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A Statistically Validated Electrochemical (Conductometric) and Spectroscopical Study of Some Metal Ligand Complexes for Prospective Biological Action

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Abstract

The present electrochemical study deals with the evaluation of a complexation process involving different metal and ligands and its physicochemical properties to find out the ligand- metal ratio of complex in solution. For the determination of complexing nature "Monovariation method" have been used to ascertain the ligand metal ratio in the complex. The stability constant of the formed complexes were calculated by conductance measurement using Modified Job's method (Turner Anderson Method). The analysis had been carried out by using conductometric principle and the final outcome of the experiment may influenced by temperature and with the ionic concentration of metal and ligand solution. Free energy change values were determined to find out the feasibility of the complexation process. One of the method was validated statistically by using system and method precision parameters. Lastly the complexes was been assayed by using a specific chromogenic agent with a specific spectroscopic technique that may give biological action in future.

Keywords: Conductometry; Monovariation method; Modified Job's method; Chelation; Metal ion.

INTRODUCTION

Complexometric Titration

In complexometric titration the complexes consist of one or more molecules bound to a *central cation*. The molecules bound to the central cation are called *Ligand*. The maximum number of groups bound to an ion is called the *coordination number* of the ion. Coordination number concept comes because of the *coordinate bond* present between the Ligand and metal ion in the structure of complexes as the metal ion (Lewis Acid) accepts the lone pair of electron and the Ligand (Lewis Base) molecule donates it. Thus in a ligand molecule there is presence of at least one lone pair of electrons through which co-ordinate linkage takes place with the metal ion. Importantly electron has two types i.e. bonding pair of electrons and lone pair of electrons. Bonding pair of electrons is accountable for bond formation between the atoms (single bond) whereas lone pair will be accountable for the formation of coordinate bond as it will be not accountable for the bond formation as the bonding pair of electrons. E.g. coordinate bond formation between NH_3 and H^+ ion. Ligands those are bound to the ion at only one point (because of having and attachment of a single functional group which are sharing only one lone pair of electron) are called *unidentate* (one toothed) two points are called *bidentate* (two teethed because a ligand is sharing two lone pair of electron) and so on. Many Ligand contains more than one group capable of bonding to the metal ion with more number of lone pair of electron as such ligands are called *multidentate*.

So denticity only depends on the number of lone pair of electron shared by the ligand with the metal ion. A single ligand can be unidentate or multidentate (e.g. bidentate, hexadentate etc.).

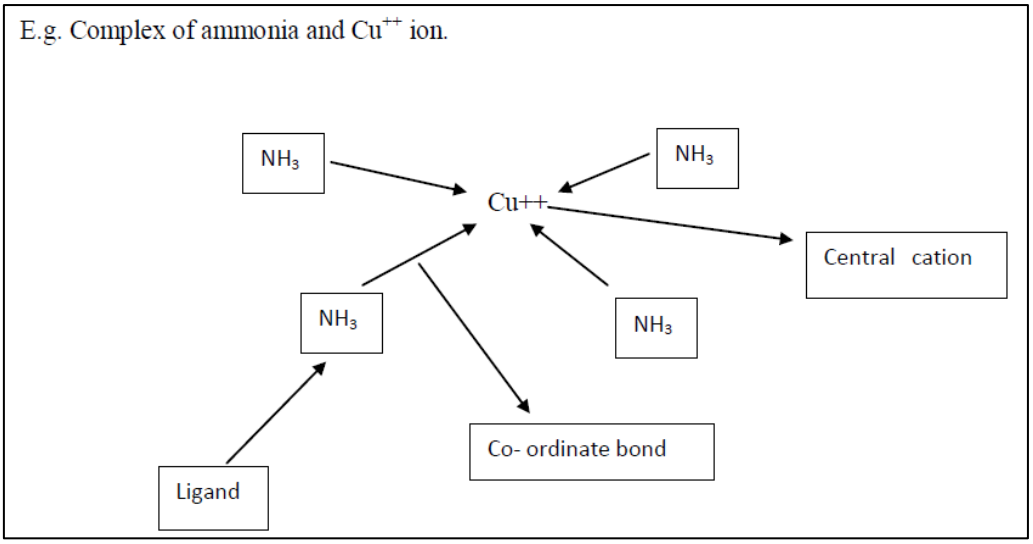


Fig 1: Structure of Ammonia and Cu^{++} ion complex

Electrochemistry

It is a branch of chemistry that deals with the chemical changes produced by electricity and the production of electricity by chemical changes. These reactions involve electrical charge moving between electrodes and electrolytes.

Principle of Conductometric Titration

The electrical current through a chemical cell is carried out by the ionic species in the solution conductometric. The ease with which current is conducted through a solution (under the influence of potential difference applied across two electrodes) depends up on the concentrations and kind of ions in the solution. If two suitable electrodes are present in a solution and potential difference is applied across those electrodes then current will flow through the solution. During progress of a titration changes in the conductivity of the solution usually occur and at the end point involving neutralization or precipitation reaction the conductivity of the solution will be minimum. The principle of conductometric titration is based on the fact that during the titration, one of the ions is replaced by the other and invariably these two ions differ in the ionic conductivity with the result that conductivity of the solution varies during the course of titration. The equivalence point may be located graphically by plotting the change in conductance as a function of the volume of titrant added.

Conductance: Solutions of electrolytes normally obey Ohm's law.
R=V/I.

R= resistance of the solution.
V= applied potential difference.
I= current through the solution.

The conductance (G) of a solution is reciprocal of resistance.

G=1/R. The unit of conductance is ***Siemens (S)***. The conductivity of a solution is measured by an instrument known as conductometer.

Conductivity: If we think the solution in between two electrodes as a slab of material then the conductance of that material is found to be directly proportional to the area of cross section (A) of the solution and inversely proportional to the length (L) between the electrodes.
Therefore G= K x L/A where K= conductivity of the material= specific conductance of the material.
Therefore K= G x L/A.

So conductivity of a material is defined as the conductance of a cube of material having a surface area of $1m^2$ and length of 1m. Unit of conductivity is S/m or S/cm or µS/cm.

Practically Conductivity = Conductance X Cell constant of that particular experimental conductometric cell or electrode which is constant for that particular electrode and independent for any electrolytic solution but it depends only on the temperature of the electrolytic solution whom the cell will measure the conductance.

Validation Parameter

Precision

Precision is the measurement of how close the data values to each other for a number of measurements under the same analytical conditions. Precision may be considered at three levels according to ICH.

Repeatability

System Precision

Precision under same operative conditions (within a laboratory over a short period of time using the same analyst with the same equipment) was determined. Mean, SD and % RSD were calculated from data. The system precision is checked by using standard chemical substance to ensure that the

analytical system is working properly. In this retention time and area of six determinations is measured and % RSD should be calculated.

Acceptance criteria: % RSD should be in between 98%- 102%.

Method Precision

In method precision, a homogenous sample of single batch should be analysed 6 times. This indicates whether a method is giving consistent results for a single batch. In this analysis the sample has been analysed six times with the calculation of % RSD.

Acceptance criteria: % RSD should be in between 98%-102%.

Reproducibility

Precision between laboratories/intermediate precision can be considered during the standardization of a procedure before it is submitted to the pharmacopoeia. A simple logic behind this parameter was some degree of inconsistency (Occurrence of random error which is a type of error that is basically untraceable or beyond the control of analyst) was allowed for every analytical measurement. But, the extent depends on steps involved (Weighing, dilution etc.), technique used in other expected variables (Stability) and intended use of the procedure.

Intermediate Precision (Ruggedness)

Precision under different laboratory conditions (within-laboratory variation, as on different days, or with different analysts, or equipment within the same laboratory) has been carried out. This experiment is done to see whether the test results are coming under the acceptance criteria or not.

Acceptance criteria: % RSD should be in between 98%-102%.

Robustness

Here the closeness of the values are seen in small changes of different parameters like solvent, temperature, pH etc. Here the mean, SD, % RSD is calculated.

Acceptance criteria: % RSD should be in between 98%-102% [1].

Stability Constant

Stability or formation or binding constant is the type of equilibrium constant used for the formation of metal complexes in the solution. Acutely, stability constant is applicable to measure the strength of interactions between the ligands and metal ions that are involved in complex formation in the solution [2]. A generally stability constant equations are expressed as the following ways: equilibrium constants, and these equilibrium constants are known as overall stability constants or overall formation [3]. Any metal complexes will be of greater stability if its stability constant has the higher value. Sometimes the 1/k values are alternative values of stability constant, and now this is called as instability constant.

Thermodynamic stability

In a chemical reaction, chemical equilibrium is a state in which the concentration of reactants and products does not change over time. Often this condition occurs when the speed of forward reaction becomes the same as the speed of reverse reaction. It is worth noting that the velocities of the forward and backward reaction are not zero at this stage but are equal. For the formation of metal chelates, the thermodynamic technique provides a very significant information. Thermodynamics is a very useful technique in distinguishing between enthalpic effects and entropic effects. The bond strengths are totally effected by enthalpic effect, and this does not make any difference in the whole solution in order/disorder. Based on thermodynamics the chelate effect below can be best explained. The change of standard Gibbs free energy for equilibrium constant is response:

Free energy change:
$\Delta G = -2.303\ RT \log K$

R = gas constant
T = absolute temperature
Log K= Stability Constant For metal complexes

Thermodynamic stability and kinetic stability are two interpretations of the stability constant in the solution. If reaction moves from reactants to products, it refers to a change in its energy as shown in the above equation. But for the reactivity, kinetic stability is responsible for this system, and this refers to ligand species [4].

Factors affecting the stability constant of metal complexes

Equilibrium concentration of the free metal ion
Temperature
Analytical concentration of the ligand.
pH of the solution

MATERIAL AND METHODS

Chemicals

All the chemicals used were of Analytical Grade. Ferric Chloride, Ferrous Chloride, Ferrous Sulphate (Lobachem, India, 0.1 M,0.05M) and EDTA, Oxalic acid, PABA, (Merck, India, 0.1M,0.05M) solution were prepared by using double distilled water in the 100ml volumetric flask for several times and has been used in subsequent times for different methods. Pure Curcumin (Lobachem, India, 0.1M, and 0.05M) and 1, 10-Phenanthroline (Lobachem, India) solution was also prepared for several types in this experimental process.

Calibration of conductivity meter

A Systronics model 306 CONDUCTIVITY METER with Conductivity Cell and a simple weight machine from EAGLE and a BOROSILICATATE GLASS. In electrode method conductance reading was noted which having the unit called Siemens.

0.7 gm KCl was dissolved in 100 ml of distilled water to prepare 0.1M solution and conductance reading was noted in Milli Siemens (mS) unit to calibrate the instrument.

Table 1: Observation found out in calibration process

Concentration at 25^0C	Conductance at 25^0 C (mS)
0.1 M KCl	12.88
0.01 M KCl	1.413
0.001 M KCl	0.146

Monovariation method (Mole ratio method/Yoe-Jones method)

This method was introduced by Yoe and Jones [5]. A series of solutions are prepared in which the total concentration of the metal is kept constant and the concentration of the ligand is varied under similar condition. A plot was prepared of conductance value as a function of the ratio of moles of ligand to moles of the metal. The corresponding point where end point was visualized as the occurrence of decrease or increase (lowest or highest) in conductance value followed by the increase or decrease in value gave the idea about molar metal: ligand complexation process.

Conductometric titration for detection of Metal-Ligand ratio (Monovariant method) [6]

Metal salt solution having strength 0.1M was prepared with distilled water. Similarly, 0.1 M of ligand solution was prepared by using the same method as the previous one. Metal salt solution was taken in a beaker and this was titrated conductometrically against 0.1M ligand solution that was taken in a burette.

Modified Job's method (Method of Continuous Variations)

The modification of the Job's [7] continuous variation method performed by Vesburgh and Cooper [8] was applied to find the stoichiometric and formation constant of the complex. The case of co-ordination may be described by:

$mM + nL = M_mL_n$

A series of solutions are prepared in which the sum of total concentration of M and L is kept constant but their proportions are continuously varied. The conductance values of the series is plotted against the mole fraction of the ligand. The ratio of the stoichiometric coefficients is determined from the mole fraction at the point of decrease or increase (lowest or highest) in conductance value followed by increase or decrease takes place. That value corresponding to the mole metal: ligand ratio will be the endpoint of the titration.

Equimolar (0.1M) solutions of ligand and metal solutions were prepared and three series C1, C2, C3 of solutions were made. In set C1 metal salt solution was filled with volume 0.0ml to 12.0ml and total volume was made to 12.0ml in each. Similarly, in C2 ligand solution was filled and set C3 was prepared by mixing metal salt solution from 0.0ml to 12.0ml and ligand solution from 12.0ml to 0.0ml. Conductance values was recorded for each solution. Δ Conductance was calculated as "C1+C2-C3" [9]. Graphs was plotted between Conductance values and mole metal-ligand ratio. The composition and stability constant can be determined from the equivalence point in the graph [10, 11].

