Highly Selective and Sensitive Stripping Voltammetric Determination of Cobalt with Ammonium 2-Aminocyclohexene-1-dithiocarboxylate and Nitrite

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A sensitive stripping voltammetric determination of Co(II) is described, based on the selective accumulation of its complex with ammonium 2-aminocyclohexene-1-dithiocarbamate on HMDE and subsequent reduction of the complex in the presence of nitrite as a catalyst. The peak potential is about -1.35 V vs. Ag/AgCl reference electrode at pH = 10.0. The calibration graph was linear for the range of 0.01 – 40 ng/ml, with a limit of detection of 8.0 pg/ml. The relative standard deviation for ten replicate analyses of 1.0 ng/ml of Co(II) is 2.6%. The method is very selective and has been applied for the determination of cobalt in natural water and serum samples.

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Cobalt is a biologically essential trace element.¹ It serves its paramount function as an essential component of vitamin B₁₂. Although cobalt is required only in minute amounts for maintaining normal health, a lower or higher concentration may lead to deficiency or toxicity states, respectively, resulting in impairment or abnormalities of health. Since the concentration of cobalt is extremely low in tap water and biological samples, a highly sensitive method is necessary for its determination.¹ Many papers have been published on the determination of cobalt in various samples. The investigations have been carried out by various analytical techniques, such as atomic absorption spectrometry,^{2,3} neutron activation analysis,^{4,5} electrophoresis^{6,7} and electrochemical methods. For cobalt determination, different modern polarographic techniques such as differential pulse, stripping voltammetry and adsorption differential pulse voltammetry have been used as well as various cobalt complexes such as those of nioxime,⁷ α -benzil dioxime,⁸ dimethylglyoxime,⁹ 1,10-phenanthroline,¹⁰ diphenylglyoxime,¹¹ thiocyanate,¹² 2,2'-bipyridine,¹³ and 2-(5'-bromo-2'-pyridylazol)-5-diethylaminophenol.¹⁴ However none of those procedures are sensitive for direct determination of cobalt at sub ppb level, because such levels are comparable to the detection limits of the methods (Table 1). In this paper the catalytic-adsorptive stripping voltammetry of the complex of Co(II) with ammonium 2-amino cyclohexene-1-dithiocarbamate at a hanging mercury drop electrode (HMDE) was investigated.

Experimental

Reagents and chemicals

All chemicals were of analytical grade unless otherwise stated. Doubly distilled water was used throughout. The buffers were constructed of a mixture of 0.2 M NH₃ and 0.2 M NH₄Cl solution at suitable ratios.

A standard cobalt solution (1.00 mg/ml) was prepared by

dissolving cobalt(II)-nitrite in distilled water, this mixture was standardized complexometrically by EDTA. Standards of working solutions were made by appropriate dilution as required.

Sodium nitrite solution (1.5 M) was prepared daily by dissolving 10.350 g NaNO₂ (Merck) in water in a 100 ml volumetric flask. Traces of cobalt in blank electrolyte were removed using Chelex-100 resin.

The Chelex 100 was purified by shaking three times with distilled methanol, twice with distilled water, then once with ammonia buffer and finally twice with distilled water.

Ammonium 2-aminocyclohexene-1-dithiocarbamate (AADC) was prepared and purified according to Takeshima *et al.*¹⁵ An aliquot (1.0×10^{-3} M) of AADC solution was made in water by dissolving 0.0190 g of the ligand in 100 ml water in a volumetric flask. This solution was prepared daily.

Apparatus

A PAR Model 384 B polarographic analyzer was employed in connection with a Model 303 SMDE (EG & G, PAR, USA); a Model DMP 40-44 plotter and a Model 305 stirrer. In all experiments, conventional three electrode system, containing a medium size HMDE with a surface area of 1.8 mm² as a working electrode, an Ag/AgCl (3 M KCl) reference electrode and platinum wire counter electrode was used. Surface preconcentration of the cobalt complex was achieved using a fast stirring rate and a medium drop size. All potentials reported in this paper are referred to the Ag/AgCl electrode. Solutions were de-aerated with pure nitrogen for 4 min prior to each determination.

