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Determination of Vitamin B12 Using Differential Pulse Polarography

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Abstract

Vitamin B12 is a type of vitamin also known by the name cobalamin. B12 is involved in many metabolism activities, including DNA synthesis, nervous system, red blood formation and immune system. Therefore, we chose the Differential Pulse Polarography (DPP) method is that has a high sensitivity for the determination of vitamin B12. This determination was possible with cobalt present in vitamin B12 structure. Since Co(III) is formed from the oxidation of the vitamin, its polarographic behavior had to be determined in various electrolytes such as acetate, borate, phosphate and ammonia. The polarograms of Co(III) were taken in these electrolytes in which 1.0 M NH_3/NH_4^+ (pH = 9.8) and 1.0 M $AcOH/AcO^-$ (pH = 4.0) were found as the most suitable electrolytes. This method was successfully applied vitamin of B12 determination in a 1 mL ampoule with high precision. The LOD was found as 3.7×10^{-7} for instead of (S/N = 3). Besides Co(III), interference effects of Zn(II), Ni(II), Cr(III), Fe(III), Cu(II), Cd(II) and Se(IV) were also studied. It was found that only Zn(II) peak had an overlap Co(III) peak in ammonium buffer. This problem could be solved by working in 1.0 M $AcOH/AcO^-$ (pH = 4.0) buffer. B12, which is 1000 μg in 1 mL vitamin ampoule, was found for 4 measurements as 999 ± 15 μg as a result of 95% confidence interval.

Keywords

Vitamin B12, Determination, Cobalt, Differential Pulse Polarography, Interferences Studies

1. Introduction

Vitamin B12 is a type of vitamin also known as cobalamin, and it is a water-soluble vitamin like other B vitamins. He is involved in many metabolism ac-

tivities, especially DNA synthesis, nervous system, it formation and immune system. Its chemical structure is quite complex. There is a very rare biochemical cobalt mineral in the central region, and this cobalamin is named after this mineral. B12 is made using the cobalt analysis. Electrochemical methods are preferred because of their high sensitivity and low interference effect in the determination of cobalt.

Differential pulse polarography has some advantages. It is a method that does not require enrichment and extraction in DPP for the determination of trace amounts of elements. These kinds of determinations are rarely possible in many analytical methods [1] [2]. Some interference studies can easily be done with DP polarography; the reproducibility is very high when compared with different methods [3] [4] [5]. Same cations with several oxidation numbers can easily be determined with DPP. Determination of Sn(II) with Sn(IV) and determination of As(III) with As(V) can give for such examples [6] [7]. Some molecules such as ascorbic acid [8], vitamin K3 [9], uric acid [10] and nitrite [11] which are not so easy to determine with the above given methods, can also be determined with our DP polarographic methods without any difficulty.

B12 in vitamin B12 tablets were determined by detection by a high performance liquid chromatography [12]. Vitamin B12, both in foods and in premixes, was determined by reversed-phase liquid chromatography with UV detection [13]. A new method was reported for the direct determination of B12 in seawater based on C18 solid-phase extraction and quantification by Reversed-Phase High-Performance Liquid Chromatography (HPLC) with an UV-V is detector [14].

UV-visible spectrophotometric, adsorptive stripping voltammetry and capillary electrophoretic study of metal ions have been studied and application was made to the determination of Co(III) in vitamin B12 [15]. The semi-derivate voltammetric peak current method was applied to determine the content of vitamin B12 in pharmaceutical preparations using gold modified electrode [16]. Cyclic voltammetry (CV) and square wave adsorptive stripping voltammetry (SWAdSV) were used to investigate the performance of an *ex situ* plated bismuth-film electrode (BiFE) employed to study the electrochemical behavior and the electroanalytical determination of vitamin B12 [17]. Trace Cobalt in vitamin B12 is determined by polarography using a complex of Co(II)-thymolphthalein-nitrite. With this adsorptive wave it was possible to determine cobalt in vitamin B12 [18]. A Self-Catalytic Carbon Paste Electrode for the detection of vitamin B12 has been constructed and it was used for the quantification of vitamin B12 in pharmaceutical products and biological matrix media [19].

