C H A P T E R - V

DETERMINATION OF IRON

5.1 INTRODUCTION

Iron is one of the industrially important elements and plays a prominent role in metallurgy, in technology, in health science, and in the evolution of and development of living forms. Without iron life, in any form, is in all likelihood impossible. It is also used in medicine, in alloys and in therapy.

Among those elements essential for life, iron enjoys a status of extra-ordinary importance. It is involved in storage and transport of oxygen, in the metabolism of Nitrogen and Hydrogen, in electron transport, in the decomposition or utilization of H_2O_2, in the reduction of ribotides to deoxyribotides (Precursors of DNA), and in oxidation and hydroxylation of a host of inorganic and organic metabolites.

Iron is a major constituent of whole earth (35%). Earth Crust contains 5% iron. It is universally distributed throughout the solar system and commonly occurs in meteorites. Nickel and iron constitute the core of earth. The sea water contains 10^{-6} to 10^{-7} gm atom per litre iron.

Several methods were applied for the determination of micro to macro amount of iron. Salicylaldehyde guanylhydrazone (SAG) has distinct advantage over the previous methods. The present chapter deals with application of SAG for photometric determination of iron.

Numerous colorimetric reagents were reported for determination of trace amount of iron. Ammonium sulphocynide, 2,2'-bipyridine[1] and 1,10-phenanthroline[2] are known as colorimetric reagents for iron. Several reagents including protocatechuric acid,[3] cupferron,[4] pyramidone,[5] salicylaldoxime,[6] salicylic acid[7] and sulphosalicylic acid[8] are used in the estimation of iron.

Methods involving violuric acid,[9] 2-thenoyl trifluoroacetone,[10] dinitrosoresorcinol,[11] O-hydroxy acetophenone oxime[12] and morin[13] as reagents are not selective. In case of reagents like O-dianisidine,[14] catechol,[15] formaldoxime,[16] quinisatin oxime,[17] 2-pyridyl glyoxime,[18] and dimethyl triketone,[19] colour formation is very slow and requires longer duration of time. In case of α-pyridyl hydrazone,[20] glycine cresol red,[21] pyridyl-β-monoxime[22] and 2-benzoyl pyridine hydrazone[23] heating is necessary due to slow rate of formation of complex. Complexes of p-amino-NN-dimethylaniline,[24] 5-sulpho-β-resorcylic acid[25] and 8-hydroxy-7-nitrosoquinoline-5-sulphonic acid,[26] are only stable for 10-20 minutes. Whereas sensitivity of the methods is very low in case of O-hydroxy acetophenone oxime[12] and chromotrope 2R,[27] Recently, some thiosemicarbazones[28-35] are reported for the determination of iron at tracer level. Some other reagents like pyrazinyl methyl ketone,[36] 1-(2-pyridylazo)-2-naphthol[37] are also used. Iron is determined in synthetic fatty acids by sodium salts of diethyldithiocarbamate.[38]

The proposed reagent, salicylaldehyde guanylhydrazone (SAG) forms a brownish yellow complex with iron at pH 8.85. The method has the desired sensitivity for colorimetric determination of iron in micro quantities.

5.2 EXPERIMENTAL

5.2.1 Standard Solution :

Standard iron(II) solution :

A stock solution of Fe(II) (1 mg/ml = 0.01792 M) was prepared by dissolving A.R. grade ferrous ammonium sulphate hexahydrate in distilled water followed by 2-3 drops of concentrated sulphuric acid and was standardised volumetrically[39] by using literature procedure.

Reagent solution :

A stock solution of 0.01 M reagent was prepared by dissolving 0.178 gms of SAG in 100 ml distilled water (1.78 mg/ml).

Buffer solution :

Buffer solution of pH 9.0 was prepared by dissolving appropriate amount of borax in distilled water.

5.2.2 Recommended Procedure :

An aliquot of the solution containing about 100 µg of iron(II) was taken in a 10 ml volumetric flask. Then 1 ml of

0.01 M reagent (SAG) solution was added which provides desired excess quantity to complete the complex formation. The pH of the solution was adjusted to 8.85 by adding 1 ml of borax buffer solution of pH 9.0 and was adjusted upto the mark with distilled water. The absorbance of the complex was measured at 425 nm against reagent blank. The concentration of iron was read out from a calibration curve.