Serum preparation

Mineralization of 2 ml of the samples was carried out for 3 h at 160°C in a PTFE vessel with the addition of 4 ml of concentrated nitric acid. Samples containing a high concentration of cobalt were analyzed directly after dilution with water to a suitable volume. Samples containing low concentrations of cobalt were digested by UV irradiation for 3 h

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Table 1 Comparison of the previous stripping voltammetric and AES methods with the proposed method for the determination of Co(II)

Reagent used	Limit of detection/ng ml ⁻¹	Linear range/ng ml ⁻¹	RED, %	Interferent	Ref.
Nioxime-NO ₂	19	63 – 630	4 (n=5)	Zn ²⁺ , Ni ²⁺ , Fe ³⁺	7
α-Benzil dioxime	2.6	6.3 – 630	2.8 (n=8)	Zn ²⁺ , Fe ³⁺ , Ni ²⁺	8
Dimethylglyoxime	10	100 – 6000	2.5 (n=8)	Zn ²⁺ , Mn ²⁺ , Fe ²⁺ , Fe ³⁺	9
1,10-Phenanthroline	1	5 – 1000	2.3 (n=8)	Zn ²⁺ , Fe ³⁺ , Ni ²⁺ , Mn ²⁺	10
Diphenylglyoxime	200	220 – 2000	2.4 (n=6)	—	11
Thiocyanate	4.5	20 – 5900	1.6 (n=10)	Fe ²⁺ , Fe ³⁺ , MoO ₄ ²⁻ , Cu ²⁺ , Zn ²⁺ , Sn ²⁺	12
2,2'-Bipyridine	0.6	2.9 – 118	4.9 (n=25)	Zn ²⁺ , Ni ²⁺ , Cr ³⁺ , Fe ²⁺	13
BPDA	30	32 – 6300	3.5 (n=10)	Zn ²⁺ , Ni ²⁺ , Pb ²⁺	14
AAE ^a	3	3 – 100	2.7 (n=10)	—	3
ICP-AES method	3	—	—	—	20
Proposed method	0.008	0.010 – 40	2.6 (n=10)	Zn ²⁺	

BPDA, 2-(5'-bromo-2'-pyridylazol)-5-diethylaminophenol. a. Nitroso-R-salt as a preconcentration reagent.

using 1 kW lamp after dilution with water to 50 ml and addition of 0.20 ml hydrogen peroxide (30%) prior to the cathodic stripping voltammetric analysis.¹⁴ Samples containing low concentrations of cobalt must be digested by UV for 3 h.

Recommended procedure

The supporting electrolyte of 0.02 M NH₃/NH₄Cl, pH = 10.0 (10 ml), containing 8.0×10^{-6} M AADC and 0.30 M NO₂⁻, was pipetted into the cell. The solution was deaerated for 4 min by bubbling nitrogen. A mercury drop was then dialed on the HMDE and pre-electrolysis was carried out at -1.2 V for 60 s with stirring. After a 5 s setting period, the solution was cathodically polarized at a scan rate of 12 mV/s from -1.0 to -1.5 V. This gave the voltammetric response of the ligand. Then aliquots of the cobalt standard solution (less than 400 ng) were introduced into the cell while maintaining a nitrogen atmosphere over the solution. The above procedure was repeated to give responses from the Co(II)-AADC. All data were obtained at room temperature. Calibration graphs were prepared by plotting peak current against Co(II) concentration. The sample peak current is labeled as (*I*_p)_s and the blank peak current as (*I*_p)_b. The net signal, equal to (*I*_p)_s - (*I*_p)_b = Δ*I*_p, was used for construction of a calibration graph.

Results and Discussion

Ammonium 2-aminocyclohexene-1-dithiocarbamate is a complexing agent with -N and -S donors that is used for the spectrophotometric analysis of Ni(II) and Ni-Co-Cu mixture by the spectrophotometric method.¹⁶⁻¹⁸ This paper was the first report about the application of this ligand in voltammetric procedures.

Figure 1 shows the stripping voltammograms for the AADC-nitrite, and AADC-nitrite-Co(II) in 0.02 M ammonium buffer solution at pH = 10.0. As can be seen in Fig. 1a, for the solution containing AADC-nitrite alone, no obvious current peaks were observable between -1.0 to -1.5 V, whereas nitrite produces a large reduction current beyond -1.55 V vs. Ag/AgCl electrode. Figure 1b shows the stripping voltammogram for the Co(II)-AADC-nitrite system in ammonium buffer (pH = 10.0). The reduction of the adsorbed Co(II)-AADC complex takes place on HMDE around -1.35 V vs. Ag/AgCl electrode. When Co(II) concentration is about 50 ng/ml, no peak current was observed. On the other hand, with the addition of a small amount of nitrite when scanning the electrode potential in the negative direction, a new cathodic peak located at about -1.35 V was obtained for cobalt concentration of 6.0 ng/ml. This suggests a catalytic

