

A method of high sensitivity and *in situ* determination of trace cobalt(II) in water samples with salicyl fluorone

Zhe Chen and Hua Li

ABSTRACT

Based on the sensitive reaction of Co(II) and salicyl fluorone with the presence of cetylpyridinium bromide in basic solution, a new method of flow injection micelle-solubilized spectrophotometry was developed for the determination of cobalt. The reaction was monitored for the absorbance of Co(II) at 540 nm. The reagents, flow injection variables and effects of foreign ions were investigated and the optimum conditions were established. At the optimum test conditions, the developed method provides a linearity range of 3–40 $\mu\text{g L}^{-1}$ with a detection limit of 0.1 $\mu\text{g L}^{-1}$ at about 60 h^{-1} sampling frequency. Relative standard deviation of 0.91, 0.95 and 0.89% were obtained for the determination of 3, 15 and 30 $\mu\text{g L}^{-1}$ Co(II) solution. The proposed method has been successfully applied for analysis of trace amounts of cobalt in water samples.

Key words | cobalt, flow injection, micellar solubilization, salicyl fluorone

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INTRODUCTION

Cobalt is one of the essential micronutrients owing to its biological importance. The level of cobalt is important to the health of mammals because it plays a part in biological and physiological functions mainly by forming vitamin B₁₂. The appropriate amount of cobalt can cure infectious anemia and kidney disease, while loss of cobalt will affect the normal metabolism to cause diseases. Excessive cobalt inhibits bone marrow oxidase to cause polycythemia, affects the thyroid to cause goiter, and is toxic to the myocardium leading to heart failure and even death.

Cobalt pollution mainly comes from human activities. Cobalt is widely used in areas such as smelting, chemistry, ceramics, catalysis, magnetic materials and cemented carbide. For example, the ash, slag and wastewater, which often contains high concentrations of cobalt from the production process in chemical industries, get into bodies of water directly or indirectly to cause environmental pollution. So it is important to determine trace amounts of cobalt for environmental protection, chemical processes and public health studies.

Because of the extensive use of cobalt and its low content in samples, various methods for quantitative analysis of cobalt have been developed, including spectrophotometry, polarography, chemiluminescence, high performance liquid chromatography, inductively coupled plasma mass spectrometry, inductively coupled plasma emission

spectrometry, flame atomic absorption spectrometry, and graphite furnace atomic absorption spectrometry. Although the above methods mentioned are of high sensitivity and accuracy, shortcomings also exist. For example, the apparatus is expensive, the operation is time consuming, skilled operators with necessary knowledge are demanded and the working environment is highly demanding. Moreover, these methods could not be applied to *in situ* determination.

Flow injection analysis (FIA) is a continuous analytical technology that was introduced in 1975. Simplicity, reliability, and reproducibility as well as ease of automation and sample throughput are its specific characteristics. So it is an ideal method for automatic monitoring and process analysis. It will greatly improve the analytical ability when combined with certain determination technologies (Ruzicka and Hansen 1975, 1998). In this paper, the FIA equipped with micelle-solubilized spectrophotometry (MSS), which was a new spectrophotometric method that been brought forward in the late 1960s. Compared with previous methods, this combined technology is simpler, faster, cheaper, more sensitive and more environmentally friendly. Furthermore, the instruments are compact and easy to carry, featuring the implementation of *in situ* determination. So it has been generally applied in the detection of samples among various fields such as food and environment (Ensafi *et al.* 2002;

Ruengsitagoon 2008; Nouroozi & Mirshafian 2009; Mrazek *et al.* 2012; Abdel-Azeem *et al.* 2013).

Over the past two decades, new highly sensitive and selective chromogenic reagents have emerged (Themelis *et al.* 1995; Daopei & Yuehui 1998; Amin and Ahmed 2001). Salicyl fluorone is a kind of fluorescent ketone reagent with high sensitivity and selectivity for the detection of metal ions, which has been successfully applied in the determination of germanium in plant samples (Huilin 2006), indium in lead (Yanhui *et al.* 2009), tungsten in alloy steel (Jun *et al.* 2004), ferrum in tap water (Shijie & Yingjie 2008), titanium in cement (Jiahua & Miao 2011), germanium in Chinese traditional herbs (Jianbo 2007), gallium in coal (Yanhui *et al.* 2008), etc. The current study found that with the existence of hexadecyl pyridine bromide (CPB), cobalt and salicyl fluorone (SAF) have a high-sensitive reaction in alkaline medium. Accordingly, a new method of flow injection MSS to determine Co(II) has been established, serving analytical advantages such as simplicity, sensitivity, rapidity, low instrumentation cost and *in situ* determination.

