

**SOLID PHASE
SPECTROPHOTOMETRIC
DETERMINATION OF COPPER IN
WATER BY USING IMMOBILIZED
ZINCON IN A SEPHADEX A25 RESIN**

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ABSTRACT

A new simple, sensitive and selective solid phase spectrophotometric method has been developed for determination of copper in water. A sensitive analytical zone was prepared by immobilization of zincon in a Sephadex A25 resin, in which copper reacts selectively, at pH 7, to form a colored complex on the surface of the resin. Absorbances can be read directly on the solid phase at 621.5 nm. The batch mode was adopted in this work, because the resin is not reusable due the reaction

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product that is retained irreversible on it. Physico-chemical variables of the method were optimized in order to find the best analytical conditions for the determination. Under the selected conditions copper can be accurately determined on the range 0.4–300 ng/mL, with a detection limit of 0.12 ng/mL and a repeatability, expressed as the relative standard deviation, lower than 3.8%. The recovery in the analysis of a certified reference material was 100.4%. By changing the pH of the sample, other metals also react (Fe, Cd, Ni, Zn, Hg, Pb), indicating that this system also can be used for screening to determine the possible presence of other trace metals in waters.

Key Words: Immobilized zincon; Solid phase spectrophotometry; Copper determination; Water; Screening system

INTRODUCTION

The chemical reactivity of metals with the organic reagent 2-carboxy-2'-hydroxy-5'-sulfoformacylbenzol (zincon) has been previously exploited for the development of analytical methods (1–5). From an analytical point of view, if 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 simultaneous determination of analytes in mixtures. For example, the different rate of the reaction between copper and zinc with the common reagent, zincon, has served as the basis for the resolution of their binary mixtures by using a flow injection analysis (FIA) differential kinetic method (1). Similarly, copper and zinc can be sequentially determined in a continuous flow process based on the variation of the stability of their zincon complexes with the pH (2).

On the other hand, it has been observed that the reagent zincon can be easily and strongly immobilized on solid supports, like ion exchange resins, generating a sensitive surface for preconcentration and reaction in the solid phase with metals. This reaction between zincon and the metallic analyte gives rise to a change in the color of the solid surface, which can be detected directly by spectrophotometry in the solid phase.

The solid phase spectrophotometry approach has been applied since 1976 (6) and it presents the advantage that the sensitivity is considerably higher than the corresponding spectrophotometry in liquid phase. Further the selectivity is also increased because the implicit separation of the analyte

from the matrix can be reached selecting the appropriate conditions. Different methods by solid phase spectrophotometry have been developed by using both batch (7–11) and continuous flow modes (12–17). This last mode is only feasible if the final product of the heterogeneous reaction can be removed from the solid support in order to re-use the sensitive zone for further determinations.

In this work, by using the reagent zincon immobilized on a Sephadex A25 resin, copper can be selectively determined when the heterogeneous reaction is produced at pH 7.0. If the reaction is carried out at other pH values other species like iron, zinc, lead, nickel, cadmium and mercury also react, indicating that this system can also be useful as a screen for detecting the presence of other heavy metals in the sample. The batch mode was adopted in this work, because the reaction product is irreversibly retained in the resin.

EXPERIMENTAL

Reagents

All chemicals used were of analytical-reagent grade unless stated otherwise. De-ionized water (NANOpure ultrapure water system; Barnstead, Dubuque, IA, USA) was used throughout. Working standard solutions of copper were prepared by dilution of aqueous 1000 mg/ml stock standard solution. A 8.25×10^{-4} M solution of 2-carboxy-2'-hydroxy-5'-sulfoformacylbenzol (zincon) was prepared in 0.02 M sodium hydroxide. A 0.1 M phosphate buffer ($\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$) pH 7.0 was also prepared as the medium for immobilization of the reagent and the subsequent heterogeneous reaction.

A SP-Sephadex A-25 resin without pre-treatment was used as the solid support for immobilization of zincon.

Instruments and Apparatus

Absorbances were measured at 621.5 nm with a Shimadzu (Kyoto, Japan) UV-1603 spectrophotometer equipped with a glass cell (1-mm optical path length). The pH measurements were carried out with an Orion (Cambridge, MA, USA) Model 701 digital ion-analyzer with a combined glass-saturated calomel electrode. A Magnetic stirrer/hot plate HI190 M HANNA Instruments with velocity and temperature control was also used.

Procedure

Immobilization of the Reagent

A portion of 100 mL of zincon solution was transferred into a 250 mL beaker and 10 mL of phosphate buffer (pH = 7.0) and 5.0 g of SP-Sephadex A-25 resin were added. The mixture was stirred for 1 h. The resin beads were then collected by filtration, washed with water and finally dried at 30°C.