Turner Anderson Method for determination of stability constant

Turner and Anderson [12] have modified Job's method and have successfully used for determination of stability constant. By plotting a continuous variation curve for a given range of compositions and then repeating the procedure for more dilute solutions.

Stability Constant equation according to Turner Anderson Method is-

$x=a2b2-a1b1/ (a2-a1) + (b2-b1)$ …….. (1)

$k= x/ (a-x) (b-x)$……. (2)

k= stability constant, (a-x) and (b-x) are the concentration of (product-reactant) in case of ligand and metal if calculated from Modified Job's Method.

x= concentration of complex

a,b= initial concentration of metal and ligand (0.1M).

a1, b1= concentration of metal and complex for 0.1M metal and ligand solution.

a2, b2= concentration of metal and complex for 0.05M metal and ligand solution.

After evaluating the value of x from equation no. 2, it can be substituted in equation no. 1 to find out the stability constant value.

Free energy change

Free energy change of all the complexes that were formed was evaluated by the following equation:

$\Delta G= -2.303\ RT \log K$

R = gas constant

T = absolute temperature

Log K= Stability Constant For metal complexes

Assay of Metal- Chromogenic agent

In the present experiment a chromogenic agent (1, 10- phenanthroline) had been added which will indicate the metal content present by imparting colour in free metal state. This concept can help for the experiment in-vivo in different disease state i.e. Thalassemia where excess haemoglobin staged up inside the body and excess ferrous ion has to be removed from the body to cure the disease. In that case a ligand molecule can bind with that ferrous ion of haemoglobin as ligand-metal complex and can be removed from the body. So that concept can be experimented in-vitro by plotting a standard curve of metal-chromogenic agent complex followed by taking the metal-ligand complex solution after reaching the endpoint (Using Monovariation Method) and find out the concentration of the solution with the help of spectroscopic principle. Importantly that metal-ligand solution was added after addition of the chromogenic agent that will give the colour to the resulting solution and will indicate the metal content in the solution.

RESULT AND DISCUSSION

M= Metal, S= Solvent, L= Ligand

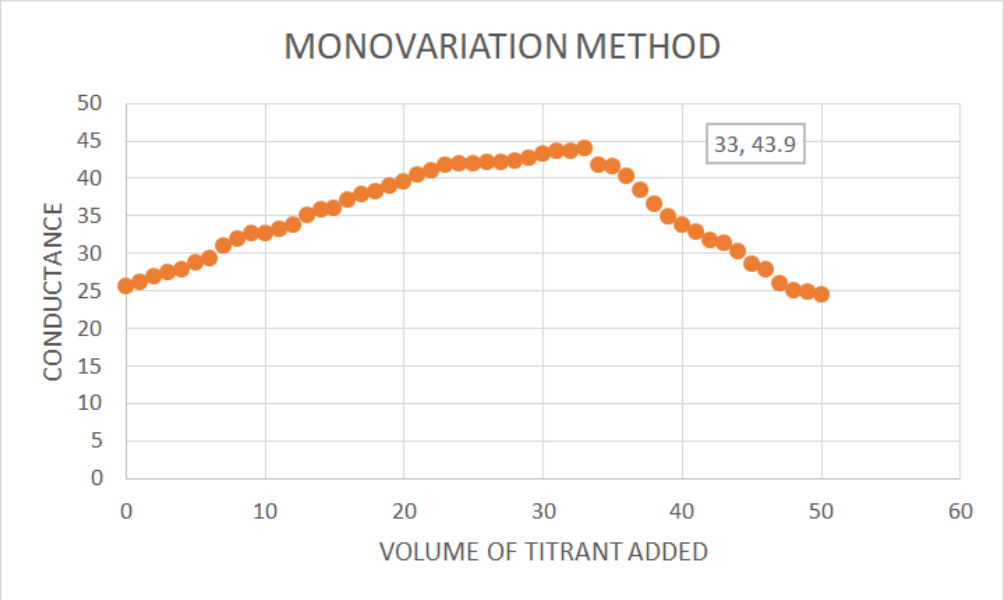
Ferric Chloride vs EDTA

Monovariation method

Table 2: Monovariation Method

Volume of titrant (ml)	Conductance
0	25.6
1	26.2
2	26.9
3	27.5
4	27.8
5	28.8
6	29.3
7	30.9
8	32
9	32.6
10	32.7
11	33.3
12	33.7
13	35
14	35.8
15	36
16	37.2
17	37.8
18	38.2
19	39
20	39.5
21	40.4
22	41
23	41.8
24	41.9
25	42
26	42.1
27	42.2
28	42.3
29	42.7
30	43.2
31	43.6
32	43.7
33	43.9
34	41.8
35	41.5
36	40.2
37	38.5
38	36.6
39	34.9

Volume of titrant (ml)	Conductance
40	33.7
41	32.9
42	31.8
43	31.3
44	30.2
45	28.6
46	27.8
47	25.9
48	25.1
49	24.8
50	24.5

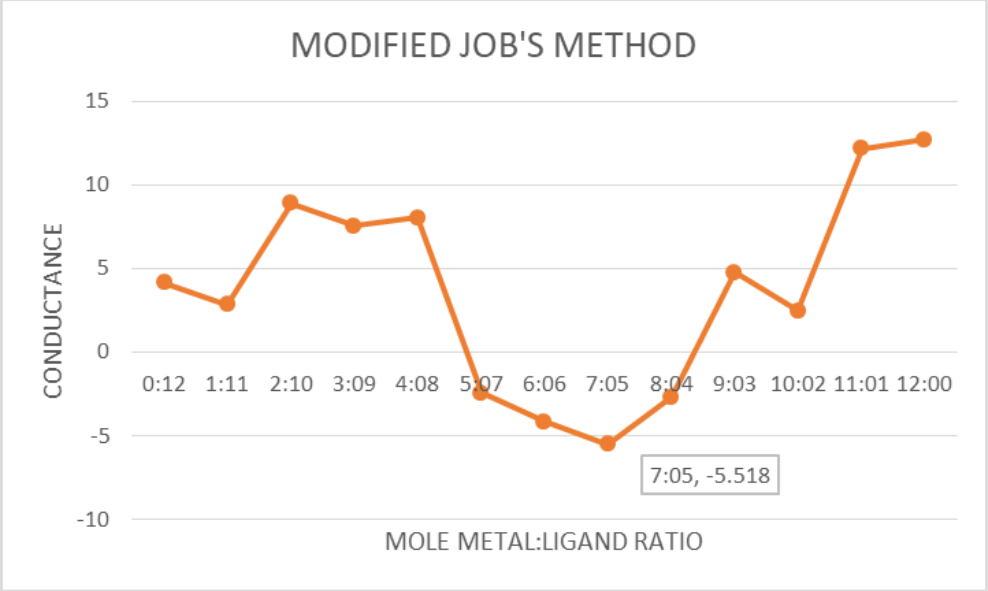


Graph 1: Monovariation Method

VIS1= V2S2
V1S1= Volume and Strength of EDTA
V2S2= Volume and strength of Ferric Chloride
33XS1=50X0.1
S1= 50X0.1/33= 0.151 M
So approx., Metal: Ligand Ratio = .1:.151.
Modified Job's Method

Table 3: Modified Job's Method

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	2.41	2.04	0.285	4.165
01:11	3.41	1.36	1.92	2.85
02:10	7.9	1.41	0.405	8.905
03:09	8.5	0.319	1.29	7.529
04:08	9.25	0.232	1.45	8.032
05:07	3.08	1.14	6.67	-2.45
06:06	4.3	1.53	9.99	-4.16
07:05	2.09	0.482	8.09	-5.518
08:04	3.51	0.475	6.66	-2.675
09:03	6.35	0.512	2.08	4.782
10:02	4.66	0.36	2.56	2.46
11:01	6.6	7.23	1.64	12.19
12:00	6.9	7.66	1.84	12.72



Graph 2: Modified Job's Method

The end point was found out at 7:5 of Metal: Ligand ratio.

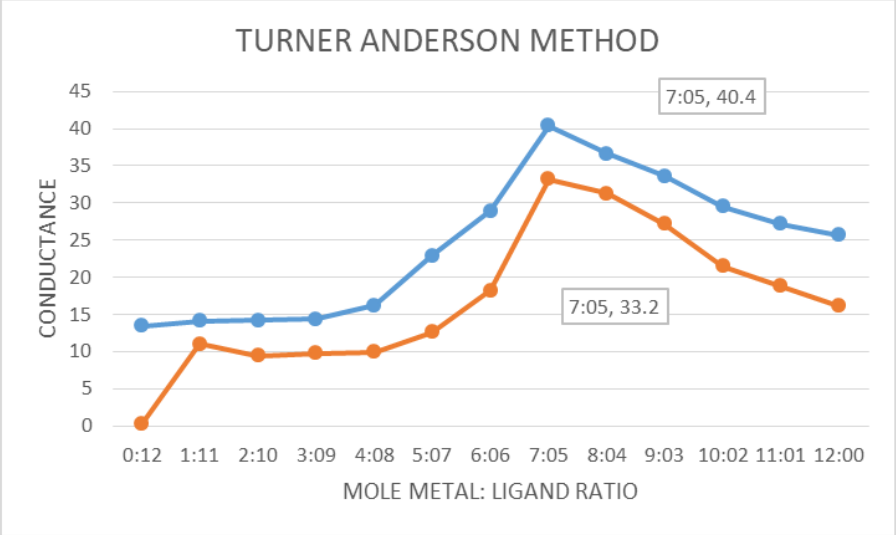
Turner Anderson Method

Table 4: Turner Anderson Method (0.1M)

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	6.73	13.9	7.15	13.48
01:11	4.23	12.6	2.73	14.1
02:10	7.14	11.8	4.74	14.2
03:09	9.65	11.1	6.35	14.4
04:08	13.3	10	7.1	16.2
05:07	15.5	8.48	1.08	22.9
06:06	17.8	8.33	-2.78	28.91
07:05	19.9	7.09	-13.41	40.4
08:04	22.3	6.2	-8.1	36.6
09:03	24.4	5.21	-3.99	33.6
10:02	26.4	4.17	1.07	29.5
11:01	28.2	3.11	4.11	27.2
12:00	30.3	0.748	5.348	25.7

Table 5: Turner Anderson Method (0.05M)

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.399	7.5	7.55	0.349
01:11	7.18	7.01	3.19	11
02:10	6.8	7.17	4.49	9.48
03:09	6.42	7.97	4.65	9.74
04:08	5.93	8.81	4.83	9.91
05:07	4.86	10.8	3.01	12.65
06:06	3.97	14.2	-0.03	18.2
07:05	2.43	22.7	-8.07	33.2
08:04	2.2	21.9	-7.2	31.3
09:03	1.98	20.6	-4.62	27.2
10:02	1.47	18.3	-1.73	21.5
11:01	1.12	17.5	-0.18	18.8
12:00	0.41	16.7	1.01	16.1



Graph 3: Turner Anderson Method

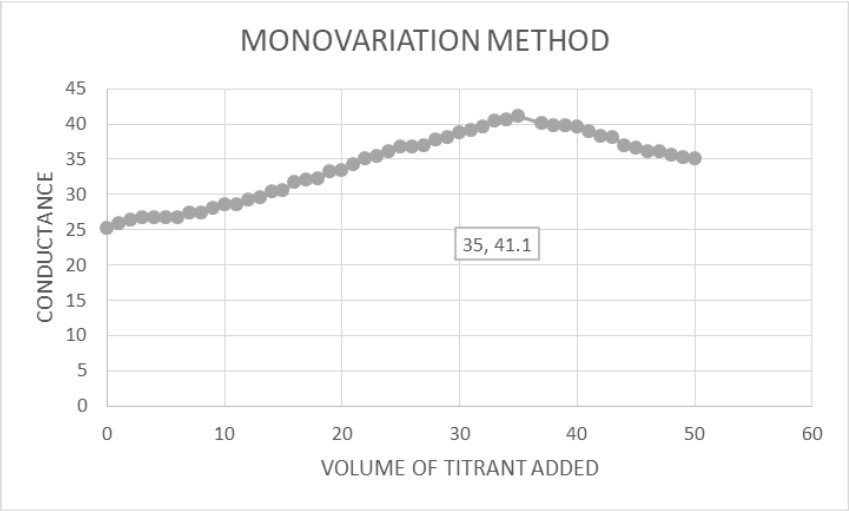
End point were observed at 7:5, for 0.1M and for 0.05M also.

