A simple continuous flow system for mole ratio determination and photometric titration

Yuthapong Udnan[1*], Jaroon Jakmunee[2,3] and Kate Grudpan[2,3]

[1]Department of Chemistry, Faculty of Science, Naresuan University, Phitsanulok, 65000, Thailand
[2]Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand
[3]Institute of Science and Technology Research and Development Chiang Mai University, Chiang Mai, 50200, Thailand
*Corresponding author. E-mail: yuthapongu@nu.ac.th

ABSTRACT

A simple continuous flow system for mole ratio determination and photometric titration was developed and applied for the study of some iron complexes behaviors in various pH solutions. By using photometric measurement it was found that pH of the solutions affect the wavelength of maximum absorption (λ_{max}), molar absorptivity and the mole ratio of iron complexes, especially the Fe(III)-salicylate complex. As the pH of the solution increases it was found that the maximum absorption of the Fe(III)-salicylate complex was shifted to a shorter wavelength and the mole ratio of the complex changed from 1:1 in highly acidic condition to 1:2 and 1:3 in the higher pH solutions. Whereas pH had less effect on these parameters of Fe(II)-1,10-phenanthroline, Fe(III)-EDTA and Fe(III)-thiocyanate complexes. The system was also applied for direct and indirect photometric titration between Fe(III) and EDTA. It was found that high accuracy and precision were obtained.

Keywords: Fe(III); Salicylate; Small scale; mole ratio, photometric titration, Job's method

INTRODUCTION

Complex formation reactions are important reactions used in spectrophotometric flow injection analysis. To study their behaviors, mole ratio and maximum absorption measurements at various pH values of the complexes are necessary. Many colorimetric analyses, particularly for metals, depend upon the formation of colored complex ions or molecules. It is frequently important to know the mole ratio of metal to the reagent in the complex. This can be ascertained from photometric data by three different procedures (Ewing, 1985; Hill and MacCarthy, 1986; Christian and O'Reilly; 1986) (1) the mole-ratio method introduced by Yoe and Jones, (2) the method of continuous variations (Hill and MacCarthy, 1986) attributable to Job and modified by Vosburgh and Cooper and (3) the slope-ratio method of Harvey and Manning. In the mole-ratio method, the absorbances are measured for a series of solutions which contain varying amounts of one constituent with a constant amount of the other. A plot is prepared of absorbance as a function of the reagent to metal mole ratio. This is expected to give a straight line from the origin to the point where equivalent amounts of the constituents are present. The curve will become horizontal,

because all of one constituent is used up, and the addition of more of the other constituent can produce no more of the absorbing complex.

The method of continuous variations requires a series of solutions of varying mole fractions of the two constituents, wherein their sum is kept constant. The difference between the measured absorbance and the absorbance calculated for the mixed constituents on the assumption of no reaction between them is plotted against the mole fraction of one of the constituents. The resulting curve will show a maximum (or minimum) at the mole fraction corresponding to that in the complex.

The sharpness of the breaks in the curves of both of these methods of identification of a complex depends on the magnitude of the stability constant. The Job plot, even when it shows a large degree of curvature in the region of its maximum, nevertheless usually approaches both the zero and unity mole-fraction points in a linear manner. The ratio of the slopes of these two linear portions will equal the ratio of the constituents in the complex. This is known as the slope-ratio method.

Among three of these methods, Job's was more frequently used and modified for the determination of mole ratio, stoichiometry or formula of a complex [Collum, *et al*., 2013]. Beside, this method was also applied for the determination of mole ratio in cobalt thio-violuric acid (Chawla and Singh, 1970) and lead-Rutin complex (Radovic and Malesev, 1985).

It has been reported that there is a typical semi continuous flow technique used for mole ratio determination (Hill and MacCarthy, 1986). In this work a small scale continuous flow system was developed for mole ratio and photometric titration. This technique differs from the classical method used by using flow system to circulate the solution between a mini reaction cell and detection system. A small amount of ligand or titrant was continuously added into the reaction cell using a micropipette. This helps shorten the analysis time and reduce chemical consumption.