5.3 RESULTS AND DISCUSSION

5.3.1 Spectral Characteristics :

The absorption spectrum of Iron(II)-SAG complex of the solution containing 100 µg Fe(II) [1.0 ml of 0.1 mg/ml i.e. 1.792×10^{-3} M] and 1.0 ml of 1.0×10^{-2} M of the reagent (SAG) was recorded at pH 8.85 against reagent blank. The complex shows absorption maximum at 425 nm. The molar extinction coefficient at 425 nm is 4.240×10^{3} l $mole^{-1}$ cm^{-1}. The reagent does not absorb in this region. Absorption spectra of the complex and reagent are shown in figure 5.1. Observations are given in table 5.1.

Table 5.1 : Molar extinction coefficients of the Fe(II)-SAG complex and the reagent (SAG)

Wavelength λ,nm	Molar extinction coefficients, ε	
	Fe(II)-SAG complex $\varepsilon x10^4$ l $mole^{-1}cm^{-1}$	Reagent, SAG $\varepsilon x10^4$ l $mole^{-1}cm^{-1}$
380	0.2901	0.001
390	0.3181	0.000
400	0.3626	-
410	0.3962	-
415	0.4129	-
420	0.4185	-
425	0.4240	-
430	0.4185	-
435	0.4165	-
440	0.4129	-
450	0.3906	-
460	0.3628	-
470	0.3237	-
480	0.2901	-
490	0.2651	-
500	0.2372	-
510	0.2148	-
520	0.1925	-
530	0.1814	-
540	0.1674	-
550	0.1618	-
560	0.1535	-
570	0.1479	-
580	0.1423	-
600	0.1283	-
620	0.1144	-

5.3.2 Effect of pH :

To study the effect of pH of the solution on the absorbance of the complex, a series of solutions containing 1 ml of 0.1 mg/ml of Fe(II) [1.792 x 10^{-3}M] and 1 ml of 0.01 M reagent (SAG) was prepared as per the recommended procedure over a range of pH 2.5 to 13. The absorbances were measured against corresponding reagent blank. It was found that the complex has maximum and constant absorbance over the pH range 8.0 to 9.7. Therefore, pH 8.85 was selected as the optimum pH for further studies. The observations are given in table 5.2 (Fig. 5.2).

Table 5.2 : Effect of pH

pH	Absorbance at 425 nm
2.5	0.05
3.0	0.07
4.0	0.20
5.0	0.40
6.0	0.61
7.0	0.63
8.0	0.75
8.5	0.75
9.0	0.75
9.7	0.75
10.8	0.62
11.7	0.35
12.0	0.25