Ferric Chloride vs Oxalic acid Monovariation method

Table 6: Monovariation method

Volume of titrant (ml)	Conductance
0	25.2
1	25.8
2	26.4
3	26.7
4	26.7
5	26.7
6	26.7
7	27.4
8	27.4
9	28
10	28.5
11	28.5
12	29.3
13	29.5
14	30.4
15	30.6
16	31.7
17	32
18	32.3
19	33.3
20	33.4
21	34.2
22	35
23	35.4
24	36
25	36.7
26	36.8
27	37
28	37.8
29	38.1
30	38.8
31	39.1
32	39.6

Volume of titrant (ml)	Conductance
33	40.4
34	40.6
35	41.1
37	40.1
38	39.8
39	39.7
40	39.6
41	39
42	38.3
43	38.1
44	37
45	36.6
46	36.1
47	36
48	35.5
49	35.2
50	35.1

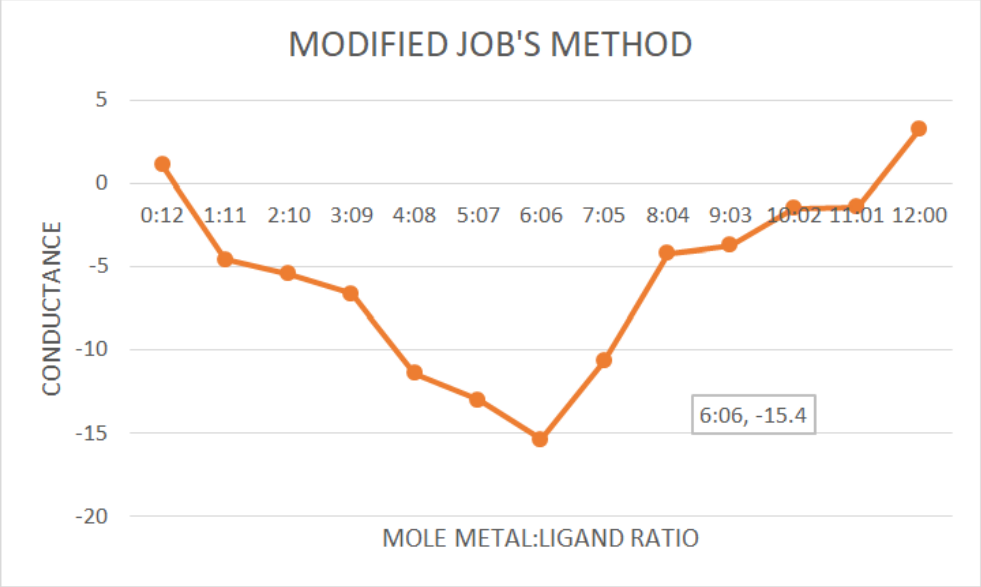


Graph 4: Monovariation method

VIS1= V2S2
V1S1= Volume and Strength of Oxalic Acid
V2S2= Volume and strength of Ferric Chloride
35XS1=50X0.1
S1= 50X0.1/35= 0.142 M
So approx., Metal: Ligand Ratio = .1:.142.
Modified Job's Method

Table 7: Modified Job's Method

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.517	24	23.4	1.117
01:11	3.4	22.5	30.5	-4.6
02:10	6.76	21.1	33.3	-5.44
03:09	9.67	20.1	36.4	-6.63
04:08	12.2	18.2	41.8	-11.4
05:07	14.4	16.4	43.8	-13
06:06	16.1	15.2	46.7	-15.4
07:05	18	12.7	41.4	-10.7
08:04	19.6	11.1	34.9	-4.2
09:03	21.4	9.17	34.3	-3.73
10:02	22.6	6.9	31	-1.5
11:01	24.1	3.67	29.2	-1.43
12:00	26.3	5.19	28.2	3.29



Graph 5: Modified Job's Method

The end point was found out at 6:6 of Metal: Ligand ratio

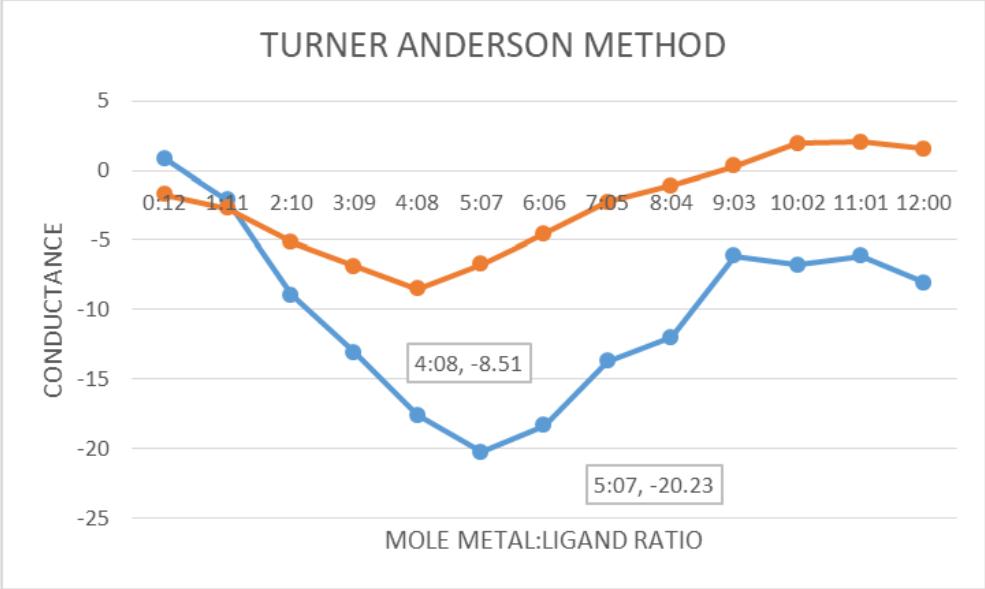
Turner Anderson Method

Table 8: Turner Anderson Method 0.1M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.885	24.1	24.1	0.885
01:11	4.05	23.1	29.3	-2.15
02:10	4.76	21.3	35	-8.94
03:09	6.33	19.9	39.3	-13.07
04:08	7.98	18.1	43.7	-17.62
05:07	8.67	16.7	45.6	-20.23
06:06	9.84	15.2	43.4	-18.36
07:05	11	14.8	39.5	-13.7
08:04	12	11	35	-12
09:03	13.1	9.04	28.3	-6.16
10:02	13.9	6.33	27	-6.77
11:01	14.9	4.03	25.1	-6.17
12:00	15.7	0.25	24	-8.05

Table 9: Turner Anderson Method 0.05 M

RATIO	**M:S(C1)**	**S:L(C2)**	**M:L(C3)**	**C1+C2-C3**
00:12	0.46	15.2	17.4	-1.74
01:11	2.67	18.2	23.6	-2.73
02:10	4.56	11.2	20.9	-5.14
03:09	6.22	10.3	23.4	-6.88
04:08	6.83	9.36	24.7	-8.51
05:07	9.3	8.56	24.6	-6.74
06:06	10.5	7.67	22.7	-4.53
07:05	11.7	6.7	20.7	-2.3
08:04	13.1	5.28	19.5	-1.12
09:03	14.1	4.31	18.1	0.31
10:02	15.8	3.14	17	1.94
11:01	16.1	2.14	16.2	2.04
12:00	17.6	0.24	16.3	1.54



Graph 6: Turner Anderson Method

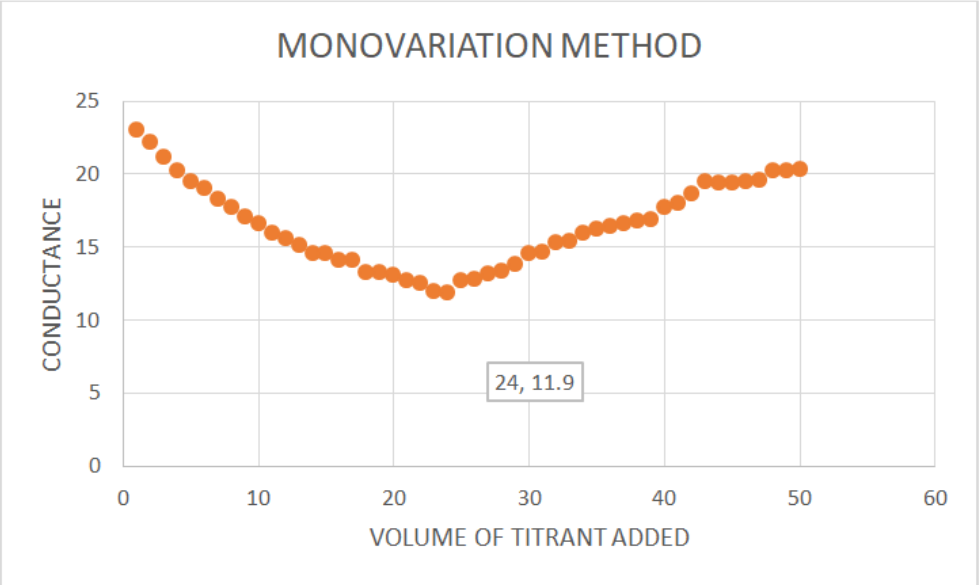
End point were observed at 7:5, for 0.1M and for 0.05M also

Ferric Chloride vs Curcumin Monovariation Method

Table 10: Monovariation Method

Volume of titrant (ml)	Conductance
0	22.9
1	23
2	22.2
3	21.2
4	20.2
5	19.5
6	19
7	18.3
8	17.7
9	17.1
10	16.6
11	16
12	15.6
13	15.1
14	14.6
15	14.6
16	14.1
17	14.1
18	13.3
19	13.3
20	13.1
21	12.7
22	12.5
23	12
24	11.9
25	12.7
26	12.8
27	13.2
28	13.4
29	13.8
30	14.6
31	14.7
32	15.3
33	15.4
34	16

Volume of titrant (ml)	Conductance
35	16.2
36	16.4
37	16.6
38	16.81
39	16.91
40	17.75
41	18
42	18.62
43	19.5
44	19.36
45	19.43
46	19.48
47	19.57
48	20.21
49	20.22
50	20.29

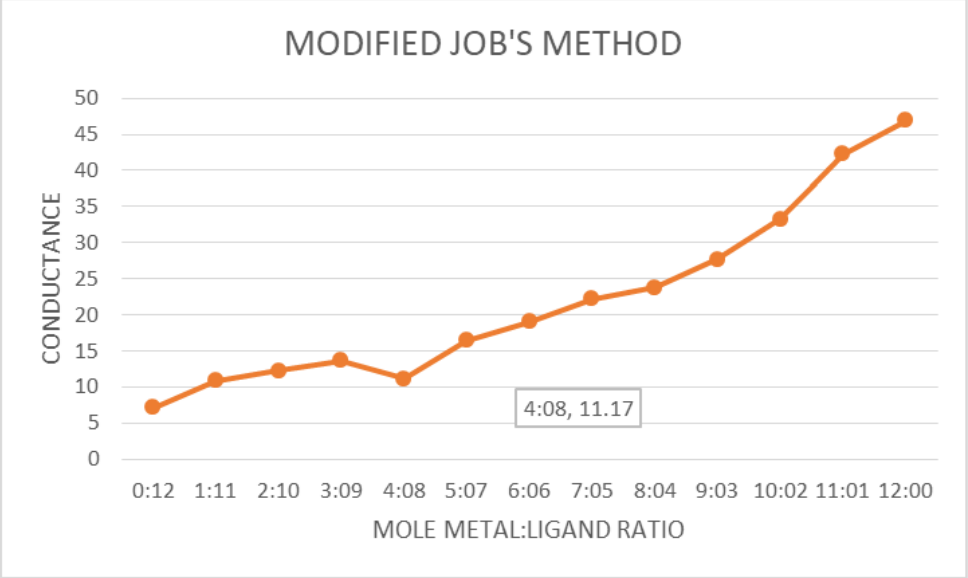


Graph 7: Monovariation Method

VIS1= V2S2
V1S1= Volume and Strength of Curcumin
V2S2= Volume and strength of Ferric Chloride
24XS1=50X0.1
S1= 50X0.1/24= 0.208 M
So approx., Metal: Ligand Ratio = .1:.0.208.
Modified Job's Method

Table 11: Modified Job's Method

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.393	8.13	1.41	7.113
01:11	3.79	8.5	1.43	10.86
02:10	6.45	8.66	2.81	12.3
03:09	8.73	10.2	5.26	13.67
04:08	7.9	11.3	8.03	11.17
05:07	12.8	12.1	8.46	16.44
06:06	14.6	14.1	9.67	19.03
07:05	16.7	16.4	10.9	22.2
08:04	18.2	18.9	13.3	23.8
09:03	19.9	22.5	14.7	27.7
10:02	21.3	29.6	17.6	33.3
11:01	23	39.2	19.9	42.3
12:00	24.4	47.1	24.6	46.9



