コバルト(II)による鉄(III)の還元反応に及ぼす Nitro-PAPS の効果とそれを
利用するコバルト(II)のフローインジェクション分析

Effect of Nitro-PAPS on reduction of iron(III) with cobalt(II) and flow injection analysis of cobalt(II) in real samples

田中　美穂[†], 渡辺　靖之[†], 大野　慎介[†], 田丸　貴臣[†], 手嶋　紀雄[†], 酒井　忠雄[®†]
Miho TANAKA, Yasuyuki WATANABE, Shinsuke OHNO, Takaomi TAMARU, Norio TESHIMA and Tadao SAKAI

Abstract The reduction reaction of iron(III) with cobalt(II) easily occurs in the presence of 2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)phenol (nitro-PAPS) at pH 3.5, in which iron(II)-Nitro-PAPS chelate is formed, and the chelate has a specific absorption maximum at 790 nm. Cobalt(II) can be thus determined by measuring absorbance of the complex at above wavelength. The redox reaction is introduced into a two-channel flow system for the determination of cobalt(II). The calibration curve is linear in the concentration range of 2.5×10^{-7} M～1×10^{-5} M cobalt(II) with a sample throughput of 70 samples h^{-1}. The detection limit (S/N = 3) is 1×10^{-7} M and the relative standard deviation for 10 determinations is 0.3% for 2.5×10^{-6} M cobalt(II). The proposed flow-injection spectrophotometric method is applied to analyses of cobalt(II) in cobalt alloy (NIST SMR 862; High Temperature Alloy L 605) and pepperbush (NIES standard, No. 1) and to the indirect determination of vitamin B_{12} in pharmaceuticals.

1. Introduction

A cobalt alloy contains chromium, tungsten, or molybdenum, and it has some advantages such as high hardness and the endurance against oxidation [1]. This alloy is used as medical instruments [2], glass frame, scissors, and accessory and so on. It is also known that cobalt exists in the center of vitamin B_{12} which participates in blood formation in human body. Thus, indirect determination of Vitamin B_{12} can be possible by detecting cobalt [3]. Since the decrease of Vitamin B_{12} in human body may cause neuralgia, pernicious anemia, and eyestrain [4], this vitamin is compounded into supplements and eye lotions. Therefore, highly sensitive analytical method for the determination of cobalt is required for the quality control.

The standard redox potential of Co(III)/Co(II) system is 1.81 (vs. NHE) [5], and therefore Co(III) rarely exists in aqueous solution. This means that Co(III) obtained by electrolysis can be used as a strong oxidation reagent [6]. Co(II) is easily oxidized to Co(III) in the presence of an appropriate ligand which forms the corresponding stable complex with Co(III). Vydra and Pribil [7,8] reported that the conditional redox potential of $Co(phen)_3^{3+}/Co(phen)_3^{2+}$ at pH 2 was 0.37 V. Teshima et al. developed the novel redox system of Co(II) with V(V), Cr(VI) [9], and Ce(IV) [10] in the presence of 2,2'-bipyridyl or phen and reported a titration method for the simultaneous determination of Cr(VI) and Fe(III) in high carbon ferrochrome using phen[11].

Water-soluble pyridylazo compound such as 2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)phenol (nitro-PAPS) [12] was synthesized as a sensitive chromogenic reagent for the determination of metal ions. Makino et al. [13, 14] and Ohno et al. [15] have reported the sensitive colorimetric determination of heavy metal using nitro-PAPS in serum. The nitro-PAPS is used as pre-column chelating agent in HPLC [16-18] and is also utilized for the determination of iron [19] or vanadium [20] by FIA. However, the effect of ligand on the redox reaction of metal ion has been never discussed quantitatively so far.

In this chapter, the redox reaction of Co(II) with Fe(III) is investigated titrimetically in the presence of nitro-PAPS. As a result, it is proved that Co(II) reduces Fe(III) to Fe(II) in the presence of nitro-PAPS. The rapid and highly sensitive FI system is developed for the determination of Co(II) by measuring the specific absorption of the produced Fe(II)-nitro-PAPS complex. This method is successfully applied to the determination of Co(II) in cobalt alloy, pepperbush, and eye lotions.