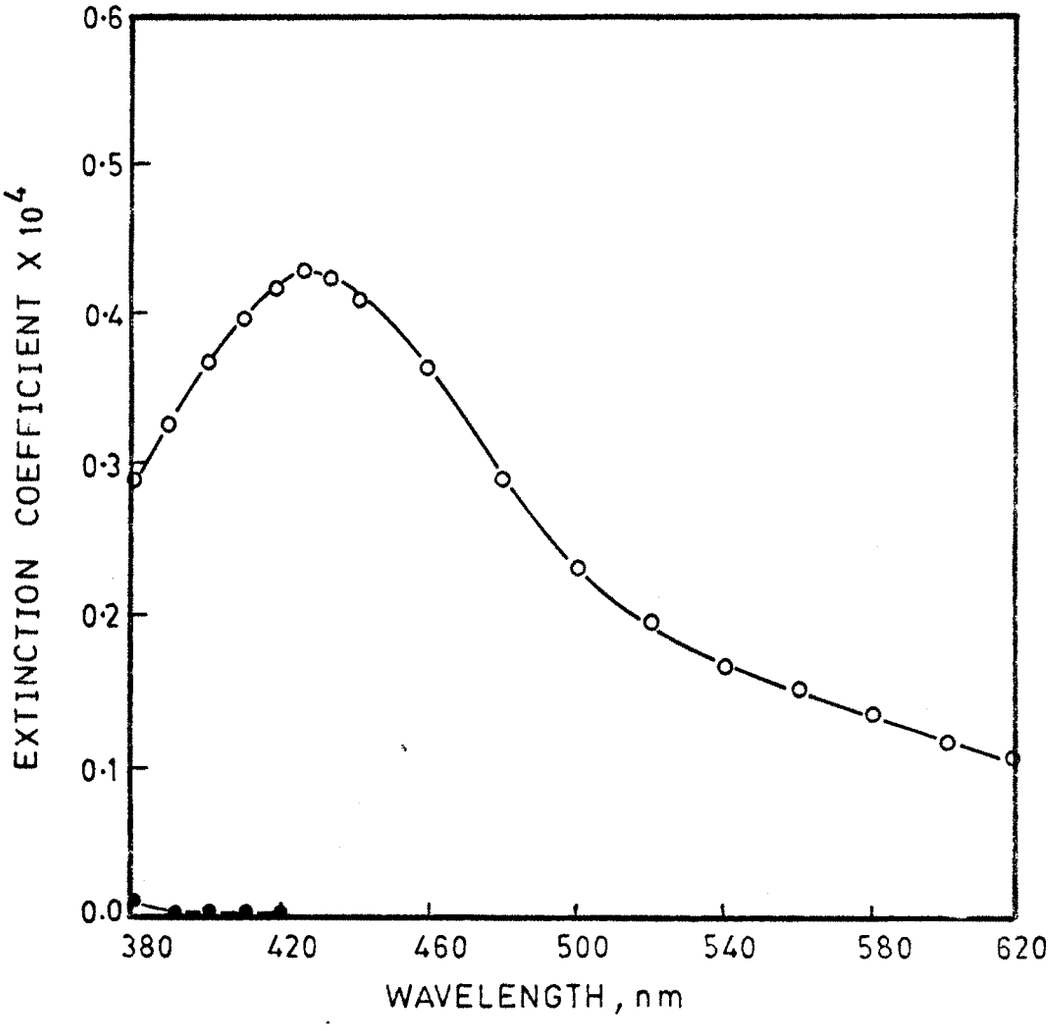


FIG.5·1 o—o ABSORPTION SPECTRUM OF IRON-SAG COMPLEX.
●—● ABSORPTION SPECTRUM OF REAGENT (SAG).