Heterogeneous Analytical Reaction and Spectrophotometric Measurements

A 300 mL water sample was transferred into a beaker and 25 mL of phosphate buffer and 100 mg of resin containing the immobilized zincon were added, 25 mL of a 0.1 M tartrate solution was also added if the sample contains mercury. The mixture was stirred for 10 min at 50°C, the resin beads collected by filtration and the resin slurry transferred to the spectrophotometric cell with the aid of a pipette. For all spectrophotometric measurements a blank was required, which was prepared in the same way as described for the sample, but using deionized water instead of sample. The absorbance was measured at 621.5 nm.

RESULTS AND DISCUSSION

In order to select the more appropriate solid support for the method, the following resins were checked: SP-Sephadex A-25, Chelex-100, Dowex 1-X1, Dowex 2-X8 and Dowex 21K. Chelex-100 was the only resin which did not retain the complex. Dowex 1-X1 and Dowex 21K were discarded because, despite both resins retain the complex, they presented a high difficulty for packing into the spectrophotometric cell. On the other hand, Dowex 2-X8 and SP-Sephadex A-25 retain the complex, but the latter was the selected resin because the signal is at least two times more sensitive.

Figure 1 shows the absorption spectra of the copper-zincon complex retained, under the conditions described, in the SP-Sephadex A-25 resin. The band centered at 612 nm, in aqueous solution, was shifted to 621.5 nm when the spectrum was recorded in the solid phase. Further, as can be seen in Figure 1, the sensitivity is increased considerably due to the preconcentration effect in solid phase. It is important to establish that in the case of Figure 1A the copper solution is 10 times more diluted than in

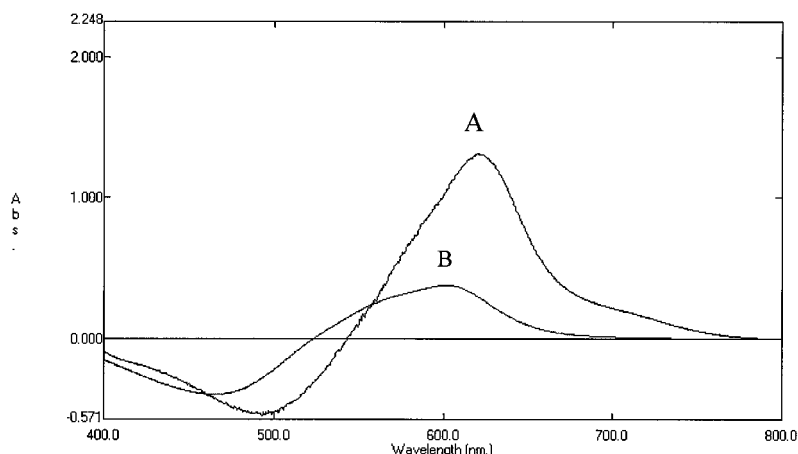


Figure 1. (A) Absorption spectra of the Cu-zincon complex on solid and liquid phase. (A) Solid phase Sephadex A25; Cu 100 ng/ml, 1-mm spectrophotometric cell, (B) Liquid phase; Cu 1000 ng/mL, 10-mm spectrophotometric cell. Each spectrum was read against the corresponding reagent (resin) blank.

Figure 1B, consequently, the sensitivity increased at least 35 times when solid phase spectrophotometry is used.

The effect of pH on the analytical signal of copper was studied between pH 2 and 12. Below pH 3 the extent of the reaction is negligible. Over pH 3 the signal increased until a maximum was reached in the range pH 9–11.5. Over this pH value, the signal decreases considerably. The optimum pH for the method was selected considering also that the reactivity of zincon is strongly pH-dependent as described earlier (2). It was found that at pH 7 this reagent is only reactive for copper. Mercury also reacts to some extent, but, its interference can be eliminated by using tartrate as masking agent. In this context, this pH was selected for analytical purposes, which was adjusted by using a 0.1 M phosphate buffer.

The immobilization of the zincon into the resin shown a behavior of saturation with the concentration of the reagent (Figure 2). The optimum amount of zincon for immobilization was 0.0165 mmol zincon per gram of solid support by using a time for reagent immobilization of 60 min.

Because the reaction between copper and zincon is relatively slow at room temperature (1), a study of the effect of temperature on the analytical reaction was carried out. It was observed that the signal increased with increasing temperature until it reached a constant value at 45°C. The temperature selected for the heterogeneous reaction was 50°C (Figure 3).