METHODS

Reagents

All reagents used were of analytical reagent grade and solutions were prepared with distilled water. The FIA reagents were prepared as follows.

Cobalt standard stock solution was prepared by dissolving 0.4770 g of cobalt sulfate ($\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$) in water, then adding 10 mL of (1 + 1) sulfuric acid diluted with water to make 1,000 mL. Now the solution concentration is 0.1 mg mL^{-1} , which was then diluted to $1 \mu\text{g mL}^{-1}$ Co (II) standard solution for later use.

Hexadecyl pyridine bromide solution was prepared by weighing 30 g of CPB to dissolve in water, then moving into a 500 mL volumetric flask to make the concentration of 60 g L^{-1} .

Salicyl fluorone solution ($1 \times 10^{-3} \text{ mol L}^{-1}$) was prepared by weighing 84.0 mg of reagent in a small beaker, and dissolving with hydrochloric acid solution and absolute ethyl alcohol, then moving into a 250 mL brown volumetric flask with ethanol for storage.

Borax solution was selected as the buffer solution.

Apparatus

A 721 spectrophotometer (Spectrum Instrument, Shandong, China) with a flow cell was used as the detector. The

spectrophotometer was controlled and the absorbance output was recorded by a PC through its serial port. In the present system the main device is a CMFIA-I flow injection system (Analysis Instrument, Shandong, China), which was equipped with one injection valve and two peristaltic pumps. The rotation speed of the two pumps, their stop-and-go intervals and the actuation of the valve were programmed and controlled by the same computer as used for controlling the spectrophotometer.

Experimental arrangements

A flow injection manifold designed for the research is shown in Figure 1. In this work, a double-channel flow system was employed, and all the channels were equipped with the same 0.5 mm inner diameter. According to the flow configuration, Co(II) standard solution or sample solution and buffer solution were injected by the host peristaltic pump respectively, and the auxiliary peristaltic pump sampled the color reagent. The sample solution flowed into the chemical block directly, while buffer solution mixed with color reagent prepared by SAF and CPB flowed through a multichannel sampling valve, and then to the chemical block subsequently. The mixed solution was sent to the reaction coil, where Co(II) reacted with SAF in the presence of CPB and borax buffer solution. Then the instrument was switched to the analysis state; the colored solution was carried to the spectrophotometric detector cell in which the absorbance of the product was selectively measured at 540 nm and recorded continuously on the computer. Then the program returned to the sampling state to start a new analysis, so as to realize the rapid and continuous determination.

RESULTS AND DISCUSSION

Principle discussion

SAF is a kind of three ketone hydroxyl fluorescence chromogenic reagent, containing chromophore (quinone) and

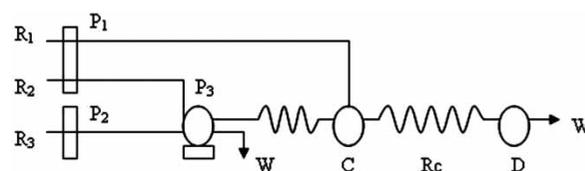


Figure 1 | Schematic diagram of the FIA system. R1: sample; R2: buffer solution; R3: color reagent; P1: host peristaltic pump; P2: auxiliary peristaltic pump; P3: multichannel sampling valve; C: chemical block; Rc: reaction coil; D: detector; W: waster.

auxochrome (hydroxyl). It also has great rigidity, and a plane and conjugated π bond structure, making it easy to absorb visible light and ultraviolet light. Surfactant was used because of its functions of solubilization, sensitization and autostabilization in aqueous solution. The using of surfactant highly improved the sensitivity, contrast and selectivity of the color reaction through the production of a ternary complex, which was less affected by the reagent composition and has the advantages of relatively high molar absorption coefficient, good reproducibility and selectivity (Daopei & Yuehui 1998).

In the presence of an appropriate amount of CPB, there is an interaction between Co(II), SAF and CPB in basic solution, forming a ternary micellar complex. Due to the effect between the ligands and the role of the ligands and central ions, the solubility of the ternary complex in water increases, the maximum absorption peak has a redshift to 540 nm and the molar absorption coefficient (ϵ) increases, whose value usually is between 10^5 and 10^6 , enhancing the sensitivity and selectivity of color reaction effectively.