Fig. 1 Stripping voltammogram for (a): AADC-NO₂⁻ system at pH 10; (b): AADC-NO₂⁻-Co(II) system at pH 10; conditions: NO₂⁻, 0.30 M; AADC, 8.0×10^{-6} M; Co(II), 7.0 ng/ml; scan rate, 12 mV/s; accumulation potential, -1.20 V vs. Ag/AgCl; accumulation time, 60 s.

process, because without addition of Co(II), there is no peak around -1.35 V vs. Ag/AgCl electrode for the AADC-NO₂⁻ system. In addition, as we know, nitrite may disproportionate to nitrate and nitrogen monoxide in aqueous media.¹⁹ When nitrite was not added, no catalytic current was observed for the electrode process. On the other hand, when nitrate was substituted for nitrite, no obvious peak current for the Co(II)-AADC was observed with 50 ng/ml Co(II). Thus the intense signal for Co(II)-AADC-NO₂⁻ system for 6.0 ng/ml Co(II) indicates the remarkable sensitivity associated with the catalytic process. The influence of Triton X-100 as a surface-active substance at a concentration of 0.1 mg/ml causes a 70% decrease of the catalytic peak. The same effect of surface-active substances such as sodium dodecyl sulfate on the decreasing of the catalytic peak current was observed. On the other hand, the peak current decreased when the temperature increased from 25 to 55°C. These phenomena show that the complex is strongly adsorbed on HMDE.

Influence of variables

The effect of pH, AADC and nitrite concentration,

Fig. 2 Influence of pH on the peak current. conditions: AACD, 4.0×10^{-5} M; NO_2^- , 0.10 M; Co(II), 25.0 ng/ml; scan rate, 20 mV/s; accumulation potential, -1.1 V; accumulation time, 40 s.

Fig. 4 Influence of nitrite concentration on the peak current. Conditions same as Fig. 2 with 8.0×10^{-6} M AACD at pH of 10.0.

Fig. 3 Effect of AACD concentration on the peak current. Conditions same as Fig. 2 at pH of 10.0.

Fig. 5 Influence of accumulation potential on the peak current. Conditions: AACD, 8.0×10^{-6} M; NO_2^- , 0.30 M; Co(II), 25.0 ng/ml; pH 10.0; scan rate, 20 mV/s; and accumulation time of 40 s.

accumulation potential, time and scan rate in the sensitivity were studied in the presence of 25 ng/ml Co(II) for sample reaction at room temperature.

Our experiments showed that the peak shape was improved in the presence of ammonium chloride. A mixture of ammonia and ammonium chloride was used as a buffer to produced buffers in the pH range of 8.0 to 10.2. Figure 2 shows the influence of pH on the peak current. The results show that by increasing pH up to 9.0, the peak current increases, whereas from pH 9.0 to 10.2, the peak current slightly decreased. On the other hand, for pH values about 8.5–9.5, the shape of the peak is not suitable (broaden peak produced). Thus a pH of 10.0 was selected as the optimum for the analysis as this is conveniently buffered by ammonia.

Figure 3 shows the influence of AACD concentration for the range of 0.4–30 μM . The results show that by increasing AACD concentration up to 8.0 μM , the current increases, but at ligand concentration above 8.0 μM , the peak current decreased slightly. This effect is due to the competitive adsorption of AACD.

Variation of the nitrite concentration on peak current for 25

ng/ml Co(II) in the presence of 8.0 μM AACD at pH = 10.0 is shown in Fig. 4. The results show that the nitrite concentration had a strong effect on the peak current. With increases in nitrite concentration, the peak current increases at a strong rate. Due to the reduction of nitrite at potentials more negative than -1.5 V, a rather broad reduction peak was observed at higher nitrite concentration of 0.30 M. Thus a nitrite concentration of 0.30 M was used for further experiments. In addition, in order to minimize the cobalt concentration in the reagent blank (as an impurity), the nitrite was cleaned with Chelex-100 resin.

Variation of the deposition potential showed that the peak height increased with the deposition potential increasing from -0.2 to -1.2 V, whereas greater values cause decreasing in peak current (Fig. 5). Therefore, all measurements were carried out using a deposition potential of -1.2 V.

The influence of deposition time on the peak current showed that the peak current increased linearly with the deposition time within 10–120 s (Fig. 6). The cathodic stripping voltammetric sensitivity is therefore significantly improved by extending the deposition period. A deposition time of 60 s was selected by comparison between sensitivity and time of analysis. In

Fig. 6 Influence of accumulation time on the peak current. Conditions same as Fig. 5 with accumulation potential of -1.2 V.