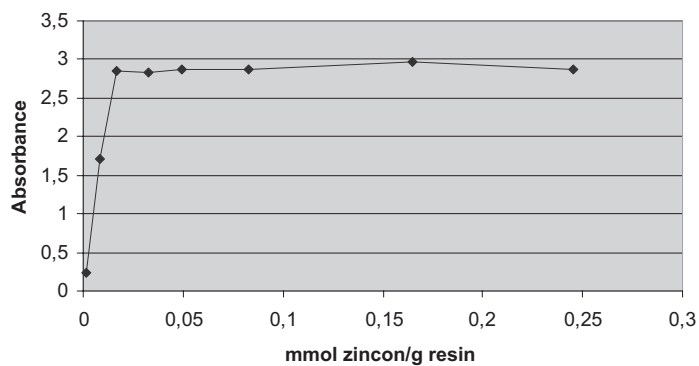


Figure 2. Retention behavior of zincon on a resin Sephadex A25. Absorbance was read directly on solid phase at 630 nm corresponding to the absorption maximum of zincon.

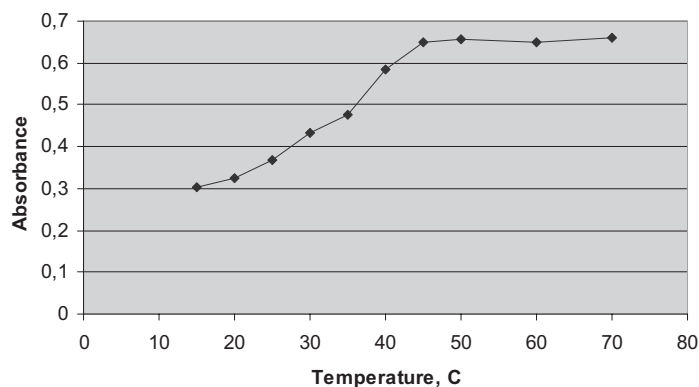


Figure 3. Effect of the temperature on the complex formation on the solid phase. Copper concentration 50 ng/mL.

One of the most important advantages of solid phase spectrophotometry is its inherent capability of preconcentration by increasing the volume of the liquid sample taken for analysis. This effect was studied by measuring the absorbance of the resin (100 mg portion), which was previously equilibrated with different volumes of a solution containing the same concentration of the analyte. As can be seen in Figure 4, the sensitivity of the method can be increased linearly with the volume of sample by a factor of 0.58 milliabsorbance units per mL of sample.

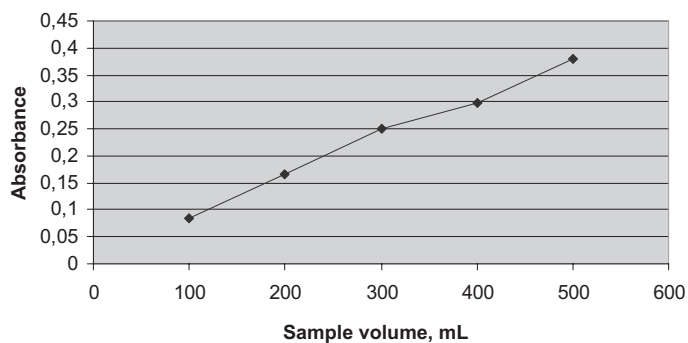


Figure 4. Effect of the volume of sample on the analytical signal. Copper concentration 20 ng/mL.

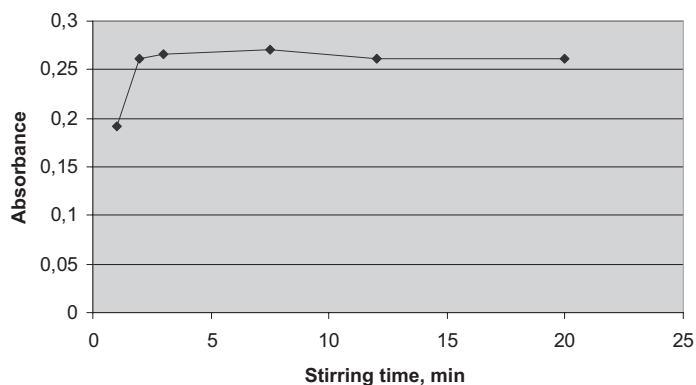


Figure 5. Effect of the stirring time on the analytical signal. Copper concentration 20 ng/mL.

With all other variables optimized, the effect of the stirring time for the heterogeneous complex formation was studied and it is shown in Figure 5. As can be seen, the quantitative formation of the complex in the resin is very fast under the conditions selected. We selected 10 min as the optimum stirring time for all volumes studied.

Features of the Method

Under the optimum conditions selected, a linear response of absorbance vs. copper concentration between 0.4 and 300 ng/mL was

obtained. The equation obtained by applying the least-squares method was as follows:

$$A = 0.01277[\text{Cu}]_{\text{ng/mL}} + 0.018 \quad (r = 0.9986)$$

The repeatability of the method, expressed as the relative standard deviation ($n = 11$), was 2.5% and 3.8%, for 150 and 50 ng/mL of copper, respectively. By using the 3σ recommendation the detection limit was found to be 0.12 ng/mL.