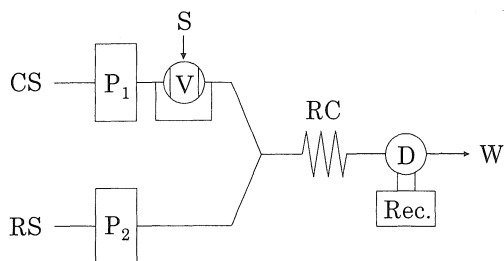## 2. Experimental

2.1. Reagents

[†]愛知工業大学　工学部　応用化学化（豊田市）

Fig. 1 Flow system for the determination of cobalt(II). CS: 0.01 M HCl; RS: a mixed solution of 2.0×10^{-5} M Fe(III), 2.5×10^{-4} M Nitro-PAPS and 0.5 M acetate buffer (pH 3.5); P1 and P2: pump (flow rate 1.5 ml/min each pump); RC: reaction coil (3 m×i.d. 0.5 mm); D: spectrophotometer; S: sample (200 μl); V: six-way valve; Rec.: recorder; W: waste.
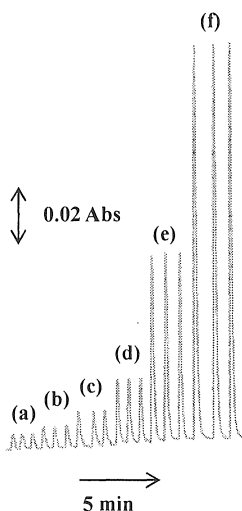

Fig. 3 Effect of Nitro-PAPS on the titration of Fe(III) with Co(II). Titrand (20 ml taken): a mixed solution of 5×10^{-4} M iron(III), 1.5×10^{-3} M Nitro-PAPS and 0.25 M acetate buffer (pH 3.5); Titrant: 5×10^{-3} M cobalt(II).

Fig. 2 Flow signal for cobalt(II). (a), 5.0×10^{-7} M; (b), 7.5×10^{-7} M; (c), 1.0×10^{-6} M; (d), 2.0×10^{-6} M; (e), 5.0×10^{-6} M; (f) 1.0×10^{-5} M. Other conditions are the same as in Fig. 1.

A 1 M acetic acid solution was prepared by diluting 28.6 ml of acetic acid (Sigma Aldrich Japan, Tokyo) to 500 ml with water. A 1 M sodium acetate solution was prepared by dissolving 8.203 g of sodium acetate (Nakalai Tesque, Kyoto) with water and made up to 100 ml with water. These solutions were mixed to prepare a buffer solution at pH 3.5.

2.2. Apparatus

A Mitsubishi Chemical Model GT-07 automatic titrator was used for the potential difference measurement. A Horiba Model F-22 pH/mV meter was used for pH adjustment. A V-560 double-beam spectrophotometer was used with 10 mm light-path cells for absorbance measurements.

The system was consists of an intelligent HPLC pump (PU-1580, Jasco corp.), a six-way injection valve (FLOM), a spectrophotometer (S-3250, Soma Optics) with a flow cell (cell volume: 8μl) and a FIA monitor (F·I·A corp.) for record. All connecting lines and reaction coils were made from 0.5 mm i.d. Teflon tubing.

2.3. Procedure

A carrier solution (CS, 0.01 M hydrochloric acid) and a Reagent solution (RS, 0.5 M acetate buffer at pH 3.5, 2×10^{-5} M Fe(III), 2.5×10^{-4} M nitro-PAPS) were delivered at 1.5 ml min^{-1} by pump 1 (P$_1$). The sample (200 μl) was injected to carrier solution and merged the R$_1$ solution. The absorbance of the Fe(II)-nitro-PAPS complex formed in the reaction coil (RC) at 3 m was monitored at 790 nm using a double-beam spectrophotometer and recorded on a recorder. Fig. 1 and Fig. 2 shows flow system and flow signals for cobalt(II).

All of the reagents were of analytical-reagent grade and were used without further purification. All solutions were prepared with deionized water purified with an Advantec Aquarius GSH-210 system.

A 2.5×10^{-3} M nitro-PAPS solution was prepared by dissolving 0.126 g of nitro-PAPS (Dojin) in water and made up to 100 ml with water.

A 2.0×10^{-2} M cobalt(II) solution was prepared by dissolving 2.380 g of cobalt(II) chloride hexahydrate (Wako) in 5×10^{-2} M sulfuric acid and made up to 500 ml. The stock solution was prepared by suitable dilution with 0.01 M hydrochloric acid.

A 2.0×10^{-2} M iron(III) solution was prepared by dissolving 4.822 g of ammonium iron(III) sulfate decahydrate (Wako) in 0.5 M sulfuric acid and made up to 500 ml.