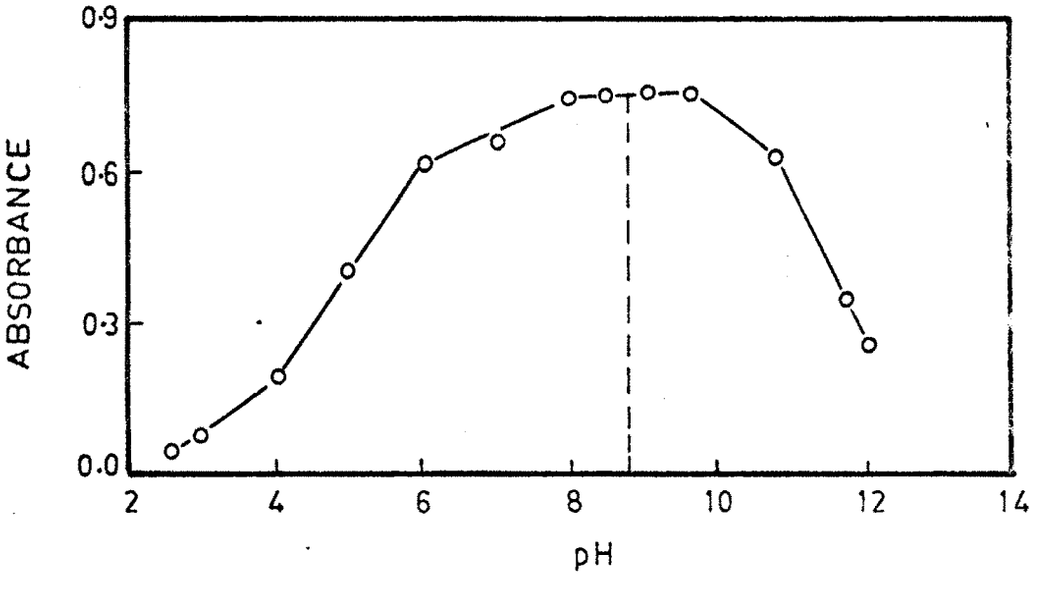


FIG. 5·2 EFFECT OF pH

5.3.3 Effect of reagent concentration :

A series of solutions containing same amount of iron (1.792×10^{-4}M) and different amounts of reagent (2.0×10^{-4}M to 1.4×10^{-3}M) were prepared at pH 8.85. The complex was developed as per the recommended procedure. Results in the table 5.3 show that six fold molar excess of the reagent is sufficient for full colour development.

Table 5.3 : Effect of reagent concentration
[Fe(II)] = 10.0 ppm; [SAG] = 1.0×10^{-2}M

ml of reagent	Absorbance at 425 nm
0.2	0.17
0.4	0.32
0.6	0.63
0.8	0.75
1.0	0.76
1.2	0.76
1.4	0.76

5.3.4 Stability and reaction rate :

Iron(II)-SAG complex is stable for several hours and complex formation is instantaneous.

5.3.5 Validity of Beer's Law :

For the study of validity of Beer's law, the solutions containing different amounts of iron and same amount of reagent (SAG) 3.0 ml of 0.01 M were taken. The absorbance measurements were recorded at 425 nm against corresponding reagent blank. It was found that Beer's law is valid upto 16 ppm (Fig.5.3). The Ringbom plot[40] indicated that optimum concentration range is 3.0 to 8.0 ppm of Fe(II) at 425 nm (Fig. 5.4).

5.3.6 Composition of the complex :

The composition of the complex Fe(II)-SAG was determined by Job's continuous variation method,[41] mole ratio method[42] and slope ratio method.[43] The complexes were developed as per the recommended procedure and absorbances were measured. The plot of absorbances against the mole fraction of the reagent indicated the existence of 1:2 complex with respect to metal and ligand (Fig.5.5). For mole ratio method, solutions containing same concentration of reagent (2.688×10^{-4} M) and varying amounts of iron ranging from 4.48×10^{-5} M to 2.688×10^{-4} M were prepared and absorbances of solutions were measured. The plot of absorbance against metal to reagent ratio (Fig.5.6) shows the formation of 1:2 complex which confirms the results of Job's method. Slope ratio method also confirms the 1:2 complex of Fe(II)-SAG.

