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Spectrophotometric Determination of Cobalt(II) and Cyanocobalamin with Vanillilfluorone and Its Applications

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Spectrophotometric determination of cobalt(II) was accomplished with vanillilfluorone (VF) in the presence of dimethylbenzyltetradecylammonium chloride (Zephiramine, Zep). In the determination of cobalt(II), Beer's law was obeyed in the range of 24—470 ng/ml, with an effective molar absorption coefficient (at 575 nm) and relative standard deviation of 1.35×10^5 l mol^{-1} cm^{-1} and 0.66% ($n=5$), respectively. The composition ratio of the colored complex was determined by the mole ratio and continuous variation methods, and it was found to be Co(II) : VF : Zep = 1 : 2 : 4. Analysis of cyanocobalamin by the same procedure showed that cyanocobalamin could be determined in the concentration range of 0.5—0.11 µg/ml using the proposed method.

Key words spectrophotometry; cobalt(II); vanillilfluorone; cyanocobalamin

Cobalt, having a Clarke number of 4×10^{-3}, is produced along with nickel, and a small amount of cobalt is also contained in copper zinc ore. Cobalt is present in steel as an impurity and is used as an additive in alloy steel in the refinery process. Additionally, cobalt is one of the essential trace elements. Cobalt is contained in blood at a concentration of 0.2—2 µg/l, and it also forms a part of the structure of the vitamin B_{12}. Therefore, the microanalysis of cobalt is very important.

Numerous studies have attempted to quantify cobalt(II) using atomic absorption spectrochemical analysis, flame photometry, inductively-coupled plasma optical emission spectrometry, fluorescent X-ray analysis, neutron activation analysis, calorimetry, and spectrophotometry. In addition, organic reagents including nitroso-R, 1-nitroso-2-naphthol, 2-nitroso-1-naphthol, nitrosocresol, 2-nitroso-1-naphthol-4-sulfonic acid, salts of thiocyanic acid, 1-(2-pyridylazo)-2-naphthol, and diethyldithiocarbamic acid have been used in the spectrophotometric determination of cobalt(II).[1—8] However, most of these methods are organic solvent extraction methods, and they suffer from the disadvantages that the use of organic solvents is not eco-friendly and the procedures are difficult and complicated. This report describes a simple and highly sensitive method for the determination of cobalt(II) and the application of this method to the determination of cyanocobalamin using vanillilfluorone (VF), which has a relatively facile synthetic procedure relative to other xanthene dyes.

Experimental

Synthetic procedure: vanillilfluorone According to a method described in literature,[9] 1,4-benzoquinone was dissolved in 20 ml of acetic anhydride and 1 ml of sulfuric acid and maintained at 30—40 °C for 30—60 min. The reaction mixture was poured into 500 ml of water to afford a white precipitate that was filtered and washed with water. 1,2,4-Benzenetriol triacetate was obtained after drying the precipitate under reduced pressure for 2 h. In the synthesis of VF, 3 g of vanillin and 10 g of 1,2,4,-benzenetriol triacetate were dissolved in 100 ml of 20% ethanol and 1 ml of concentrated sulfuric acid. The color of the solution changed to deep red upon heating in a steam bath for 3 h. This solution was subsequently allowed to stand in a cool, dark place for 3 weeks. The obtained crude VF was then collected and dissolved in methanol saturated with sodium bicarbonate, on a steam bath. The solution was then filtered hot into 10% acetic acid and allowed to stand in a cool, dark place for several days. The precipitate was filtered and washed with pure water to yield pure VF that was dried under reduced pressure. [^{1}H-NMR δ: 7.04 (d, 1H, J=8.05 Hz), 7.03 (sd, 1H, J=0.55 Hz), 6.86 (dd, 1H, J=8.05, 1.65 Hz), 6.83 (s, 2H), 6.68 (s, 2H), 3.80 (s, 3H)]. [High resolution-mass spectra (HR-MS) FAB-MS m/z: 367.082 (Calcd for $C_{20}H_{15}O_7$: 367.0818). The synthetic scheme is shown in Fig. 1.

