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Sequential fluorescent determination of copper (II) and cobalt (II) in food samples by flow injection analysis

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Abstract

Copper (II) can react with a new fluorescence reagent, 5-(4-chlorophenylazo)-8-aminoquinoline (APAQCL), forming a fluorescence product only in slightly acidic medium, while the fluorescence product of cobalt (II) with APAQCL forms only in basic medium and in the presence of H_2O_2. Based on the difference of the reaction between cobalt (II) and copper (II) with APAQCL, a new flow injection method for the sequential determination of cobalt and copper in binary mixtures by use of a fluorimetric detector and APAQCL as reagent was proposed. Linear calibration graphs were obtained over the concentration range 0.010–1.20 (μg/ml) of cobalt (II) and 0.050–5.00 (μg/ml) of copper (II) with the detection limit of 0.0030 (μg/ml) of cobalt (II) and 0.010 (μg/ml) of copper (II), respectively. The applicability of the method to the sequential determination of cobalt (II) and copper (II) in food samples was demonstrated by investigating the potential interference and by the analysis of synthetic mixtures of copper (II) and cobalt (II). The method was successfully applied to the determination of copper and cobalt in food samples. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Copper and cobalt are two important elements, which have been received widespread attention in the physiological and clinic studies. Some investigations shown that cobalt-deficiency or copper-deficiency in the human body chronically is one of the reasons for resulting in the Cardiovascular disease (Qiu, 1979), and copper can cause poisoning if it is excessively accumulated in the human body (Wang, 1994). Therefore, copper and cobalt especially for copper are the elements which must be analyzed in food detection (Lin and Zhao, 1995). The large number of spectrophotometric and spectrofluorimetric methods have been developed for the individual determination of copper (Chimpalee, Chimpalee, Lohwitee, Nakwatchara, & Burns, 1996; Cao, Zhao & Xu, 1993) and cobalt (Mori, Fujita, Toyoda, Hamada, & Akagi, 1992; Burns, Hanprasopwattana, & Kheawpintong, 1983). In contrast to the individual determination, less works have been done on their sequential (Giovannetti, Bartocci, Ferraro, Gusteri, & Passamonti, 1995; Thompson, Patchan, Huang, & Fierke, 1996; Djebil, Guermouche, & Meklati, 1995) or simultaneous determination (Vereda, Rios, & Valcarcel, 1997), and these proposed methods are either time-consuming (Giovannetti et al., 1995; Thompson et al., 1996; Djebil et al., 1995) or not sufficiently sensitive to assess cobalt quantitatively in the nanogram level (Giovannetti et al., 1995; Djebil et al., 1995).

In the last two decades, continuous-flow methods, in particular flow-injection analysis (FIA), have become a firmly established analytical tool. This methodology considerably simplifies the analytical procedures, improves the reproducibility and shortens the analysis time, etc. Fluorimetry, due to its inherent high sensitivity, can provide a highly sensitive detector. So a rapid, highly sensitive and more exact analytical method can be obtained by the combination of the flow injection analysis and a fluorimetric detector.

In this paper, we used the reactions of copper (II) and cobalt (II) with a new fluorescence reagent 5-(4-chlorophenylazo)-8-aminoquinoline (APAQCL), which was firstly synthesized and used as a fluorescence reagent for the determination of Cr (VI) by Sou, Xu, and Zhao (1990), proposing a new sequential method for the determination of copper and cobalt. The method based on the fact that copper (II) reacts with APAQCL forming a fluorescence product only in slightly acidic medium and the fluorescence product of cobalt (II) with APAQCL forms only in basic medium and in the presence of H_2O_2.

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The method is sensitive, rapid and of high selectivity and was used to the determination of copper and cobalt in food samples with satisfactory results.

2. Experimental

2.1. Apparatus

Fluorescence measurements were performed on a Hitachi (Japan) M-850 fluorescence spectrometer equipped with a 150 w Xenon lamp and a flow cell (18 μl). All of the fluorescence data were given with correction. The FIA system used in this work was a Tecator 5020 analyzer with a Tecator chemifold (II) and a Model 5101 thermostat, as shown schematically in Fig. 1. The pump tubes are Tygon. All other tubes are Teflon (i.d. 0.5 mm). Sample solutions were aspirated into 120 μl sample loop and then injected into a carrier stream. A model of pHs-2 pH-Meter (Shanghai, China) was also employed for the pH measurements.

2.2. Reagents

All chemicals were of analytical reagent grade; doubly distilled water was used throughout.

APAQCL (its structure was shown in Fig. 2) was synthesized according to the procedure of Sou et al. (1990), and APAQCL solution (1.0×10^{-4} mol/liter) was prepared by dissolving it in 1:1 aqueous ethanol solution.

