

# Determination of Iron Content in Different Hemoglobin Samples from Some Patients by UV-Visible Spectrophotometer

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**Abstract** A validated, simple, accurate and precise spectrophotometric method has been proposed for the determination of iron in hemoglobin in human blood. The method is based on the reaction of iron with ammonium thiocyanate after the wet digestion of the blood samples with  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$ . The optimum experimental conditions have been studied carefully. The absorption maxima was occurred at 475 nm and molar absorptivity of  $0.03602 \text{ L mol}^{-1} \text{ cm}^{-1}$ . The linear regression line was obtained in the range of 0.5 - 60  $\mu\text{g/mL}$  for iron content in hemoglobin. The detection limit and the limit of quantification were found to be 0.0583 and 0.1768  $\mu\text{g/mL}$  respectively for the iron, and with  $R^2$  value of 0.998. And recovery in range of 98.42 - 99.72%. Effects of pH, temperature, standing time and thiocyanate concentration on the determination of iron in human hemoglobin have been examined.

**Keywords** Iron, Hemoglobin, Determination, UV spectrophotometer

## 1. Introduction

Iron is a fundamental element for human and animals. Iron exists in the living body as ferritin and hemosiderin which are considered to be proteins containing iron [1]. Iron participates in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. About 70% of the body's iron is bound to hemoglobin in red blood cells [2]. Iron deficiency is one of the leading risk factors for disability and death worldwide, affecting an estimate of two billion people [3]. Anemia and iron deficiency anemia must always be taken into consideration for they have a clear impact on the patient's quality of life and they can be the consequence of severe diseases [4]. The literature shows various methods for determination of iron in blood and hemoglobin. Among these methods are colorimetric determination of iron and hemoglobin in blood which based on the comparison of the color of iron reacted with sulfocyanate [5] or using o-phenanthroline [6], spectrophotometric by the use of sodium hypochlorite solution [7]. Body iron can be quantitatively estimated by the method based on the ratio of the serum transferrin receptor to serum ferritin [8]. Atomic-absorption-spectrophotometric was used for iron determination in whole blood for the measurement of

hemoglobin content by Lötterle [9]. Iron in hemoglobin also was determined by using atomic absorption spectroscopy [10]. In this work, a reliable and reproducible spectrophotometric method for the assay of iron content in hemoglobin in human blood, using thiocyanate as derivatizing agents after the samples were wet digested by nitric acid and hydrogen peroxide was reported.

## 2. Materials and Methods

### 2.1. Sampling and Area of the Study

15 blood samples of different hemoglobin content from some patients were brought from Baljurashi General Hospital, Saudi Arabia.

### 2.2. Chemicals and Apparatus

Chemicals reagents used in this work were of high analytical grade, and deionized water was used to prepare all solutions. All absorbance measurements were made with a double beam UV-VIS Spectrophotometer (SP- 3000 Plus model, Optima, Tokyo, Japan) ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cells. A pH meter (model 3305, Jenway Ltd., United Kingdom) was used. Digital water bath (Model LWB-122D, Daihan Labtech Co. Ltd., Indonesia) was also used.

### 2.3. Preparation of Buffers and Standard Solutions

#### 2.3.1. Preparation of Buffer Solutions

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A series of different buffer solutions of pH ranged from 3 to 11 were prepared. A buffer solutions of pH 3.0 and 4.0 was prepared by mixing 0.2 M Acetic acid with 0.2 M sodium acetate, solutions of pH 5.0, 9.0, 10.0 and 11.0 were prepared by mixing 0.10 M  $\text{Na}_2\text{CO}_3$  solution with 0.10 M  $\text{NaHCO}_3$  solution and solutions of pH 6.0, 7.0 and 8.0 were prepared by mixing 0.20 M  $\text{NaH}_2\text{PO}_4$  solution with 0.20 M NaOH solution, each buffer solution was prepared in 100 mL volumetric flask and adjusted by a pH meter.

#### 2.3.2. Preparation of Standard Ammonium Thiocyanate Solution

It was prepared by dissolving an accurately weighed 40.0 g of  $\text{NH}_4\text{SCN}$  in deionized water, then transferred into a 100 ml standard flask and diluted to the mark and thoroughly mixed to get 40% (w/v). Then a series of dilutions of this solution from 0.5 to 40% (w/v) were prepared in order to assign the best concentration for analysis.