Graph 8: Modified Job's Method

The end point was found out at 4:8 of Metal: Ligand ratio

Turner Anderson Method

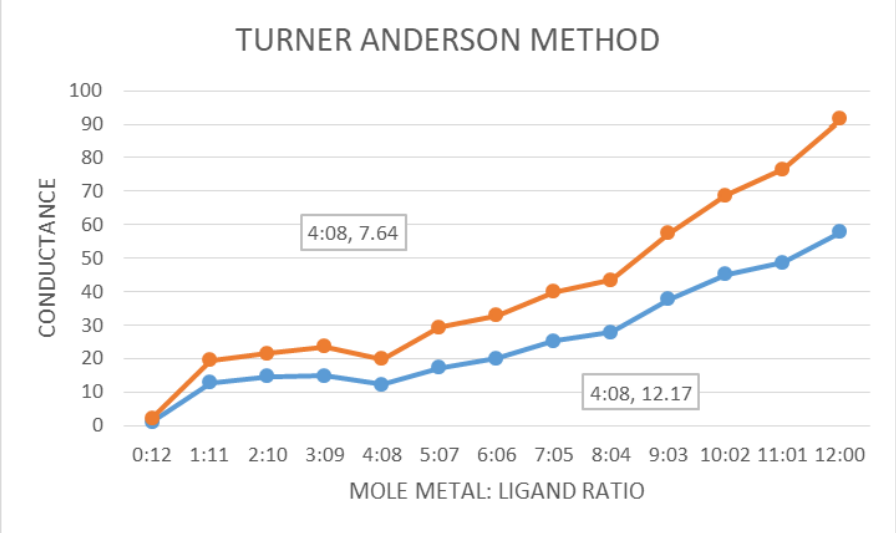
Table 12: Turner Anderson Method 0.1M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.392	8.11	7.4	1.102
01:11	3.77	10.4	1.4	12.77
02:10	8.76	7.76	1.82	14.7
03:09	9.73	10.4	5.26	14.87
04:08	10.9	11.3	10.03	12.17
05:07	13.8	12.1	8.56	17.34
06:06	16.6	13.1	9.57	20.13
07:05	19.7	15.4	9.9	25.2
08:04	23.2	16.9	12.3	27.8
09:03	29.9	21.5	13.7	37.7
10:02	31.1	29.6	15.6	45.1
11:01	33	35.2	19.6	48.6
12:00	34.3	47	23.6	57.7

Turner Anderson Method

Table 13: Turner Anderson Method 0.05 M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.387	8.1	7.3	1.187
01:11	3.65	9.23	6.1	6.78
02:10	5.93	10.21	9.21	6.93
03:09	7.86	12.1	11.2	8.76
04:08	6.34	11.3	10	7.64
05:07	10.3	11.9	10.2	12
06:06	11.1	12	10.3	12.8
07:05	12.2	13.5	10.9	14.8
08:04	12.9	14.1	11.3	15.7
09:03	16.3	15.3	11.9	19.7
10:02	19.8	16	12.1	23.7
11:01	23.6	17	12.6	28
12:00	29.3	18.6	13.9	34



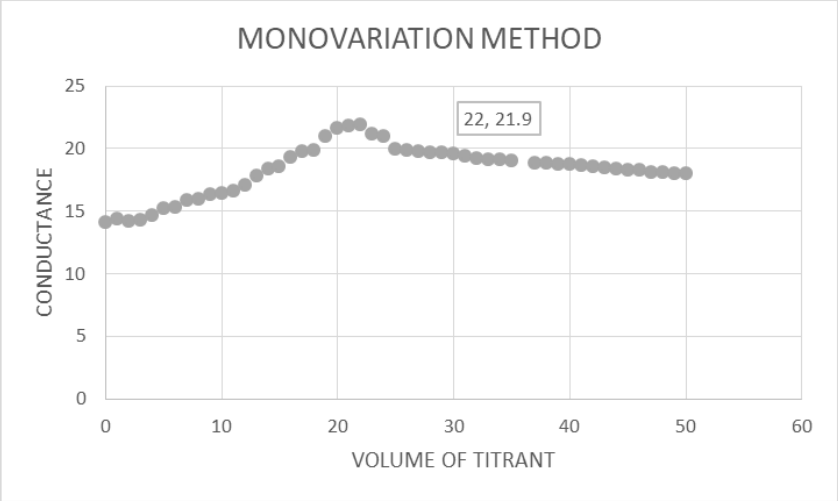
Graph 9: Turner Anderson Method

PABA vs Ferric Chloride
Monovariation Method

Table 14: Monovariation Method

Volume of titrant (ml)	Conductance
0	14.1
1	14.4
2	14.2
3	14.3
4	14.7
5	15.2
6	15.3
7	15.9
8	16
9	16.3
10	16.4
11	16.6
12	17.1
13	17.8
14	18.4
15	18.6
16	19.3
17	19.8
18	19.9
19	21
20	21.6
21	21.8
22	21.9
23	21.2
24	21
25	19.95
26	19.9
27	19.78
28	19.71
29	19.64
30	19.54
31	19.39
32	19.22

Volume of titrant (ml)	Conductance
33	19.14
34	19.13
35	19.03
37	18.88
38	18.83
39	18.79
40	18.7
41	18.63
42	18.54
43	18.42
44	18.4
45	18.3
46	18.32
47	18.11
48	18.1
49	18.03
50	17.98



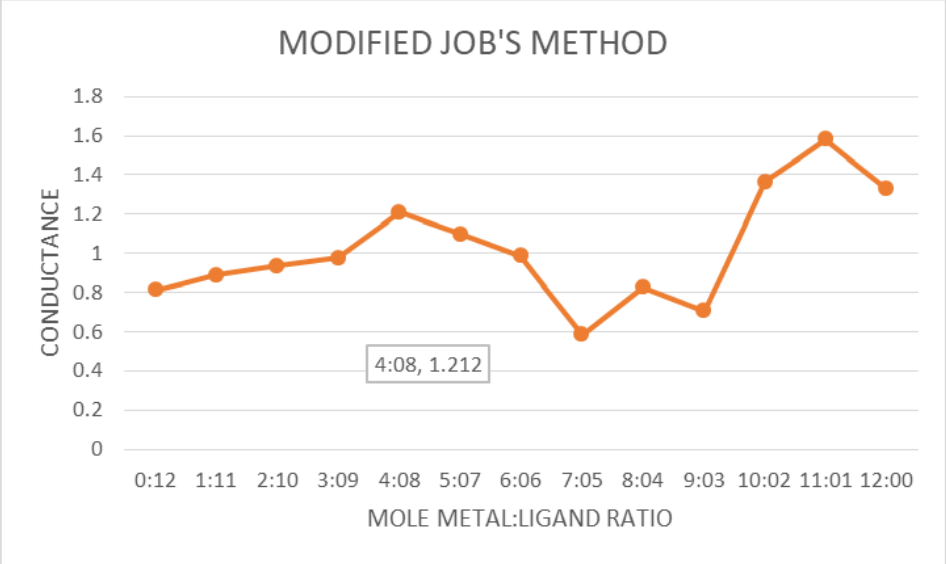
Graph 10: Monovariation Method

VIS1= V2S2
V1S1= Volume and Strength of PABA
V2S2= Volume and strength of Ferric Chloride
22XS1=50X0.1
S1= 50X0.1/22= 0.227 M
So approx., Metal: Ligand Ratio = .1:.0.227

Modified Job's Method

Table 15: Modified Job's Method

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.627	0.74	0.553	0.814
01:11	2.83	0.749	2.69	0.889
02:10	4.63	0.716	4.41	0.936
03:09	5.77	0.706	5.5	0.976
04:08	6.73	0.702	6.22	1.212
05:07	7.93	0.677	7.51	1.097
06:06	7.93	0.666	7.61	0.986
07:05	9.44	0.656	9.51	0.586
08:04	10.71	0.616	10.5	0.826
09:03	11	0.604	10.9	0.704
10:02	12.6	0.563	11.8	1.363
11:01	13	0.581	12	1.581
12:00	14.9	0.127	13.7	1.327



Graph 11: Modified Job's Method

The end point was found out at 4:8 of Metal: Ligand ratio

Turner Anderson Method

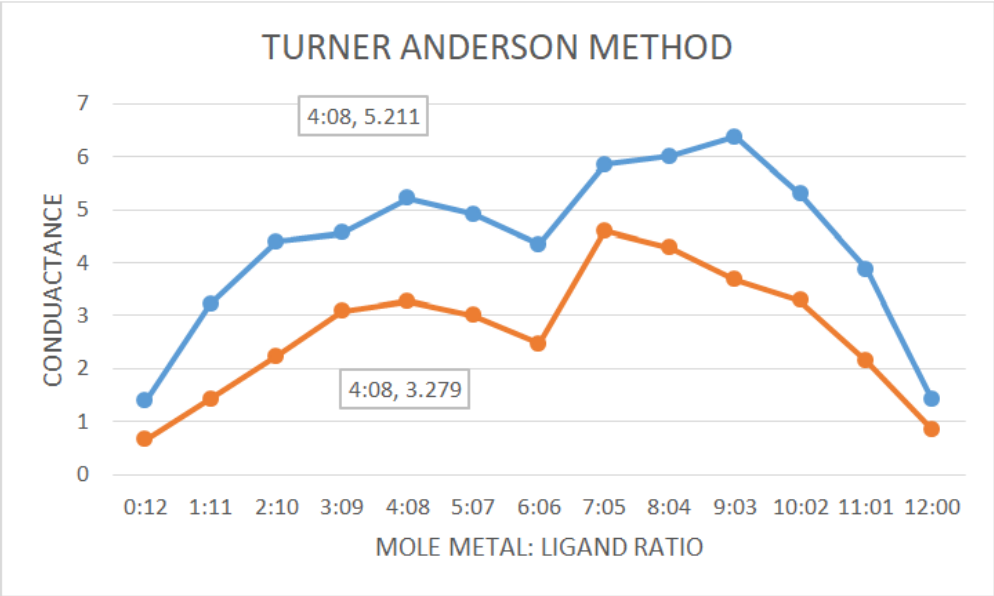
Table 16: Turner Anderson Method 0.1M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.68	0.8	0.083	1.397
01:11	2.69	0.88	0.343	3.227
02:10	4.81	0.103	0.519	4.394
03:09	5.34	0.114	0.891	4.563
04:08	6.42	0.141	1.35	5.211
05:07	6.64	0.173	1.89	4.923
06:06	6.77	0.212	2.64	4.342
07:05	9.23	0.224	3.6	5.854
08:04	10.4	0.267	4.66	6.007
09:03	11.9	0.313	5.84	6.373
10:02	12.7	0.383	7.79	5.293
11:01	13.9	0.47	10.5	3.87
12:00	14.2	0.628	13.4	1.428

Turner Anderson Method

Table 17: Turner Anderson Method 0.05M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.654	0.039	0.037	0.656
01:11	1.55	0.041	0.156	1.435
02:10	2.35	0.06	0.176	2.234
03:09	3.19	0.069	0.18	3.079
04:08	3.9	0.088	0.709	3.279
05:07	3.65	0.09	0.738	3.002
06:06	2.96	1.12	1.61	2.47
07:05	6.13	0.189	1.72	4.599
08:04	6.57	0.214	2.51	4.274
09:03	7.35	0.288	3.96	3.678
10:02	7.91	0.341	4.96	3.291
11:01	8.36	0.461	6.67	2.151
12:00	8.7	0.663	8.5	0.863



