CHAPTER 3

RESULTS AND DISCUSSION

Purities of Amaranth, Ponceau 4R and Erythrosine were examined by paper chromatographic technique, ultraviolet-visible, infrared, and atomic absorption spectrophotometric techniques before they were used for the study of compound and complex formations with metal ions.

3.1 Dyes

3.1.1 Purity and identification

3.1.1.1 Paper chromatography

The paper chromatogram of Amaranth, Ponceau 4R, or Erythrosine showed a well defined spot (see Figure 1) whose R_F value was found to be 0.20, 0.49, or 0.56, respectively. These R_F values are slightly different from the literature values (0.19 for Amaranth, 0.51 for Ponceau 4R, and 0.52 for Erythrosine(17)) owing to a slight difference in experimental conditions.

3.1.1.2 Absorption spectrophotometry

The ultraviolet-visible spectra of Amaranth, Ponceau 4R and Erythrosine in aqueous solution were compared to the ones obtained from the literature(18), as shown in Figures 2A-2C. The spectrum of each dye studied indicated an insignificant difference from the literature one. The maximum peaks of Amaranth in an acidic solution, of Ponceau 4R in an acidic solution, and of Erythrosine in an basic solution illustrated at 521, 505, and 525 nm, respectively (see Table 1). Their molar absorptivities were determined from the sloped of the curves of absorbances of dyes against their concentrations (see Figures 3 and 4(C)). The molar absorptivity of each dye was found in the order of 10^4 (see Table 1) which indicated the strong absorption of the dye in the visible range.

The IR spectra of Amaranth, Ponceau 4R and Erythrosine in solid KBr pellets were shown in Figures 5A-5C. The spectrum of Erythrosine indicated an insignificant difference from the literature one(19) as well as the spectra of Amaranth and Ponceau 4R showed the azo -N=N- (1630 cm^{-1}), phenolic OH (3458 cm^{-1}), aromatic (1450 cm^{-1} and 1500 cm^{-1} - skeleton carbon stretching, and 750 cm^{-1} - aromatic out of plane bending) and ionic sulfonate (1200 cm^{-1} and 1080 cm^{-1}) characters (see Figures 5A and 5B).

By atomic absorption spectrophotometric study, no absorption of Amaranth, Ponceau 4R, and Erythrosine solutions at the wavelengths where Hg(II), Cd(II), Fe(II), Fe(III) and Pb(II) ions absorbed were observed.

Thus, evidences from paper chromatographic and absorption spectrophotometric data of dyes investigated indicated that the purities of Amaranth, Ponceau 4R and Erythrosine are high enough for use in the study of compound and complex formations with metal ions.

3.1.2 Solubilities of dyes in the buffer systems studied

Amaranth and Ponceau 4R are very soluble in every buffer system studied. However, in the acidic solution at pH 1-2 the acidic form of Erythrosine precipitates completely, at the pH 3.0-4.4 it is very soluble in every buffer system studied except in the acetate buffers pH 4.4 and 6.0 Erythrosine is soluble to some extent.

3.1.3 Dependence of absorbances on concentrations of the dye solutions

A linear relationship between absorbances and concentrations of each dye in the aqueous solution without any buffer was obtained in the range of concentrations of 4.00x10^{-6} - 6.00x10^{-5}M Amaranth, 1.00x10^{-5}M - 1.00x10-^{4}M Ponceau 4R, or 1.00x 10^{-6}M - 1.40x10^{-5}M Erythrosine. In the acetate buffer pH 4.4, the absorbance of Erythrosine solution is directly proportional to its concentration in the range of 1.00x10^{-6}M - 8.00x10^{-6}M as well as in the nitric acid solution pH 3.3 the absorbance of Erythrosine solution is directly proportional to its concentration in the range of 4.00x10^{-7}M - 1.00x10^{-6}M. At higher concentrations, the absorbances tended to decrease and a curvature was shown in the relationship of the absorbance to the concentration (see Tables 2-a, 2-b and Figures 3 and 4).

3.1.4 Dissociations constants of dyes

Ultraviolet-visible spectra of dyes investigated as seen in Figures 2A-2C indicated the wavelengths of the maximum absorption peaks of Amaranth, Ponceau 4R and Erythrosine shifted with the pH of solutions. Therefore, the dissociation constant of each dye was evaluated. The absorbances of each dye solutions at various pH (using hydrochloric acid, acetate buffer and sodium hydroxide for adjusting pH) were measured (see Tables 3-a to 3-c) and the absorbance of the solution was plotted against its pH. The curves of absorbances vs pH of Amaranth, Ponceau 4R and Erythrosine solutions showed the inflection points at pH 10.0, 11.0, and 4.2, respectively (see Figures 6A, 6B and 6C). Thus, the pK_a of Amaranth, Ponceau 4R and Erythrosine are, 10.0, 11.0 and 4.2, respectively.