#### 2.3.3. Preparation of Stock and Standard Solutions of Iron

A stock solution of iron of concentration of 1000  $\mu\text{g/mL}$  was prepared by dissolving an accurately 2.9045 g of  $\text{FeCl}_3$  in deionized water transferred into a 1000 mL volumetric flask and completed to the mark and thoroughly mixed. Then a series of standard solutions iron were prepared by dilution to get different concentrations ranged from 0.5- 75  $\mu\text{g/mL}$ .

#### 2.4. Job's Method for the Reaction of Iron and Thiocyanate

The Job's method of continuous variation [11] was applied by taking equal concentration of each of prepared standard iron solution and  $\text{NH}_4\text{SCN}$  and prepared in complementary attributions (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10), and heated at temperature of 40°C to for 10 minutes.

#### 2.5. Preparation of Standard Calibration Curve of Iron

Standard calibration curve of iron was established by graphing concentrations versus absorbance of iron standard

solutions by taking 2 mL of each of standard solutions and put in a test tube, then 2 mL of buffer solution of pH 3.0 and 1 mL of 8.0%  $\text{NH}_4\text{SCN}$  were added. This solution was heated at temperature of 40°C to for 10 minutes. The absorbance of this standard solutions were read at 475 nm against blank.

#### 2.6. Digestion of Blood Samples for Analysis

Each of the 15 of blood samples were accurately taken as 1.0 mL for each sample (equivalent to 1.07 g), and then were digested by transferring them into a 100 mL calibrated beaker, and dissolved in about 6.0 mL of concentrated  $\text{HNO}_3$  and 2.0 ml of  $\text{H}_2\text{O}_2$  and covered by watch glass. The contents of the beakers were heated 110°C in a hotplate for 30 minutes and then transferred to 25 mL volumetric flasks, and then completed to volume with deionized water. The contents were mixed well and filtered. The prepared solution was used to obtain a suitable concentration for the analysis.

### 3. Results and Discussion

#### 3.1. Determination of the Absorption Spectra

The maximum absorption wavelength ( $\lambda_{\text{max}}$ ) for the products which obtained by the reaction of iron of prepared blood samples and ( $\text{NH}_4\text{SCN}$ ) occurred at 475 nm against the blank as in Figure 1.

#### 3.2. Determination of the Ideal Conditions of the Reaction Parameters of the Method

The ideal conditions for this method were determined by varying the reaction parameters and recording the influence on the absorbance. The reaction parameters studied were the effects of pH, volume of buffer, temperature, reaction time and  $\text{SCN}^-$  concentration as shown in Figures 2-5. The ideal conditions for the reaction of prepared blood sample with  $\text{NH}_4\text{SCN}$  with respect to pH, volume of buffer, temperature, reaction time and  $\text{SCN}^-$  concentration were found to be 3.0, 2 ml, 40°C, 10 minutes and 8.0% respectively.