Optimization of experimental conditions of FIA

The flow systems were optimized by varying the chemicals and the instrumental factors, to establish the best conditions for the determination of cobalt(II).

Effect of concentration of SAF

The concentration of SAF was found to be a very important factor affecting the formation of the ternary complex, and the influence was tested by varying the concentration of SAF in the range of $4.0 \times 10^{-5} \text{ mol L}^{-1}$ to $2.0 \times 10^{-4} \text{ mol L}^{-1}$. As can be seen from Figure 2, the sensitivity of determination increased gradually with the concentration of SAF up to $1.6 \times 10^{-4} \text{ mol L}^{-1}$ and then decreased slightly. Therefore, this concentration of the reagent was chosen as optimum.

Effect of concentration of CPB

The effect of various concentrations of CPB ($1.2\text{--}6.0 \text{ g L}^{-1}$) on the absorbance signals was examined. The analytical signals increased with the increasing concentration of CPB up to 3.6 g L^{-1} ; thereafter the analytical signals decreased and leveled off. This is maybe because the CPB has functions of solubilization, sensitization and stabilization, but when there is a tremendous amount of CPB in the reaction liquid, discoloration occurs in the micellar region. Due to the quasi-homogeneous extraction package effect of micelle

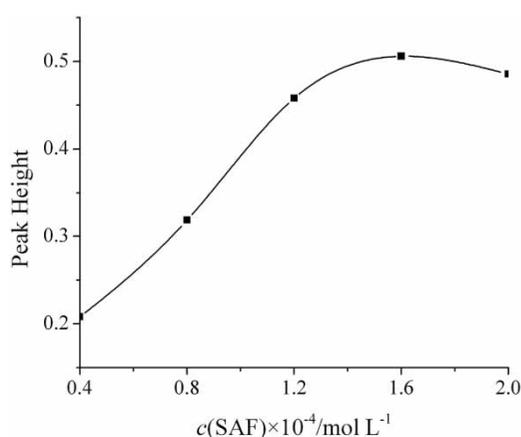


Figure 2 | Effect of concentration of SAF on peak height.

with chromogenic agent, the concentration of free color liquid in the solution is reduced and the balance of the formation of complex compound is damaged, causing color fading and signal peak height reduction. Thus, the optimum concentration was chosen as 3.6 g L^{-1} for the further experiments (Figure 3).

Effect of pH

The studies were carried out with various pH values of the borax buffer solution, and it was found that the best pH range for the reaction in an aqueous medium lies between 9.4 and 10.4. The correct adjustment of this variable is necessary to improve the formation of the complex. It was shown that the highest analytical signal was obtained when the pH value was 10.0. Afterwards, the signals decreased at higher pH values. These results indicate that the maximum concentration of the complex occurs when the pH value was about 10, which was chosen for subsequent studies (Figure 4).

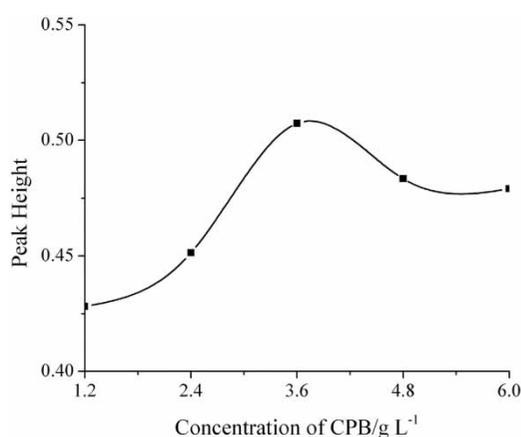


Figure 3 | Effect of concentration of CPB on peak height.

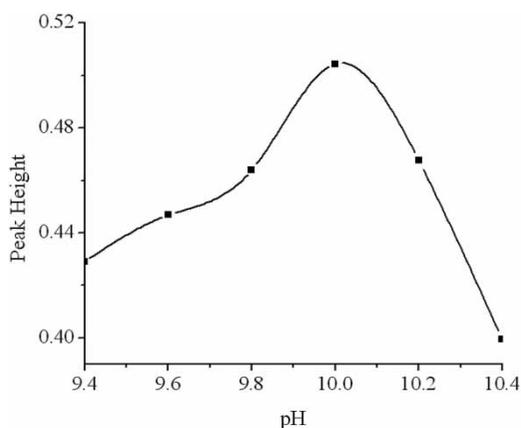


Figure 4 | Effect of pH on peak height.