METHODOLOGY
Instrumentation

The instruments used in this study are illustrated in Figure1.
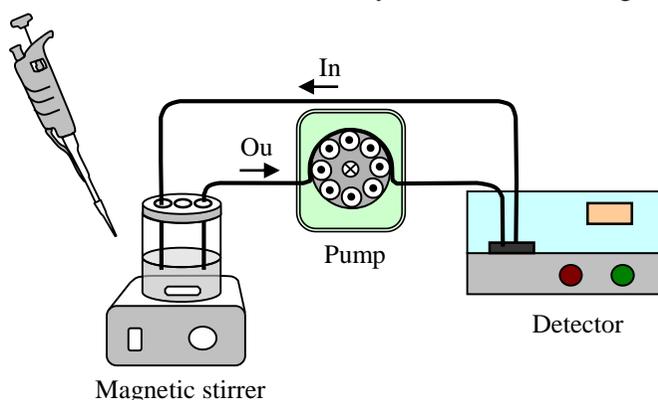
Figure 1. The apparatus for small scale mole ratio determination and photometric titration

A laboratory-made flow system, consisting of peristaltic pumps (Masterflex, USA.), a magnetic stirrer and a spectrophometric detector (Unicam model 400, USA). A titration glass vessel was made of a 15 mL vial. A mini magnetic bar was used to stir the mixed solution. A 100 µL micro pipette (Nicrio, Japan) was used to add the solution of ligands or titrant to the reaction vessel. Tubing of 1.5 mm i.d. Tygon and 0.5 mm i.d. PTFE were used for pump tubing and the flow system, respectively.

Reagents

All solutions were prepared from analytical grade reagents and distilled water. The 100 mL of 0.0100 M Fe(III) solution was prepared from 0.4822 g of $FeNH_4(SO_4)_2 \cdot 12H_2O$ (BDH) and 0.5 mL of concentrated H_2SO_4. The 100 mL of 0.0100 M of each ligand solution (sodium salicylate, potassium thiocyanate, ethylene diamine tetraacetic acid disodium salt (EDTA), 2,4- pyridylazoresorcinol and 1,10-phenanthroline) was prepared. A 0.10 M acetate buffer was used as an intermediate solution and control the pH of solutions.

Procedure
Effect of pH on Fe(III)-salicylate spectrum

The pH of the Fe(III)-salicylate complex in acetate buffer media was varied from 1.5-6.0. A series of solutions was prepared in 10 mL volumetric flasks. A 0.10 mL portion of 0.0100 M Fe(III) was pipetted into a 10 mL volumetric flask followed with 1.00 mL of 0.0100 M sodium salicylate. Acetate buffer of required pH was used to adjust the volume of the complex solutions. The absorption spectra of the complex solutions were measured from 400-700 nm using acetate buffer as a reference solution.

Effect of pH on Fe(III)-salicylate mole ratio

A 0.50 mL portion of 0.0100 M Fe(III) was pipetted into the reaction vessel of Fig. 1 followed with 9.50 mL of 0.100 M acetate buffer of required pH. A small magnetic bar was put into the reaction vessel on a magnetic stirrer of suitable speed. The solution was continuously passed through the detector by the peristaltic pump and returned back to the reaction vessel. The solution of non-ligand added was used as blank solution. The absorbance of the flowed solution was measured at 520 and λ_{max} for each pH. The flow rate was 5 mL/min. As portions of 0.1 mL of 0.0100 M sodium salicylate were pipetted into the reaction vessel, the steady state absorbance was recorded in 30 second. The corrected absorbances (calculated by equation 1) were plotted against the mole ratio between Fe(III) and salicylate.

$$A_{corr} = A_{obs} (V+v)/V \ldots\ldots\ldots\ldots(1)$$
A_{corr} = Corrected absorbance
A_{obs} = Observed absorbance
V = Initial volume
v = Collected volume of ligand added

Mole ratio of Fe(II) and Fe(III) with other ligands

Mole ratio determinations of Fe(II) and Fe(III) with other ligands were performed in the same way as for the Fe(III)-salicylate complex, except for the concentrations of the reagents used as showed in table 1.

Table 1 The concentration of Fe ions and each ligand used for the mole ratio determination.

Concentration of Fe ion and each ligand for mole ratio determination	
0.001 M Fe(II)	0.003 M 1,10-phenanthroline
0.001 M Fe(III)	0.001 M EDTA
0.001 M Fe(III)	0.001 M PAR
0.010 M Fe(III)	0.010 M Salicylate
0.010 M Fe(III)	0.010 M thiocyanate

Photometric titration of Fe(III) with EDTA

The other application of the instrument setup is photometric titration. The experiment was performed in the same way as the mole ratio determination. Photometric titration of Fe(III) with EDTA can be performed by direct and indirect methods and is discussed below.