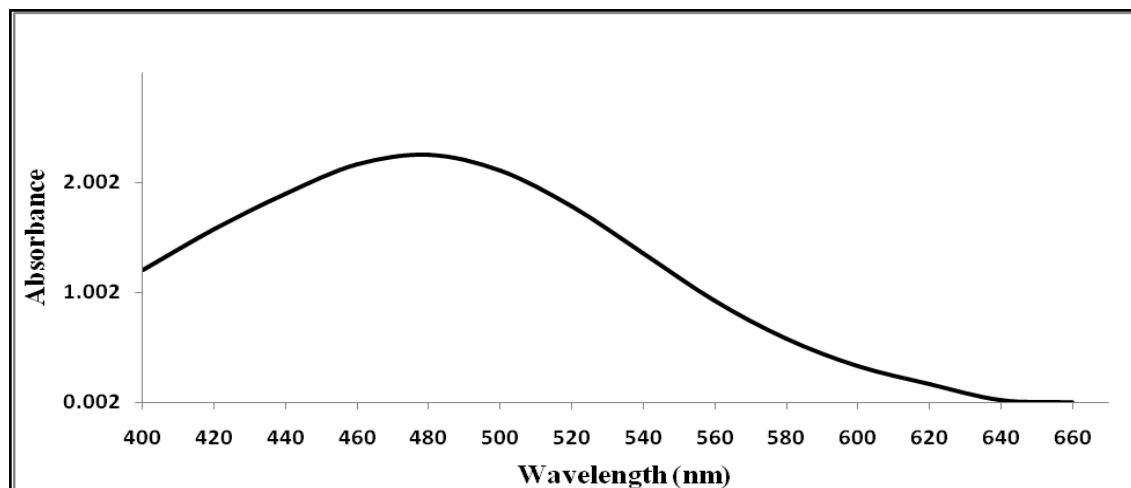


Figure 1. The  $\lambda_{\text{max}}$  of reaction of prepared blood sample (10  $\mu\text{g/mL}$ ) with ( $\text{NH}_4\text{SCN}$ , 8.0% w/v)

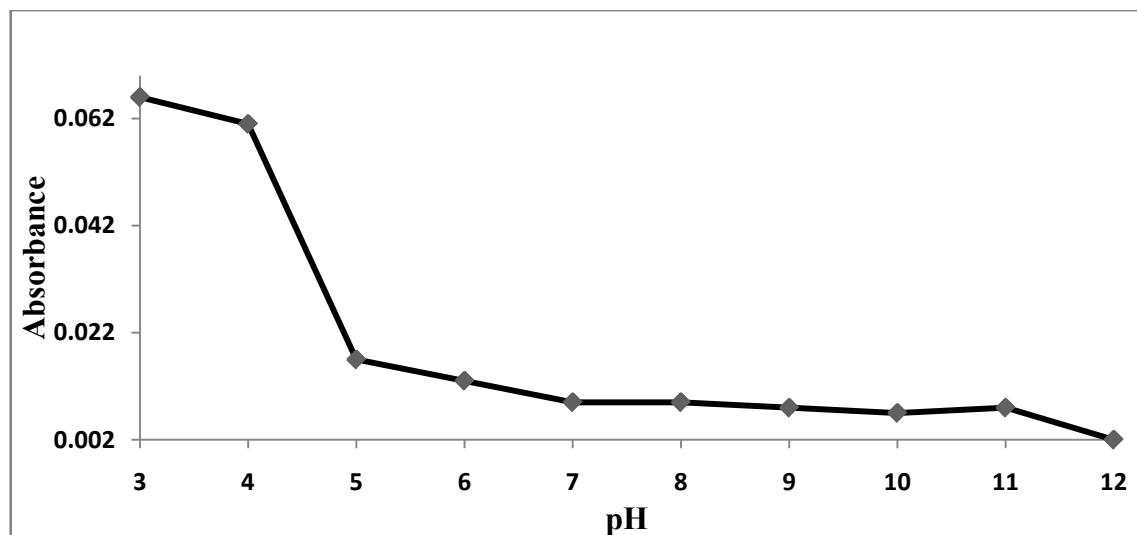


Figure 2. Effect of pH on the reaction of standard iron solution with  $(\text{NH}_4\text{SCN})$