Copper (II) and cobalt (II) stock solution (1.00 mg/ml) were prepared by dissolving 0.1000 g of pure copper and cobalt (99.9%) in 10 ml of 1:1 nitric acid solution, respectively. The solution was heated gently to remove the nitrogen oxide. After cooled, the solution was diluted to 100 ml with water. Working solution of 2.00 (μg/ml) of Cu (II) and Co (II) was prepared by diluting with water daily.

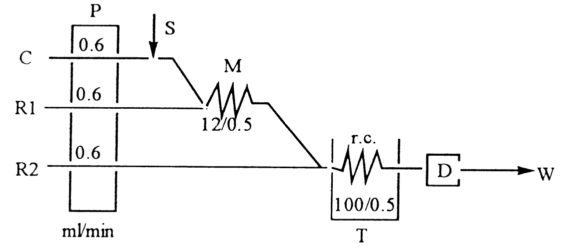

Fig. 1. Manifold of the flow injection systems for the determination of Cu (II) and Co(II). C, Carrier stream; R1, APAQCL and Tween-80 mixing solution; R2, buffer solution; M, mixing coil; r.c., reaction coil; P, peristaltic pump; T, thermostat; D, detector (spectrofluorimeter); S, sample; W, waste.

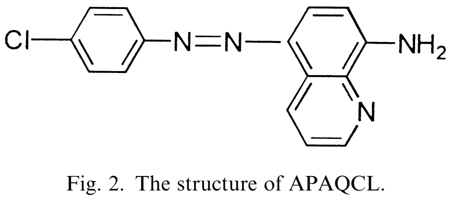

Fig. 2. The structure of APAQCL.

Buffer solution was prepared with 0.1 M $Na_2B_4O_7$ solution and 0.1 M HCl solution and 0.1 M NaOH.

Tween-80 aqueous solution 3.0% (v/v) and H_2O_2 (1.5%) aqueous solution were used in this work.

2.3. Samples treatment

An appropriate amount of tea (obtained from Yunnan, China), flour and fresh vegetable samples (obtained from Lanzhou, China) were firstly carbonized at low temperature, then burned in the muffle furnace at 600°C for about 3 h. When the residue was cooled to room temperature, it was extracted by heating in 2.0 ml of 9 M H_2SO_4, then an excessive amounts of NH_4OH was added to the solution under agitation to precipitate iron and other transition metal ions as hydroxide. The mixture was filtered and the filtrate was heated to remove NH_3, the solution was allowed to cool, then diluted to 25 ml with water.

Mineral water was treated as follows: 250 ml mineral water (Hanzhou, China) was concentrated to nearly dry, then diluted to 25 ml with water.

2.4. Procedure for the FIA

The FIA system and conditions for the determination of Co (II) and Cu (II) were all given in Fig. 1 and Table 1. After the temperature of the Model 5101 thermostat reached 90°C and a steady baseline was obtained, a sample or standard solution, which was injected into the sample loop, was carried into the flow system by carrier stream (C). Then the carrier stream and sample or standard solution were mixed with the mixture solution of APAQCL and Tween-80 (R1) in the mixing coil (M). Finally the flow was mixed with the buffer solution (R2) in the reaction coil (r.c.). The fluorescence intensity was measured at $\lambda_{ex} / \lambda_{em} = 330$ nm/370 nm.

2.5. Procedure for the sequential determination of Co (II) and Cu (II)

Because Cu (II) and Co (II) did not interfere each other, the process for the sequential determination of cobalt and copper was shown as follows: under the optimum conditions for the individual determination of cobalt and copper, a series of Co (II) or Cu (II) standard solution was injected into the FIA system to obtained a linear calibration graph of Co (II) and Cu (II), respectively. Then the sample solution instead of standard solution was injected into the FIA system to determine cobalt under the optimum condition for Co (II) and copper under the optimum condition for Cu (II). The concentration of cobalt and copper in the sample was finally calculated from the linear calibration graph of themselves, respectively.

Table 1
Conditions for FIA

	FIA for Cu (II)	FIA for Co (II)
Composition and flow rate of C (ml/min)	Redistilled water, 0.6	1.5% (v/v) H_2O_2, 0.6
Composition and flow rate of R1 (ml/min)	2:1 APAQCL (1.0×10^{-4} M) and Tween-80 (3.0%), 0.6	2:1 APAQCL (1.0×10^{-4} M) and Tween-80 (3.0%), 0.6
Composition and flow rate of R2 (ml/min)	pH 4.5 buffer solution,0.6	pH 11.0 buffer solution, 0.6
Sample volume (μl)	120	120
Mixing coil length (cm)	12	12
Reaction coil length (cm)	100	100
Temperature (°C)	90	90