Reagents and Apparatus A stock (1.0×10^{-3} M, 1 M = 1 mol dm^{-3}) Co(II) standard solution (Wako Pure Chem. Co., Ltd., Japan) was prepared in water. Working solutions were prepared by suitable dilution of this stock solution as required. A solution of VF, which had been synthesized according to a method described in the literature,[9] was prepared in a 1.0×10^{-3} M methanol solution. A 1.0% aqueous solution of dimethylbenzyltetradecylammonium chloride (Zephiramine, Zep, Dojindo Laboratories Co., Ltd., Japan) was prepared by dissolving Zep in water and was filtered using a 0.65-µm membrane filter. A buffer solution of pH 9.2 was prepared by dissolving borax (Nacalai Tesque Co., Ltd., Japan) in water and filtered using a 0.65-µm membrane filter. Reagent-grade chemicals were used throughout. Pure water was prepared by purifying deionized water using a Milli-Q Labo system just before use. A Shimadzu spectrophotometer (Model UV-160) with 1.0-cm matched silica cell was used for absorbance measurements. The pH measurements were made using a Horiba (F-11) pH meter in combination with a calomel glass electrode.

Standard Procedure for Determination of Cobalt(II) The following components were mixed in a 10-ml volumetric flask: a stock solution containing cobalt ions, 1.0 ml of 1.0% Zep solution, 2.0 ml of 0.05 M borax solu-

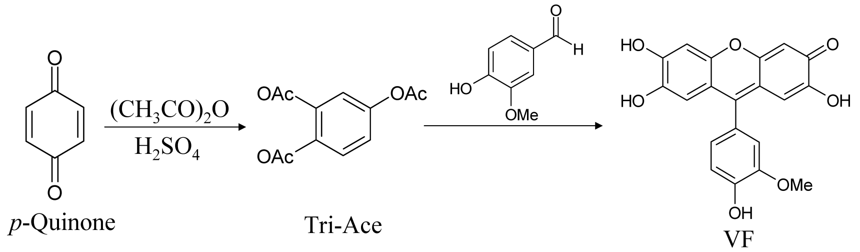

Fig. 1. Synthesis of VF

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tion (pH 9.2), and 0.5 ml of a 1.0×10^{-3} M VF solution. The mixture was diluted to 10 ml with water, transferred to a test tube, mixed well, and kept at room temperature for 10 min. The absorbance of the resultant solution was measured at 575 nm against a reagent blank without cobalt ions.

Results and Discussion

Effect of Buffer Solution and pH The effect of pH on the color reaction of the solution containing cobalt(II) was examined using weakly basic solutions. The color intensity and the reaction rate of cobalt(II) with VF were maximal in the weakly basic solution at room temperature. The effects of the buffer solution on the color reaction were examined by using buffers with buffering activities in the weakly basic range. These buffers included 0.05 M borax solution, 0.1 M Tris/HCl solution, 0.2 M ammonia/ammonium chloride solution, 0.1 M $NaHCO_3/Na_2CO_3$ solution, and 0.1 M glycine/NaOH solution. It was found that borax buffer at a pH of approximately 9.2 was the most appropriate for the determination of cobalt. Thus, 2.0 ml of the 0.05 M borax solution (pH 9.2) was used for the pH adjustments.

Effect of Surfactants The effects of dispersing agents on the cobalt(II)-VF reaction was examined using cationic surfactants (Zep, stearyltrimethylammonium chloride (STAC), cetyltrimethylammonium chloride (CTAC), cetylpyridinium chloride (CPC)), anionic surfactants (sodium dodecylsulfate (SDS)), amphoteric surfactants (Swanol AM-301), and non-ionic surfactants (poly(*N*-vinyl pyrolidone) (PVP), polyoxyethylene sorbitan monooleate (Tween 80), polyethylene glycol mono-*p*-isooctylphenyl ether (Triton X-100), polyoxyethylene lauryl ether (Brij 35) (Table 1). The maximal, constant absorbance was obtained by adding more than 0.5 ml of 1.0% Zep to 10 ml of the cobalt(II)-VF solution.

Effect of VF Concentration The optimal VF concentration was studied in the presence of 4.0×10^{-6} M cobalt(II). The maximal, constant absorbance at 575 nm was obtained by adding more than 0.5 ml of the 1.0×10^{-3} M VF solution to the final volume of 10 ml.

Stability of Coloring Complex The changes in the absorbance with time were examined in the presence of the borax solution and Zep. The coloring complex became stable immediately upon combining the reagents, and the absorbance remained stable for at least 60 min. Therefore, a 10-min standing time at room temperature was selected for all of the measurements to determine cobalt(II).

Table 1. Effect of Surfactants

Surfactant	λ_{max} (nm)	Absorbance
None	525	0.009
Zep	575	0.536
Benzalkonium chloride	575	0.534
STAC	575	0.522
CTAC	575	0.520
CPC	575	0.449
SDS	525	0.129
Swanol (AM 301)	570	0.164
PVP (K-30)	590	0.371
PVP (K-90)	590	0.405
Triton X-100	535	0.074
Brij 35	420	0.265

Co(II): 4.0×10^{-6} M; 5.0×10^{-5} M; surfactants: 0.1%; pH 9.2; reference: reagent blank solution.