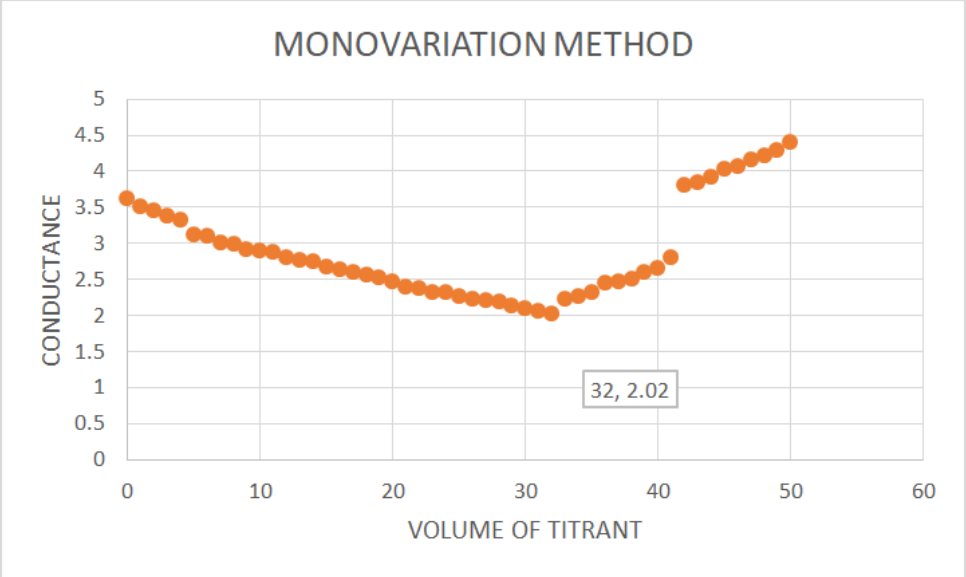
Graph 12: Turner Anderson Method

Ferrous Sulphate Vs EDTA
Monovariation Method

Table 18: Monovariation Method

Volume of titrant (ml)	Conductance
0	3.61
1	3.5
2	3.45
3	3.38
4	3.32
5	3.12
6	3.09
7	3
8	2.98
9	2.92
10	2.9
11	2.87
12	2.81
13	2.77
14	2.74
15	2.68
16	2.63
17	2.6
18	2.55
19	2.52
20	2.47
21	2.4
22	2.38
23	2.32
24	2.31
25	2.26
26	2.22
27	2.21
28	2.18
29	2.14
30	2.1
31	2.06
32	2.02
33	2.22
34	2.27

Volume of titrant (ml)	Conductance
35	2.31
36	2.45
37	2.47
38	2.51
39	2.59
40	2.65
41	2.81
42	3.8
43	3.85
44	3.92
45	4.02
46	4.06
47	4.15
48	4.22
49	4.29
50	4.4



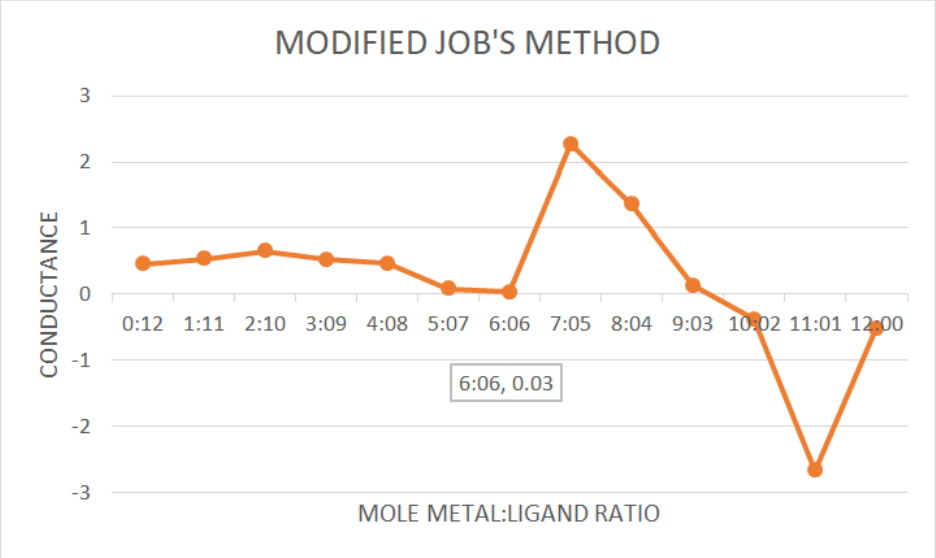
Graph 13: Monovariation Method

VIS1= V2S2
V1S1= Volume and Strength of EDTA
V2S2= Volume and strength of Ferrous Sulphate
32XS1=50X0.1

S1= 50X0.1/32= 0.156 M
So approx., Metal: Ligand Ratio = .1:.156.
Modified Job's Method

Table 19: Modified Job's Method

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.75	12.3	12.6	0.45
01:11	0.83	11.8	12.1	0.53
02:10	1.15	11.1	11.6	0.65
03:09	0.92	10.4	10.8	0.52
04:08	0.72	9.48	9.74	0.46
05:07	1.17	8.64	9.73	0.08
06:06	0.92	7.49	8.38	0.03
07:05	2.8	6.74	7.27	2.27
08:04	3.09	5.43	7.16	1.36
09:03	2.62	4.37	6.86	0.13
10:02	2.8	3.29	6.47	-0.38
11:01	3.08	2.07	7.82	-2.67
12:00	3.3	0.7	4.52	-0.52



Graph 14: Modified Job's Method

The end point was found out at 6:6 of Metal: Ligand ratio

Turner Anderson method

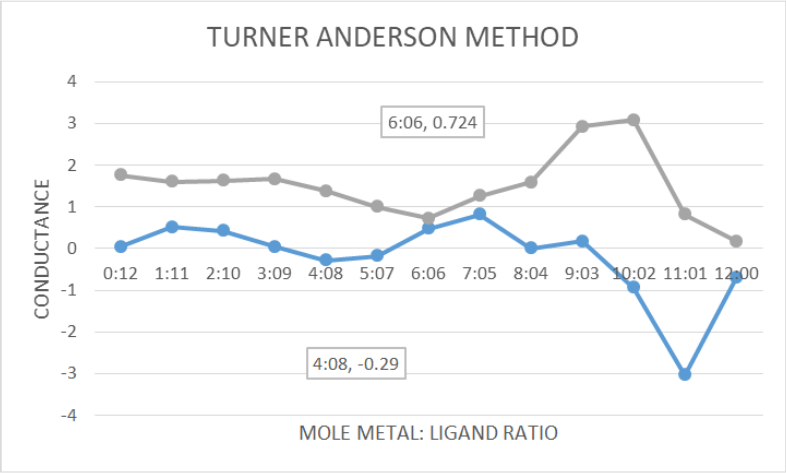
Table 20: Turner Anderson method 0.1M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.743	11.6	12.3	0.043
01:11	0.81	11.71	12	0.52
02:10	0.796	12.43	12.8	0.426
03:09	0.979	9.66	10.6	0.039
04:08	1.2	8.71	10.2	-0.29
05:07	1.51	7.62	9.31	-0.18
06:06	1.71	6.93	8.17	0.47
07:05	1.95	6.22	7.36	0.81
08:04	2.11	5.11	7.21	0.01
09:03	2.46	4.4	6.69	0.17
10:02	2.61	2.86	6.41	-0.94
11:01	2.77	1.91	7.71	-3.03
12:00	2.93	0.8	4.43	-0.7

Turner Anderson method

Table 21: Turner Anderson method 0.05M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.757	12.1	11.1	1.757
01:11	0.707	11.7	10.8	1.607
02:10	0.731	11.3	10.4	1.631
03:09	0.865	10.6	9.8	1.665
04:08	0.885	9.8	9.3	1.385
05:07	0.903	8.7	8.6	1.003
06:06	0.924	8.3	8.5	0.724
07:05	1.16	7.9	7.8	1.26
08:04	1.29	7.4	7.1	1.59
09:03	1.43	6.9	5.4	2.93
10:02	1.58	6.6	5.1	3.08
11:01	1.73	4.19	5.1	0.82
12:00	1.87	3.16	4.86	0.17



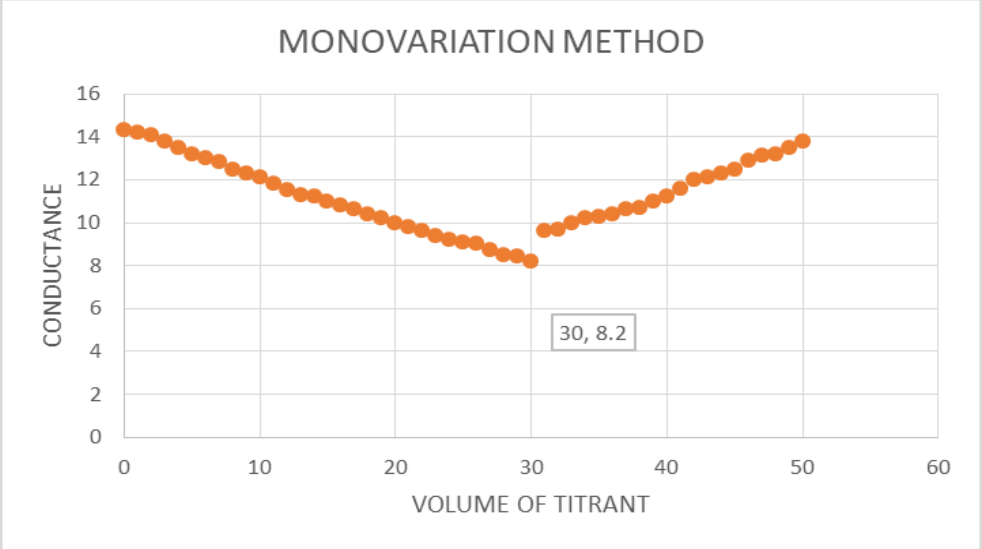
Graph 15: Turner Anderson method

Oxalic acid vs Ferrous Chloride
Monovariation Method

Table 22: Monovariation Method

Volume of titrant (ml)	Conductance
0	14.3
1	14.2
2	14.1
3	13.8
4	13.5
5	13.2
6	13
7	12.8
8	12.5
9	12.3
10	12.1
11	11.8
12	11.5
13	11.3
14	11.2
15	11
16	10.8
17	10.6
18	10.4
19	10.2
20	10
21	9.8
22	9.6
23	9.4
24	9.2
25	9.1
26	9
27	8.7
28	8.5
29	8.4
30	8.2
31	9.6
32	9.7
33	10
34	10.2
35	10.3

Volume of titrant (ml)	Conductance
36	10.4
37	10.6
38	10.7
39	11
40	11.2
41	11.6
42	12
43	12.1
44	12.3
45	12.5
46	12.9
47	13.1
48	13.2
49	13.5
50	13.8



Graph 16: Monovariation Method

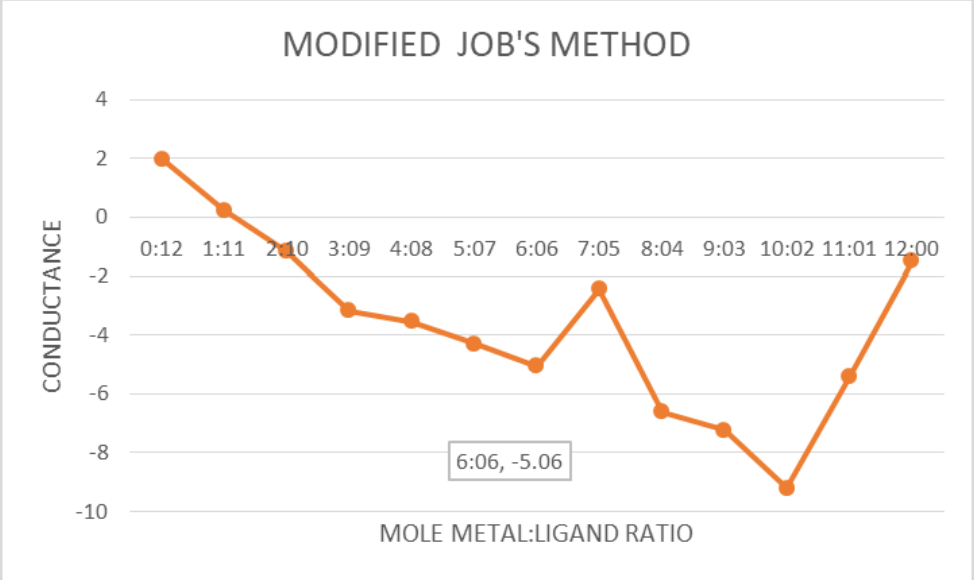
VIS1= V2S2
V1S1= Volume and Strength of Oxalic Acid
V2S2= Volume and strength of Ferrous Chloride
30XS1=50X0.1

S1= 50X0.1/30= 0.166 M
So approx., Metal: Ligand Ratio = .1:.166.