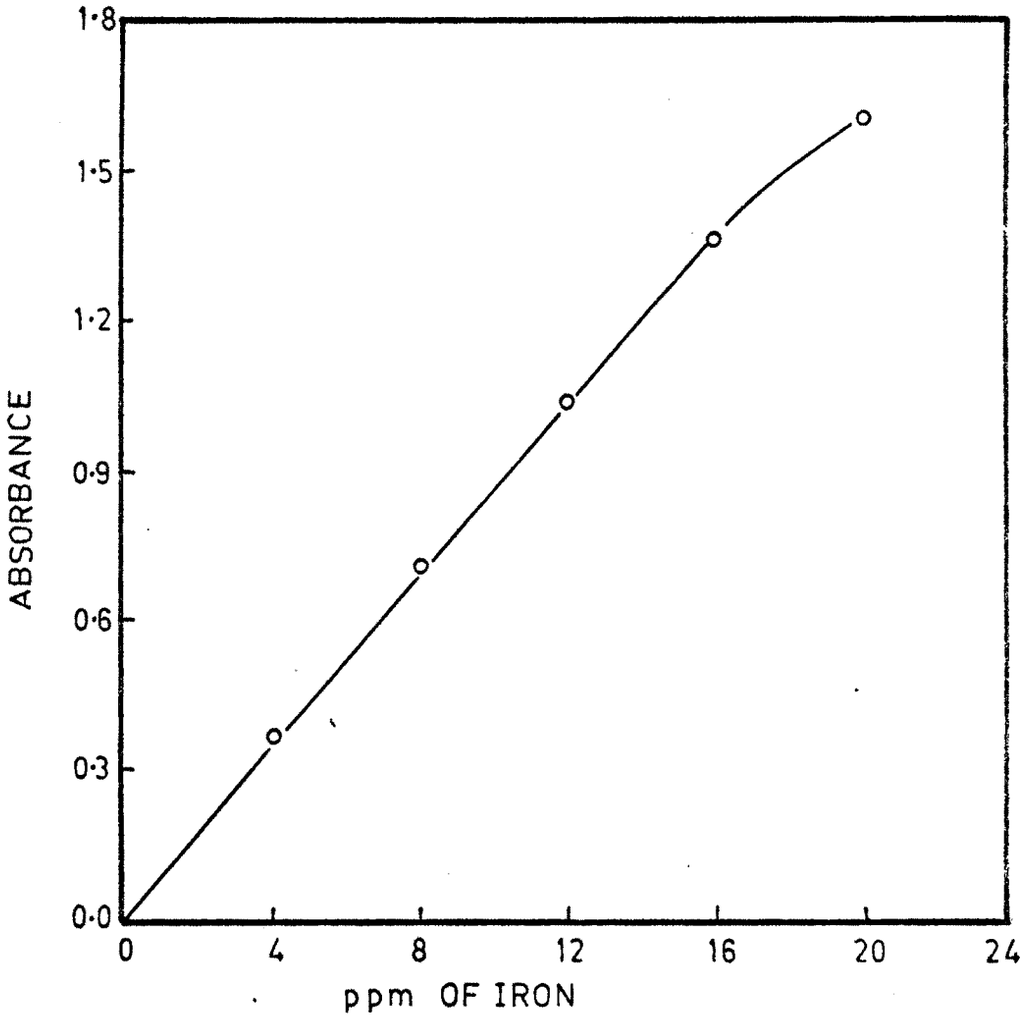


FIG.5·3 VALIDITY OF BEER'S LAW

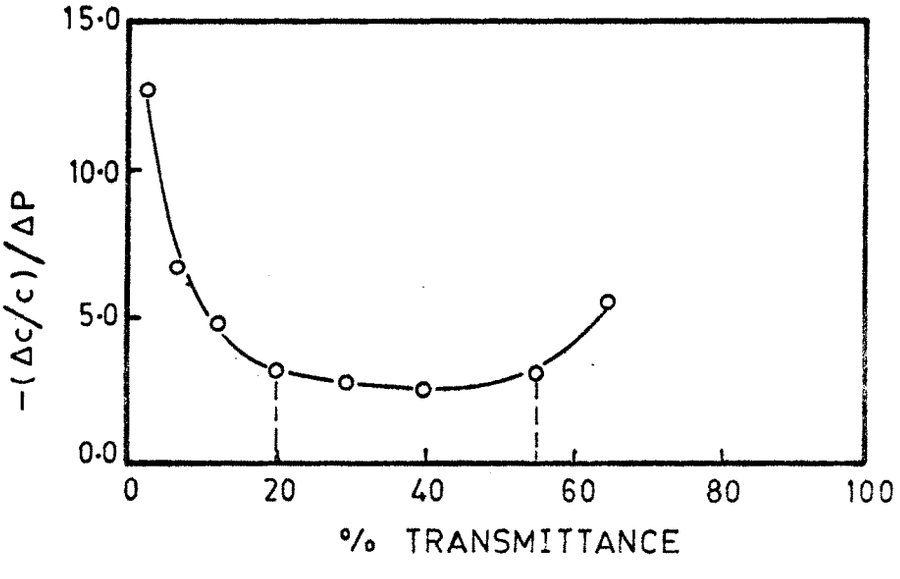


FIG. 5·4 RINGBOM PLOT

Table 5.4 : Job's continuous variation method

$[Fe(II)] = [SAG] = 1.792 \times 10^{-3}$ M

Iron(II) ml	Reagent (SAG) ml	Molar Ratio M:L	Absorbances at, λ		
			425 nm	440 nm	450 nm
0.00	1.50	-	-	-	-
0.25	1.25	1:5	0.138	0.100	0.072
0.50	1.00	1:2	0.180	0.168	0.130
0.75	0.75	1:1	0.160	0.140	0.110
1.00	0.50	2:1	0.125	0.105	0.080
1.25	0.25	5:1	0.065	0.040	0.025
1.50	0.00	-	-	-	-

