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Colorimetric Determination of Cyanocobalamin (VitaminB12) by indirect method with Ferrous ion and 1,10 phenanthroline

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Abstract

CyanoCobalamin (Vitamin B12) was determined using a simple, reliable, and affordable colorimetric method. According to this method, excess Ferrous sulfate (FeSO4.7H2O, or FS) is used to reduce vitamin B12, and the excess FS reacts with the reagent 1,10-phenanthroline to produce a soluble red color complex. The greatest wavelength of the spectrum of this complex was 510 nm. The concentration linearity range was 5-100 µg/mL. The RSD% values were 0.107-0.259% for intraday and 1.085-0.1378% for interday, while the Rec% values ranged from 95 to 100.833%. LOD and LOQ values were respectively 0.016 µg/mL and 0.053 µg/mL. The method was used to determine the presence of vitamin B12 in medicinal preparations with success.

Keywords: Colorimetric method, CyanoCobalamin, Ferrous Sulfate ,1,10-phenanthroline,Vitamin B12.

Introduction

CyanoCobalamin uses cell in the body, a water-soluble, dark pink vitamin for metabolism. Its name comes from the presence of cobalt[1]. Its structural formula is depicted in Fig. 1, and its scientific name is -(5,6-dimethylbenzimidazolyl) cobamidcyanide. Its chemical formula is $C_{63}H_{88}CoN_{14}O_{14}P$, and its molecular weight is 11355.4 g/mol[2]

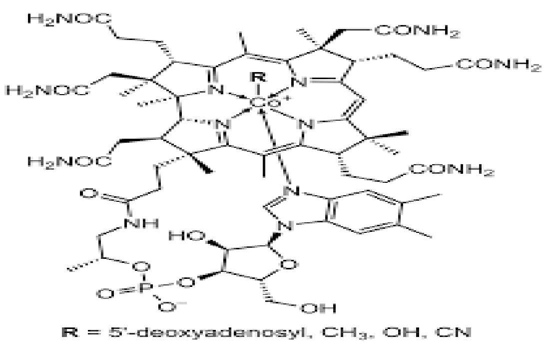

Figure 1. The chemical structure of cyanocobalamine

The production of DNA, proper red blood cell creation, and myelination of the central nervous system all depend on vitamin B12[3,4,] . vitamin B12 Functions as Methionine Synthase and L-Methylmalonyl-CoA Mutase, supporting their enzymatic activities[5]. Before it can be absorbed, vitamin B12 which is attached to the protein in meals must be released when saliva and food are combined in the mouth, The process begins. The Cobalamin-binding protein haptocorrin in the saliva then binds with the vitamin B12 that has been released. The hydrochloric acid and gastric protease in the stomach cause more vitamin B12 to be liberated from its dietary matrix, where it subsequently binds to haptocorrin[4] . Vitamin B12 is released from haptocorrin by digestive enzymes in the duodenum, where it binds with intrinsic factor, a transport and delivery binding protein secreted by the parietal cells of the stomach. By means of receptor-mediated endocytosis, the resultant complex is absorbed in the

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distal ileum. vitamin B12 is already in free form when it is added to fortified meals and nutritional supplements, thus the separating stage is not necessary.

Measurements of serum or plasma vitamin B12 levels are commonly used to determine vitamin B12 status. However, most laboratories define subnormal serum or plasma readings as those lower than 200 or 250 pg/mL (148 or 185 pmol/L), which is the cutoff between normal vitamin B12 levels and deficiency[6]. Megaloblastic anemia, which is distinguished by large, abnormally nucleated red blood cells, as well as low counts of white and red blood cells, platelets, or a combination of these, glossitis of the tongue, fatigue, palpitations, pale skin, dementia, weight loss, and infertility are all symptoms of vitamin B12 deficiency. Additionally, neurological abnormalities including tingling and numbness in the hands and feet might manifest[7]. Since these neurological symptoms might manifest even in the absence of anemia, prompt identification and treatment are essential to prevent irreparable harm[8]. Additionally, some research has linked depression to vitamin B12 deficiencies or insufficient intakes, Lack of vitamin B12 during pregnancy and nursing may result in neural tube abnormalities, developmental delays, failure to thrive, and anemia in the offspring[9].