RESULTS AND DISCUSSION

Mole ratio determination of salicylate complexes

From the study it was found that the wavelength of maximum absorption (λ_{max}) of Fe(III)-salicylate complex was dependent on the pH of the media solution. The wavelength of maximum absorption will shift to shorter wavelength as the pH increases (Figure 2 and Table 2).
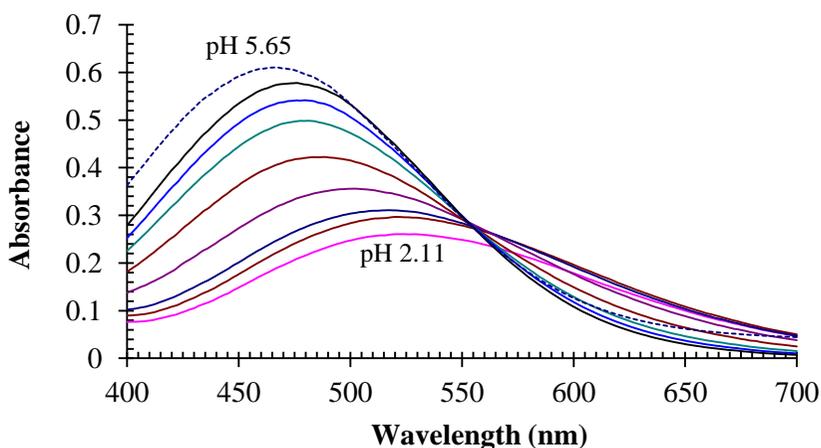
Figure 2 The absorption spectrum of Fe(III) salicylate complex at various pH values.

Table 2 Effect of pH on λ_{max} of Fe(III)-salicylate complex

pH	λ_{max} (nm)	pH	λ_{max} (nm)
2.11	524	4.05	488
2.65	521	4.61	481
2.99	517	5.04	480
3.48	502	5.65	476

It can be explained that salicylic acid, the structure shown in the equation (2), is a weak acid for which the dissociation constants, K_{a1} and K_{a2}, are 1.05×10^{-3} and 4.0×10^{-14} (Fritz, and Schenk, 1987).

$$\text{H}_2\text{SAL} \quad \xrightleftharpoons{K_{a1}} \quad \text{HSAL}^- \quad + \text{H}^+ \quad \ldots\ldots\ldots(2)$$

$$\text{HSAL}^- \quad \xrightleftharpoons{K_{a2}} \quad \text{SAL}^{2-} \quad + \text{H}^+ \ldots\ldots\ldots(3)$$
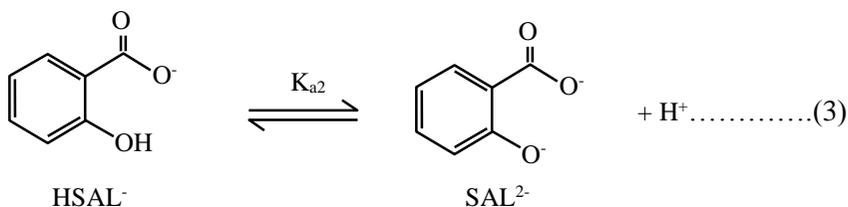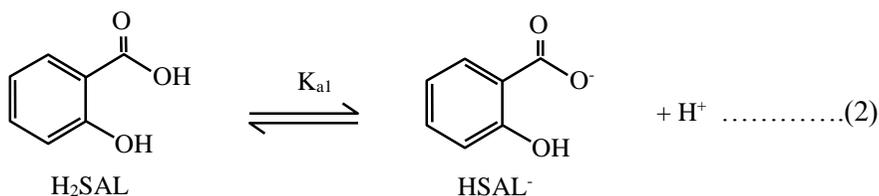
The reactive forms of salicylic acid which are able to react with Fe(III) is HSAL$^-$ and SAL$^-$. As the pH of media solution is less than 2.0 the dominant form of salicylic acid is H$_2$SAL, whereas there is a small amount of the reactive species of the salt forms (HSAL$^-$ and SAL^{2-}). Consequently, the possible mole ratio of the complex should be 1:1 rather than 1:2 or 1:3. As the pH of the media solution is increased, the reactive salt forms are increased so the tendency of 1:2 and 1:3 complex formation is higher as illustrated in equations 5 and 6.