Optimization of instrument parameters

To realize high sensitivity, minimum baseline noise, small baseline drift, the widest detection range and the shortest analysis time, the instrument parameters were optimized with $20 \mu\text{g L}^{-1}$ of Co(II) standard solution for testing.

Higher sample flow rate could cause shorter retention time and incomplete reaction for the target analyte; on the other hand, lower flow rate could cause higher dispersion and a broader peak. So the effect of sample flow rate on the signal was investigated. Injection flow rate of the host peristaltic pump was studied between 5 and 9 mL min^{-1} ; the analytical signals were observed to decrease at concentrations greater than 7 mL min^{-1} , the best signal height being obtained with a 7 mL min^{-1} of flow rate (Figure 5(a)). As is shown in Figure 5(b), the flow rate of the auxiliary peristaltic pump was selected in the range from 1 to 5 mL min^{-1} , and the greatest signal height was obtained at the rate of 1 mL min^{-1} . Figure 5(c) shows the influence of injection time of valve on the sensitivity for the range of 5–25 s. Since there was no improvement in sensitivity above 15 s, this time was employed. The length of the knotted reaction coil was tested in a range between 1 and 3 m. A 2 m knotted reaction coil is enough to obtain the maximum analytical peak height. Reactors of less than 2 m did not allow a good sample–reagents mixing, and longer reactors caused an increase of the reaction plug dispersion (Figure 5(d)).

Performance of the FIA system

On the basis of the fixed optimal analysis conditions, the developed method was validated in terms of linearity, detection limit, precision and anti-interference ability.

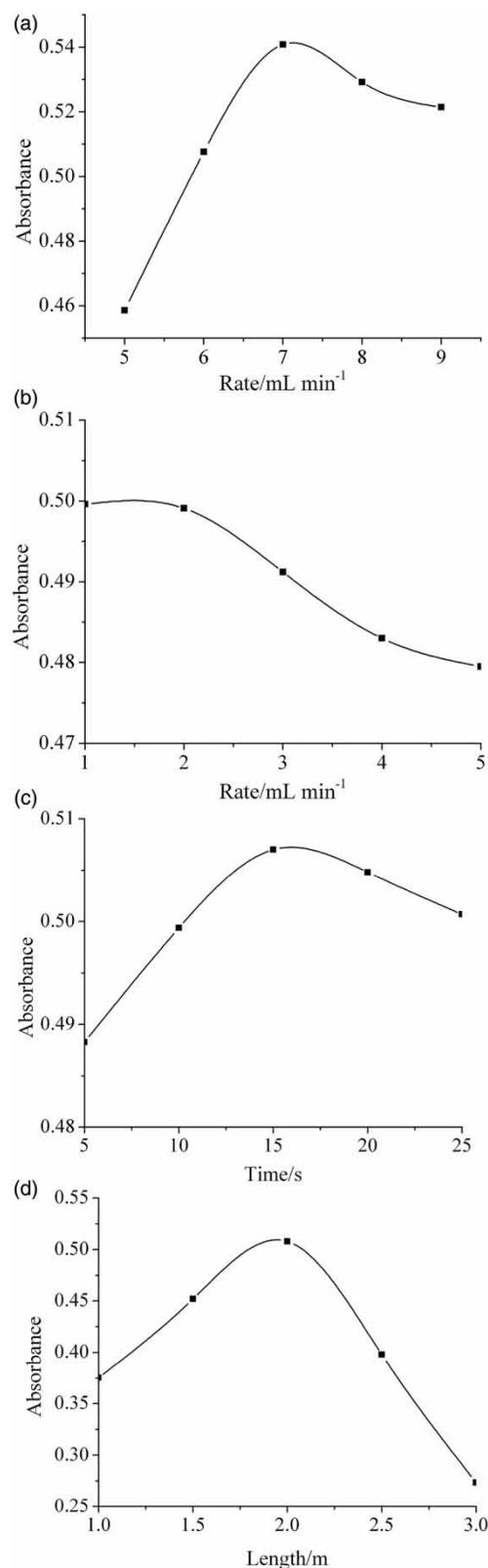


Figure 5 | (a) Effect of flow rate of host peristaltic pump. (b) Effect of flow rate of auxiliary peristaltic pump. (c) Effect of injection time of valve. (d) Effect of length of reaction coil.

Range of linearity

Using the proposed assay for Co(II) determination under the optimum conditions, a linear calibration curve over the range of 3–40 $\mu\text{g L}^{-1}$ cobalt(II) was established, which can be expressed by the regression equation $A = 0.006c + 0.3693$ ($r = 0.9995$), where A represents the peak height in absorbance and c is cobalt(II) concentration in $\mu\text{g L}^{-1}$ after subtraction of blank.