Modified Job's Method

Table 23: Modified Job's Method

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.69	22.3	21	1.99
01:11	1.63	21.3	22.7	0.23
02:10	2.63	20.1	23.9	-1.17
03:09	3.03	18.6	24.8	-3.17
04:08	5.06	17.3	25.9	-3.54
05:07	6.29	15.4	26	-4.31
06:06	7.94	13.5	26.5	-5.06
07:05	13.16	11.2	26.8	-2.44
08:04	10.4	8.78	25.8	-6.62
09:03	13	5.47	25.7	-7.23
10:02	13.2	3.19	25.6	-9.21
11:01	11.4	1.49	18.3	-5.41
12:00	15.2	0.517	17.2	-1.483



Graph 17: Modified Job's Method

The end point was found out at 6:6 of Metal: Ligand ratio
Turner Anderson Method

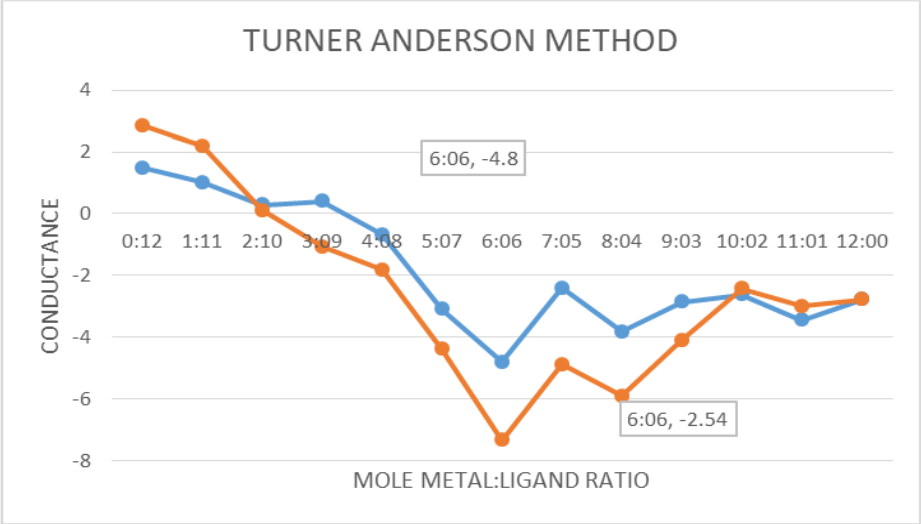
Table 24: Turner Anderson Method 0.1M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.581	21.7	20.8	1.481
01:11	1.61	21	21.6	1.01
02:10	2.78	20.4	22.9	0.28
03:09	5.31	19.3	24.2	0.41
04:08	6.32	18.6	25.6	-0.68
05:07	7.11	16.6	26.8	-3.09
06:06	8	14.1	26.9	-4.8
07:05	9.29	12.9	24.6	-2.41
08:04	10.36	9.91	24.1	-3.83
09:03	11.98	7.46	22.3	-2.86
10:02	13.16	4.81	20.6	-2.63
11:01	14.3	1.36	19.1	-3.44
12:00	14.9	0.523	18.2	-2.777

Turner Anderson Method

Table 25: Turner Anderson Method 0.05M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	0.675	12.8	12.1	1.375
01:11	1.18	12.9	12.9	1.18
02:10	1.62	11.61	13.4	-0.17
03:09	2.11	10	13.6	-1.49
04:08	3.1	9.37	13.6	-1.13
05:07	3.57	8.83	13.7	-1.3
06:06	4.13	6.83	13.5	-2.54
07:05	4.77	5.96	13.2	-2.47
08:04	5.44	5.19	12.7	-2.07
09:03	6.26	4.31	11.8	-1.23
10:02	7.44	3.7	10.93	0.21
11:01	8.09	1.92	9.56	0.45
12:00	8.39	0.591	8.99	-0.009



Graph 18: Turner Anderson Method

Stability Constant

Table 26: Stability constant Calculation

Metal- Chelate Complex	Stability Constant Value (logK) (at 29.8^{0}C)
1. Ferric Chloride- EDTA	1.28072381
2. Ferric Chloride- Oxalic Acid	1.99215164
3. Ferric Chloride- Curcumin	1.17248713
4. Ferric Chloride- PABA	1.172487
5. Ferrous Sulphate-EDTA	1.17425422
6. Ferrous Sulphate-PABA	1.172487

Free energy Change
Free energy change: ΔG= -2.303 RT log K

Table 27: Free energy change calculation

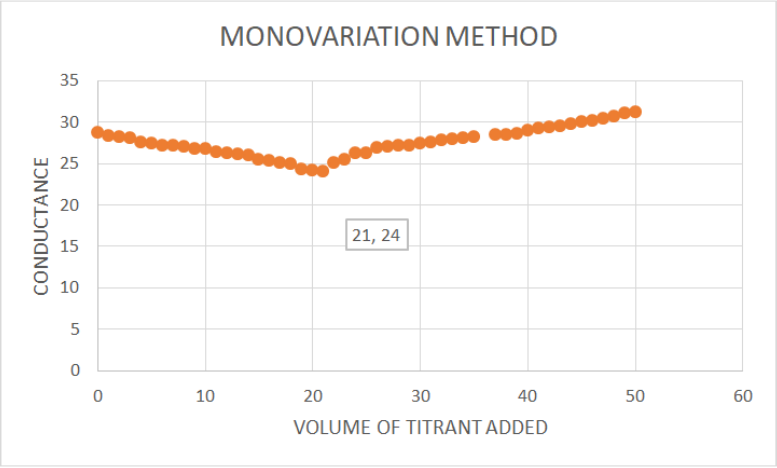
Metal- Chelate Complex	Free Energy Change (Kcal/mole)
1. Ferric Chloride- EDTA	-6694.96
2. Ferric Chloride- Oxalic Acid	-10413.9
3. Ferric Chloride- Curcumin	-6129.16
4. Ferric Chloride- PABA	-6129.16
5. Ferrous Sulphate-EDTA	-6138.4
6. Ferrous Sulphate-PABA	-6129.16

Assay of Metal-Chromogenic agent
Ferrous Sulphate- 1, 10- Phenanthroline
Monovariation method

Table 28: Monovariation method

Volume of titrant (ml)	Conductance
0	28.7
1	28.3
2	28.2
3	28
4	27.5
5	27.4
6	27.2
7	27.1
8	27
9	26.8
10	26.7

Volume of titrant (ml)	Conductance
11	26.4
12	26.2
13	26.1
14	26
15	25.4
16	25.3
17	25.1
18	25
19	24.3
20	24.1
21	24
22	25.1
23	25.4
24	26.2
25	26.3
26	26.9
27	27
28	27.1
29	27.2
30	27.4
31	27.5
32	27.8
33	27.9
34	28.1
35	28.2
37	28.4
38	28.5
39	28.6
40	29
41	29.2
42	29.3
43	29.5
44	29.7
45	30
46	30.2
47	30.4
48	30.7
49	31
50	31.2

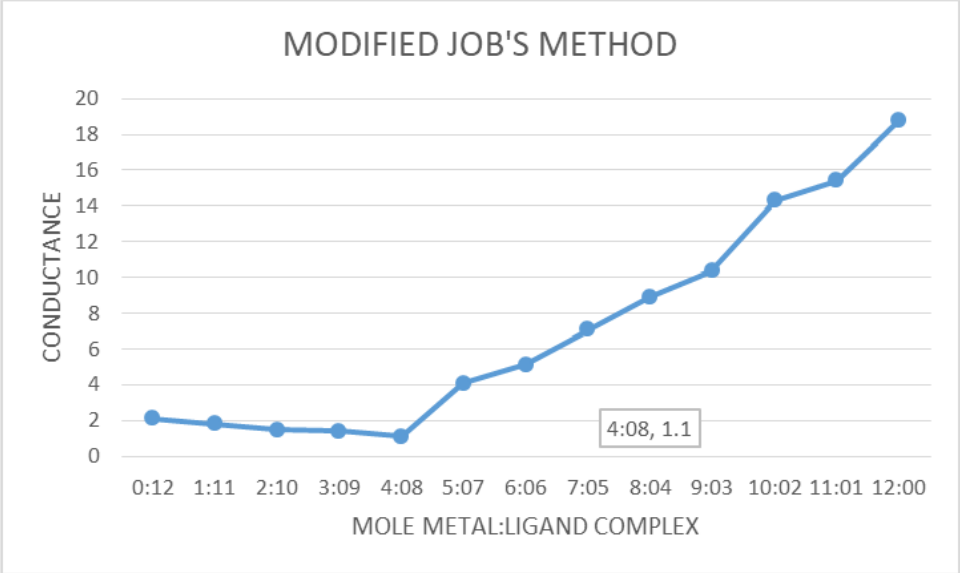


Graph 19: Monovariation method

Modified Job's Method

Table 29: Modified Job's Method

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	5.23	15.2	18.3	2.13
01:11	8.42	16.6	23.2	1.82
02:10	12.2	11.9	22.6	1.5
03:09	13.11	13.4	25.1	1.41
04:08	16	13.1	28	1.1
05:07	16.3	14	26.2	4.1
06:06	17.32	14.2	26.4	5.12
07:05	18.6	14.5	26	7.1
08:04	19.2	15	25.3	8.9
09:03	20.2	15.3	25.1	10.4
10:02	23.3	16	25	14.3
11:01	27.2	16.32	28.1	15.42
12:00	28	17	26.2	18.8



Graph 20: Modified Job's Method

The end point was found out at 4:8 of Metal: Ligand ratio

Turner Anderson Method

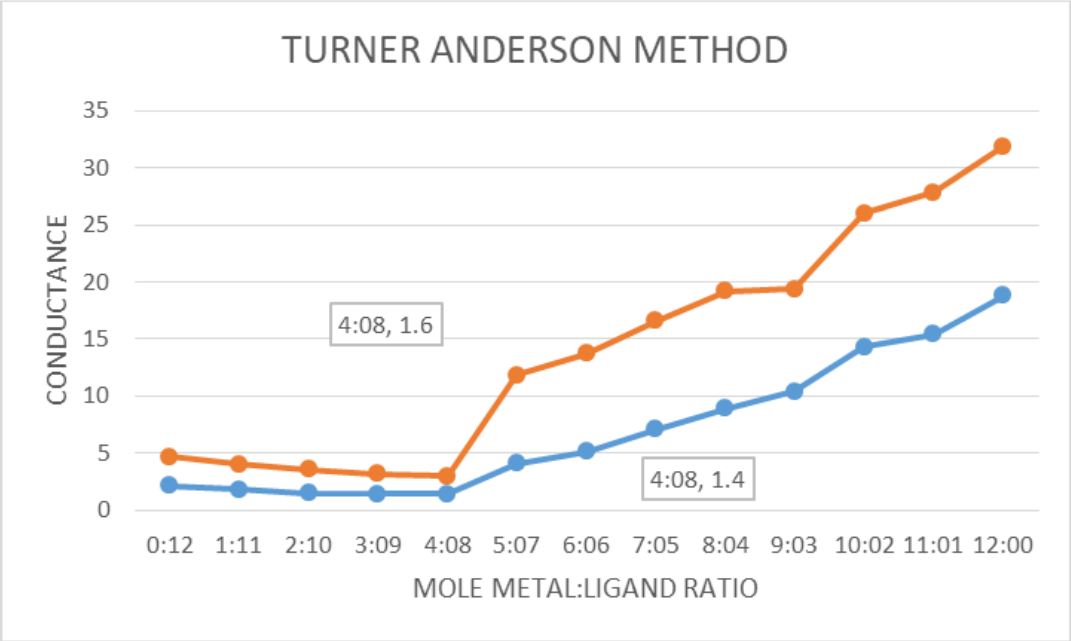
Table 30: Turner Anderson Method 0.1M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	5.23	15.2	18.3	2.13
01:11	8.42	16.6	23.2	1.82
02:10	12.2	11.9	22.6	1.5
03:09	13.11	13.4	25.1	1.41
04:08	16	13.4	28	1.4
05:07	16.3	14	26.2	4.1
06:06	17.32	14.2	26.4	5.12
07:05	18.6	14.5	26	7.1
08:04	19.2	15	25.3	8.9
09:03	20.2	15.3	25.1	10.4
10:02	23.3	16	25	14.3
11:01	27.2	16.32	28.1	15.42
12:00	28	17	26.2	18.8

Turner Anderson Method

Table 31: Turner Anderson Method 0.05M

RATIO	M:S(C1)	S:L(C2)	M:L(C3)	C1+C2-C3
00:12	2.52	15.3	15.3	2.52
01:11	5.1	16.3	19.2	2.2
02:10	8.2	15.2	21.3	2.1
03:09	10.2	15.3	23.7	1.8
04:08	12.1	15.7	26.2	1.6
05:07	14	15	21.3	7.7
06:06	15.2	16.3	22.9	8.6
07:05	15.3	17.6	23.4	9.5
08:04	16.2	19.2	25.1	10.3
09:03	18.3	17	26.3	9
10:02	20.3	18.3	26.9	11.7
11:01	21.4	19	28	12.4
12:00	22.3	20.1	29.3	13.1