Effect of Additional Order The effect of additional order was examined. The maximum absorbance was obtained when the cobalt(II) solution was added last. Therefore, the order of addition was Zep, buffer, VF, and cobalt(II).

Absorption Spectra, Calibration Curve, Sensitivity, and Accuracy The absorption spectra of the cobalt(II)-VF and VF solutions were acquired according to standard procedures, and these are shown in Fig. 2. The calibration curve produced using the standard procedure showed a good linear relationship in the concentration range of 24—470 ng ml^{-1} of cobalt(II) ion. The effective molar absorption coefficient (ε) was $1.35\times10^{5}\,dm^3\,mol^{-1}\,cm^{-1}$. The sensitivity of this method was more than 4-fold that of the Nitroso-R method ($\varepsilon=3.3\times10^{4}\,dm^3\,mol^{-1}\,cm^{-1}$),[10,12] 3-fold that of the 1-nitroso-2-naphthol method ($\varepsilon=4\times10^{4}\,dm^3\,mol^{-1}\,cm^{-1}$),[13] 8-fold that of the 2-nitroso-1-naphthol method ($\varepsilon=1.6\times10^{4}\,dm^3\,mol^{-1}\,cm^{-1}$),[14] 100-fold that of the thiocyanate method ($\varepsilon=1.2\times10^{3}\,dm^3\,mol^{-1}\,cm^{-1}$),[15] and 6-fold that of the 1-(2-pyridylazo)-2-naphthol method (PAN method, $\varepsilon=2\times10^{4}\,dm^3\,mol^{-1}\,cm^{-1}$).[16] Therefore, the proposed method was superior to the other methods not only in terms of sensitivity but also with regard to speed and simplicity. The relative standard deviation (RSD) of the procedure was below 0.66% ($n=5$), indicating that the reproducibility of the proposed method was good. For reference, the other metal ions were also measured under the standard procedure used for cobalt. The results shown in Table 2 indicate that λ_{max} of cobalt was red-shifted compared with those of other metal ions except for iron(II).

Effects of Coexisting Ions The effects of coexisting metal ions on the proposed method were examined by using 235 ng ml^{-1} of cobalt(II). Because this method was conducted under basic conditions, the presence of metal ions in-

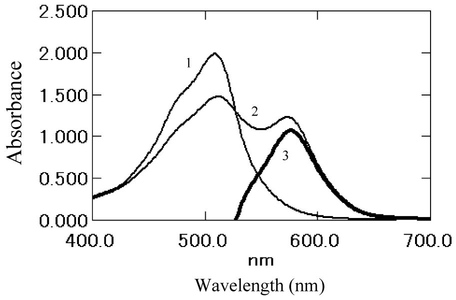

Fig. 2. Absorption Spectra Obtained by Standard Procedure

Co(II): 4.0×10^{-6} M; VF: 5.0×10^{-5} M; Zep: 0.1%; pH: 9.2; reference: reagent blank solution. 1: VF solution against water, 2: VF-Co(II) solution against water, 3: VF-Co(II) solution against VF solution.

Table 2. Comparison of Wavelength of Metal Ions Used

Metal ion	λ_{max} (nm)
Co(II)	575
Cu(II)	565
Ni(II)	545
Mn(II)	550
Zn(II)	540
Fe(II)	575
Al(III)	540

Metal ion: 4.0×10^{-6} M; VF: 5.0×10^{-5} M; Zep: 0.1%; pH: 9.2; reference: reagent blank solution.