In this study, vitamin B12 was determined with the cobalt contained in it. In the analysis, high sensitivity differential pulse polarography was used. In this analysis, the interference effects of other cations were examined. The appropriate medium for vitamin B12 determination was investigated and the vitamin B12 in the ampoule was analyzed in this medium.

2. Experimental

2.1. Apparatus

A PAR (Model 174A) polarographic analyzer system equipped with a PAR mercury drop timer was used. The natural drop time of the mercury electrode was in the range of 2 - 3 s (2.37 mg/s). A Kalousek electrolytic cell with a reference saturated calomel electrode (SCE), separated by a liquid junction, was used in the 3-electrode configuration, so that the IR drop can be eliminated. The counter electrode was platinum wire. The polarograms were recorded with a Linseis (LY1600) X-Y recorder. DP polarograms were recorded under the conditions of a drop life of 1 s, a scan rate of 5 mV/s, and a pulse amplitude of 50 mV.

2.2. Reagents

Various supporting electrolytes including ammonium, acetate, borate and phosphate buffer with or without EDTA were used over a wide pH range.

1.0 M AcOH/AcONa electrolyte: It was prepared by adding 6 g of solid NaOH, washed with distilled water in order to remove the carbon-ate formed, into 57 mL of 1.0 M AcOH, and diluting into 1 L with distilled water. The pH was adjusted with the addition of acid or base to the de-sired value using a pH meter.

1.0 M Borate buffer: 12.4 g H_3BO_3 was dissolved in small amount of distilled water in a 100 mL volumetric flask, then 4.0 g NaOH was added and all of them was dissolved with distilled water. The pH was adjusted by the addition of an appropriate amount of 1.0 M NaOH to the desired value using a pH meter.

1.0 M NH_3/NH_4Cl buffer: 8 mL of concentrated NH_3 is added into a 100 mL volumetric flask, after addition of 5.0 g of NH_4Cl and about 100 mL distilled water, the pH was adjusted to 9.8, by the addition of 1.0 M NaOH using a pH meter.

1.0 M Phosphate buffer: 15.6 g of Na $H_2PO_4\cdot2H_2O$ and 7.8 g of $Na_2HPO_4\cdot2H_2O$ are dissolved in water and diluted into 100 mL in a volumetric flask. Then 50 mL is taken from this solution and its pH was adjusted by the addition of 1 M NaOH to the required pH value using a pH meter.

0.1 M Mo(VI) solution: 0.88 g of $(NH_4)_6Mo_7O_{24}\cdot4H_2O$ is dissolved in distilled water and diluted into 50 mL.

0.1 M Se(IV) solution: 0.28 g of SeO_2 is dissolved in hot distilled water and diluted into 50 mL.

Standard 0.1 M Co(II), Pb(II), Zn(II), Ni(II), and Cu(II) solutions were prepared from their standard nitrate solutions. However, Fe(III), Cr(III) and Cd(II) solutions, their chloride salt was used.

Preparation of B12 vitamin solution: Since Cobalt is present in the organic structure of vitamin B12, it had to be decomposed with concentrated acid mixture as given below, so that the organic structure was decomposed and Co(III) ions could be dissolved in the solution. Thus, it was possible to determine vitamin B12 from the dissolved Co(III). For the destruction of B12 molecule, so that Co(III) can be determined, 0.5 mL H_2SO_4 and 5 mL HNO_3 was added to 1mL of

B12 vitamin taken from the ampoule. It was left wait until the solution was clear. Then it was warmed up while shaking the solution, until the solution left was about 0.5 mL. When it was cool the final solution was made 1 mL using distilled water. By the addition of these acids, the organic structure will decompose and Co(III) ions will become free.