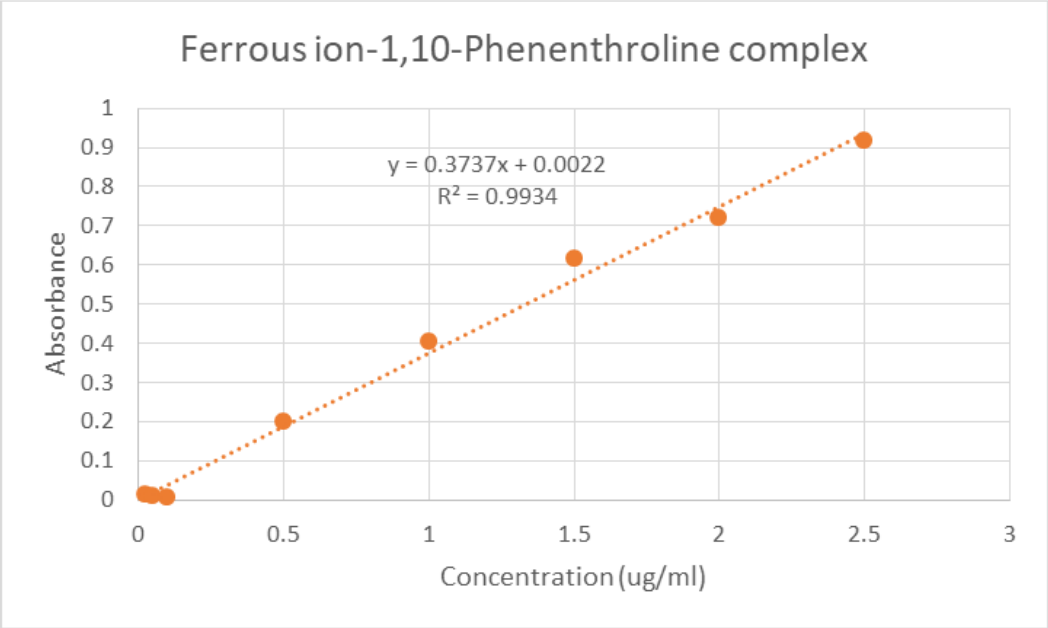


Graph 21: Turner Anderson Method

Spectroscopic estimation of Ferrous- 1, 10-Phenanthroline complex (λ_{max}- 509.6 nm)

Table 32: Spectroscopic data of Ferrous-1, 10-Phenanthroline complex

Conc. (ug/ml)	absorbance
0.025	0.0126
0.05	0.0114
0.1	0.0073
0.5	0.1982
1	0.4043
1.5	0.6143
2	0.7198
2.5	0.9175



Graph 22: Standard Curve of Ferrous-1, 10-Phenanthroline complex

From the standard curve it was seen that-

Ferrous- EDTA complex having the concentration of 0.523129 ug/ml where absorbance of that solution was 0.582

Ferrous- PABA complex having the concentration of 0.337129 ug/ml where absorbance of that solution was 0.396.

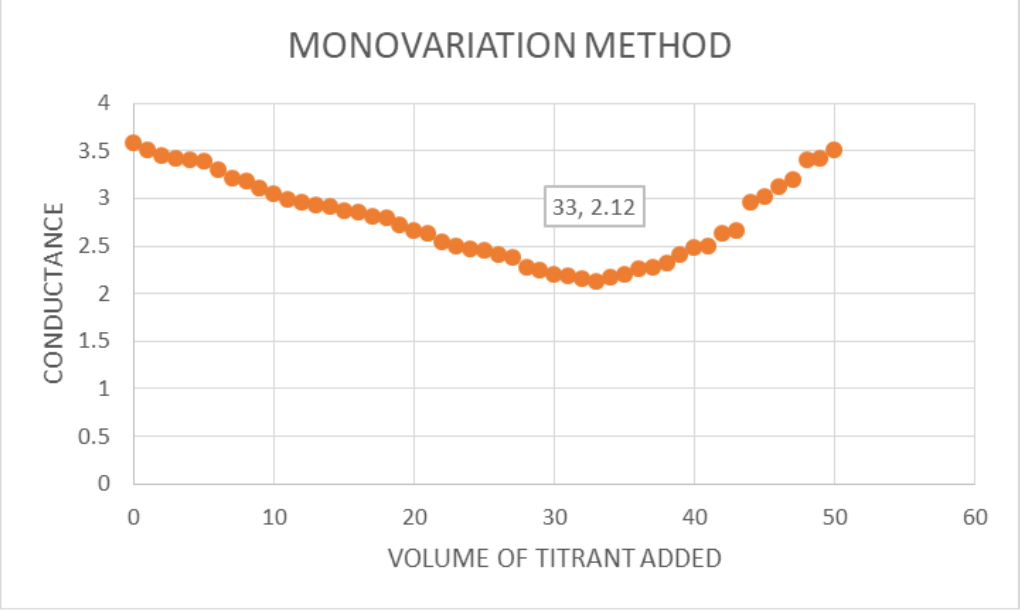
Ferrous –Oxalic Acid complex having the concentration of 0.682129 ug/ml where absorbance of that solution was 0.741.

Ferrous Sulphate-EDTA + 1, 10 - Phenanthroline Monovariation Method

Table 33: Monovariation Method

Volume of titrant (ml)	Conductance
0	3.58
1	3.51
2	3.45
3	3.41
4	3.4
5	3.38
6	3.29
7	3.21
8	3.18
9	3.11
10	3.05
11	2.99
12	2.95
13	2.93
14	2.91
15	2.87
16	2.85
17	2.8
18	2.79
19	2.72
20	2.65
21	2.62
22	2.54
23	2.5
24	2.47
25	2.45

Volume of titrant (ml)	Conductance
26	2.41
27	2.38
28	2.27
29	2.24
30	2.2
31	2.18
32	2.15
33	2.12
34	2.17
35	2.19
36	2.25
37	2.27
38	2.31
39	2.4
40	2.48
41	2.5
42	2.62
43	2.65
44	2.96
45	3.02
46	3.12
47	3.19
48	3.4
49	3.42
50	3.5



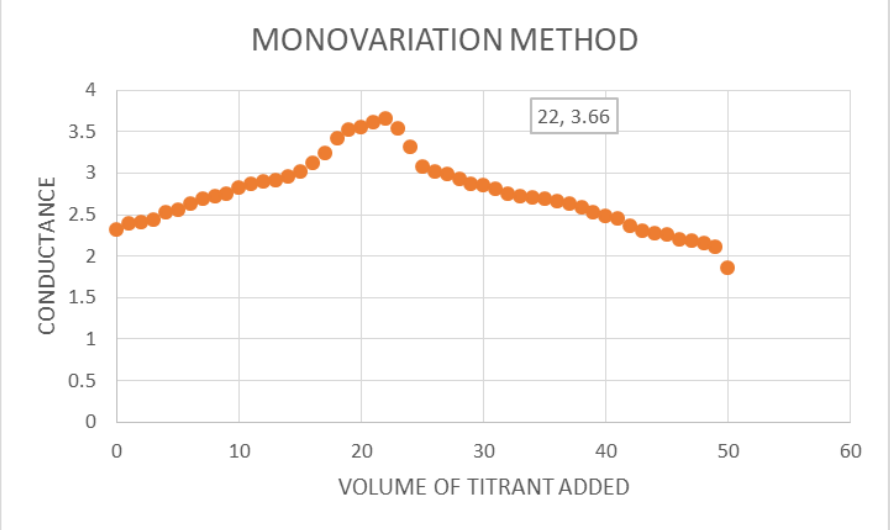
Graph 23: Monovariation Method

After addition of 33 ml of ligand solution to the metal solution where endpoint was seen 1 ml of 1, 10- phenanthroline was added and was estimated with spectroscopic technique.

Ferrous Sulphate-PABA +1, 10 –phenanthroline Monovariation Method

Table 34: Monovariation Method

Volume of titrant (ml)	Conductance
0	2.32
1	2.39
2	2.41
3	2.43
4	2.52
5	2.55
6	2.62
7	2.68
8	2.71
9	2.74
10	2.82
11	2.87
12	2.9
13	2.91
14	2.96
15	3.02
16	3.12
17	3.23
18	3.41
19	3.52
20	3.55
21	3.61
22	3.66
23	3.54
24	3.31
25	3.08
26	3.01
27	2.98
28	2.92
29	2.87
30	2.85
31	2.81
32	2.75
33	2.72
34	2.7
35	2.68
36	2.65
37	2.62
38	2.58
39	2.52
40	2.48
41	2.45
42	2.36
43	2.3
44	2.27
45	2.25
46	2.2
47	2.18
48	2.15
49	2.1
50	1.85



Graph 24: Monovariation Method

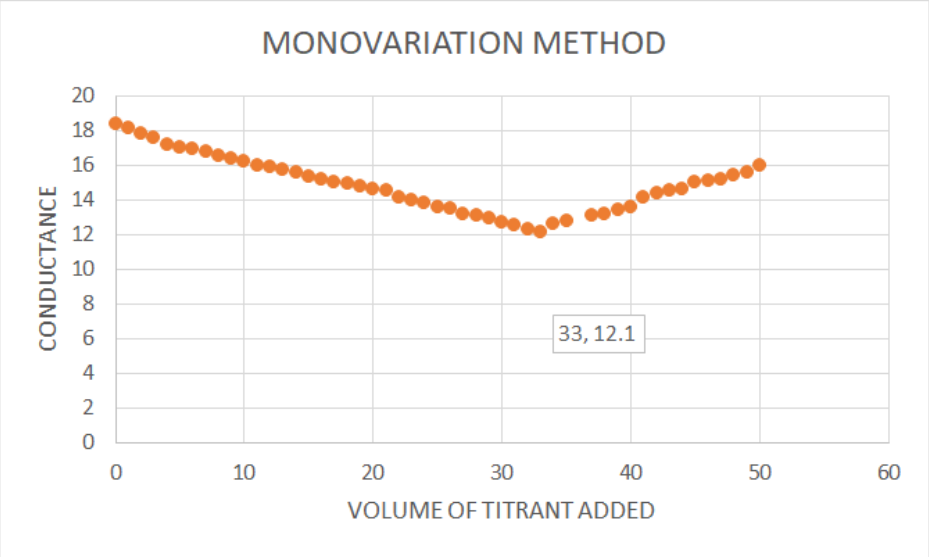
After addition of 22 ml of ligand solution to the metal solution where endpoint was seen 1 ml of 1, 10- phenanthroline was added and was estimated with spectroscopic technique.

Ferrous Sulphate- Oxalic Acid +1, 10- Phenanthroline Monovariation Method

Table 35: Monovariation Method

Volume of Titrant added	Conductance
0	18.4
1	18.1
2	17.8
3	17.6
4	17.2
5	17
6	16.9
7	16.8
8	16.5
9	16.4
10	16.2
11	16
12	15.9
13	15.7
14	15.6
15	15.3
16	15.2
17	15
18	14.9
19	14.8
20	14.6
21	14.5
22	14.1
23	14
24	13.8
25	13.6
26	13.5
27	13.2
28	13.1
29	12.9
30	12.7
31	12.5

Volume of Titrant added	Conductance
32	12.3
33	12.1
34	12.6
35	12.8
37	13.1
38	13.2
39	13.4
40	13.6
41	14.1
42	14.4
43	14.5
44	14.6
45	15
46	15.1
47	15.2
48	15.4
49	15.6
50	16



Graph 25: Monovariation Method

After addition of 33 ml of ligand solution to the metal solution where endpoint was seen 1 ml of 1, 10- phenanthroline was added and was estimated with spectroscopic technique.