Table 5.5 : Molar ratio method

$[Fe(II)] = [SAG] = 1.792 \times 10^{-3}$M

Iron(II) ml	Reagent (SAG) ml	Molar Ratio M:L	Absorbances at, λ	
			425 nm	450 nm
0.0	1.50	-	-	-
0.25	1.50	1:6	0.100	0.065
0.50	1.50	1:3	0.180	0.130
0.75	1.50	1:2	0.240	0.180
1.0	1.50	2:3	0.270	0.220
1.25	1.50	5:6	0.300	0.240
1.50	1.50	1:1	0.310	0.255

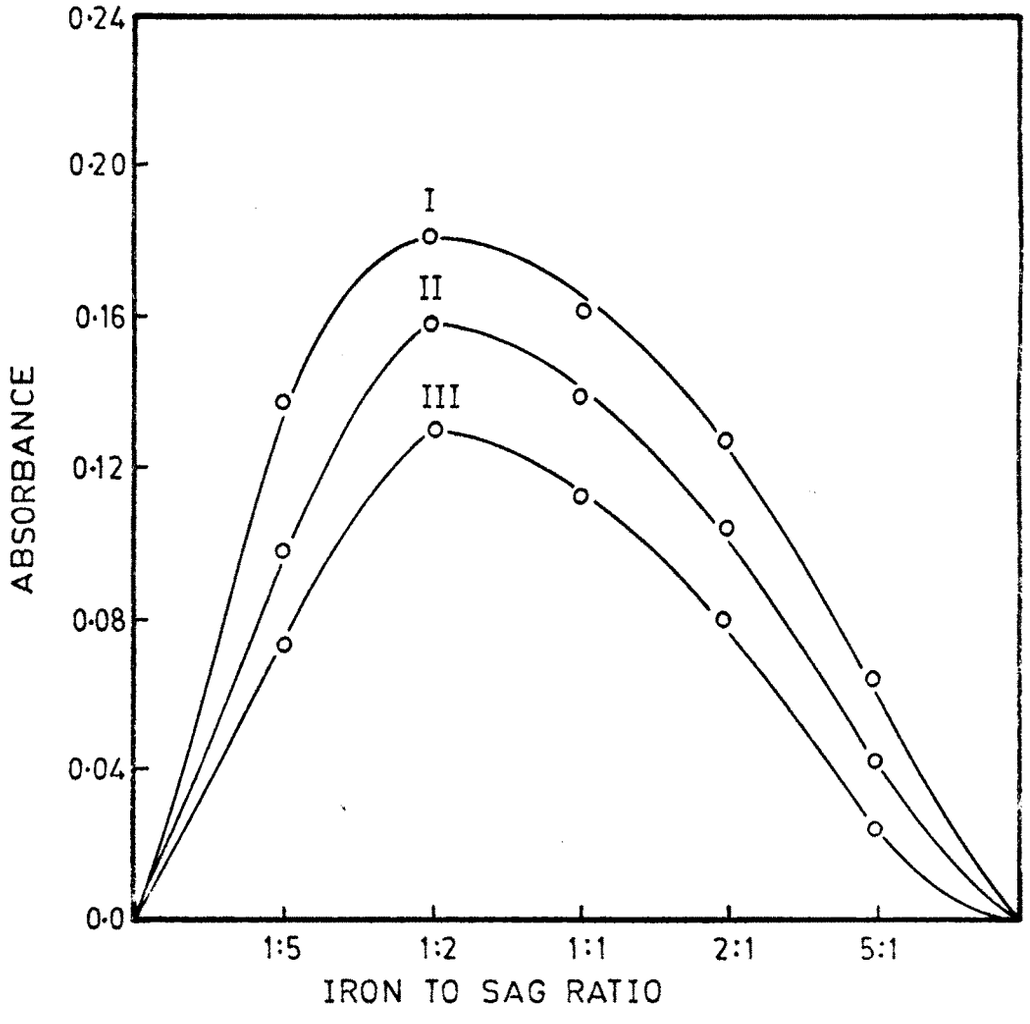


FIG.5·5 JOB'S CONTINUOUS VARIATION METHOD