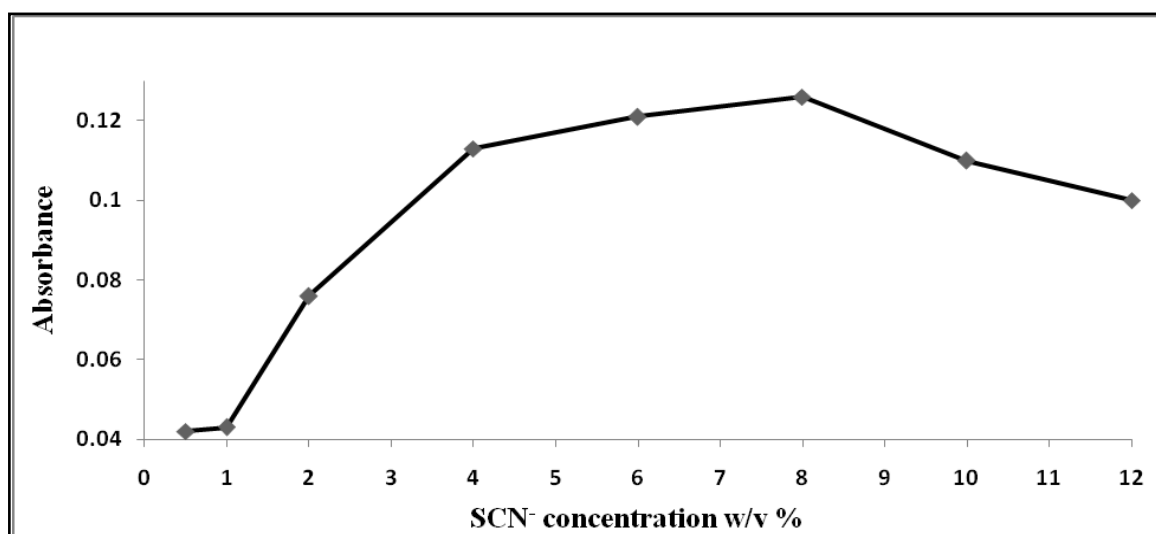


Figure 3. Effect of  $(\text{NH}_4\text{SCN})$  concentrations on the reaction of prepared blood sample with  $(\text{NH}_4\text{SCN})$

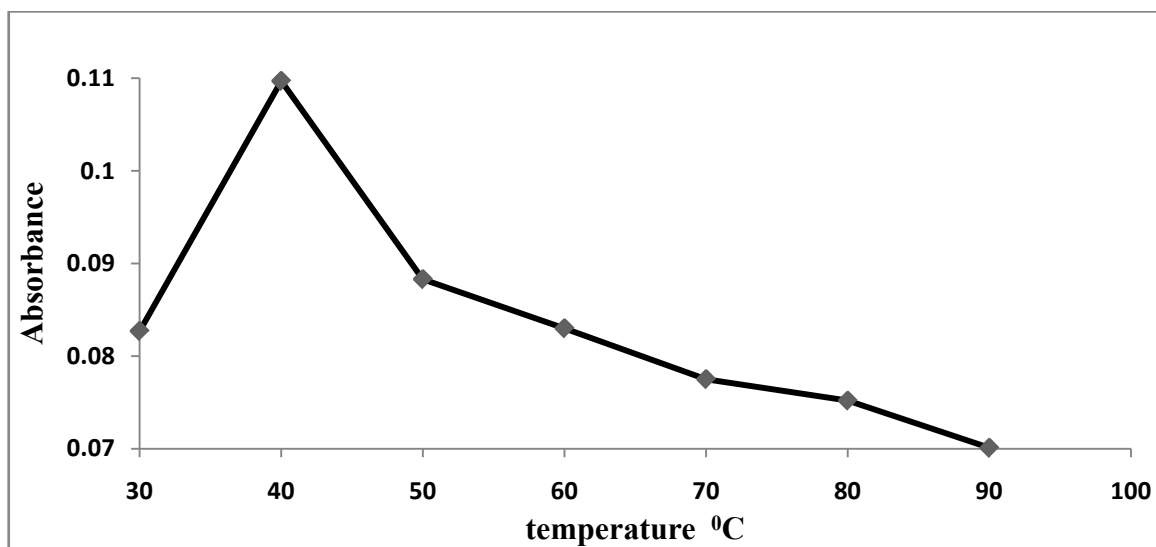


Figure 4. Effect of temperature on the reaction of prepared blood sample with  $(\text{NH}_4\text{SCN})$