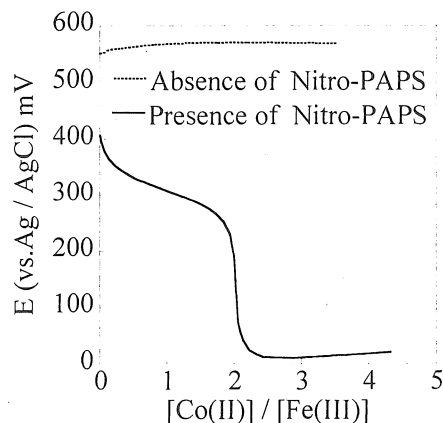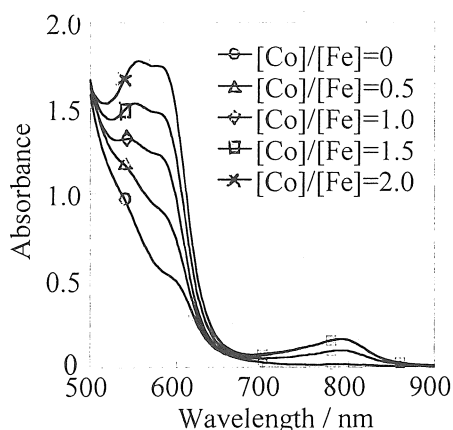Cobalt(II) and iron(III) were standardized by EDTA titration.

3. Results and discussion

Fig. 4 Absorption spectra of iron-nitro-PAPS chelate after reduction with Co(II). [Fe(III)]: 2.5×10^{-6} M; [Nitro-PAPS]: 4×10^{-4} M; [acetate buffer]: 0.25 M (pH 3.5); molar ratio ([Co(II)]/[Fe(III)]): (○) 0 (Fe(III) only); ( ) 0.5; ( ) 1.0; (□) 1.5; (×) 2.0.

3.1. The reduction of iron(III) with cobalt(II)

The equilibrium constant (K_{Co-Fe}) is calculated to be $10^{-17.6}$ from the standard redox potentials of $E°_{Co}$ (1.81 V vs. NHE) and $E°_{Fe}$ (0.771 V vs. NHE) [5]. Thus, this reduction of Fe(III) with Co(II) can not proceed.

$$Co(II) + Fe(III) \xrightleftharpoons{K_{Co-Fe}} Co(III) + Fe(II)$$
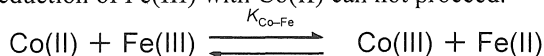
If a suitable ligand that can form a stable complex with Co(III) and Fe(II) exists, the equilibrium constant can be improved. Thus, the effect of nitro-PAPS on the reduction was studied. The result is shown in Fig. 3. Since the potential break did not appear in the absence of nitro-PAPS, Co(II) can not reduce Fe(III) to Fe(II). However, a potential break was obtained in the presence of nitro-PAPS. The effect of pH on the titration curve was studied. The end point was detected at the equilibrium point in the range of pH 2.5 - 3.5. The conditional equilibrium constant of this redox system estimated from the conditional redox potential was $10^{4.9}$. Although the conditional equilibrium constant is not enough for the determination of cobalt (or iron) by titrimetry, it was enough to apply to spectrophotometric analysis.

3.2. Absorption spectrum of iron-nitro-PAPS complex

The effect of Co(II) concentration on the Fe(II)-nitro-PAPS complex formation was studied. The result is shown in Fig. 4. The absorbance at around 560 nm increased with increasing the molar ratio. When the ratio was over 1, the absorbance continued to increase. This indicated that the complex formation of Co(II) with nitro-PAPS proceeded at the ratio over 1. Although the absorbance at 790 nm increased with increasing the concentration of Co(II), the absorbance was kept constant at molar ratio over 1. Thus, the absorption at 790 nm is depended on the only Fe(II)-nitro-PAPS complex.
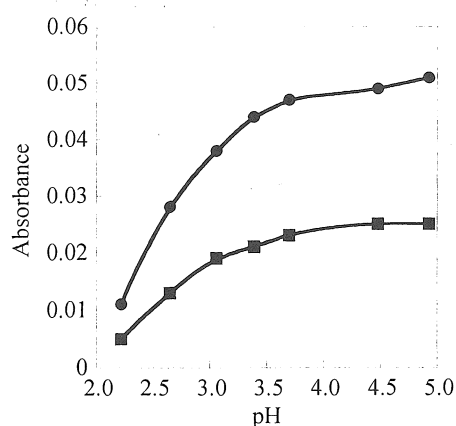