Table 3. Effect of Foreign Substances

Substance	Conc. (ng/ml)	Mole ratio (substance/ Co^{2+})	Absorbance at 575 nm	Recovery (%)
None	—	—	0.536	100.0
Ca(II)	1.60×10^2	1	0.536	100.0
Ni(II)	2.35×10^2	1	0.666	124.0
Fe(II)	2.23×10^2	1	0.735	137.0
Fe(II)+NaF	—	—	0.536	100.0
Fe(III)	2.23×10^2	1	0.585	109.0
Fe(III)+Na_2HPO_4	—	—	0.536	100.0
Cu(II)	2.54×10^2	1	0.995	186.0
Zn(II)	2.62×10^2	1	0.604	113.0
Cd(II)	4.50×10^2	1	0.594	111.0
Ti(IV)	1.91×10^2	1	0.667	124.0
Ti(IV)+NaF	—	—	0.536	100.0
NaCl	2.34×10^4	100	0.536	100.0
KBr	4.76×10^4	100	0.536	100.0
NaI	6.00×10^4	100	0.536	100.0
KNO_3	4.04×10^4	100	0.536	100.0
$NaNO_2$	2.76×10^4	100	0.536	100.0
NH_4Cl	2.14×10^4	100	0.536	100.0
Na_2SO_4	5.68×10^4	100	0.536	100.0
KH_2PO_4	5.44×10^4	100	0.536	100.0
Sodium citrate	1.18×10^4	—	0.536	100.0
Sodium succinate	4.72×10^4	100	0.536	100.0
Sodium oxalate	5.36×10^4	100	0.536	100.0
Sodium tartarate	9.20×10^4	100	0.536	100.0
Uric acid	6.72×10^3	10	0.536	100.0
Urea	2.40×10^4	100	0.536	100.0
Creatinine	4.52×10^4	100	0.536	100.0
Glycine	3.00×10^4	100	0.481	92.3
Lysine	5.85×10^4	100	0.536	100.0
Glucose	7.21×10^4	100	0.536	100.0
Caffeine	7.77×10^4	100	0.536	100.0
Thiamine	1.06×10^4	10	0.491	94.2
Pyridoxal hydrochloride	8.15×10^3	10	0.499	95.7
Ascorbic acid	7.04×10^3	10	0.536	100.0
Histamine	4.45×10^2	1	0.499	95.8
HSA	1.00×10^3	—	0.536	100.0

Co(II): 235 ng/ml; VF: 5.0×10^{-5} M; Zep: 0.1%; pH 9.2; reference: reagent blank solution.

Table 4. Recovery Tests on Tap Water and Rain Water

Sample	Recovery (%)
Tap water	99.5
Rain water	98.2

Co(II): 4.0×10^{-6} M; VF: 5.0×10^{-5} M; Zep: 0.1%; pH: 9.2; reference: reagent blank solution.

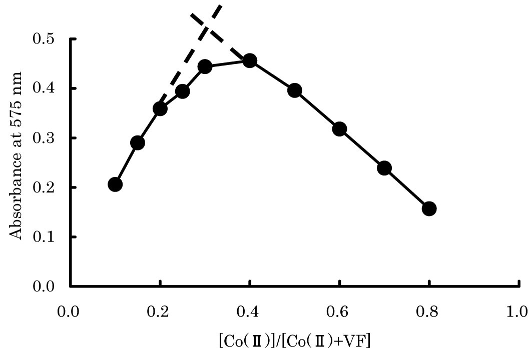

Fig. 3. Composition of Complex by Continuous Variation Method

[Co(II)+VF]=2.0×10^{-5} M; Zep: 0.1%; pH: 9.2; reference: reagent blank solution.

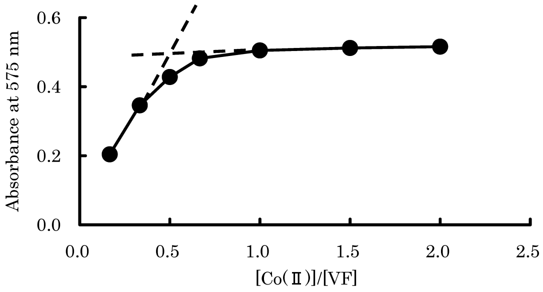

Fig. 4. Composition of Complex by Molar Ratio Method

Co(II): [2.0×10^{-4} M$\times$X] ml/10 ml; VF: 2.0×10^{-5} M; Zep: 0.1%; pH: 9.2; reference: reagent blank solution.

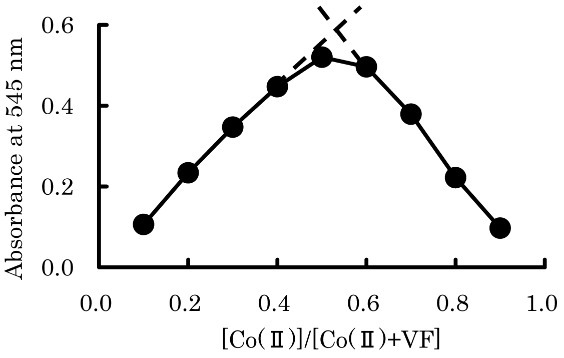

Fig. 5. Composition of Complex by Continuous Variation Method

[Co(II)+VF]=8.0×10^{-5} M; Tween 80: 0.1%; pH: 9.2; reference: reagent blank solution.

cluding Ni(II), Fe(II), Fe(III), Cu(II), Zn(II), Cd(II), and Ti(IV) at a concentration similar to that of Co(II) positively interfered with this technique. In the experiments carried out with these metals, Fe(II) and Ti(IV) were completely masked by 1.0 ml of 0.1 M NaF, and Fe(III) was completely masked by 1.0 ml of 0.2 M Na_2HPO_4. On the other hand, no interference was observed with coexisting organic acid ions and organic compounds, including sodium oxalate, urea, glucose, caffeine, and HSA. The results of the effects of foreign substances are summarized in Table 3.