I — 425 nm , II — 440 nm , III — 450 nm

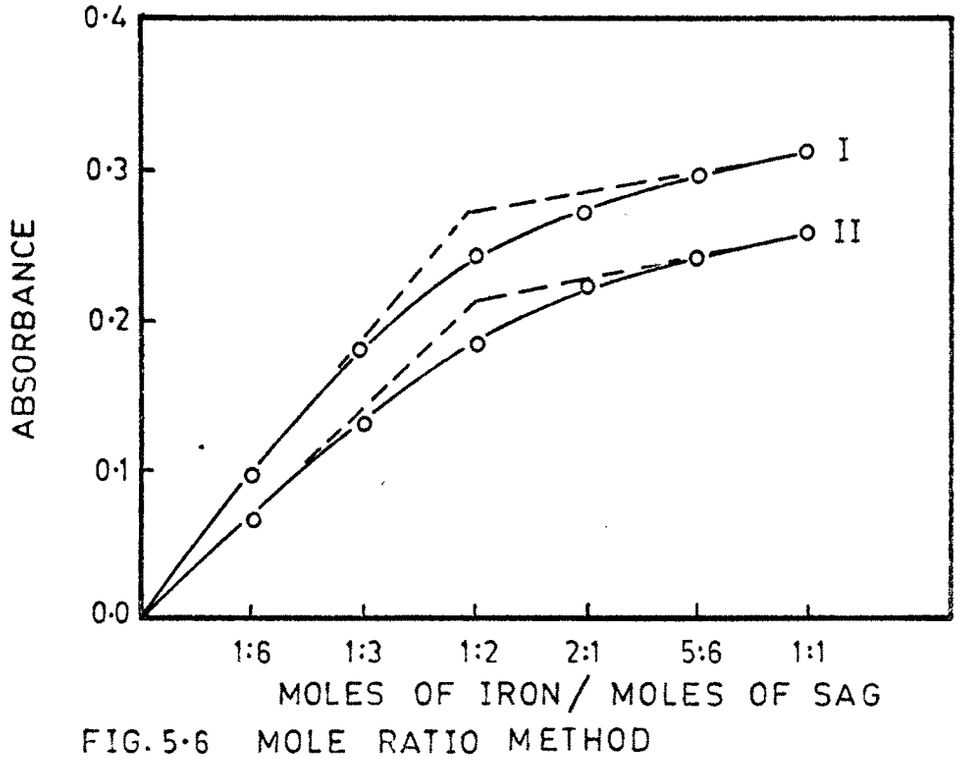
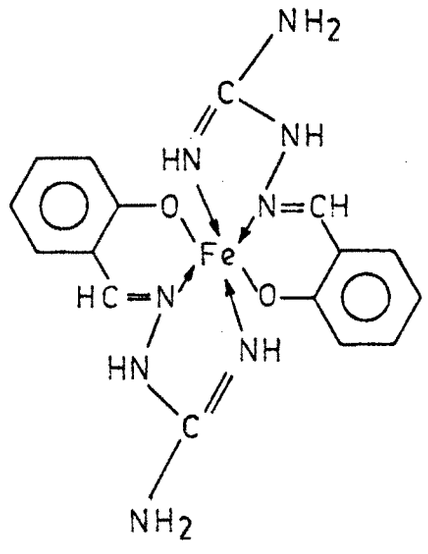


FIG. 5·6 MOLE RATIO METHOD
I — 425 nm , II — 450 nm

The probable structure for Fe(II)-SAG may be shown as :



5.3.7 Sensitivity of the method :

The photometric sensitivity of the method was calculated by Sandell's method[44] and was found to be 0.09665 $\mu g/cm^2$ at 425 nm.