Fig. 5 Effect of pH on iron(II)-nitro-PAPS complex. (■) 2.5×10^{-6} M; (●) 5.0×10^{-6} M; other conditions as in Fig. 1.
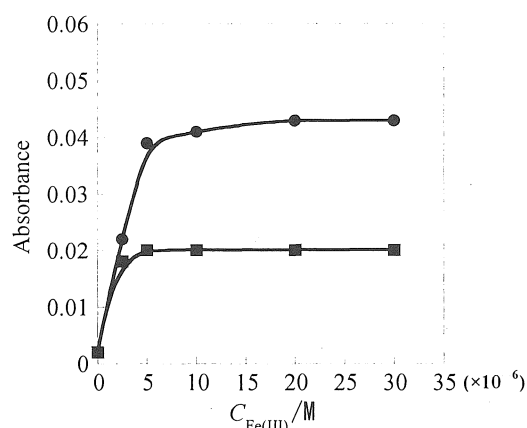

Fig. 6 Effect of iron(III) concentration on the peak height of cobalt(II). (■) 2.5×10^{-6} M; (●) 5.0×10^{-6} M; other conditions as in Fig. 1.

Although the maximum wavelength of Co(III)-nitro-PAPS complex is at 560 nm, the various metal ions such as copper, zinc, and nickel may form the chelate with nitro-PAPS, and these chlates have the absorption maximum at around 560 nm. Taking into account the selectivity, we selected the monitoring wavelength of 790 nm for the determination of Co(II) in this proposed method.

3.3. Optimization of FIA conditions

The effect of pH was investigated over the range of 2.2 – 4.9. This result is shown in Fig. 5. The absorbance increased with increasing pH up to 3.5 and continued to increase slightly above 3.5. The pH 3.5 was selected by taking into consideration the result obtained by the potentiometric titration of iron(III) with Co(II) in the presence of nitro-PAPS shown in Fig. 3.

The effect of Fe(III) concentration was varied from 2.5×10^{-6} to 3.0×10^{-6} M. The result is shown in Fig. 6. The absorbance of was maximum and almost constant over 5×10^{-6} M. Thus, the 2.0×10^{-5} M Fe(III) was
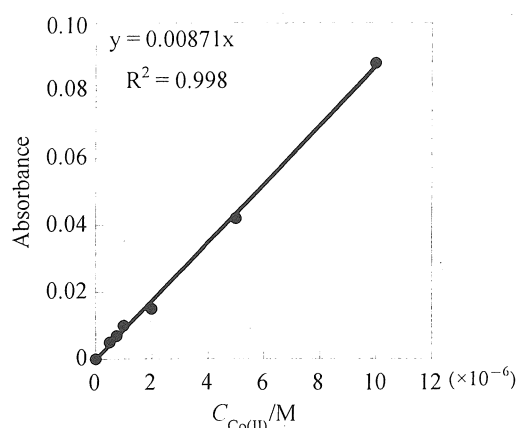
Fig. 7 Calibration curve for cobalt(II) in the optimum conditions. Conditions as in Fig. 1.

chosen.

The effect of nitro-PAPS concentration was studied. The peak height was constant more than 2×10^{-4} M. Thus, 2.5×10^{-4} M nitro-PAPS was selected for the determination of Co(II).

The length of RC was varied from 0.5 to 5 m. The peak height was almost constant over the length of RC examined. The length of RC was used as 3 m.

The effect of the flow rate on the peak height was examined in the range $0.5 - 2.0$ ml min^{-1}. The peak height of Co(II) was almost constant more over 0.75 ml min^{-1}. The flow rate was selected as 1.5 ml min^{-1} for the sake of the sampling rate.

The sample volume was studied in the range from 120 to 280 μl. The peak height increased with increasing the injected volume. The sample volume was selected to be 200 μl.

3.4. Calibration curve for cobalt(II)

The calibration curve for Co(II) was prepared under the optimum condition. The result is given in Fig. 7. The dynamic range was $2.5\times10^{-7} - 1.0\times10^{-5}$ M for Co(II) ($R^2 = 0.998$). The sampling rate was 70 sample h^{-1}. The relative standard deviation (RSD) (n = 10) was 0.3 % for 2.5×10^{-6} M. The detection limit (S/N = 3) was 1×10^{-7} M for Co(II).