3. Results and discussion

3.1. Fluorescence spectra

The complexes of Cu (II)–APAQCL and Co (II)–APAQCL-H_2O_2 precipitated out of aqueous solution in the absence of a surfactant. In order to develop methods based on solution fluorescence measurement, excitation and emission spectra in different surfactants were recorded. The shape of spectra were similar in different surfactants, such as Tween-80, Tween-60, Tween-40, Tween-20, OP, PVA, Triton X-100 and arabic gum, but the fluorescence intensity was higher in Tween-80 for the two systems than that in the others. So Tween-80 was selected to carry out all subsequent researches. The maximum wavelength of excitation and emission for the systems of copper and cobalt were at $\lambda_{ex}/\lambda_{em} = 328$ nm/368 nm and 330 nm/370 nm, respectively. The maximum wavelengths of excitation and emission for the corresponding reagent blanks were the same as their complex systems. The fluorescence intensity for the two systems were finally determined at $\lambda_{ex}/\lambda_{em} = 330$ nm/370 nm.

3.2. Optimum conditions for FIA

It was shown from the experimental results that the longer the reaction coil, the higher was the sensitivity. The reaction coil length in the Model 5101 thermostat is 100 cm and cannot be changed conveniently. In addition, the sensitivity is not sensitive to variations in the length of mixing coil. Hence, 100 and 12 cm were used as the length of reaction coil and mixing coil, respectively. In a certain range, the sensitivity increases when injection volume increases, but at the same time the sampling frequency decreases with increasing injection volume. 120 μl was chosen as the injection volume because it provided the best compromise between sensitivity and sampling frequency. The optimum flow rate was found to be 1.8 ml/min. In the experimental conditions mentioned above, sampling frequency was 52 h^{-1}. The optimum FIA conditions for the two systems were the same.

3.3. Effect of acidity

Effects of acidity on the reaction of copper and cobalt with APAQCL were studied. It pointed out that the reaction between copper and APAQCL is difficult to carry out and the fluorescence intensity of the system is very low in the strongly acidic ($pH < 2.0$) and strongly basic ($pH > 11$) medium. The optimum pH range was found to be between 4.0 and 6.5. On the other hand, the optimum pH range for the determination of cobalt (II) is over 9.5–12.0. When the pH of the medium was out of these ranges, the fluorescence intensity decreased rapidly. In the acidic ($pH < 5.0$) medium, there is almost no fluorescence product formed in the system. Therefore, to avoid the interference to each other, $pH = 4.5$ and $pH = 11.0$ obtained with $Na_2B_4O_7$–HCl and $Na_2B_4O_7$–NaOH buffer solution were used to the determination of copper and cobalt, respectively.

3.4. Effect of reagent concentration

The fluorescence intensity increased with increasing the concentration of APAQCL for both the systems. The maximum fluorescence intensity was obtained in the concentration range of APAQCL 2.5×10^{-5}–8.0×10^{-5} mol/liter and 6.0×10^{-5}–1.2×10^{-4} mol/liter for the determination of Cu (II) and Co (II), respectively. 6.6×10^{-5} mol/liter APAQCL was finally recommended for both the systems.

3.5. Effect of the surfactant

The complexes of the two systems all precipitated out of aqueous solution in the absence of a surfactant. The best additive for both the systems was found to be Tween-80. The maximum and constant fluorescence intensity was obtained in the concentration range of Tween-80 between 0.5 and 1.5% and between 0.1 and 1.0% (v/v) for the determination of Cu (II) and Co (II), respectively. So 1.0% Tween-80 was selected in the further experiments for the two systems.

3.6. Effect of H_2O_2 concentration

In contrast to the fact that the sensitivity for the determination of Co (II) improved remarkably in the presence of H_2O_2, the sensitivity for that of Cu (II) decreased after H_2O_2 was added. So H_2O_2 was not used in the system for the determination of Cu (II). The optimum concentration range of H_2O_2 for the determination of Co (II) is over 0.6–2.1% (v/v). Subsequent determination of Co (II) was therefore carried out in 1.5% H_2O_2.

3.7. Effect of the temperature

The rate of the reaction of the two systems was very slow at room temperature. The fluorescence intensity increased with increasing the reaction temperature, and it reached the maximum when the reaction temperature was above 80°C. So the reaction temperature used in the experiment was 90°C, provided by a Model 5101 thermostat.

3.8. Effect of foreign ions

The effects of interfering ions on the determination of 2.00 μg/ml Cu (II) and 1.0 μg/ml Co (II) were all summarized in Table 2. The tolerance limit was taken as maximum concentration of the interference ion causing about a ±5% error in the determination. For the determination of Co (II) and Cu (II), they did not interfere each other at 100-fold concentration of themselves. Moreover, many kinds of foreign ions did not interfere at 10–1000-fold concentration of Cu (II) and Co (II). Fe (III) causes negative interference when its concentration exceeds five fold that of Cu (II) and Co (II) in this work. However, the interfering effect of Fe (III) can be effectively removed by precipitating it with NH_4OH.