Effect of Foreign Species and Masking Agents

Different metal species, which have shown reactivity with zincon, were tested in this study. Because the reactivity of zincon toward metals is strongly pH-dependent (2), this study was carried out at pH 5, 7 and 9 and using different masking agents (Table 1).

By using the reagent zincon immobilized on a Sephadex A25 resin, copper can be selectively determined when the heterogeneous reaction is produced at pH 7.0. The only species which interferes at this pH value is mercury, which can be easily masked in the presence of tartrate and thiocyanate, decreasing on this media the sensitivity for copper less than 7%. At pH 5 iron interferes, but in presence of tartrate and citrate this interference is eliminated. If the reaction is carried out in more basic media (pH 9.0), in addition to copper, other species like cadmium, nickel, zinc, mercury and lead also react, and the use of masking agents at this pH value do not provide improvement of selectivity. Considering both selectivity and sensitivity for the analyte determination, pH 7 was selected for analytical purposes.

On the other hand, the pH-dependence of the selectivity of the method can be useful from an analytical point of view, because by changing the pH of the solution this system can also be useful as a screening tool for detecting the presence of other heavy metals in the sample.

Validation and Application of the Method

In order to check the analytical application of the present method, validation was achieved by analysis of a certified reference material (CRM). The CRM GBW 08607 "Metal Element in Water" from the Laboratory of the Government Chemist, LGC, UK, which contains

Table 1. Study of Interferences

Analyte and Foreign Species Relative Signals								
pH Value	Masking Agent (MA)	Cu	Cd	Hg	Ni	Zn	Fe	Pb
pH = 5	No MA	1.00	0	0.09	0.03	0	0.13	0
	Citrate	0.82	0	0.01	0	0	0	0
	Nitrite	0.73	0	0	0	0	0.09	0
	Thiosulfate	0.18	0	0	0.01	0	0.15	0
	Tartrate	1.00	0	0.06	0	0	0.04	0
	Thiourea	0.75	0	0	0.02	0	0.17	0
pH = 7	No MA	1.00	0	0.40	0.01	0	0	0
	Citrate	0.61	0	0.54	0	0	0	0
	Nitrite	0.90	0	0.47	0.01	0.01	0	0
	Thiocyanate	0.94	0	0	0.03	0.01	0	0
	Tartrate	0.93	0	0	0.02	0	0	0
	Fluoride	0.97	0	0.35	0.02	0	0	0
pH = 9	No MA	1.00	0.15	0.20	0.52	0.99	0	0.10
	Citrate	0.03	0	0.15	0	0.19	0.01	0
	Nitrite	0.84	0.02	0.02	0.28	0.36	0.01	0.03
	Thiosulfate	0.77	0	0	0.25	0.39	0	0.01
	Thiocyanate	0.96	0.06	0	0.43	0.96	0.01	0.05
	Tartrate	0.95	0	0.24	0.06	0.35	0	0
	Thiourea	0.71	0.11	0	0.47	0.93	0	0.08

For each pH value, all responses were normalized to copper absorbance signal without masking agent. Copper concentration 50 ng/mL, concentration of foreign species 100 ng/mL. Concentration of masking agent 0.1 M. All anions used as masking agent contain sodium as counter-ion.

a certificate of copper concentration of 1.02 µg/mL, was diluted and analyzed by the proposed method. The average amount found was 1.024 ± 0.019 µg/mL, indicating that the proposed method can be applied to water analysis.

Determination of the analyte was then carried out in drinking water (Ñuñoa, Santiago, Chile, March 2000). Because the concentration was very low in this case a volume of 1000 mL of sample was used. The concentration found was 5.2 ± 1.2 ng/mL. The recovery for an addition of 100 ng/mL of copper on this sample was $104 \pm 3\%$.

These analytical features indicate that the proposed method is reliable, selective and sensitive for determination of copper in water.

CONCLUSIONS

A new application of solid-phase extraction as a system to concentrate copper as a coloured complex from a solution and the direct measure in the solid phase by spectrophotometry was developed. In this method the reagent zincon was immobilized in a Sephadex A25 resin, in which copper reacts selectively, at pH 7.0. By changing the pH of the sample other metals also reacts indicating that this system also can be used for screening to determine the possible presence of other trace metals in waters. The proposed method allows the determination of the analytes at ultratrace levels without the use of sophisticated instrumentation. In the proposed method the preconcentration on the solid phase increases the sensitivity by the formation and preconcentration of complexes on solid phase previous to the instrumental measurement. In this context, the proposed method is more sensitive than others where the determination of the analyte is carried out directly in aqueous solution or by liquid-liquid extraction. The method is fast and simple because the equilibrium involves the formation and retention of the complexes are rapid and kinetic phenomena are not present. The proposed method was successfully applied to a certified reference material and real samples.

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