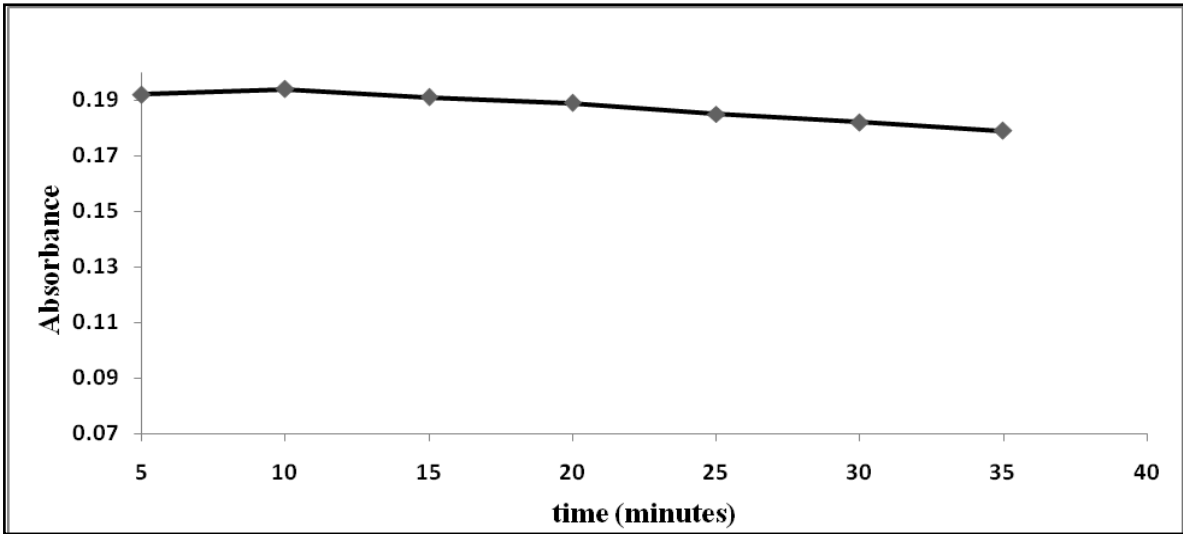


Figure 5. Effect of the reaction time on the reaction of prepared blood sample with (NH<sub>4</sub>SCN)

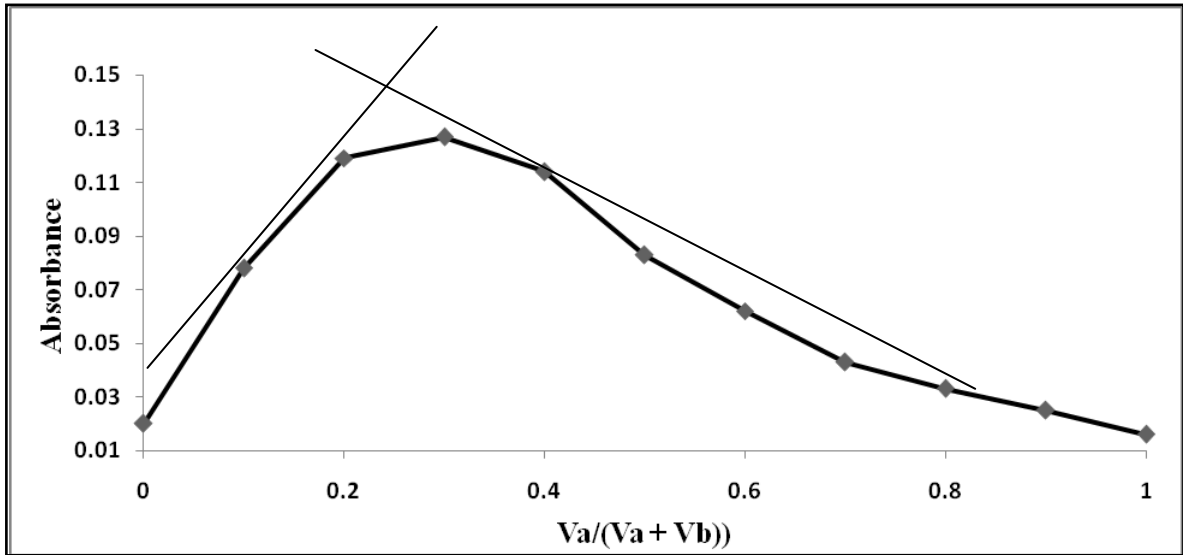


Figure 6. Stoichiometry by Job's method for (SCN-) with prepared blood samples: Va: (SCN-) and Vb: (iron of blood samples)