3.9. Calibration

The calibration graph for the determination of Cu (II) and Co (II) were investigated under the optimum conditions described above. The results were shown in Table 3. It pointed out that the methods are of relatively high sensitivity and precise.

Table 2
Tolerance limits of the foreign ions (μg) in the determination of 2.0 μg Cu (II) and 1.0 μg Co (II)

Ion added	For Cu (II)	For Co (II)
Ca^{II}, Mg^{II}, Cr^{III}, PO_4^{3-}, F^-, NH_4^+, NO_2^-, NO_3^-	2000	2000
Cr^{VI}	1000	1000
Pb^{II}, Sb^{III}	400	300
SiO_3^{2-}	200	200
Al^{III}, Zn^{II}, Cd^{II}	150	200
Hg^{II}, Ni^{II}, Mn^{II}, Pt^{IV}, Pd^{II}, Sn^{II}, Sn^{IV}	100	100
Bi^{III}	50	30
Ga^{III}, In^{III}, Th^{IV},	10	20
Fe^{III}	10(200)[a]	5(200)[a]
Co^{II}	100	
Cu^{II}		100

[a] After removed as described in the text.

3.10. Applications

To demonstrate the applicability of the proposed method to the successive determination of cobalt (II) and copper (II), a series of synthetic mixtures of copper (II) and cobalt (II) were analyzed by it. The results were given in Table 4. It can be seen that the results are satisfactory.

Due to the concentration of cobalt in the food samples is very low and that of copper is high, the sample

Table 3
The analytical characteristics for the determination of Cu (II) and Co (II)

Analyte	Linear regression equation[a]	r	Linear range (μg/ml)	LOD[b] (μg/ml)	RSD[c] (%)
Cu (II)	$F=0.452+3.497C$	0.9995	0.050–5.0	0.010	1.10
Co (II)	$F=0.170+3.152C$	0.9989	0.010–1.2	0.0030	2.47

[a] F is the relative fluorescence intensity and C is the concentration of Cu (II) or Co (II) expressed as μg/ml.

[b] Limit of detection, calculated from three times the standard deviation of nine blank determinations.

[c] Relative standard deviation [for 2.0 μg/ml Cu (II) and 1.0 μg/ml Co (II), $n=7$, respectively.]

Table 4
Analysis of Co (II) and Cu (II) in the synthetic mixtures of Co (II) and Cu (II)

Composition	Value added (μg/ml)		Value found (μg/ml)		RSD (%, $n=7$)	
	Co	Cu	Co	Cu	Co	Cu
1:1	1.00	1.00	0.98	0.98	2.15	1.96
1:5	1.00	5.00	1.02	4.94	2.78	2.32
1:10	1.00	10.00	1.06	9.89	1.97	1.97
1:25	1.00	25.00	0.96	25.01	2.45	2.11
1:50	1.00	50.00	1.08	49.76	2.63	2.71

Table 5
Determinations of copper and cobalt in food samples

Samples	Determination of copper ($n=5$)			Determination of cobalt ($n=5$)		
	Found (μg/g)	RSD (%)	Recovery (%)	Found (μg/g)	RSD (%)	Recovery (%)
Flour	16.4	1.59	102	1.76	1.79	91
Tea	11.1	2.14	101	2.13	2.15	106
Chrysanthemum	1.56	2.17	100	0.099	2.95	108
Spinach	3.06	1.95	97	0.18	3.05	90
Swamp cabbage	2.25	2.79	105	0.076	2.65	96
Mineral water[a]	1.26	2.85	100	0.060	2.13	95

[a] Expressed as μg/liter.

solution was used to the determination of cobalt by the proposed method directly, but before the analysis of copper, the sample solution had to be diluted. The results were shown in Table 5. The mineral water sample was used to analyze the copper and cobalt in it directly. The results were also shown in Table 5. The recovery was tested by making five different standard addition to the sample solution. The recovery for all the determinations was between 89 and 109%. The average recovery was given in Table 5, which alluded to that the method is more accurate.

4. Conclusions

The automatic FIA method with a fluorescence detector based on the use of a new fluorescence reagent APAQCL permits a sequential determination of copper and cobalt in different food samples with high sampling frequency. Against the method of Thompson et al. (1996); Djebil et al. (1995) and Vereda et al. (1995), this one is of high sensitivity and does not require a previous separation of the samples; additionally, the method is rapid and more selective. The results obtained with synthetic mixtures of Co (II) and Cu (II) and the recovery assays carried out on real samples are satisfactory.

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