3.1.5 Dependence of absorbances on concentrations of the metal ion solutions

A linear relationship between absorbances and concentrations of Hg (II) ion solutions or Fe(III) ion solutions was obtained in the range of concentrations 50.00-200.00 $\mu g/cm^3$ Hg(II) or 2.00-20.00 $\mu g/cm^3$ Fe(III) (see Table 4-a and 4-c, and Figures 7A and 7B). Even in the acetate buffer pH 4.4 the absorbances of Hg (II) ion solutions did not differ from those obtained in nitric acid (see Table 4-a). The lines drawn in Figures 7A and 7B are least squares lines.

3.2 Compound and complex formations between dyes and metal ions

A study of compound and complex formations between each dye and each metal ion was performed by mixing the dye solution with the metal ion solution at various concentration ratios within an buffer system or without any buffer system.

3.2.1 Mixtures of Amaranth and Hg (II), Pb(II), Fe(II), Fe(III) or Cd(II)

The measurements of visible absorbances of Amaranth concentrations in the mixtures of Amaranth (8.00×10^{-5}M) and various concentrations of each metal ion were made at the wavelength of 521 nm as well as the measurements of absorbances of concentrations of Hg(II), Pb(II), Fe(II), Fe(III) or Cd(II) ion in the mixture solutions were performed by atomic absorption spectrophotometric technique at the wavelengths of 253.7, 217.0, 248.3,248.3, or 228.8 nm, respectively. These absorption values are listed in Tables 5-a to 5-p. It can be seen that the absorption value of either Amaranth or the metal ion studied does not change in each mixture system. In addition, no physical change in each mixture system, such as color or precipitate was observed except some precipitates of hydrous metal oxides were formed at the pH higher than 6. This meant that no reaction between Amaranth and Hg(II), Pb(II), Fe(II), Fe(III) or Cd(II) ion was occurred in each mixture system. Thus, no compound or complex was formed in the solution mixture of Amaranth and Hg(II), Pb(II), Fe(II), Fe(III) or Cd(II) ion either within an buffer system or without any buffer system.

3.2.2 Mixtures of Ponceau 4R and Hg(II), Pb(II), Fe(II), Fe(III) or Cd(II)

Absorbances of Ponceau 4R concentrations in the mixtures of Ponceau 4R (6.00×10^{-5}M) and various concentrations of each metal ion were measured in the visible region at 505 nm as well as absorbances of concentrations of Hg(II), Pb(II), Fe(II), Fe(III) or Cd(II) ion in the mixture solutions were measured as the same conditions as mentioned above (3.2.1). The absorption values are shown in Tables 6-a to 6-p. The same phenomena as mentioned in 3.2.1 were observed. Therefore, no compound or complex formation between Ponceau 4R and Hg(II), Pb(II), Fe(II), Fe(III) or Cd(II) ion was occurred in any mixture system either within an buffer system or without any buffer system.

3.2.3 Mixtures of Erythrosine and Hg(II), Pb(II), Fe(II), Fe(III) or Cd(II)

Mixtures of this dye and the five metal ions were performed in two series.

3.2.3.1 The first series

Various concentrations of the metal ion solutions desired were added to $2.00x10^{-5}$M Erythrosine solutions which were in a buffer system at some pH. Absorbances of Erythrosine in these mixtures were measured in visible region at 525 nm as well as absorbances of concentrations of Hg(II), Pb(II), Fe(II), Fe(III), or Cd(II) ion were measured as the same conditions as mentioned in 3.2.1 The absorption values are shown in Tables 7-a to 7-o. The same phenomena as notified in 3.2.1 were shown. Therefore, no compound or complex was formed between Erythrosine and Hg(II), Pb(II), Fe(II), Fe(III) or Cd(II) ion in every mixture of this series.

3.2.3.2 The second series

The sequence for mixing the solution was an addition of the metal ion to the dye solution and following with a buffer system if it was desired. This series was performed since the physical changes were observed when Erythrosine solution was mixed with a metal ion solution, such as Hg(II), Fe(II), Fe(III), Cd(II), or Pb(II) ion solution. The red precipitate was formed and the red color of Erythrosine turned to other colors depending on the metal ion mixed. The Hg(II), Fe(II), Fe(III), and Pb(II) ions formed red precipitates with Erythrosine solution. However, Cd(II) ion did not react with Erythrosine since no physical change appeared and visible absorbances of the mixtures at the wavelength 525 nm did not change too (Table 8). The absorptions of Erythrosine and the metal ion in these mixtures were measured from their filtrates and the molar ratio plot served for determining composition of the compound or complex formed.