$$\text{HSAL}^- + [\text{Fe}(\text{H}_2\text{O})_6]^{3+} \rightleftharpoons [\text{FeSAL}(\text{H}_2\text{O})_4]^{2+} + 2\text{H}_2\text{O} + \text{H}^+ \ldots\ldots\ldots (4)$$

$$[\text{FeSAL}(\text{H}_2\text{O})_4]^{2+} + \text{HSAL}^- \rightleftharpoons [\text{Fe}(\text{SAL})_2(\text{H}_2\text{O})_2]^{2+} + 2\text{H}_2\text{O} + \text{H}^+ \ldots\ldots (5)$$

$$[\text{Fe}(\text{SAL})_2(\text{H}_2\text{O})_2]^{2+} + \text{HSAL}^- \rightleftharpoons [\text{Fe}(\text{SAL})_3]^{2+} + 2\text{H}_2\text{O} + \text{H}^+ \ldots\ldots\ldots (6)$$
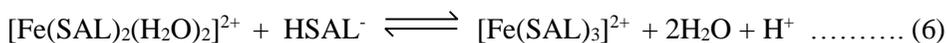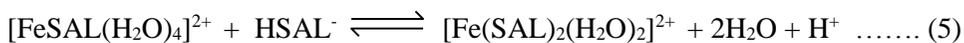
The interaction of radiation of each form of the complexes and the shift in absorbance maxima (Table 2) can be explained by ligand field theory. Salicylate is a strong field ligand compared with water. As salicylate was bonded with Fe(III), its d-orbital was separated as shown in the energy diagram.
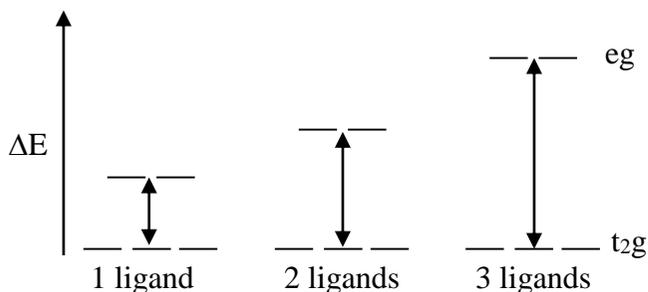
As the amount of bonding ligands increase from 1 to 3 the strength of the ligand field was increased. This results in a wider gap between t_2g and eg (ΔE), so the maximum radiation absorption (λ_{max}) of the Fe(III)-salicylate complex was shifted to lower wavelengths as the pH increased.

From the study of the effect of pH on the mole ratio of the Fe(III)-salicylate complex, it was found that at pH less than 2 (1.5) the graph plotted between mole ratio and corrected absorbance was a straight line without a bending point (Figure 3), the point used for mole ratio determination.
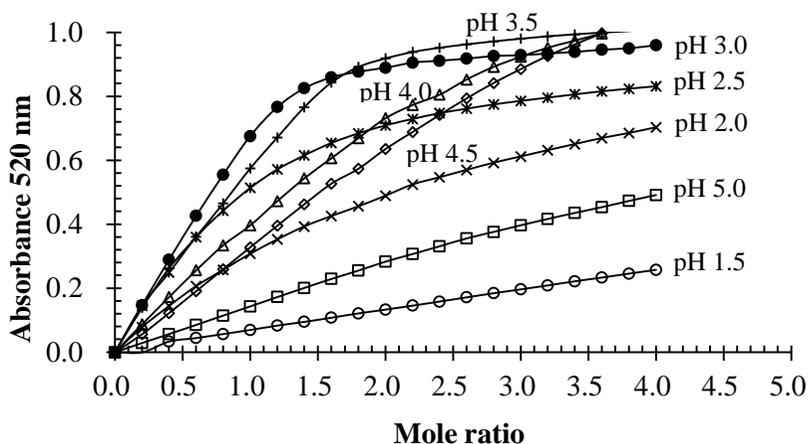
Fig. 3 The effect of pH on mole ratio of Fe(III)-salicylate complex.

Table 3 log K_f of each ligand with Fe(II) and Fe(III) (Martell, 1971)

Ligands	log K_f	
	Fe^{2+}	Fe^{3+}
thiocyanate	-	2.95
		3.36
EDTA	14.33	24.23
1,10-phenanthroline	9.08	5.85
	11.45	11.4
	21.30	23.5
Salicylic acid	6.55	16.48
	11.25	28.12
	-	36.80
2,4-pyridylazoresorcinol	-	-