5.3.8 Degree of dissociation and instability constant :

The degree of dissociation was obtained by the method of Harvey and Manning.[43] The value of α, degree of dissociation was found to be 0.1273.

The apparent instability constant[45] was found to be 1.708×10^{-10} for Fe(II)-SAG complex. The change in free energy[46] of the system is - 13.320 k cals/mole.

5.3.9 Precision and accuracy of the method :

In order to test the accuracy and precision of the method, different amounts of iron were determined in six identical samples. The results in table 5.6 show that there is good agreement in the experimental values. The coefficient of variation and standard deviations of methods are also given in table 5.6.

Table 5.6 : Reproducibility of the method

Iron(II) ppm	Mean absorbance of six observations	Standard deviation	Coefficient of variation %
3.5	0.30	0.0020	0.6667
7.0	0.59	0.0038	0.6440
10.5	0.88	0.0055	0.6250
14.0	1.18	0.0071	0.6017

5.3.10 Effect of diverse ions :

Various ions were added to a sample containing a fixed amount of iron (5 ppm) and the colour was developed and measured as per recommended procedure. The tolerance limit was assumed to be the amount of foreign ion needed to cause an error less than 2% in absorbance values. It is found

that V(IV), Pd(II), Ag(I) and $EDTA^{-4}$ interfere seriously, while Ca(II) is tolerated upto 65.0 ppm. The tolerance limits for the ion are listed in table 5.7.

Table 5.7 : Effect of diverse ions

[Fe(II)] = 5.0 ppm [SAG] = 5.0×10^{-3} M

Foreign ions	Added as	Tolerance limit, ppm
Cations		
Cr(III)	$CrCl_3 . 6H_2O$	1.0
V (IV)	$VOSO_4 . H_2O$	none
Ba(II)	$BaCl_2 . 2H_2O$	5.0
Al(III)	$AlCl_3 . 6H_2O$	3.0
Ca(II)	$Ca(NO_3)_2 . 4H_2O$	65.0
Pd(II)	$PdCl_2$	none
Co(II)	$CoSO_4 . 7H_2O$	0.1
Ag(I)	$AgNO_3$	none
W (VI)	$Na_2WO_4 . 2H_2O$	45.0
Pb(II)	$Pb(NO_3)_2$	5.0
Zn(II)	$ZnSO_4 . 7H_2O$	13.0
Mn(II)	$MnSO_4 . H_2O$	1.0
Anions		
Citrate	Citric acid	13.0
Thiourea	Thiourea	50.0
Tartrate	Tartaric acid	10.0
$EDTA^{-4}$	Disodium salt	none
Oxalate	Potassium Oxalate	11.0
Thiosulphate	Sodium thiosulphate	15.0
Phosphate	Potassium dihydrogen orthophosphate	2.0

5.4 APPLICATIONS

Analysis of Vitcofol syrup

0.38 ml of commercially available Vitcofol syrup was taken in a 50 ml conical flask. To this, 2-3 ml perchloric acid was added and was evaporated almost to dryness. It was then dissolved in distilled water and diluted to 25 ml with distilled water.

1.0 ml (100 ppm) of above diluted solution was taken in a 10 ml volumetric flask. To this 3.0 ml of 0.01 M reagent solution was added and pH was adjusted to 8.85 with buffer solution. It was then diluted upto the mark with distilled water and absorbance was measured at 425 nm against reagent blank. Same procedure was repeated by using 0.8 ml of above diluted solution of Vitcofol syrup. Results are given in table 5.8.

Table 5.8 : Analysis of Vitcofol syrup

Syrup	Amount used	Certified value of Fe	Experimental value of Fe	Relative standard deviation for six observations
Vitcofol	10 ppm	0.65 %	0.644 %	0.05
	8 ppm	0.65 %	0.640 %	0.045

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