) of the other injection valve (IV_2) was filled with 0.1 M nitric acid. After the pre-concentration time, which depended on the concentration of the analytes in the samples, valves IV_1 and IV_2 were sequentially switched in that order with an interval of 2 s. The nitric acid solution passed through the microcolumn and the concentrated metal ions were quantitatively eluted. Depending on the position of the selecting valve (SV), the sample zone was merged and mixed in L_1 ($25 \text{ cm} \times 0.56 \text{ mm id}$) with a buffer system of pH 5 or 9 at a flow rate of 2.0 ml min^{-1} , and the analytical reaction of the analytes with zincon (R) occurred subsequently in L_2 ($150 \text{ cm} \times 0.56 \text{ mm id}$) at a flow rate of 6.0 ml min^{-1} . The signal obtained at pH 5 was used to calculate the copper content in the sample. The copper contribution was subsequently subtracted from the signal at pH 9 in order to determine the zinc concentration. The pre-concentration system inside the dotted section of Fig. 1 can be excluded from the manifold when the analyte concentration in the samples is $> 0.3 \mu\text{g ml}^{-1}$. In this case, direct injection of the samples gave rise to well defined signals.

Results and Discussion

McCall *et al.*¹² reported that the stabilities of Cu– and Zn–zincon complexes are different and pH dependent. It is well known that the principal factor affecting the formation of chelates in practical situations is the acidity of the solutions. This can be explained by considering the conditional equilibrium constants for both Cu– and Zn–zincon complexes, which

are strongly pH dependent.¹⁴ For instance, the pK values for the Zn–zincon complex are 7.9 and 0.6 at pH 9 and 5, respectively, which indicates that complexation of Zn at pH 5.0 is negligible. On the other hand, the true stability of the Cu–zincon complex must be considerably higher than that of the Zn complex, because at pH 5.0 the copper complex is still stable. Based on this fact, McCall *et al.*¹² suggested that the copper concentration can be determined by measuring the absorbance of a solution containing both copper and zinc in the presence of zincon at pH 5.2, where the absorbance resulted entirely from the Cu complex. The total concentration of the two analytes was found by measuring the signal of a similar solution at pH 9.0, where the absorbance was due to both complexes. However, McCall *et al.* could not obtain satisfactory results because at pH 5.2 precipitation of the reagent occurred with most samples, which made it impossible to measure accurately the signal due to the copper content. In order to avoid problems associated with precipitation of the reagent, McCall *et al.* preferred to form the complexes at pH 8.5–9.5 to determine the total concentration of the two elements and subsequently to destroy selectively the complex of one of the analytes without affecting the color intensity of the other. Similarly, selective masking of copper at pH 9 was implemented for the determination of both species by FIA.¹¹

We found that zincon shows very low solubility in aqueous acidic media and, consequently, it precipitates at pH 5.0. However, it was observed that the reagent becomes soluble and perfectly stable in the temperature range $20 \pm 5 \text{ }^\circ\text{C}$ when a zincon solution stream is adjusted continuously to pH 5.0 by merging, at a similar flow rate, with a buffer system of pH 5.0 prepared in a mixed water–ethanol (70 + 30 v/v) medium. In view of this, the difference in stability between the analyte–zincon complexes at different pH values was the basis of the method reported here. The continuous flow manifold depicted in Fig. 1 permits the alternate flow of two streams with different pH values (5.0 and 9.0). A stream solution buffered at pH 5 was prepared in the presence of 30% of ethanol, as indicated above, and the pH 9.0 buffer system was prepared in water, because the reagent does not precipitate under these conditions.

Fig. 2 shows typical analytical signals obtained for copper and zinc at pH 5.0 and 9.0. At pH 5.0 only the Cu–zincon complex is formed, and consequently successive injections of standard solutions of zinc do not show any variation in absorbance. In contrast, at pH 9.0, both elements gave rise to similar FIA signals. When citrate is included in the buffer streams, which favors the masking of interference from species such as iron, aluminum and manganese, the signal at pH 9.0 is almost completely due to the Zn–zincon complex, because citrate at pH 9.0 also masks copper almost completely.