Limit of detection

Under the optimized conditions, the detection limit was calculated according to the IUPAC recommendations, as $K \times S_b/a$, where K is the coefficient according to a certain confidence level (the recommended value is 3), S_b represents the standard deviation of the blank measurements ($n = 20$) and a is the slope of the calibration graph. It was found to be 0.1 $\mu\text{g L}^{-1}$ cobalt(II), which is considered a satisfactory value for the determination of low concentration of samples.

Degree of precision

The within-day precision of the proposed method was evaluated by repeated injections ($n = 11$) of cobalt standard solutions. The relative standard deviations of Co(II) solutions at 3, 15 and 30 $\mu\text{g L}^{-1}$ were 0.91%, 0.95% and 0.89% respectively, showing good and reproducible analytical results.

Study of interferences

Some potential interferences have been investigated under optimum conditions. Various concentrations of foreign ions were spiked into a standard solution of 30 $\mu\text{g L}^{-1}$ Co^{2+} . An interfering concentration was considered as the concentration that caused variation in percentage recovery higher than $\pm 5\%$. Tolerance limits of some interfering ions (calculated in multiples) are as follow: I^{2+} (3), Cd^{2+} (4), Fe^{3+} (2), Zn^{2+} (2), Cu^{2+} (1) and Ni^{2+} (1.5). When Cu^{2+} , Cd^{2+} , Zn^{2+} , Ni^{2+} and Fe^{3+} are over their allowances and the interference occurs, adding 20 g L^{-1} of thiourea, 10 g L^{-1} of sodium fluoride and 20 g L^{-1} of potassium sodium tartrate is an effective way to mask them. The effect of a large amount of K^+ , Na^+ , SO_4^{2-} , NO_3^- , Cl^- , F^- , Ca^{2+} and Mg^{2+} was also investigated, and it was found that they did not interfere with the determination. Therefore the most common coexisting ions did not affect

Table 1 | Determination results of Co(II) in samples using the proposed method and reference method

Sample	Added/ $\mu\text{g L}^{-1}$	Found/ $\mu\text{g L}^{-1}$ ($n = 3$)		Recovery/%	
		Proposed FIA	AAS method	Proposed FIA	AAS method
Pond water	20	28.30	27.10	102.0	104.2
Lake water	12	15.35	15.70	97.5	96.8
Paddy field water	10	13.60	13.90	103.6	104.3
Purified drinking water	5	4.88	4.73	98.0	95.5
Black tea water 1	10	13.26	13.33	102.3	104.0
Black tea water 2	10	13.38	13.36	103.1	103.9
Black tea water 3	10	13.23	13.11	98.6	96.4
Green tea water 1	10	13.45	13.41	97.7	96.0
Green tea water 2	10	13.51	13.25	102.6	103.2
Green tea water 3	10	13.65	13.47	104.0	104.5

the determination, confirming the satisfactory selectivity of the proposed procedure.

Analytical application

Cobalt is commonly monitored for environmental protection purposes in water, agriculture and food control (Jadhav *et al.* 1998; Park & Cha 1998). To evaluate the applicability and validate the accuracy of the developed method, it was applied to determine Co(II) in 10 kinds of water samples in this study. All the samples were prepared with a simple pretreatment by adding an appropriate masking agent. Also an intercomparison experiment was carried out for the proposed method and atomic absorption spectrometry (AAS) at the same time. The results are presented in Table 1. Obviously, the recoveries in the spiked samples plus the results from the proposed and the reference method are in good agreement, showing the excellent performance of the method for the examined samples.

CONCLUSIONS

A simple, rapid and selective flow injection procedure was developed for the determination of cobalt(II) with micelle-solubilized spectrophotometric detection. The main advantages of the method are simplicity, reproducibility, high speed and sensitive analysis, making it possible to determine cobalt in water samples with satisfactory results. Thus this

research can be applied in the fields of environmental monitoring and the food service industry, where online, real-time and automatic monitoring of varying concentrations of Co(II) in potential polluted water can be realized to provide information of the trace element and ensure the safety of drinking water.

ACKNOWLEDGEMENTS

The authors are very grateful for the sponsorship of the Scientific Research Fund of Sichuan Provincial Education Department (grant no. 08zd1101). We are also thankful to the anonymous reviewers.

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First received 9 April 2014; accepted in revised form 31 July 2014. Available online 12 August 2014