Fig. 7 Effect of scan rate on the sensitivity. Condition same as Fig. 5 with accumulation potential of -1.2 V and accumulation time of 60 s.

addition, if we select a long accumulation time, the interference effect of potential interfering species in real samples may be increased.

The effect of scan rate on the sensitivity was studied for 25 ng/ml Co(II). Figure 7 shows the influence of scan rate on the peak current. The results show that maximum sensitivity was achieved at a scan rate of 12 mV/s, whereas at a scan rate greater than 12 mV/s, the peak current decreased. Thus a scan rate of 12 mV/s was selected.

Analytical data

Under the optimum conditions described before, the calibration graph is linear in the range from 0.01 to 40.0 ng/ml for accumulation time of 60 s. The regression equation is $\Delta I_p = 0.096 + 61.4C_{\text{Co(II)}}$ with $r = 0.9998$, when ΔI_p is nA and C is the Co(II) concentration in ng/ml. The relative standard deviation for ten replicate measurements of 1.0 and 25.0 ng/ml of Co(II) is 2.6% and 1.6%, respectively.

The detection limit, estimated from the blank signal plus 3 times its standard deviation, was found to be 0.008 ng/ml.

Interference study

In order to evaluate the selectivity of the proposed method, the influence of 36 foreign ions on the determination of 25 ng/ml

Table 2 Interference study for the determination of Co(II) at the optimum conditions

Added ion	Tolerance limit
Cs ⁺ , K ⁺ , Li ⁺ , Cu ²⁺ , Mn ²⁺ , Hg ₂ ²⁺ , Ba ²⁺ , Ca ²⁺ , La ³⁺ , Cr ³⁺ , Pb ²⁺ , Pd ²⁺ , S ₂ O ₈ ²⁻ , UO ₄ ²⁻ , B ₄ O ₇ ²⁻ , HCO ₃ ⁻ , WO ₄ ²⁻ , CH ₃ COO ⁻ , ClO ₃ ⁻ , I ⁻ , Br ⁻ , Cl ⁻ , BrO ₃ ⁻ , S ₂ O ₃ ²⁻ , NO ₃ ⁻ , CrO ₄ ⁻	1000
Th ⁴⁺ , TeO ₃ ²⁻ , SCN ⁻ , Ce ⁴⁺	500
Cd ²⁺ , Ni ²⁺ , Fe ²⁺ , Fe ³⁺ , IO ₃ ⁻	200
Zn ²⁺	75

Table 3 Analysis of real samples at the optimum conditions

Sample	Co(II) added/ ng ml ⁻¹	Co(II) found/ ng ml ⁻¹	Recovery, %
River water	0.0	0.23±0.03	—
River water	10.0	10.35±0.05	101.2
River water	20.0	20.40±0.06	101.0
Tap water	0.0	0.15±0.02	—
Tap water	10.0	10.20±0.03	100.5
Tap water	20.0	20.25±0.04	100.5
Blood	0.0	0.93±0.02	—
Blood	10.0	10.85±0.04	99.3
Blood	15.0	15.80±0.06	99.2

a. $n = 5$.

Co(II) was studied. The results are shown in Table 2. The results show that the determination of cobalt was free from the interference of Ni²⁺ up to 200-fold and Zn²⁺ up to 75-fold, which is of particular significance considering their major interference in all of the other adsorptive catalytic voltammetric measurements.

Analysis of real samples

In order to evaluate the applicability of the method to real sample analysis, the method was applied to determination of cobalt in natural water and human blood with sample pretreatment after addition of different amounts of Co(II) to them. The cobalt content of human blood after vitamin B₁₂ administration was determined using the optimized condition. The results are shown in Table 3.

The catalytic-adsorptive voltammetric method described here is suitable for determining low cobalt concentrations in real samples. However, for serum samples, a thorough mineralization procedure consisting of acid digestion followed by digestion by UV irradiation has to be used to eliminate interferences from residual organic matter. Because the detection limit mainly depends on the cobalt content of the blank, particular attention should be paid to the use of purified reagents.

The present study demonstrates that the catalytic adsorptive stripping voltammetric determination of cobalt is excellent for determination of ultra trace concentration in real samples. The method is inexpensive and is a convenient alternative for high cost mass spectrometry, neutron activation^{4,5} and ICP-AES²⁰ analyses. The limit of detection of the method is much lower than that of FIA-preconcentration-atomic absorption spectrometry.³

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