However, it could be that the mole ratio of the Fe(III)-salicylate complex at this pH is 1:1. The K_f of the 1:1 complex (Table 2) and K_a of salicylic acid are very different (3.02×10^{16} and 1.05×10^{-3}) so the concentration of Fe(III) in solution is much more than the concentration of reactive $HSAL^-$. So 1:1 Fe(III)-salicylate complex can be formed continuously as salicylate ligand is added. That results in a straight line graph. When the pH of the solution increased from 2.0 to 3.5, the plotted graphs were bending lines. At pH 2.0, the line did not show the bending point clearly. But at pH 2.5, 3.0 and 3.5, the two linear equations can be used to calculate the mole ratio of the Fe(III)-salicylate complex at each pH, which was observed to be 1:1.24, 1:1.39 and 1:1.71, respectively. From the results it can be explained that when the pH of the solution was increased, the concentration of $HSAL^-$ was increased too, and then the 1:2 complex could be formed. The solution now should consist of 1:1 and 1:2 complexes mixtures, which resulted in the calculated mole ratios of 1:1.24 (pH 2.5), 1:1.39 (pH 3.0) and 1:1.71 (pH 3.5), respectively. At pH 4.0, the calculated mole ratio was 1:2.02 which indicated that most of the entire complex was in the 1:2 form. At pH 4.5, there was no 1:1 form left and some of 1:3 complex can be formed. The mole ratio of Fe(III)-salicylate complex at this pH was 2-3 (from calculation = 2.7). At pH 5.0, the mole ratio curve plotted was nearly a straight line and could not be used to investigate the mole ratio of the complex. However, it can be predicted that the ratio of Fe(III)-salicylate complex at this pH was 1:3. The reason for the explanation was similar to that for pH 1.5.

Mole ratio determination of other complexes

The set up was also applied for determination the mole ratio of other complexes of Fe(II) and Fe(III) in the media without pH control. As it was a flow system so the pH of solution cannot be stated. The results were depicted in Figure 4 and Table 4.
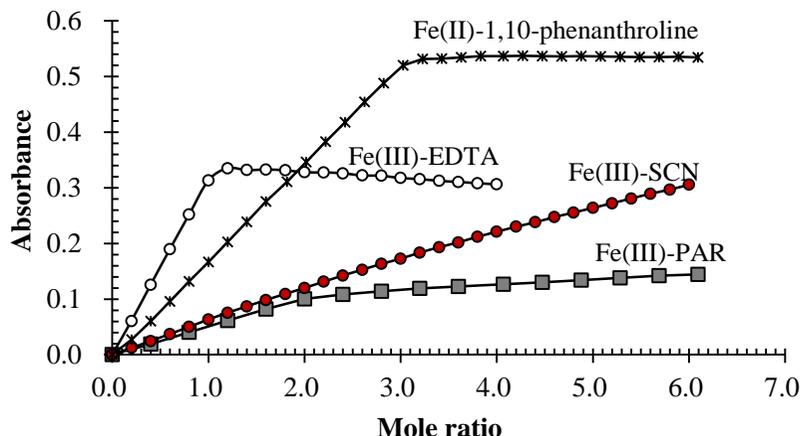
Figure 4 Mole ratio of Fe(II) and Fe(III) complexes with various ligands.

Table 4 The mole ratio of each ligand with Fe(II) and Fe(III)

Ligands	Mole ratio	
	Fe(II)	Fe(III)
thiocyanate	-	-
salicylate	-	1,2,3
EDTA	-	1
PAR	-	2
1,10-phenanthroline	3	-

Photometric titration

The other advantage of the described system for mole ratio determination is for efficient photometric titration. The system was applied for the titration of Fe(III) with EDTA. The experiment can be carried out in two ways namely, direct and indirect measurements. For the indirect measurement, Fe(III) was first reacted with sodium salicylate as a reagent to form a violet complex of Fe(III)-SAL. As the complex formation constant of this complex is less than that of Fe(III)-EDTA (see table 3) so the color disappear when EDTA was added to the end point. The conditions used for both direct and indirect methods are summarized in Table 5.

Table 5 The condition for direct and indirect method for photometric titration of Fe(III) with EDTA

Method	Measured wavelength (nm)	Color developing agent used
Direct	260 (UV)	none
Indirect	450 (Visible)	Sodium salicylate

For the direct method, 0.50 mL of 0.0100 M Fe(III) was added to the vessel followed by 9.00 mL of deionised water and the solution was subsequently titrated

with 0.0100 M EDTA. The absorbance of the solution was measured at 260 nm, wavelength of maximum absorption of Fe(III)-EDTA complex, for each addition of EDTA. For the indirect method, the solution was prepared similarly for the direct titration, except for using 9.00 mL of 0.01 M sodium salicylate instead of deionised water and the absorbance of the solution was measured at 450 nm. The reactions involve in the indirect titration may be illustrated as:

Fe(III) + Salicylate $\longrightarrow$ Fe(III)-Salicylate (λ_{max} = 450 nm)
Fe(III)-Salicylate + EDTA $\longrightarrow$ Fe(III)-EDTA (colorless)
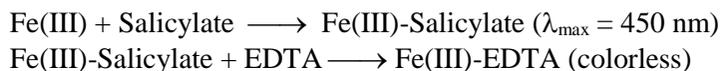
As the K_f of Fe(III)-Salicylate complex is less than that of the Fe(III)-EDTA complex (see table 2), so salicilate can be replaced by EDTA during the titration. At the end point of the titration the violet color of Fe(III)-Salicylate complex disappeared. The results of both titration methods are shown as titration graphs in Figure 5.
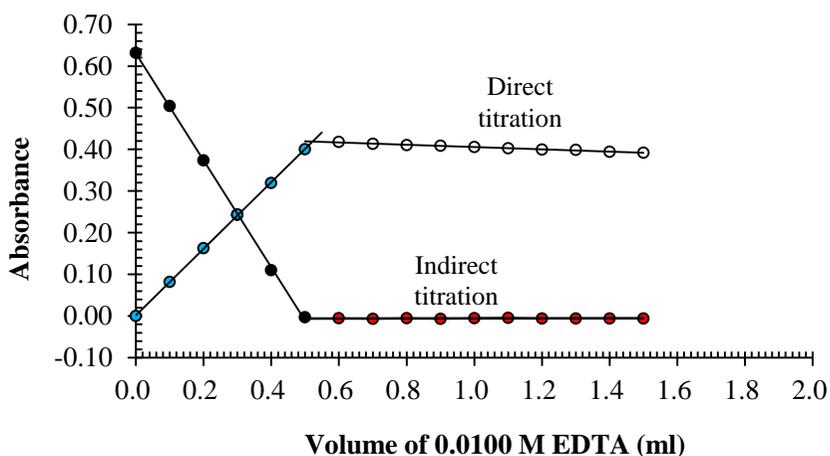
Figure 5 The titration curve between Fe(III) and 0.0100 M EDTA

From the results of triplicate titration, it was found that the calculated concentrations by direct and indirect methods were nearly equal to the real concentration. The relative accuracy of the direct methods was 110% and relative standard deviation was 4.7% whereas those of the indirect method were 110% and 1%, respectively. However, it was found that the indirect method was better than the direct method according to the stability of the signal and the measured wavelength used, which was available for a general laboratory spectrometer.

CONCLUSIONS

A simple continuous flow system for mole ratio determination and photometric titration was developed. In this system the circulation of the solution between a reaction flask and a detection system is performed by using a magnetic stirrer. The advantages of the developed method over the previous method and conventional method are more rapid, less time consumption and small volume of solution used. It was applied for the mole ratio determination of Fe ion with salicylate, EDTA, 1,10-phenanthroline, PAR and thiocyanate and photometric titration between Fe(III) and EDTA. It was found that high accuracy and precision were obtained. The system consumed less chemical and environmentally friendly.

REFERENCES

Ewing, G. W. (1985). *Instrumental Methods of Chemical Analysis 5th ed*. McGraw-Hill, New York, 1985, 67-68.

Hill, Z. D. and MacCarthy, P. (1986). Novel approach to Job's method: An undergraduate experiment. *J Chem Educ. 63*, 162.

Christian, G.D. and O'Reilly, J. E. (1986). *Instrumental Analysis 2nd ed*. Allyn and Bacon, Boston, 1986, 185-187.

Collum, D. B., Renny, J. S., Tomasevich, L. L. and Tallmadge, E. H. (2013). Method of Continuous Variations: Applications of Job Plots to the Study of Molecular Associations in Organometallic Chemistry. *Angew. Chem. Int. Ed*. 52, 2–18.

Chawla, R. S. and Singh, R. P. (1970). A Spectrophotometric Study of Cobalt Complex with Thio-violuric Acid. *Microchim. Acta*, *58*, 332-336.

Radovic, Z. and Malesev, D. (1985) Spectrophotometric Investigations of the Complex of Lead^{2+} and Rutin. *Microchim. Acta*, *86*, 247-252.

Fritz, J. S. and Schenk, G. H. (1987). *Quantitative Analytical Chemistry*. Allyn and bacon, Massachusetts.

Martell, E. (1971). *Stability Constants of Metal-ion Complexes*. Alden & Mowbray, Oxford.