There are a variety of analytical techniques that have been used to estimate vitamin B12[10], including chromatographic techniques, such as HPLC-UV and HPLC-ICP-OES [11], HPLC [12-15], and Liquid chromatography[16]. Affordable analytical technique based on spectrophotometry[17] or based on ultra-nano solvents was developed, and it allowed for the separation of vitamin B12 from infant formula, dietary supplements, and dairy products using the chromatographic technique in conjunction with mass spectrometry[18,19]. The effects of metal ions on the loss of Vitamin B12 during the preparation or storage of meals and dietary supplements with oxidizing and reducing agents were also investigated using an electrical method[20-22].

By reducing vitamin B12 with an excess of ferrous (II) ion and using the reaction between unreacted ferrous ion and the reagent 1,10-phenanthroline, it is an important reagent used in the determination of many compounds[23], the current study intends to establish a new, simple, and affordable colorimetric method for the indirect determination of vitamin B12.

Materials and Methods

Apparatuses

A dual-band ultraviolet-visible instrument made by Shimadzu Company, model 1650, with quartz cells 1 cm, was used to record the absorption spectra for all measurements. The wavelength scanned from 400 to 800 nm, and it had a medium scan speed. Having a slit width of 2 nm, and a sampling interval of 0.1 nm. A LabTech-Korea ultrasonic water bath to aid in the samples' dissolution.

Chemicals

pure substances

Cyanocobalamin, reagent 1,10-phenanthroline, and pure components of analytical grade were acquired from the manufacturing of the Indian company Merind, and ferrous sulfate, $FeSO_4.7H_2O$, from the American company Pharbest. A 100 ml volumetric flask containing 0.1 gm of each ingredient was dissolved in distilled water to make a solution that contained 1000 $\mu g.ml^{-1}$ of vitamin B12 standard, ferrous sulfate $FeSO_4.7H_2O$, and 1,10-phenanthroline.

pharmaceuticals

The dosage of 500 mcg of the vitamin B12 medication, manufactured by the American company Century, was utilized. Twenty of the medication's tablets weighed 6.234 grams, while each individual tablet weighed 0.3117 grams and each tablet contained 0.5 mg of vitamin B12. The tablets were then crushed, thoroughly combined in a ceramic mortar, diluted in an amount of distilled water, and filtered using Whatman No. 42 filter paper to remove any insoluble materials. Then, using a volumetric flask with a 100 ml capacity, fill the volume to the mark with distilled water. that 20 tablets give a concentration of 100 $\mu g.ml^{-1}$ of vitamin B12.

The method's principle

Vitamin B12 is reduced ferrous II ion by after being heated to a boil when Fe^{+2} is oxidized to Fe^{+3}. Non-oxidized ferrous sulfate is present in excess, it reacts with the reagent 1,10-phenanthroline to produce a red soluble solution. When this solution was scanned between 300 and 800 nm against the suitable blank, the wavelength with the maximum absorption was 510 nm.

Selecting optimum conditions

Selection of the optimal amount of ferrous sulfate. Using multiple Volumes of $FeSO_4.7H_2O$ ranging from (0.5-5.5) ml with a concentration of 1000 µg.ml^{-1}, the optimal amount of ferrous sulfate was chosen. Each volume's absorbance was measured in comparison to a blank. As illustrated in Fig. 2, it was discovered that 1 ml of ferrous sulfate at a concentration of 1000 µg.ml^{-1} is the optimal volume.

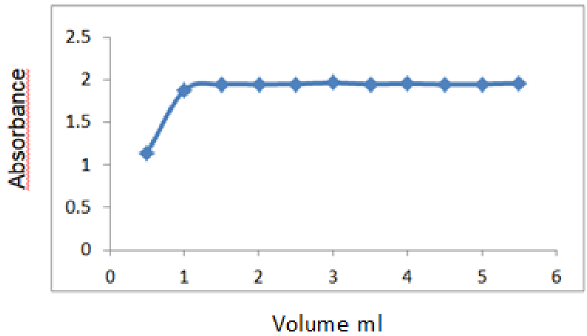

Figure 2. The optimal volume of ferrous sulfate against the blank.

Effect of 1,10-Phananthroline's volume
Employing several volumes of 1,10-phenanthroline, ranging from 0.5-5.5 ml with a concentration of 1000 µg.ml^{-1}, with an optimal amount of ferrous sulfate. Each volume's absorbance was scanned against the blank. It was found that 1 ml of 1,10-phenanthroline is the optimal volume, as shown in Fig. 3.