3. Results and Discussion

3.1. Determination of the Optimum Working Conditions for Co(III)

Since cobalt ion is present in vitamin B12 as Co(III) after destruction with acids, the best medium for its determination had to be studied. For this purpose in various electrolytes such as pH = 2, 4 and 5 AcOH electrolyte, borate, phosphate and NH_3/NH_4Cl buffer the DP polarograms were taken and the peak potentials and peak heights for 1×10^{-5} M Co(III) were determined (Table 1). As can be seen from Table 1 the most suitable electrolytes for the determination of Co(III) was found as 1.0 M NH_3/NH_4Cl (pH = 9.8) and 1.0 M $AcOH/AcO^-$ (pH = 4.0) because of their peak heights. However, only in 1.0 M NH_3/NH_4Cl (pH = 9.8) electrolyte the peak of Co(III) was high and sharp. In 1.0 M $AcOH/AcO^-$ (pH = 4.0) electrolyte on the other hand the peak was high enough but it was not as sharp as it was in ammonia. Figure 1 is given as an example for the determination of Cobalt.
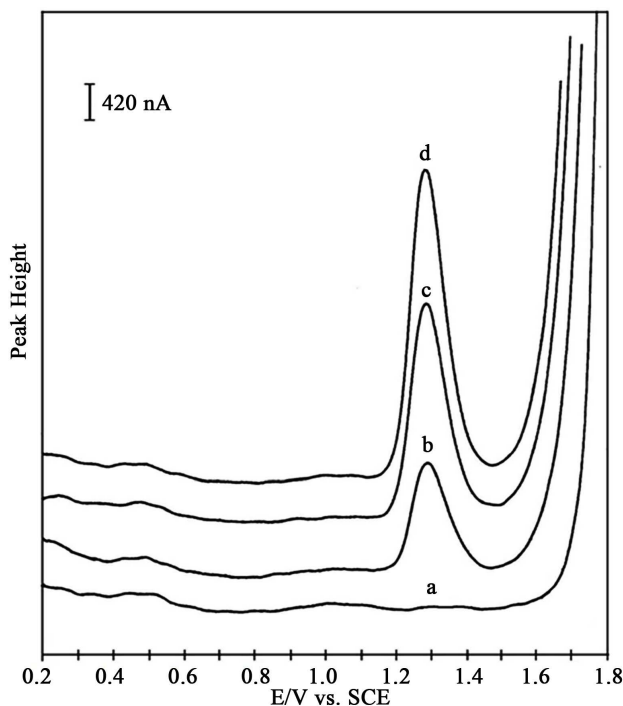

Figure 1. Determination of 1×10^{-5} M Co(III) with standard additions ((b), (c), (d)) in 1.0 M NH_3/NH_4Cl buffer (pH = 9.8). a: 10 mL 1.0 M NH_3/NH_4Cl buffer (pH = 9.8); b: a + 0.1 mL 1×10^{-3} M Co(III); c: b + 0.1 mL 1×10^{-3} M Co(III); d: c + 0.1 mL 1×10^{-3} M Co(III).

Table 1. The peak potentials and shapes of 1×10^{-5} M Co(III) in various buffers.

Medium	Ion	Peak potential (V)	Peak height (mm)	Peak shape
1.0 M Borate buffer (pH = 9.5)	1×10^{-5} M Co^{3+}	−1.43	11 ± 1	Broad
1.0 M Phosphate buffer (pH = 9.6)	1×10^{-5} M Co^{3+}	−1.64	11 ± 1	Broad
1.0 M HAc/Ac⁻ buffer (pH = 4.0)	1×10^{-5} M Co^{3+}	−1.35	33 ± 1	Broad
1.0 M NH_3/NH_4Cl buffer (pH = 9.8)	1×10^{-5} M Co^{3+}	−1.28	32 ± 1	Sharp

3.2. Interference Studies

There is no element other than Co(III) in the composition of vitamin B12 in ampoule. However, some elements may be added for some purposes. In this case, there may appear some interference.

The most common ions Fe(III), Zn(II), Ni(II), Cu(II), Cr(VI), Se(IV), Cd(II) and Pb(II), were investigated for their possible interference effect. The peak potentials of the above given ions are studied in two different electrolytes, 1.0 M NH_3/NH_4Cl (pH = 9.8) and $AcOH/AcO^-$ (pH = 4.0), since these were the best electrolytes for Co(III) determinations.