Precision
System Precision
Ferric Chloride-EDTA

Table 36: System Precision

Volume of titrant (ml)	r1	r2	r3	r4	r5	r6	Mean	SD	%RSD
1	24.4	24.5	24.5	24.6	24.6	24.6	24.53333333	0.08165	0.332811
2	24.1	24.2	24.2	24.3	24.3	24.3	24.23333333	0.08165	0.336931
3	24.2	24.5	24.6	24.6	24.7	24.7	24.55	0.187083	0.762048
4	24.7	24.8	24.7	24.7	24.7	24.7	24.71666667	0.040825	0.165171
5	25	25	25	25.1	25	25	25.01666667	0.040825	0.163191
6	25.4	25.4	25.4	25.3	25.4	25.4	25.38333333	0.040825	0.160833
7	25.7	25.7	25.6	25.7	25.7	25.7	25.68333333	0.040825	0.158955
8	25.5	25.9	26.3	26.3	26.3	26.3	26.1	0.334664	1.282238
9	26.7	26.7	26.6	26.5	26.5	26.5	26.58333333	0.098319	0.369853
10	27.1	27.1	27.1	27	27.1	27.1	27.08333333	0.040825	0.150738
11	27.2	27.3	27.3	27.3	27.3	27.3	27.28333333	0.040825	0.149633

Volume of titrant (ml)	r1	r2	r3	r4	r5	r6	Mean	SD	%RSD
12	27.9	28	28.1	28.1	28.2	28.2	28.08333333	0.116905	0.416277
13	28.6	28.5	28.5	28.5	28.5	28.5	28.51666667	0.040825	0.143161
14	28.9	29	29	29	29	29	28.98333333	0.040825	0.140856
15	29.6	29.6	29.6	29.5	29.6	29.6	29.58333333	0.040825	0.137999
16	30	30.1	30.1	30.1	30.1	30.1	30.08333333	0.040825	0.135706
17	30.5	30.5	30.5	30.3	30.5	30.5	30.46666667	0.08165	0.167997
18	30.9	31	31	31.1	31.1	31.1	31.03333333	0.08165	0.163103
19	31.3	31.6	31.7	31.7	31.7	31.7	31.61666667	0.160208	0.506721
20	31.9	31.9	31.9	31.8	31.9	31.9	31.88333333	0.040825	0.128044
21	32.2	32.3	32.3	32.3	32.4	32.4	32.31666667	0.075277	0.132936
22	32.8	32.8	32.9	32.9	32.9	32.9	32.86666667	0.05164	0.157119
23	33.3	33.4	33.4	33.4	33.4	33.4	33.38333333	0.040825	0.122291
24	33.4	33.5	33.6	33.6	33.6	33.6	33.55	0.083666	0.149377
25	33.8	33.9	33.9	33.9	33.9	34	33.9	0.063246	0.186565
26	35.1	35.1	35.1	35.2	35.2	35.2	35.15	0.054772	0.155824
27	35.2	35.3	35.3	35.3	35.3	35.3	35.28333333	0.040825	0.115706
28	35.5	35.5	35.5	35.6	35.5	35.5	35.51666667	0.040825	0.114946
29	36.1	36.1	36.1	36.3	36.1	36.1	36.13333333	0.08165	0.225968
30	36.5	36.5	36.6	36.6	36.6	36.6	36.56666667	0.05164	0.141221
31	36.7	36.7	36.8	36.8	36.7	36.8	36.75	0.054772	0.14904
32	37	37.2	37.2	37.2	37.2	37.2	37.16666667	0.08165	0.119685
33	37.4	37.6	37.6	37.6	37.6	37.6	37.56666667	0.08165	0.117346
34	37.7	37.7	37.7	37.7	37.7	37.8	37.71666667	0.040825	0.108241
35	37.1	37.1	37.2	37	36.8	36.6	36.96666667	0.225093	0.608907
36	35.7	35.6	35.6	35.6	35.7	35.7	35.65	0.054772	0.153639
37	34.7	34.8	35	35	34.9	34.6	34.83333333	0.163299	0.468802
38	32.6	32.7	32.9	33.1	32.9	32.9	32.85	0.176068	0.535976
39	37.6	37.6	37.6	37.2	37.6	37.6	37.53333333	0.163299	0.435078
40	37.7	37.7	37.6	37.6	37.6	37.7	37.65	0.054772	0.145477
41	37.7	37.6	37.6	37.6	37.6	37.6	37.61666667	0.040825	0.108529
42	37.6	37.7	37.7	37.7	37.7	37.7	37.68333333	0.040825	0.108337
43	37.6	37.6	37.6	37.7	37.6	37.6	37.61666667	0.040825	0.108529
44	37.7	37.6	37.6	37.6	37.6	37.6	37.61666667	0.040825	0.108529
45	37.7	37.6	37.7	37.7	37.7	37.6	37.66666667	0.05164	0.137097
46	37.7	37.7	37.7	37.6	37.7	37.2	37.6	0.2	0.531915
47	37.7	37.6	37.6	37.7	37.7	37.7	37.66666667	0.05164	0.137097
48	37.6	37.7	37.7	37.6	37.6	37.6	37.63333333	0.05164	0.137218
49	37.7	37.6	37.6	37.7	37.7	37.6	37.65	0.054772	0.145477
50	37.7	37.6	37.7	37.6	37.6	37.6	37.63333333	0.05164	0.137218

r= result of conductance.

All the %RSD value came within 2% so the above process is Precised Systematically.

Method Precision

Ferric Ammonium Sulphate-Oxalic Acid

Table 37: Method Precision

Volume of titrant (ml)	t1	t2	t3	t4	t5	t6	Mean	SD	%RSD
1	15.6	15.5	15.3	15.6	15.6	15.7	15.55	0.13784	0.886434
2	15.4	15.7	15.7	15.8	15.7	15.9	15.7	0.167332	1.065809
3	15.5	15.8	16.1	15.9	15.8	16	15.85	0.207364	1.308293
4	15.9	16	16.4	16	15.9	16.2	16.06666667	0.196638	1.223891
5	16.3	16	16.4	16.4	16.2	16.4	16.28333333	0.160208	0.983878
6	16.4	16.5	16.7	16.4	16.3	16.6	16.48333333	0.147196	0.892999
7	16.5	16.5	16.7	16.6	16.5	16.6	16.56666667	0.08165	0.492855
8	16.8	16.5	17	16.9	16.7	16.8	16.78333333	0.17224	1.026257
9	16.9	16.9	17.2	17.1	16.9	16.9	16.98333333	0.132916	0.782626
10	17	17.2	17.4	17.2	17	17.2	17.16666667	0.150555	0.877017

Volume of titrant (ml)	t1	t2	t3	t4	t5	t6	Mean	SD	%RSD
11	17.2	17.3	17.8	17.7	17.1	17.4	17.41666667	0.278687	1.600119
12	17.6	17.6	18.1	17.7	17.2	17.6	17.63333333	0.287518	1.630538
13	17.9	17.8	18.4	17.9	17.5	17.8	17.88333333	0.292689	1.636656
14	18.2	18.2	18.7	18.4	18.2	18.1	18.3	0.219089	1.197208
15	18.3	18.3	18.9	18.7	18.5	18.4	18.51666667	0.240139	1.296879
16	18.6	18.7	19	18.9	18.6	18.9	18.78333333	0.17224	0.916984
17	18.9	18.8	19.1	19.2	19	19.1	19.01666667	0.147196	0.774037
18	19.2	19.2	19.2	19.4	19.1	19.3	19.23333333	0.10328	0.536982
19	19.6	19.4	19.6	19.4	19.3	19.5	19.46666667	0.121106	0.62212
20	19.5	19.8	19.8	20.1	19.5	19.8	19.75	0.225832	1.143452
21	19.9	20	20.1	20.2	20.1	20	20.05	0.104881	0.523097
22	20	19.6	20.2	20	20.2	20.4	20.06666667	0.273252	1.361721
23	20.5	20.7	20.4	20.8	20.4	20.7	20.58333333	0.17224	0.836794
24	20.6	20.9	20.8	21	20.7	20.9	20.81666667	0.147196	0.707107
25	20.7	20.9	21.1	21.3	20.7	21	20.95	0.234521	1.119431
26	21	21	21.4	21.6	21	21.4	21.23333333	0.265832	1.251956
27	21.2	22	21.6	21.7	21.1	21.6	21.53333333	0.332666	1.544889
28	21.7	22.1	21.8	21.7	21.3	21.8	21.73333333	0.258199	1.188032
29	21.7	22.4	22.4	22.2	21.8	22.2	22.11666667	0.299444	1.353929
30	22	22.7	22.5	22.5	22.1	22.3	22.35	0.266458	1.192207
31	22	22.9	22.8	22.5	22.3	22.4	22.48333333	0.33116	1.472911
32	22.4	23.6	23.2	23.2	22.9	22.6	22.98333333	0.440076	1.91476
33	22.6	23.2	23.2	23.2	22.6	23.2	23	0.309839	1.347125
34	22.8	23.9	23.7	23.6	23.1	23.6	23.45	0.413521	1.763418
35	23.2	24.3	24.2	24.3	24.4	24.3	24.11666667	0.453505	1.880462
36	24	24.5	24.4	24	23.6	24.3	24.13333333	0.332666	1.37845
37	23.5	24.7	24.7	24.2	24	24.8	24.31666667	0.511534	2.103634
38	23.8	24.9	25	24.6	24.2	24.9	24.56666667	0.476095	1.937972
39	24	25.2	25.1	24.7	24.4	25.2	24.76666667	0.492612	1.989012
40	25.1	25.5	25.4	24.7	24.7	25.4	25.13333333	0.361478	1.438243
41	25	25.8	25.6	25.3	24.9	25.7	25.38333333	0.376386	1.482809
42	25.2	26.1	25.2	25.3	25	25.9	25.45	0.441588	1.73512
43	26	26.4	26.1	25.8	25.1	26.1	25.91666667	0.444597	1.715488
44	26	26.9	26.5	25.9	26	26.3	26.26666667	0.382971	1.458011
45	25.2	26.3	26.6	26.1	26	26.6	26.13333333	0.520256	1.990777
46	27	27.5	26.9	26.5	27	26.6	26.91666667	0.354495	1.317009
47	27	27.7	27.3	26.8	26.2	26.8	26.96666667	0.508593	1.886006
48	28	28	27.4	27	27	26.9	27.38333333	0.507609	1.853714
49	28	28.2	27.5	27.3	27.1	27.1	27.53333333	0.467618	1.698371
50	28.4	28.4	27.9	27.4	27.3	27.3	27.78333333	0.526941	1.896608

t= number of titration with its conductance value.

This process is precised methodically as %RSD value comes within 2%.

Difference between the observed value and expected value

Table 38: Difference between the observed value and expected value

Method	Complex	Expected value	Observed Value (Approx.)	Reason for deviation
Monovariation method Modified Job's method Turner Anderson method	Ferric-EDTA	1:1 6:6 For 0.05M- 6:6 For 0.1M- : 6:6	.1:.151 7:5 For 0.05M- 7:5 For 0.1M- : 7:5.	Temperature/Ionic concentration of metal or ligand.
Monovariation method Modified Job's method Turner Anderson method	Ferric-Oxalic acid	1:1 6:6	.1:.142 6:6	Temperature/Ionic concentration of metal or ligand.

Method	Complex	Expected value	Observed Value (Approx.)	Reason for deviation
		For 0.05M- 6:6 For 0.1M- : 6:6	For 0.05M- 4:8 For 0.1M- : 5:7	
Monovariation method Modified Job's method Turner Anderson method	Ferric- Curcumin	1:2 4:8 For 0.05M- 4:8 For 0.1M- : 4:8	.1:.208 4:8 For 0.05M- 4:8 For 0.1M- : 4:8	Temperature/Ionic concentration of metal or ligand.
Monovariation method Modified Job's method Turner Anderson method	Ferric-PABA	1:2 4:8 For 0.05M- 4:8 For 0.1M- : 4:8	.1:.227 4:8 For 0.05M- 4:8 For 0.1M- : 4:8	Temperature/Ionic concentration of metal or ligand.
Monovariation method Modified Job's method Turner Anderson method	Ferrous-Oxalic acid	1:2 4:8 For 0.05M- 4:8 For 0.1M- : 4:8	.1:.166 6:6 For 0.05M- 6:6 For 0.1M- : 6:6	Temperature/Ionic concentration of metal or ligand.
Monovariation method Modified Job's method Turner Anderson method	Ferrous-EDTA	1:1 6:6 For 0.05M- 6:6 For 0.1M- : 6:6	.1:.156 6:6 For 0.05M- 6:6 For 0.1M- : 4:8	Temperature/Ionic concentration of metal or ligand.
Monovariation method Modified Job's method Turner Anderson method	Ferrous-1,10-Phenanthroline	1:1 6:6 For 0.05M- 6:6 For 0.1M- : 6:6	.1:.0.07 4:8 For 0.05M- 4:8 For 0.1M- : 4:8	Temperature/Ionic concentration of metal or ligand

CONCLUSION

Through this analysis, it has been observed that the formation of complex of Ferric and Ferrous salt with EDTA was taken place approximately at 1:1 ratio while using 0.1M metal and ligand solution for experiment (Monovariant method). Similarly in the case of PABA and Curcumin it was seen that the complexation takes place approximately at 1:2 ratio as metal and ligand ratio. The modified Job's method of continuous variation (Turner Anderson Method) was used to calculate the stability constant of the complex and the free energy change. The value of free energy change is negative showing the feasibility of complex formation.

1, 10- Phenanthroline was used as the chromogenic agent which essentially confirmed the presence of free metal content in the solution as EDTA, PABA and Oxalic Acid were used as ligand in the assay process of metal- chromogenic agent complex. The spectroscopic data shows the concentration from the standard curve that where free metal was exist in the titration solution.

In future this method can also be used to find out the stoichiometric ratio of other metal and ligands along with the evaluation of different biological activities like in the treatment of Thalassemia when conjugation with ferrous ion takes place.

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