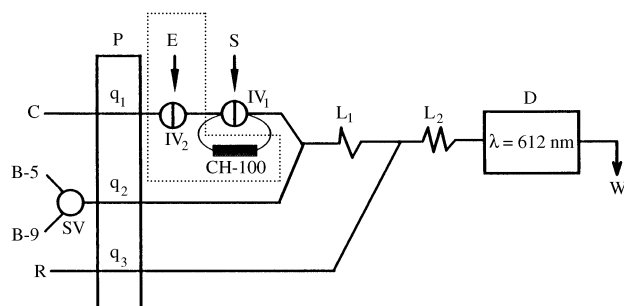


Fig. 1 Flow injection manifold for implementation of the method. P = peristaltic pump, E = eluting agent, S = sample, C = carrier (H_2O) stream, B-5 and B-9 = buffer systems of pH 5 and 9, respectively, R = reagent (zincon), q = flow rate, SV = selection valve, IV = injection valve, CH-100 = Chelex-100 microcolumn, L = mixing coil, D = detector and W = waste. The dotted section indicates the pre-concentration system.

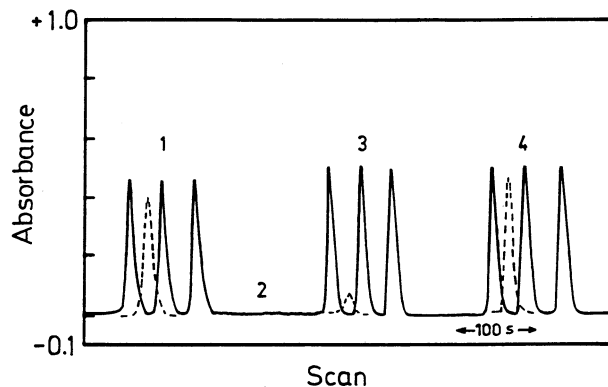


Fig. 2 Analytical signals obtained with the manifold in Fig. 1. 1, Copper at pH 5.0; 2, zinc at pH 5.0; 3, copper at pH 9.0, and 4, zinc at pH 9.0. Segmented signals were obtained under the same experimental conditions but in the presence of citrate as masking agent.

However, in all instances the small contribution of copper to the signal at pH 9.0 must be subtracted, after determining this analyte at pH 5.0, in order to calculate the zinc concentration accurately.

The chemical and flow injection variables were optimized by the univariate method, and the best analytical conditions for the determination of the two species were determined. Table 1 gives the optimum values found for the variables studied.

The presence of ethanol in the carrier stream at pH 5 was strictly necessary. However, its presence does not produce noise for eventual inadequate mixing. If the ethanol content was <30% v/v, precipitation occurred after a few minutes of continuous flow operation of the manifold.

The zincon reagent was prepared in a similar manner to that reported for the classical method,¹² although 20 times more dilute, which is sufficient for good sensitivity. Further increments in the reagent concentration increased the sensitivity, but the signals were considerably less reproducible and the possibility of precipitation increased.

Because the method is based on the different responses of the analytes of pH 9.0 and 5.0, and as the analytical signal in both instances is strongly pH dependent,^{4,12} the buffer systems used were relatively concentrated, in order to avoid changes in the pH of the sample when it meets the buffer stream and the reaction takes place. On the other hand, it was necessary to inject the samples in a carrier stream of water, which is subsequently mixed with the buffer streams, thus avoiding the noise due to

changes in refraction indices, which always occurred when the samples were injected directly in the buffer streams.

The optimum flow rates and lengths of the reactors were selected so as to obtain the maximum sensitivity for both analytes, taking into account that the formation of the Zn-zincon complex requires a development time longer than that observed for the Cu-zincon complex.⁴

Preconcentration of the analytes was necessary when their concentrations in the samples were <0.3 ppm. An on-line preconcentration unit similar to that described earlier⁸ was included in the manifold (Fig. 1). A Chelex-100 microcolumn was used to preconcentrate and separate the analytes from very diluted aqueous samples. To achieve better performance, the samples were adjusted to pH \approx 6.5 before loading on to a Chelex-100 column using a flow rate of 3.0 ml min⁻¹.^{8,15} The elements were quantitatively eluted from the Chelex-100 resin with 50 μ l of 0.1 M nitric acid. Under the selected conditions given in Table 1, when using a microcolumn containing 40 mg of Chelex-100, the maximum loading of the column was 310 ng of Cu and 250 ng of Zn.