Their peak potentials are summarized in Table 2. As can be seen there will be no peak overlap with any ion except with Zn(II) in 1.0 M NH_3/NH_4Cl (pH = 9.8). In this medium while Co(III) peak was at −1.28 V, Zn peak was at −1.32 V and both were sharp. That is, in the presence of Zn(II), the determination of Co(III) will not be correct in 1.0 M NH_3/NH_4Cl (pH = 9.8).

In AcOH electrolyte (pH = 4.0) on the other hand, Table 3, there is no peak overlap between Zn and Co(III). However, while Zn peak was sharp, Co(III) peak was broad and thus, the accuracy for Co(III) determination cannot be high. Because of the low accuracy for Co(III) in AcOH electrolyte (pH = 4.0) medium, in our further studies we preferred to use NH_3 buffer for the determination of Co(III). However, in the presence of Zn(II) ion, Co(III) can be determined in AcOH (pH = 4.0) medium but with low accuracy (Table 3).

3.3. Determination of Co(III) in Artificially Prepared Solution Containing Fe(III) Using 1.0 M NH_3/NH_4Cl (pH = 9.8) Electrolyte

For this purpose a 10 mL artificially prepared solution is prepared containing 1×10^{-3} M Co(III) and 1×10^{-3} M Fe(III) (this maybe the most possible ion added to the vitamin) from this solution 0.1 mL is taken and added into a polarographic cell containing 10 Ml NH_3/NH_4Cl (pH = 9.8) electrolyte. The polarogram obtained was as same as which was obtained for pure Co(III) solution. While no peak for Fe(III) was observed, a peak for Co(III) at −1.28 V was observed. Its quantity could be calculated by standard additions of 10^{-5} M Co(III) solution. The quantity of 1×10^{-5} M Co(III) was found with only 1.6% error and the results found are given in Table 4. The LOD was found as 3.7×10^{-7} M with S/N = 3.

Table 2. The peak potentials and shapes of Co(III), Zn(II), Ni(II), Cr(VI), Fe(III), Cu(II), Cd(II) and Se(IV) in NH_3/NH_4 buffer at pH = 9.

Medium	Ions	Peak potantial (V)	Peak height (mm)	Peak shape
1.0 M NH_3/NH_4Cl buffer (pH = 9.8)	1×10^{-5} M Co(III)	−1.28	32 ± 1	Sharp
	1×10^{-5} M Zn(II)	−1.32	30 ± 1	Sharp
	1×10^{-5} M Ni(II)	−1.09	22 ± 1	Sharp
	1×10^{-5} M Cr(VI)	−0.28	45 ± 1	Sharp
	1×10^{-5} M Fe(III)	No peak	No peak	No peak
	1×10^{-5} M Cu(II)	−0.47	17 ± 1	Sharp
	1×10^{-5} M Cd(II)	−0.78	53 ± 1	Sharp
	1×10^{-5} M Se(IV)	−1.60	35 ± 1	Broad

Table 3. The polarographic behavior of 1×10^{-5} M Co(III), Zn(II) and Fe(III) ions in 1.0 M AcOH/AcO⁻ electrolyte under various pH values.

Medium 1.0 M AcOH/AcO⁻	Ions	Peak potantial (V)	Peak height (mm)	Peak Shape
pH = 2	1×10^{-5} M Co(III)	No peak	No peak	No peak
pH = 2	1×10^{-5} M Zn(II)	−1.02	11 ± 1	Broad
pH = 2	1×10^{-5} M Fe(III)	No peak	No peak	No peak
pH = 4	1×10^{-5} M Co(III)	−1.35	33 ± 1	Broad
pH = 4	1×10^{-5} M Zn(II)	−1.02	35 ± 1	Sharp
pH = 4	1×10^{-5} M Fe(II)	No peak	No peak	No peak
pH = 5	1×10^{-5} M Co(III)	−1.43	11 ± 1	Broad
pH = 5	1×10^{-5} M Zn(II)	−1.09	20 ± 1	Broad
pH = 5	1×10^{-5} M Fe(III)	No peak	No peak	No peak

Table 4. Determination of Co(III) in a synthetic sample contaninig 1×10^{-5} M (Fe(III), Co(III)) in 1.0 M NH_3/NH_4Cl pH = 9.8 buffer.