3.5. Effect of interference ions on the determination of cobalt(II)

A large amount of iron exists in cobalt alloy and trace amount of iron exist as impurity in eye lotion. Thus, the interference of iron on the determination of cobalt(II) was examined. The maximum absorption wavelength ($\lambda_{max} = 560$ nm) of cobalt(III)-nitro-PAPS complex was compared that ($\lambda_{max} = 790$ nm) of iron(II)-nitro-PAPS complex. When the mixed solution of 2.5×10^{-6} M cobalt(II) and iron(III) was injected to the flow system, the peak height of that at 560 nm was obtained 2 times peak height of 2.5×10^{-6} M cobalt(II). But the peak height of cobalt(II) at 790 nm was subject to no

Table 1 Effect of foreign ions on the determination of 5.0×10^{-6} M cobalt(II)

$\dfrac{[Ion]}{[Co(II)]}$	Added Ion
10000	Na(I), Mg(II), Al(III), K(I), Ca(II) SO_4^{2-}, NO_3^-, CO_3^{2-}
5000	Mn(II)
1000	Cd(II)
200	Zn(II), Rb(I), Sr(II), Ba(II)
50	Si(IV)
20	$Cr_2O_7^{2-}$, Pb(II)
10	VO_2^+, Ni(II), Cu(II)
2	WO_4^{2-}
1	Fe(III)

An error of ±5% was considered to be tolerable.

Table 2 Determination of cobalt(II) in practical samples

Sample	Cobalt(II) or Vitamin B_{12} found[a]	Nominal or certified value
Vitamin B_{12}[b]	1430±10 μg / 3 tablets	1500 μg / 3 tablets
Vitamin B_{12}[c]	0.014±0.000₃ %	0.015 %
Cobalt alloy	51.1±0.1 %	51.5±0.3 %
Pepperbush leaves[d]	20.7±0.1 μg/g	23±3 μg/g

a. Average for three determinations.
b. Tablet.
c. Eye lotion.
d. The iron in pepperbush was removed by solvent extraction.

interference. Thus, the measurement wavelength at 790 nm was chosen.

Table 1 summarizes the tolerance limits for foreign ions on the determination of 5×10^{-6} M cobalt(II). An error of ±5 % was considered to be tolerable. The iron(III), tungsten(VI), nickel(II) and copper(II) showed serious positive interference on the determination of 5×10^{-6} M cobalt(II). As the concentration level of these metals does not exist in cobalt alloy, this proposed method can apply to the determination of cobalt(II) in cobalt alloy. But pepperbush includes a large amount of iron ion , so it is necessary to remove iron.

3.6. Application to real samples

The proposed method was applied to the determination of cobalt(II) in medicines, cobalt alloy, and pepperbush. The procedure for the decomposition of Vitamin B_{12}, cobalt alloy, and pepperbush leaves are depicted below.

Vitamin B_{12} : To beaker, six tablets or 10 ml of eye lotions, 20 ml of conc. hydrochloric acid were taken.

The solution was heated at 200 °C for 2 hour for decomposition. After 10 ml of nitric acid and 2 ml of perchloric acid were added to the beaker, it evaporated to dryness. The residue was dissolved by adding 30 ml of 1 M hydrochloric acid and diluted to 50 ml with distilled water. The solution was appropriately diluted for the determination.

Cobalt alloy : The cobalt alloy standard sample (NIST SMR 862; High Temperature Alloy L605) purchased from National Institute of Standard and Technology (NIST) was used. To vessel, 0.1 g of the sample, 2 ml of conc. nitric acid, 2 ml of conc. hydrochloric acid, and 1 ml of conc. hydrofluoric acid were taken. The mixture was heated at 230 °C for 30 minutes by the microwave digestion labstations (Milestone com.) for the decomposition of cobalt alloy. The solution was diluted to 100 ml with water.

Pepperbush leaves : 0.2 g of pepperbush leaves (NIES No.1) purchased from National Institute for Environmental Studies (NIES) were taken to small teflon vessel. After 2 ml of nitric acid, 0.4 ml of perchloric acid, 0.2 ml hydrofluoric acid were added to the vessel. The small vessel was placed in a large teflon vessel in where 1.5 ml of water was put. The large vessel was placed in a stainless vessel, and the solution in the stainless vessel was heated at 90 °C for 2 hours and at 130 °C for 2 hours, and then it was evaporated to dryness under an infrared lamp on the hot plate. The residue was dissolved by adding 4 ml of 0.1 M hydrochloric acid and was heated. To remove iron in pepperbush, the extraction of hinokitiorl in benzene solution was done. The solution is appropriately diluted with 0.01 M hydrochloric acid for the determination.

The analytical results are shown in Table 2. The content of Vitamin B_{12} in medicine was estimated from the determination value of cobalt. As a result, these values almost corresponded to the label values or certified values.

Acknowledgment

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