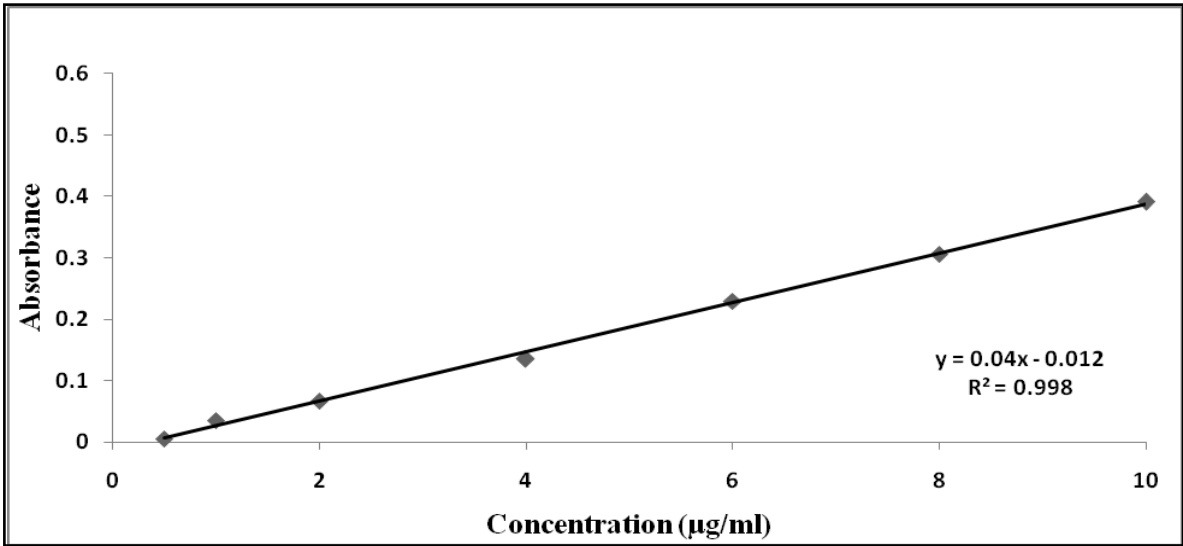


Figure 7. Calibration curve for the determination of iron

### 3.3. Determination of the Complexation of the Reaction by Job's Method

The Job's method of for the reaction under study was applied [11] as previously described. The shape of Job's plot was obtained which indicate the complexation of the reaction of  $\text{NH}_4\text{SCN}$  with iron of the prepared blood sample as seen in Figure 6 forming a red-brown complex product.

### 3.4. Method Validation

The validation parameters for this study were determined according to validation of analytical procedures guidelines described by International Conference of Harmonization [12]. Calibration curve for the determination of prepared blood sample and  $\text{NH}_4\text{SCN}$  was established by graphing concentrations against absorbance as in Figure 7. Robustness of the method was carried out and it was seen that small differences in the method parameters did not significantly influence the procedures; the percent recoveries were seen in Table 1.

**Table 1.** Robustness of the spectrophotometric method for iron of Hemoglobin sample

Condition	Iron concentration (8.0 $\mu\text{g/mL}$ )	Recovery % $\pm$ RSD*
Standard condition		
pH	2.80	99.38 $\pm$ 0.02
	3.20	100.5 $\pm$ 0.07
SCN- concentration (w/v %)	7.80	99.13 $\pm$ 0.80
	8.20	100.8 $\pm$ 0.97
Temperature ( $^{\circ}\text{C}$ )	38.0	99.69 $\pm$ 0.53
	42.0	99.38 $\pm$ 1.02
Reaction time (min.)	8.00	99.12 $\pm$ 0.68
	12.0	99.00 $\pm$ 0.86

\* RSD is the relative standard deviation.

The linear plots with  $R^2$  were obtained in the 0.5-60  $\mu\text{g/mL}$ . The detection and quantification limits were calculated based on the standard deviation of the ( $n = 5$ , standard deviation = 0.000707) and the slope of the calibration curve. Results were summarized in Table 2.

Regarding the accuracy and precision of the method were evaluated by performing three replicate analyses on standard

iron solutions at three different concentrations. The relative error (%) was 0.53- 1.45, and intraday precision expressed in relative standard deviation (RSD) (%) did not exceed 2% for the investigated samples, revealing the good accuracy and precision of the studied method. The results of this study are compiled in Table 3 and 4 reflecting the usefulness of this method in analysis of the 15 investigated blood samples in the laboratories as seen in Table 5. The good correlation between iron determination by this method and the amount of hemoglobin determined by Cell-Dyn instrument provides an accurate determination of the concentrations of hemoglobin in blood samples as shown in Table 5.

The percent recoveries ( $n=3$ ) were found to be in the range of 98.42 to 99.72% for the iron in Hemoglobin as in Table 6 which revealing good accuracies and non-interference chemical species.

