CHAPTER 12

ESTIMATION OF ELEMENTAL IRON CONTENT PER TABLET OF FERROUS SULPHATE AND FERROUS FUMARATE

This investigation was taken up to find out and confirm that each tablet of Ferrous Sulphate and Ferrous Fumarate contains EXACTLY 60 mg. of Elemental Iron. If it is proved that each tablet of both the iron preparations contains equal amount of elemental iron, and if equal number of tablets are given to the patients in either case, the basic requirement of administering EQUAL dosage in terms of elemental iron is fulfilled beyond doubt.

The main difference between this estimation of inorganic iron and the estimation of serum iron is that the amount of iron present in the tablets is much more than that present in the serum and hence it produces a very strong colour for the colorimetric reading. Secondly, the iron present in serum is in combination with proteins and hence additional steps for

precipitation of proteins are necessary in case of serum iron estimation which is not the case here.

IMPORTANT PRE-REQUISITES

(1) Iron Standards :

The iron standards must be prepared very accurately.

(2) Re-agents :

All the reagents used for the purpose must be free from iron-contamination.

(3) Glassware :

All the glassware used for the purpose must be free from iron-contamination.

(4) Water :

The water used for any purpose during the procedure viz. preparation of standards, preparation of reagents, preparation of glassware etc. must be iron-free. Triple-glass-distilled water must, therefore, be used.

PRINCIPLE

Ferric iron is allowed to react with thiocyanate to give ferric thiocynate which produces the red colour, the intensity of which is measured colorimetrically.

REAGENTS

(1) Saturated solution of Potassium persulphate:

Potassium persulphate was added to 100 ml. of triple-glass-distilled water till the full saturation was obtained.

(2) 3 M (3 N) Potassium Thiocyrate Solution (KCNS):

146 gm. of potassium thiocynate was dissolved in 500 ml. of triple-glass-distilled water.

(3) Concentrated Potassium Permanganate solution :

10 gm. of potassium permanganate was dissolved in 100 ml. of triple-glass-distilled water.

IRON STANDARDS

(1) Stock Iron Standard :

0.702 gm. of crystalline ferrous ammonium sulphate (A.R.) was accurately weighed out on an analytical balance. It was dissolved in 100 ml. of triple-glass-distilled water and 50 ml. of concentrated sulphuric acid (A.R). It was warmed slightly and concentrated potassium permanganate solution was added drop-wise till a drop produced permanant pink colour. The volume was made up to 1 litre by adding triple-glass-distilled water. The iron (Fe) content of this stock iron standard solution was 100 r per ml.

(2) Working Iron Standard :

10 ml. of stock iron standard was diluted to 100 ml. with triple-glass-distilled water (i.e. 1 in 10 dilution of stock iron standard). The iron (Fe) content of this working iron standard solution was 10 r per 100 ml. 0.5 ml., 1.0 ml., 1.5 ml., 2.0 ml., and 2.5 ml. portions of this solution were taken for standardisation.

SOLUTION OF IRON TABLETS

(1) Solution of Ferrous Sulphate Tablet :

In order to prepare the solution of Ferrous Sulphate Tablet, one tablet of ferrous sulphate was selected by "random-sampling" method. The tablet was weighed accurately on an analytical balance. Then the tablet was crushed to fine powder and the powder was mixed thoroughly to obtain the uniform mixture of the contents of the tablet (of ferrous sulphate) viz. the ferrous sulphate and the excipient.

A portion of this powder was accurately weighed on an analytical balance, and the weight was recorded. The powder was then dissolved in 5.0 ml. of concentrated hydrochloric acid by gentle boiling. The boiling was continued till the powder dissolved completely giving a uniformly transparent solution. It was allowed to cool. Then, it was diluted to 100 ml. with triple-glass-distilled water, thus giving a 1 in 100 dilution.

(2) Solution of Ferrous Fumarate Tablet :

In order to prepare the solution of Ferrous Fumarate Tablet, one tablet of Ferrous Fumarate was selected by "random-sampling" method. The tablet was weighed accurately on an analytical balance. Then the tablet was crushed to fine powder and the powder was mixed thoroughly to obtain the uniform mixture of the contents of the tablet(of ferrous fumarate) viz. the ferrous fumarate and the excipient.

A portion of this powder was accurately weighed on an analytical balance, and the weight was recorded. The powder was then dissolved in 5.0 ml. of concentrated hydrochloric acid by gentle boiling. The boiling was continued till the powder dissolved completely giving a uniformly transparent solution. It was allowed to cool. Then, it was diluted to 100 ml. with triple-glass-distilled water, thus giving a 1 in 100 dilution.

TABLE NO. 9

BALANCE READINGS

Sr. No.	Name of the Tablet	Weight of the tablet as a whole	Amount of the powdered tablet taken for iron Estimation.
1.	Ferrous Sulphate	491.0 mg.	40.0 mg.
2.	Ferrous Fumarate	486.0 mg.	80.0 mg.

PROCEDURE

Five different aliquotes of the respective "Test" solutions were taken in different labelled iron-free test tubes. Each was balanced to 7.5 ml. with triple-glass-distilled water. Similarly, a water blank and five iron standards were also carried with the different test samples. Then 0.5 ml. of saturated solution of potassium persulphate was added to each, followed by 2.0 ml. of 3 N potassium thiocynate solution. The contents of the different test tubes were mixed thoroughly by shaking. The readings were taken within 30 minutes in Klett-Summerson Photoelectric Colorimeter at 480 mu.