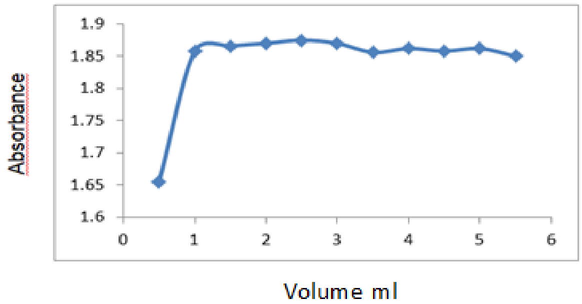

Figure 3. The optimal volume of 1,10-phananthroline against the blank.

Effect of the Temperature
Two concentrations from the calibration curve's concentrations, as given in Table 1, were selected in order to determine the optimal temperature. It was discovered that the commencement of boiling is the ideal temperature.

Table 1. The effect of the temperature on the determination of vit-B12

Concentration µg.ml^{-1}	Temperature°c			
	26	50	100	Up of 100
	Absorbance			
30	1.651	1.659	1.674	1.668
40	1.638	1.641	1.656	1.654

Effect of time on the stability of the complex
To determine the impact of time on the stability of the resultant complex, two concentrations were selected from the values on the calibration curve. After two minutes of the reaction, Table 2 demonstrates that it is steady.

Table 2. The effect of time on the determination of vit-B12

Concentration µg.ml^{-1}	Time min									
	beginning	2	4	6	8	10	12	14	16	18
	Absorbance									
30	1.673	1.67	1.67	1.67	1.671	1.67	1.66	1.66	1.66	1.66
40	1.65	1.64	1.64	1.63	1.62	1.62	1.61	1.61	1.61	1.61

Effect of the reaction's medium
Within the concentrations of the calibration curve, the effects of sodium hydroxide and hydrochloric acid on the reaction were examined for two concentrations. It was discovered that the acids and bases effect was negative. According to Table 3, optimal absorption occurs when neither acid nor base is used.

Table 3. The effect of medium's reaction on the determination of vit-B12

medium	Vitamin B-12	Absorbance of µg.ml^{-1}
	30	40
HCl	-0.0677	-0.0695
NaOH	0.0277	0.0111
without addition	1.673	1.645

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Final Absorbance Spectrum

According to the obtained optimal conditions, the final absorption spectrum of the Fe^{+2} complex with 1,10 - phenanthroline was recorded against the blank solution, which gave λmax at wavelength 510 nm, as in Fig. 4-B, while the λ_{max} for ferrous sulfate was 380 nm, for 1,10 - phenanthroline was 286 nm, and 4-c the λ_{max} at 510 nm represents to the complex of 1,10 - phenanthroline and Fe II in the presence of reduced vit B12. B12's maximum absorbance was measured at 550 nm in Fig. 4-A.

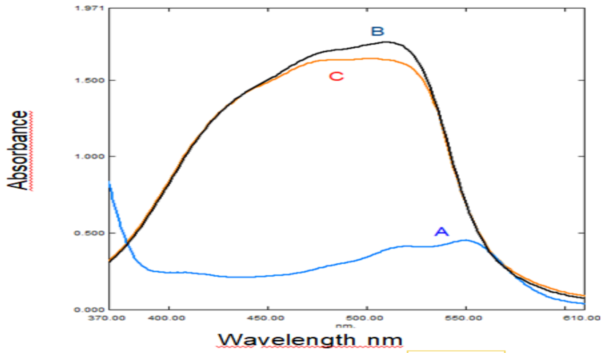

Figure 4. Represent the spectrum of A= Vit B12, B=1,10-ph+Fe(II) and C= 1,10-ph+Fe(II)+Vit B12.

Procedure and Construction of the Calibration Curve

Increasing concentrations (5-100 µg.mL^{-1}) of vit B12 with a concentration of 1000 µg.mL^{-1} were added to a series of 10-mL volumetric flasks, to which 100 µg.mL^{-1} of ferrous sulfate with was added. The mixture was heated until boiling, and then 100 µg.mL^{-1} of 1,10 - phenanthroline with was added. The volume was filled to the mark with distilled water, and the absorbance of these solutions was measured against the blank at the wavelength of 510 nm.