**Table 2.** The quantitative parameters and statistical data of the method

Parameter	Iron of prepared blood samples and ( $\text{NH}_4\text{SCN}$ )
$\lambda_{\text{max}}$ /nm	475nm
Bear's law limits ( $\mu\text{g/mL}$ )	0.500 - 60.0
Molar absorptivity, $\epsilon$ ( $\text{L mol}^{-1} \text{cm}^{-1}$ )	0.0360
Correlation coefficient ( $R^2$ )	0.998
Regression equation (Y)	$Y = 0.04 X - 0.012$
Slope	0.0400
Intercept	-0.0120
LOD ( $\mu\text{g/mL}$ )	0.0583
LOQ ( $\mu\text{g/mL}$ )	0.177

**Table 3.** Determination of the accuracy and precision of the method

Sample	Taken	Found	Relative error%	SD*	RSD%
Iron ( $\mu\text{g/mL}$ )	4.00	3.94	1.45	0.0382	0.970
	8.00	7.96	0.530	0.0144	0.180
	10.0	9.88	1.17	0.0382	0.390

\* SD is the standard deviation.

**Table 4.** Determination of Interday and Intraday Accuracy

Added ( $\mu\text{g/mL}$ )	Interday ( $n = 3$ )				Intraday ( $n = 3$ )			
	Found	Recovery %	$\pm$ SD	%RSD	Found	Recovery %	$\pm$ STD	%RSD
6	5.96	99.3	0.0760	1.28	5.96	99.3	0.0760	1.28
8	7.80	97.5	0.0500	0.641	7.99	99.9	0.0520	0.651
10	9.86	98.6	0.0630	0.639	9.93	99.3	0.0380	0.383

•SD, standard deviation; RSD, relative standard deviation.

**Table 5.** Determination of Iron of Hemoglobin samples

Blood sample #	Amount Hemoglobin of samples (g/dL)*	Amount of iron $\pm$ SD** ( $\mu$ g/mL)	Amount of iron (mg/dL)	Correlation factor for iron hemoglobin ratio***
1	9.19	28.6 $\pm$ 1.32	2.86	3.21
2	9.29	30.5 $\pm$ 1.25	3.05	3.05
3	7.15	23.0 $\pm$ 1.13	2.30	3.11
4	9.41	33.6 $\pm$ 1.12	3.36	2.80
5	7.83	24.3 $\pm$ 1.13	2.43	3.22
6	13.2	36.1 $\pm$ 2.00	3.61	3.66
7	13.0	35.0 $\pm$ 0.26	3.50	3.71
8	14.8	39.3 $\pm$ 0.36	3.93	3.77
9	13.5	35.4 $\pm$ 1.48	3.54	3.82
10	12.5	35.2 $\pm$ 1.09	3.52	3.55
11	18.3	48.5 $\pm$ 0.45	4.85	3.78
12	18.4	48.9 $\pm$ 0.45	4.89	3.77
13	9.19	33.2 $\pm$ 0.26	3.32	2.77
14	9.29	30.0 $\pm$ 0.07	3.00	3.10
15	7.17	23.4 $\pm$ 0.09	2.34	2.15
Average of Correlation factor for iron hemoglobin ratio (mean $\pm$ SD)				3.30 $\pm$ 0.490

\* The values obtained for Baljurashi General Hospital (determined by Cell-Dyn, Ruby, ABOT, USA).

\*\* Values are mean of three determinations determined by UV-VIS spectrophotometer.

\*\*\* Correlation factor for iron (mg/dL) hemoglobin (g/dL) ratio was calculated by dividing of the amount of iron (mg/dL)/ amount of hemoglobin (g/dL).

**Table 6.** Recovery of the method

sample	Sample iron content ( $\mu$ g/mL)	Standard iron Added ( $\mu$ g/mL)	Found ( $\mu$ g/mL)	Recovery (% $\pm$ RSD)*
Blood	4.00	2.00	5.98	99.7 $\pm$ 1.05
	4.00	4.00	7.98	99.7 $\pm$ 1.00
	4.00	6.00	9.87	98.4 $\pm$ 1.94

## 4. Conclusions

The present study described the successful evaluation of ammonium thiocyanate reagent in the development of simple, sensitive, selective, accurate and reproducible spectrophotometric method for the accurately determination of iron in hemoglobin. The validated method is simple. Moreover, all the chemical reagents used are cheap and available in any chemical laboratory. The method involve the measurement of stable colored compound in a shorter times of the reaction. Therefore, the method is practical and useful for application in the laboratories for analysis of iron and hemoglobin in blood sample. Suggestions that could made for this study is that further investigations is necessary particularly for the

strong correlation between the amount of iron content and the concentration of hemoglobin in blood sample which is very clearly in this work.

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