Medium	$[Co^{3+}]$ (M) in synthetic sample	Determined $[Co^{3+}]$ (M)	$\bar{x}$	s	$\bar{x} \pm ts/\sqrt{N}$
1.0 M NH_3/NH_4Cl buffer (pH = 9.8)	1×10^{-5}	1.01×10^{-5} 1.02×10^{-5} 1.02×10^{-5} 1.01×10^{-5}	1.02×10^{-5}	2.08×10^{-7}	$(1.02 \pm 0.03) \times 10^{-5}$

N = 4, 95% confidence interval.

3.4. Determination of Co(III) in a Artificially Prepared Solution Containing Zn(II) and Fe(III) Using AcOH/AcO⁻ (pH = 4.0) Electrolyte

Because of the peak overlap of Zn(II) and Co(III) in NH_3/NH_4Cl electrolyte (pH = 9.8), the Co(III) determination can be made in AcOH (pH = 4.0) electrolyte in the presence of Zn(II). However as can see from Table 3 although there is no

interference between Co(III) and Zn(II), Co(III) peak is broad and Zn peak is sharp in AcOH, pH = 4.0. To check its accuracy below given artificially prepared solution is prepared and the determination was made.

A sample was prepared containing Co(III), Fe(III) and Zn(II) each in 1×10^{-3} M (Figure 2), from this 0.1 mL was taken and added into 10 mL AcOH/AcO$^-$ (pH = 4.0) buffer in polarographic cell. A peak for Zn(II) was observed at -1.02 V and a peak for Co(III) at -1.35 V. As expected there was no peak for Fe(III), the Co(IIII) present was determined by additions of standard 1×10^{-5} M Co(III). The LOD was found as 1×10^{-6} M with S/N = 3. The results are given in Table 5.
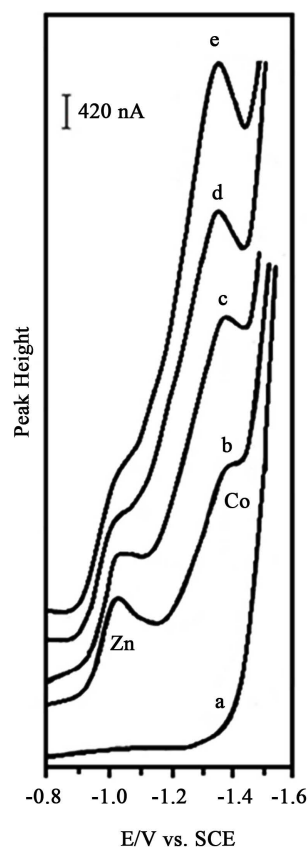

Figure 2. Determination of Co(III) in a artificially prepared solution containing 1×10^{-3} M (Fe^{3+}, Co^{3+}, Zn^{2+}) in 1.0 M AcOH/AcO$^-$ pH = 4.0 buffer. a: 10 mL 1.0 M AcOH/AcO$^-$ pH = 4.0 buffer; b: a + 0.1 mL artificially prepared solution; c: b + 0.1 mL 1×10^{-3} M Co(III); d: c + 0.1 mL 1×10^{-3} M Co(III); e: d + 0.1 mL 1×10^{-3} M Co(III).

Table 5. Determination of Co(III) in a synthetic sample contaninig 1×10^{-5} M (Fe(III), Co(III) and Zn(II)) in 1.0 M AcOH/AcO$^-$ pH = 4.0 buffer.

Medium	[Co^{3+}] (M) in synthetic sample	Determined [Co^{3+}] (M)	$\bar{x}$	s	$\bar{x} \pm ts/\sqrt{N}$
1.0 M AcOH/AcO$^-$ buffer (pH = 4.0)	1×10^{-5}	0.91×10^{-5} 0.92×10^{-5} 0.94×10^{-5} 0.91×10^{-5}	0.92×10^{-5}	2.75×10^{-7}	$(0.92 \pm 0.05) \times 10^{-5}$

N = 4, 95% confidence interval.