A standard graph was prepared by plotting the iron concentration against the optical density. A linear relationship was obtained. The values of the test (unknown) samples were read from the graph.

The actual steps of the procedure employed are as follows :

TABLE NO. 10

STANDARDISATION

	B	S_1	S_2	S_3	S_4	S_5
Standard iron solution in ml.	0.0	0.5	1.0	1.5	2.0	2.5
Triple-glass-distilled water in ml.	7.5	7.0	6.5	6.0	5.5	5.0
Potassium persulphate solution in ml.	0.5	0.5	0.5	0.5	0.5	0.5
Potassium thiocynate solution in ml.	2.0	2.0	2.0	2.0	2.0	2.0

TABLE NO. 11

TEST SAMPLE - FERROUS SULPHATE

	T_1	T_2	T_3	T_4	T_5
Ferrous Sulphate Solution in ml.	0.1	0.2	0.3	0.4	0.5
Triple-glass-distilled water in ml.	7.4	7.3	7.2	7.1	7.0
Potassium persulphate solution in ml.	0.5	0.5	0.5	0.5	0.5
Potassium thiocynate solution in ml.	2.0	2.0	2.0	2.0	2.0

TABLE NO. 12

TEST- SAMPLE — FERROUS FUMARATE

	T_1	T_2	T_3	T_4	T_5
Ferrous Fumarate Solution in ml.	0.1	0.2	0.3	0.4	0.5
Triple-glass-distilled water in ml.	7.4	7.3	7.2	7.1	7.0
Potassium persulphate solution in ml.	0.5	0.5	0.5	0.5	0.5
Potassium thiocynate solution in ml.	2.0	2.0	2.0	2.0	2.0

TABLE NO. 13

STANDARD READINGS

Sample	Iron concentration in r per ml.	Readings I	Readings II	Mean O.D.	Calculated O.D. for 5 r per ml.	Mean O.D. for 5 r per ml.
B	0.0	0.0	0.0	0.0	0.0	
S_1	6.0	22	22	22	22	
S_2	10.0	45	47	46	23	22
S_3	15.0	64	64	64	21	
S_4	20.0	90	89	89	22	
S_5	25.0	107	111	109	22	

$\therefore$ 22 O.D. $\equiv$ 5 r Iron per ml.

On plotting a graph, a straight line relationship between the iron concentration and optical density was obtained.

TABLE NO. 14

READINGS OF TEST — Fe-S SOLUTION

Sample	Readings I	Readings II	Mean O.D.	Calculated O.D. for 0.1 ml. of Fe-S Solution.	Mean O.D. for 0.1 ml. of Fe-S Solution
T_1	22	23	22	22	22
T_2	44	40	42	21	
T_3	66	66	66	22	
T_4	86	92	89	22	
T_5	118	113	115	23	

Calculations :

0.1 ml. of Fe-S Solution gives 22 O.D. ≡ 5 r (From Standard graph)

∴ 1.0 ml. ≡ 50 r Fe

Now, dilution done was 1 in 100.

∴ 100 ml. ≡ 5,000 r Fe

≡ 5 mg. Fe

This 100 ml. of Fe - S Solution contains 40.0 mg. of Ferrous Sulphate Powder.

∴ 40 mg. $FeSO_4$ powder (amount taken for experiment) contains 5 mg. Fe.

∴ 491 mg. $FeSO_4$ (weight of the whole tablet) contains 61.3 mg. Fe.

TABLE NO. 15

READINGS OF TEST — Fe-F SOLUTION

Sample	Readings I	Readings II	Mean O.D.	Calculated O.D. for 0.1 ml. of Fe-F Solution.	Mean O.D. for 0.1 ml. of Fe-F Solution
T_1	43	45	44	44	
T_2	90	95	92	46	
T_3	126	134	130	43	44
T_4	172	182	177	42	
T_5	221	229	225	45	

Calculations :

0.1 ml. of Fe-F Solution gives 44 O.D. ≡ 10 r (From standard graph)

∴ 1.0 ml. ≡ 100 r Fe

Now, dilution done was 1 in 100.

∴ 100 ml. ≡ 10,000 r Fe.

≡ 10 mg. Fe

This 100 ml. of Fe-F Solution contains 80.0 mg. of Ferrous Fumarate Powder.

∴ 80 mg. of Fe-F (amount taken for experiment) contains 10 mg. Fe.

∴ 486 mg. Fe-F (Weight of the whole tablet) contains 60.7 mg. Fe.

RESULTS

THREE such experiments were run and the procedure and the tabulated results of only one experiment are presented here. The following observations were made and the mean values of Elemental Iron content per tablet as found in all the experiments are given here.

TABLE NO. 16

ELEMENTAL IRON CONTENT

Sr. No.	Name of Tablet	Elemental Iron content per tablet		Percent error	Remarks
		Demanded	Actual		
1.	Fe-S	60.0 mg.	61.3 mg.	+ 2.1	Within experimental error
2.	Fe-F	60.0 mg.	60.7 mg.	+ 1.2	

The estimation of elemental iron content per tablet of Ferrous Sulphate and Ferrous Fumarate has been carried out by the method of Wong[59] which is modified and simplified during this study.