3.2.3.2.1 Mixtures of Erythrosine and Hg(II)

An orange-red precipitate was formed in the mixture of Erythrosine and Hg(II) ion solution as well as the color of the mixture solution was more intense (red color). The mixture solutions were performed within many buffer systems such as nitric acid at pH 3.1-6.8 (pH of the solution depending on the concentration of the metal ion used), the nitric acid pH 4.3 (pH controlled) and the acetate buffers pH 4.4 and 6.0. The absorbances of Erythrosine concentration and Hg(II) ion concentration in each filtrate were measured as shown in Tables 9-a to 9-i. The plot of the absorbances of mixture solutions v.s molar ratios are shown in Figure 8A-8D. By graphical method, the composition of the compound formed between Erythrosine and Hg(II) ion is 1:1 for Erythrosine to Hg(II) ion. The IR spectrum of Hg(II) Erythrosinate was compared to the spectrum of Erythrosine as shown in Figure 8E. Since the spectrum was performed in the range of wavelengths 650 cm^{-1} to 4000 cm^{-1}, the bond between metal ion and Erythrosine could not be seen unless the far IR spectrophotometer was used.

3.2.3.2.2 Mixtures of Erythrosine and Fe(III)

A red-orange precipitate was formed in the mixture of Erythrosine and Fe(III)ion solution as well as the color of solution was more intense. The mixture solutions were performed within many buffer systems such as sulfuric acid pH 6.8-3.3 (pH of the solution depending on the concentration of the metal ion used),the sulfuric acid pH 3.3 (pH controlled), nitric acid pH 5.5-3.5 (pH of solution depending on the concentration of the metal ion used),the nitric acid pH 3.3 (pH controlled) and the acetate buffers pH 4.4 and 6.0. The absorbances of Erythrosine concentration and Fe(III) ion concentration in each filtrate solution were measured as shown in Tables 10-a to 10-j. Since the precipitate of ferric acetate formed at higher concentrations of Fe(III) ion, the atomic absorption method was not used for determining the composition of the compound formed in the acetate buffers pH 4.4 and 6.0. The molar ratio plots are shown in Figures 9A- 9F. By graphical method, the compositions of the complexes and compound formed between Erythrosine and Fe(III) ion is 1:1, 2:1, and 3:2 for Erythrosine to Fe(III)ion. The IR spectrum of Fe(III) Erythrosinate compound was compared to the spectrum of Erythrosine as shown in Figure 9G. The spectrum showed many new absorption peaks in the wavelengths 1,300 cm^{-1} to 1,370 cm^{-1}.

3.2.3.2.3 Mixtures of Erythrosine and Fe(II)

A red-orange precipetate was formed in the mixture of Erythrosine and Fe(II) ion. Since the Fe(II) ion is easily oxidized in aqueous solution and even in an acidic solution, the concentration of Fe(II) ion cannot be as prepared. Therefore, the composition of compound formed between Fe(II) ion and Erythrosine was not determined in this study.

3.2.3.2.4 Mixtures of Erythrosine and Pb(II)

A purple-red precipitate appeared in the mixture of Erythrosine and Pb(II)ion as well as the color of the mixture solution was changed to purple-red. The mixture solutions were performed either without any buffer system or within many buffer systems such as in aqueous solution pH 6.2-5.3 (pH of the solution depending on the concentration of metal ion used) and in the nitric acid pH 5.5 (pH controlled). The visible absorption values of the mixtures at various molar ratios are listed in Table 11-a to 11-c. The molar ratio plot for the mixture system containing 1.20×10^{-5}M Pb(II) and various concentrations of Erythrosine in aqueous solution (see Figure 10A) showed a curvature at the intersection point. This indicated that the stability of the precipitate formed is not high and some precipitates are soluble in the mixture solution. The solubility of this precipitate was then studied and the result illustrated that this precipitate is very soluble in acetate buffer, soluble in acetic acid, nitric acid and Erythrosine solution. However, the composition of this precipitate was found to be 1:1 for Erythrosine to Pb(II) ion. When the concentration of Erythrosine in the mixture was held constantly and the concentration of Pb(II) ion was varied, the composition of the complex formed was found to be 1:3 for Erythrosine to Pb(II)ion. This composition value was found both the mixtures in water and in nitric acid. In addition, at higher concentrations of both Pb(II)ion (2.00×10^{-4}M) and Erythrosine (0.04×10^{-4}M - 1.20×10^{-4}M) in the mixtures the composition of the complex formed was found to be 2:3 for Erythrosine to Pb(II)ion by using atomic absorption method. This meant that polynuclear complexes could be formed in the mixture solution of Erythrosine and Pb(II) ion, especially in the mixture solution of higher concentrations of Pb(II) ion. The IR spectrum of Pb(II)Erythrosinate was compared to the spectrum of Erythrosine as shown in Figure 10c. Both spectra showed an insignificant difference in the range of wavelengths 650-4000 cm^{-1}.