Table 2 gives the analytical features of the method. Calibration graphs were obtained separately for each element at pH 9.0 and 5.0 with and without preconcentration. When preconcentration was carried out, the RSD values in Table 2 reflect the repeatability of the combined preconcentration-elution system and the FIA method. According to the slopes of the calibration graphs (Table 2), the preconcentration factors were about 100 for a preconcentration time of 2 min. Calibration graphs for each element in the presence of the other showed the same slopes as those corresponding to the individual elements, which implies that the sensitivity is not affected by the other metal. The sampling rates, considering the sequential determination of both analytes (two injections for each determination), were 70 and 14 h⁻¹ by using the manifold without or with preconcentration, respectively.

Although calibration for one element is not altered in the presence of the other, synthetic water samples were prepared in order to test the applicability of the method. The synthetic samples contained 50 ng ml⁻¹ Zn, 50 ng ml⁻¹ Cu, 50 ng ml⁻¹ Fe, 50 μ g ml⁻¹ Ca, 50 μ g ml⁻¹ Mg and 3% NaCl. The recoveries were 104.2 \pm 3.2% and 97.6 \pm 3.0% for Zn and Cu, respectively. Determination of both analytes was then carried out in a tap water sample (collected in January 1996 at Santiago, Chile). The concentrations found were Cu 6.5 \pm 0.6 and Zn 10.1 \pm 0.5 ng ml⁻¹, which were consistent with those determined by AAS.

The method was also applied to the analysis of brass. In this case, the preconcentration system was not used. Seven portions of about 7 mg of sample were accurately weighed, dissolved in 25 ml of nitric acid (1 + 3) and then diluted to 1000 ml with water. The copper and zinc contents in the sample were determined by the proposed method and the results are given in Table 3, together with those obtained by using other methods. Considering that AAS is usually recognized as a standard

Table 1 Optimization of variables

Variable	Studied range	Selected value
<i>FIA—</i>		
Injected volume (IV ₁)/ μ l	50–250	100
Delay coil (L ₁)/cm	20–250	25
(L ₂)/cm	20–250	150
Flow rate (q ₁)/ml min ⁻¹	0.6–3.0	2.0
(q ₂)/ml min ⁻¹	0.6–3.0	2.0
(q ₃)/ml min ⁻¹	0.6–3.0	2.0
<i>Chemical—</i>		
pH	3–11	5.0 and 9.0
Buffer components/M (pH 5):		
Acetic acid + sodium acetate	0.012–0.36	0.31
Citrate/M	0.01–0.3	0.1
EtOH, % v/v	5–40	30
Buffer components/M (pH 9):		
Boric acid + borate	0.02–0.60	0.125
Potassium chloride/M	—	0.05
Citrate/M	0.01–0.4	0.2
Zincon/M	1.4×10^{-5} – 1.4×10^{-3}	1.4×10^{-4}
Eluting agent, HNO ₃ /M	0.1–1.0	0.1 (50 μ l)
Chelex-100/mg	20–80	40

* Manifold without preconcentration unit.

Table 2 Features of the method

Analyte*	pH	Equation†	Correlation coefficient	Determination range/ μ g ml ⁻¹	RSD (%) (n = 11)	LOD‡/ng ml ⁻¹
Cu	5	$A = 5.82 \times 10^{-2}[\text{Cu}] + 2.3 \times 10^{-3}$	0.9997	0.30–8.0	0.72	90
Cu(P)	5	$A = 6.60[\text{Cu}] + 2.5 \times 10^{-2}$	0.9998	0.0026–0.025	1.50	0.8
Cu	9	$A = 6.34 \times 10^{-3}[\text{Cu}] + 2.8 \times 10^{-4}$	0.9997	—	1.16	—
Cu(P)	9	$A = 0.750[\text{Cu}] + 3.6 \times 10^{-3}$	0.9998	—	3.10	—
Zn	9	$A = 6.40 \times 10^{-2}[\text{Zn}] - 3.8 \times 10^{-4}$	0.9989	0.14–8.0	1.80	40
Zn(P)	9	$A = 7.65[\text{Zn}] + 5.0 \times 10^{-2}$	0.9998	0.0012–0.025	1.90	0.35