Results and discussion

Construction of calibration curve

The calibration curve was constructed at 510 nm, which is the maximum wavelength. The method's linearity was 5-100 µg.ml^{-1}, and its Linearity percentage (R^2) value was 0.9999. Its slope was -0.0024, According to Fig. 5.The molar absorptivity was 3252.96 L.mol^{-1}.cm^{-1} and the Sandell's sensitivity was 0.41667 g.cm^{-2}.

Accuracy and Precision

By calculating the percentage recovery value (Rec%) and the relative standard deviation (RSD%), respectively, for the concentrations of the calibration curves (5-100µg.ml^{-1}), the suggested methods' accuracy and precision were evaluated in accordance with the International Council for Harmonization (ICH) [24]. The results of five iterations of each measurement process, as shown in Table 4. The method has good accuracy and precision. The Rec% values ranged from 95,000 to 100.833%, and the RSD% values were between 0.107 and 0.259% for one day and between 1.085 and 0.137% for more than one day.

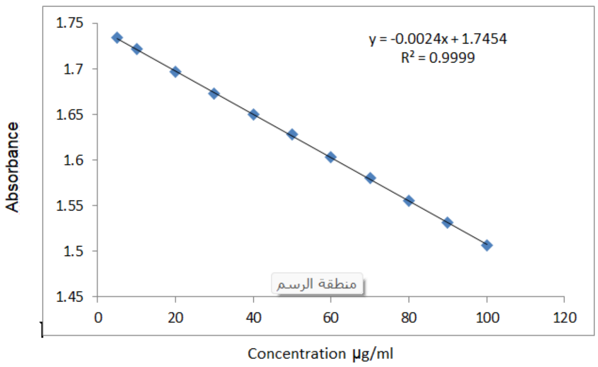

Figure 5. Calibration curve of vit B12 for concentrations 5-100 µg.ml $^{-1}$ at wavelength 510 nm.

Table 4. Results for the accuracy and precision of the method

Concentration µg/ml		RSD%		Rec%
Taken	Found	Intra-day	Inter-day	
5	4.75	0.107	0.947	95.000
10	9.75	0.314	0.610	97.500
20	20.16667	0. 187	0.393	100.833
30	30.16667	0.168	0.137	100.555

40	39.75	0.101	0.760	99.375
50	48.91667	0.165	0.307	97.833
60	59.3333	0.226	0.364	98.888
70	68.91667	0.567	1.085	98.452
80	73.3333	0.558	1.068	99.166
90	88.91667	0.158	0.543	98.796
100	99.33333	0.259	0.375	99.333

Limit of detection and limit of quantification

The following formulas were used to calculate the limits of detection (LOD) and quantification (LOQ) [25].

L O D = (3.3×S)/(X) ----------1
L O Q = (10×S)/(X) -----------2

Where X is the slope of the calibration curve and S is the standard deviation of the blank measurements (five replications),
The values for the LOD and LOQ were 0.016 gmL^{-1} and 0.053 gmL^{-1}, respectively.

Application of the Method

The proposed method was utilized to indirectly estimate the concentrations of vitamin B12 (20, 60, and 70 µg.ml^{-1}) present in the aforementioned pharmaceutical formulations by doing each measurement five times. The values for Rec% ranged from 102.29 to 104.44%. The RSD% values for intraday ranged from 0.018 to 0.846%, and the values for interday ranged from 0.121 to 0.185%, as in Table 5.

Table 5. Application results of the method

Pharmaceutical form	Concentration µg/ml		RSD%		Rec%
	Taken	Found	Intra-day	Inter-day	
VitaminB12 500 mg	20	20.166	0.0183	0.121	102.29
	60	59.333	0.129	0.185	104.44
	70	68.916	0.846	0.131	104.4

The recovery study

A concentration of 20 µg/ml was subjected to the Recovery Study method [26], and various proportions of standard concentrations (50, 100, and 150%) were added to it. The average Rec% values reached 100.0523%, while the RSD% values for intraday ranged between 0.9860 and 1.2580%, and for interday between 1.1250 and 1.4580%, as shown in Table .6. These results indicate that the study had good accuracy and agreement.