Application of Proposed Method to Real Samples This method was applied to the recovery test of tap and rain water. The results shown in Table 4 indicate that the recoveries were 98—99% and that this method can be used for the determination of cobalt(II) in real samples.

Determination of Cyanocobalamin The proposed method was applied to the determination of cyanocobalamin, a cobalt(II)-containing vitamin of the B_{12} family. Pretreatment based on the Japanese Pharmacopeia[17] was carried out by mixing 1 mg of cyanocobalamin with 0.05 g of potassium hydrogen sulfate. The mixture was fused by igniting, because cyanocobalamin did not react with VF directly, and cyanocobalamin was determined as an aqueous solution. The results show that a linear relationship was observed on the calibration curve for concentrations in the range of 0.5—11 μg ml^{-1} of cyanocobalamin.

Composition of Coloring Complex The composition ratio of cobalt(II) to VF was examined by the continuous variation method and the molar ratio method. The molar ratio of cobalt(II) to VF determined by both methods was 1 : 2, as

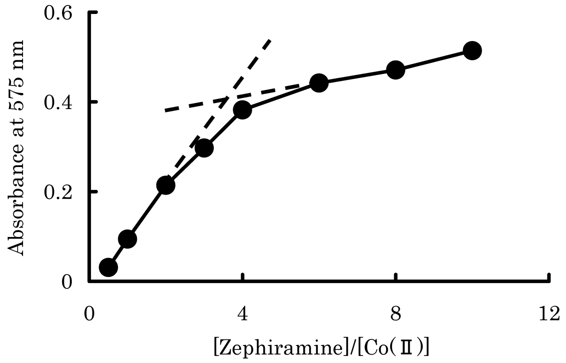

Fig. 6. Composition of Complex by Molar Ratio Method

Zep: [8.0×10^{-4} M×X] ml/10 ml; Co(II): 8.0×10^{-5} M; VF: 1.6×10^{-4} M; Tween 80: 0.1%; pH: 9.2; reference: reagent blank solution.

shown in Fig. 3 and Fig. 4, respectively. On the other hand, the composition ratio of cobalt(II) to VF was examined in the presence of Tween 80, a non-ionic surfactant, using the continuous variation method, and was found to be 1 : 1. However, subsequent determinations using Zep, a cationic surfactant, gave the high-order coloring complex. The composition ratio of [Co(II)-VF] to Zep was examined by the molar ratio method and the formation of a ternary intermetallic complex with a ratio of cobalt(II) : VF : Zep = 1 : 2 : 4 was estimated in the presence of Tween 80, as shown in Fig. 5 and Fig. 6.

Conclusion

We developed a simple and high sensitivity spectrophotometric method for the determination of cobalt(II) by using VF in the presence of borax solution and Zep, a cationic surfactant. In the determination of Co(II) by this method, Beer's law was obeyed in the range of 24−470 ng ml^{-1}, with an effective molar absorption coefficient (at 575 nm) of 1.35×10^5 dm^3 mol^{-1} cm^{-1}. The sensitivity of the proposed method was approximately 4-fold that of the nitroso-R method ($\varepsilon=3.3\times10^4$ dm^{-3} mol^{-1} cm^{-1}), approximately 3-fold that of the 1-nitroso-2-naphthol method ($\varepsilon=4\times10^4$ dm^{-3} mol^{-1} cm^{-1}), approximately 8-fold that of the 2-nitroso-1-naphthol method ($\varepsilon=1.6\times10^4$ dm^{-3} mol^{-1} cm^{-1}), approximately 100-fold that of the thiocyanate method ($\varepsilon=1.2\times10^3$ dm^{-3} mol^{-1} cm^{-1}), and 6-fold that of the 1-(2-pyridylazo)-2-naphthol (PAN) method which uses azo color ($\varepsilon=2.0\times10^4$ dm^{-3} mol^{-1} cm^{-1}). The method was applied to the recovery test for environmental samples, and a good recovery rate was obtained. This method was also demonstrated in the determination of environmental samples. In addition, cyanocobalamin, a cobalt(II)-containing complex, was also determined, and the calibration curve showed a good linear relationship for 0.5—11 μg ml^{-1}.

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