* (P): preconcentration unit included in the manifold. † A in absorbance units, analyte concentration in μ g ml⁻¹. ‡ LOD: Limit of detection for a preconcentration time of 2 min.

technique, the results obtained indicate a good level of accuracy.

Conclusions

The difference in stability between the analyte–zincon complexes at different pH values was the basis of the method reported here. Because the preconcentration increases the sensitivity about 100-fold, the method can be used to determine copper and zinc in water samples. On the other hand, determination of both metal in alloys does not require the preconcentration step. Comparison of the results with those obtained by other methods indicates that the proposed method is suitable for the analysis of these types of samples.

In contrast to the classical determination with zincon, this continuous flow method permits the determination of copper at pH 5.0 without precipitation of the reagent. The present continuous flow method is considerably faster than the classical approaches^{12,13} and the consumption of zincon is about 20 times lower.

Table 3 Determination of copper and zinc in brass

Analyte	Amount found* (%) ($\Delta\phi$) [†]		
	Proposed method	Kinetic method	AAS method
Zinc	41.3 (± 1.23)	40.1 (± 1.03)	39.3 (± 1.85)
Copper	58.1 (± 1.65)	57.9 (± 1.65)	58.2 (± 0.82)

* Mean of five determinations. [†] $\Delta\phi$ values in parentheses; ϕ = confidence interval of 99%.

The financial support of the Dirección de Investigación de la Universidad Tecnológica Metropolitana (Project 053) and FONDECYT Project 1970466 is gratefully acknowledged.

References

- 1 Valcarcel, M., and Luque de Castro, M. D., *Flow Injection Analysis. Principles and Applications*, Ellis Horwood, Chichester, 1987.
- 2 Arruda, M. A. Z., Zagatto, E. A. G., and Maniasso, N., *Anal. Chim. Acta*, 1993, **283**, 476.
- 3 Christian, G. D., and Ruzicka, J., *Anal. Chim. Acta*, 1992, **261**, 11.
- 4 Richter, P., Toral, M. I., Tapia, A. E., Ubilla, C., and Bunster, M., *Bol. Soc. Chil. Quim.*, 1996, **41**, 167.
- 5 Fernández, A., Luque de Castro, M. D., and Valcárcel, M., *Anal. Chem.*, 1984, **56**, 1146.
- 6 Richter, P., Toral, M. I., Parra, V., Fuentes, S., and Araya, E., *Bol. Soc. Chil. Quim.*, 1995, **40**, 337.
- 7 Richter, P., Toral, M. I., and Hernández, P., *Anal. Lett.*, 1996, **29**, 1013.
- 8 Richter, P., Fernández-Romero, J. M., Luque de Castro, M. D., and Valcárcel, M., *Chromatographia*, 1992, **34**, 445.
- 9 Luque de Castro, M. D., and Tena, T., *Talanta*, 1995, **42**, 151.
- 10 Hernández, O., Jiménez, F., Jiménez, A. I., and Arias, J. J., *Analyst*, 1996, **121**, 169.
- 11 Liu, R. M., Liu, D. J., and Sun, A. L., *Talanta*, 1993, **40**, 511.
- 12 McCall, J. T., Davis, G. K., and Stearns, T. W., *Anal. Chem.*, 1958, **30**, 1345.
- 13 Platte, J. A., and Marcy, V. M., *Anal. Chem.*, 1959, **31**, 1226.
- 14 Ringbom, A., *Formación de Complejos en Química Analítica*, Editorial Alhambra, Madrid, 1979.
- 15 Pai, S. C., *Anal. Chim. Acta*, 1988, **211**, 271.