3.5. Determination of Vitamin B12 in Injectable Ampoule

From 1 mL of B12 sample which was dissolved as given in section "Preparation of reagents" in Sulfuric acid Nitric acid mixture a, sample of 0.1 mL is taken and added into 10 mL polarographic cell containing 10 mL of NH_3/NH_4Cl buffer (pH = 9.8). A peak at −1.28 V for Co(III) was observed, there was no interfering ion (Figure 3). This peak increased by the additions of standard 0.1 mL 1×10^{-3} M Co(III). The results obtained for Co(III) in vitamin B12 are given in Table 6, the B12 quantity in the same ampoule was found as (999 ± 15) μg instead of 1000 μg (Table 7).
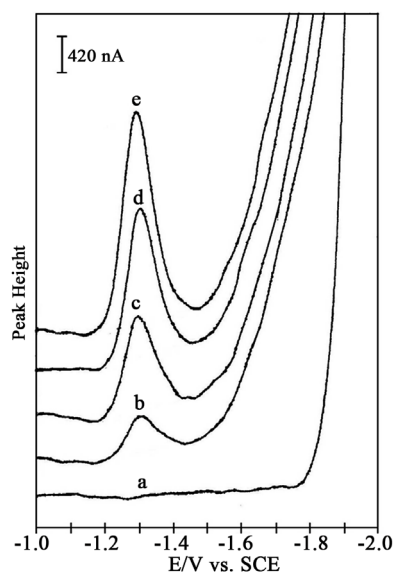

Figure 3. Determination of vitamin B_{12} in 1.0 M NH_3/NH_4Cl pH = 9.8 buffer. a: 10 mL 1.0 M NH_3/NH_4Cl pH = 9.8 buffer; b: a + 0.1 mL 1×10^{-3} M Co(III); c: b + 0.1 mL 1×10^{-3} M Co(III); d: c + 0.1 mL 1×10^{-3} M Co(III); e: d + 0.1 mL 1×10^{-3} M Co(III).

Table 6. Determination Co(III) in vitamin B_{12}.

Medium	$[Co^{3+}]$ (M) in vitamin B_{12}	Determined $[Co^{3+}]$ (M)	$\bar{x}$	s	$\bar{x} \pm ts/\sqrt{N}$
1.0 M NH_3/NH_4Cl buffer (pH = 9.8)	7.4×10^{-4}	7.2×10^{-4} 7.5×10^{-4} 7.3×10^{-4} 7.5×10^{-4}	7.4×10^{-4}	1.5×10^{-5}	$(7.4 \pm 0.2) \times 10^{-4}$

N = 4, 95% confidence interval.

Table 7. Determination of B_{12} in vitamin B_{12} ampoule sample (1000 μg).

Medium	B_{12} (μg) in B_{12} ampoule sample	Determined B_{12} (μg)	$\bar{x}$	s	$\bar{x} \pm ts/\sqrt{N}$
1.0 M NH_3/NH_4Cl buffer (pH = 9.8)	1000	976 1016 990 1016	999	24	999 ± 24

N = 4, 95% confidence interval.

4. Conclusion

In this investigation, a new and highly sensitive DPP method with high reproducibility has been established for the determination of vitamin B12. During the dissolution of vitamin B12 with concentrated acid mixture, the organic structure was decomposed and Co(III) ions could be dissolved in the solution. Thus, it was possible to determine vitamin B12 from the dissolved Co(III). For the polarographic determination of Co(III) the optimum working conditions had to be studied. It was found that in 1.0 M NH_3/NH_4Cl (pH = 9.8) buffer Co(III) and also B12 vitamin can be determined with high reproducibility. In this medium the LOD was (S/N = 3) 3.7×10^{-7} M. The interference of Fe(III), Zn(II), Ni(II), Cr(VI), Cu(II), Cd(II) and Se(IV) ions had also been studied. It was found that only there was a peak overlap with Zn(II) ion in 1 M NH_3/NH_4Cl (pH = 9.8) buffer. However, in 1.0 M $AcOH/AcO^-$ (pH = 4.0) buffer there was no peak overlap and the interference could be eliminated. In both media it was possible to determine vitamin B12 with high accuracy.

Acknowledgements

Conflicts of Interest

The authors declare no conflict of interest.

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