Table 6. the results of recovery study of 20 µg.ml^{-1}

Standard% added	Concentration µg.ml^{-1}			RSD%		Rec%	
	Taken	added	found	Intra-day	Inter-day		Average%
50%	20	10	11.083	1.0210	1.2580	103.611	
100%		20	20.166	1.2580	1.4580	100.416	100.052
150%		30	28.833	0.9860	1.1250	96.130	

Method comparison

The proposed method for the determination of vitamin B12 was compared with some of the methods used to estimate it, as in Table 7.

Table 7. Comparison of the method.

`Parameter	Reference method[12]	Reference method[27]	Current method
λmax nm	361	548	510
Linearity µg.ml^{-1}	0.167-114	96-6	-1005
Slope	2.6111	0.0117	0.0024
R^2	0.9990	0.9988	0.9999
LOD µg.ml^{-1}	0.165	0.1381	0.016
LOQ µg.ml^{-1}	0.499	0.4606	0.0534
Rec%	96.42-97.36	99.783-102.399	100.833-95
RSD% Intra-day	3.09	0.4811-0.8238	0.259-0.107
RSD% Inter-day	3.2	--------	0.1378-1.085
Molar absorptivity L/mol.cm	-------	--------	3252.96
Sandell's Index µg/cm^2	--------	--------	0.41667

By comparing the last three proposed methods for the determination of vitamin B12, it appears as in Table 7 that the vitamin B12 reduction method is better than the rest methods, since the detection limit, the quantitative limit, and the estimate limit are less than the other two methods, and the percent recovery is better than the other two methods.

T- test

When a T-test was performed, it was discovered that the calculated t-value was 1.08, which is less than 2.132, the value represented by the tabular data at a 95% reliability and five degrees of freedom, indicating that the errors connected to the analysis method are random errors.

Conclusion

According to our results, a simple and rapid method can be applied to estimate vitamin B12 in its pure form and in pharmaceutical preparations indirectly, by reducing vitamin B12 using ferrous sulfate $FeSO_4.7H_2O$ after heating it to a boil, where Fe^{+2} is oxidized to Fe^{+3}. The reaction of the excess of ferrous sulfate ($FeSO_4.7H_2O$), with the reagent 1,10–phenanthroline, and in the presence of vitamin B12 gives a red soluble solution that gave the highest absorption at the wavelength of 510 nm. This method depends on the decrease in absorption with increasing concentration of vitamin B12.

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Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and Images, which are not ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Samarra.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- No potentially identified images or data are present in the manuscript.

Author's Contribution Statement

K.F.A., contributed to proposing the research topic, monitoring the progress of the work, and suggesting solutions to the problems encountered by the researcher during the work.

S.A. Acontributed to preparing the solutions, completing the work in all its stages, writing the research, and overseeing the corrections.

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التقدير اللوني للسيتوكوبال (فيتامين ب 12) بطريقة غير مباشرة باستخدام الأيونات الحديديوز و 1،10 الفينانثرولين

سهاد عبدالمنعم مجيد ياسين , خلف فارس السامرائي

قسم الكيمياء، كلية التربية، جامعة سامراء،سامراء، العراق.

الخلاصة

تم تقدير CyanoCobalamin (فيتامين ب 12) باستخدام طريقة لونية بسيطة وموثوقة ومعقولة التكلفة. وفقًا لهذه الطريقة ، يتم استخدام زيادة من كبريتات الحديدوز (FeSO4.7H2O ، أو FS) لاختزال فيتامين B12 ، ويتفاعل FS الزائد مع الكاشف 1،10-فينانثرولين لتكوين مركب أحمر قابل للذوبان في الماء. كان الطول الموجي الأعظم للطيف لهذا المركب هو 510 نانومتر وكان مدى خطية التركيز (5-100 ميكروجرام / مل) وكانت قيم RSD% خلال اليوم الواحد 0.107-0.259% و 1.085-0.1378٪ بين يوم و اخر، في حين تراوحت قيم Rec% من 95 إلى 100.833%. كانت قيم LOD و LOQ على التوالي 0.016 ميكروغرام / مل و 0.053 ميكروغرام / مل. تم استخدام الطريقة لتقدير فيتامين ب 12 في المستحضرات الطبية بنجاح

الكلمات المفتاحية: طريقة لونية ، سيانوكوبال امين ، كبريتات الحديدوز، 1،10- فينانترولين و فيتامين ب12.