3.3 Stability constants and solubility products of the compounds formed between Erythrosine and metal ions

The stability constants and solubility products of the metal ion-Erythrosinate compounds formed under the conditions studied as mentioned in 3.2 were evaluated on the basis of the concentrations of compounds formed as precipitates were equal to unity. From the experiment, the initial concentration of the metal ion or Erythrosine as well as the concentration of the metal ion remained or Erythrosine remained in the mixture solution were determined by their absorbances. Since the molar ratio of Hg(II) or Pb(II) ion to Erythrosine to form the precipitated compound was found as minimum as 1:1, its stability constant and solubility product were evaluated for the formula of Me(Eryth) and $Me_2(Eryth)_2$ where Me is the metal ion and Eryth is Erythrosine. In addition, the stability constant and solubility product of ferric Erythrosinate precipitate were determined as $Me_2(Eryth)_3$ and $Me_4(Eryth)_6$. These values are shown in Tables12-a to 12-c.

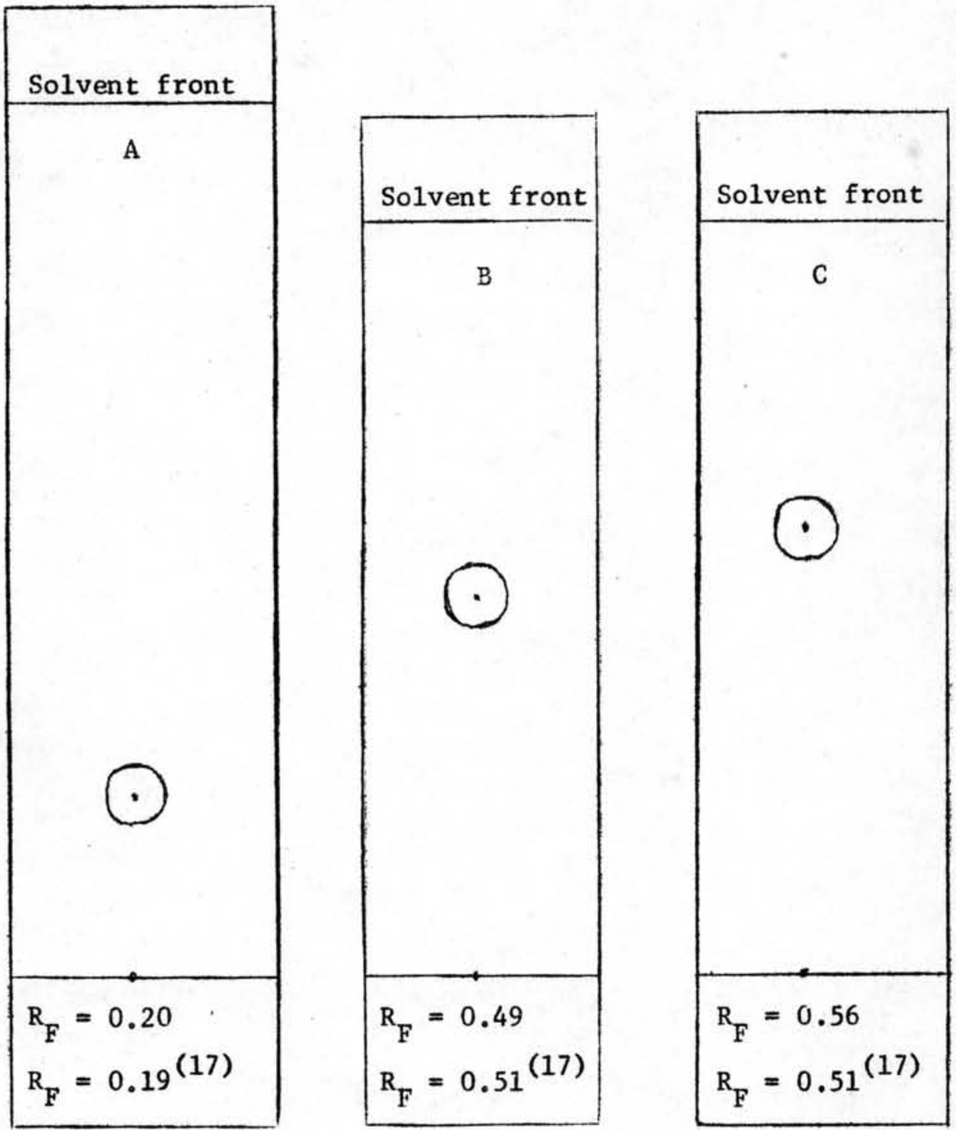

Figure 1 Paper chromatograms of dyes (true scale);

A) Amaranth, B) Erythrosine, and C) Ponceau 4 R

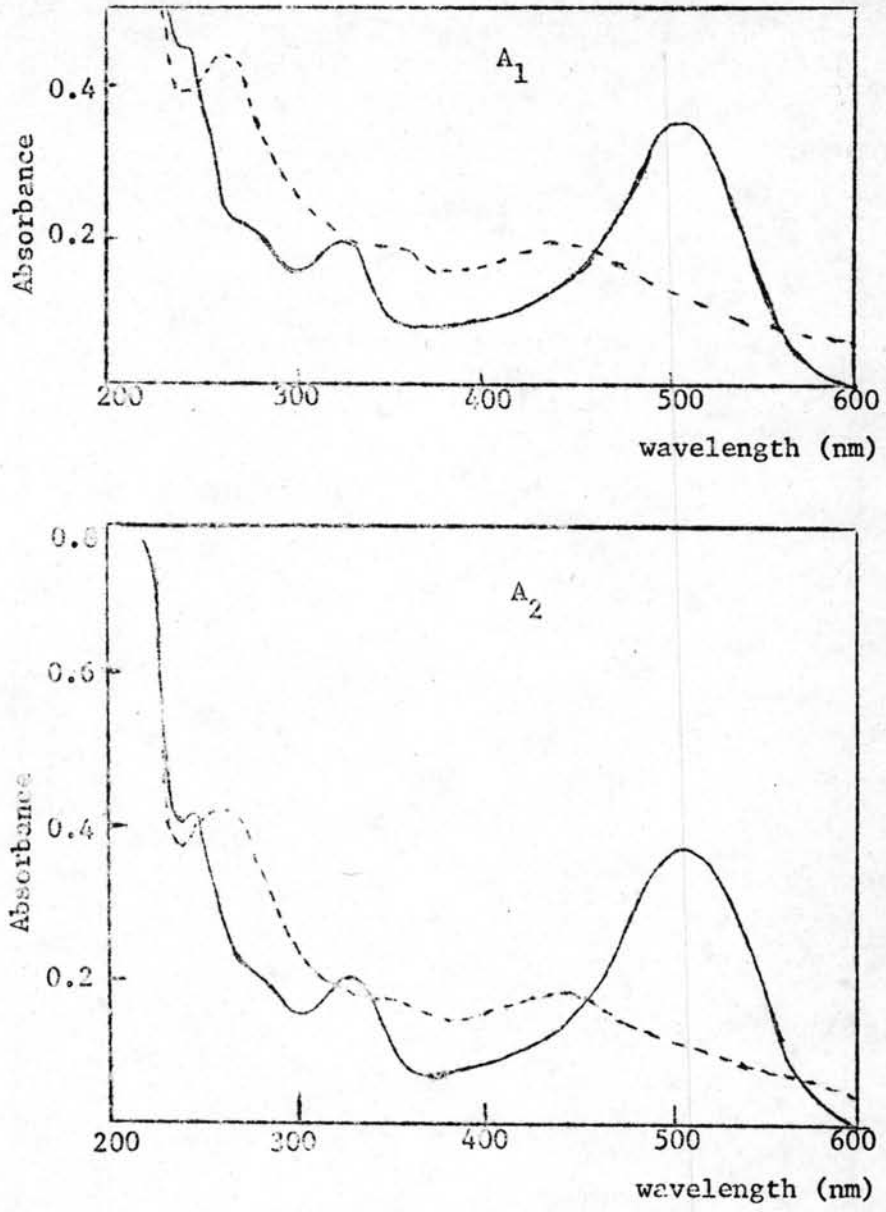

Figure 2A Comparision of ultraviolet-visible spectra of Amaranth between A$_1$) experiment and A$_2$) literature$^{(18)}$; — in $\frac{N}{10}$ HCl and ..... in $\frac{N}{10}$ NaOH

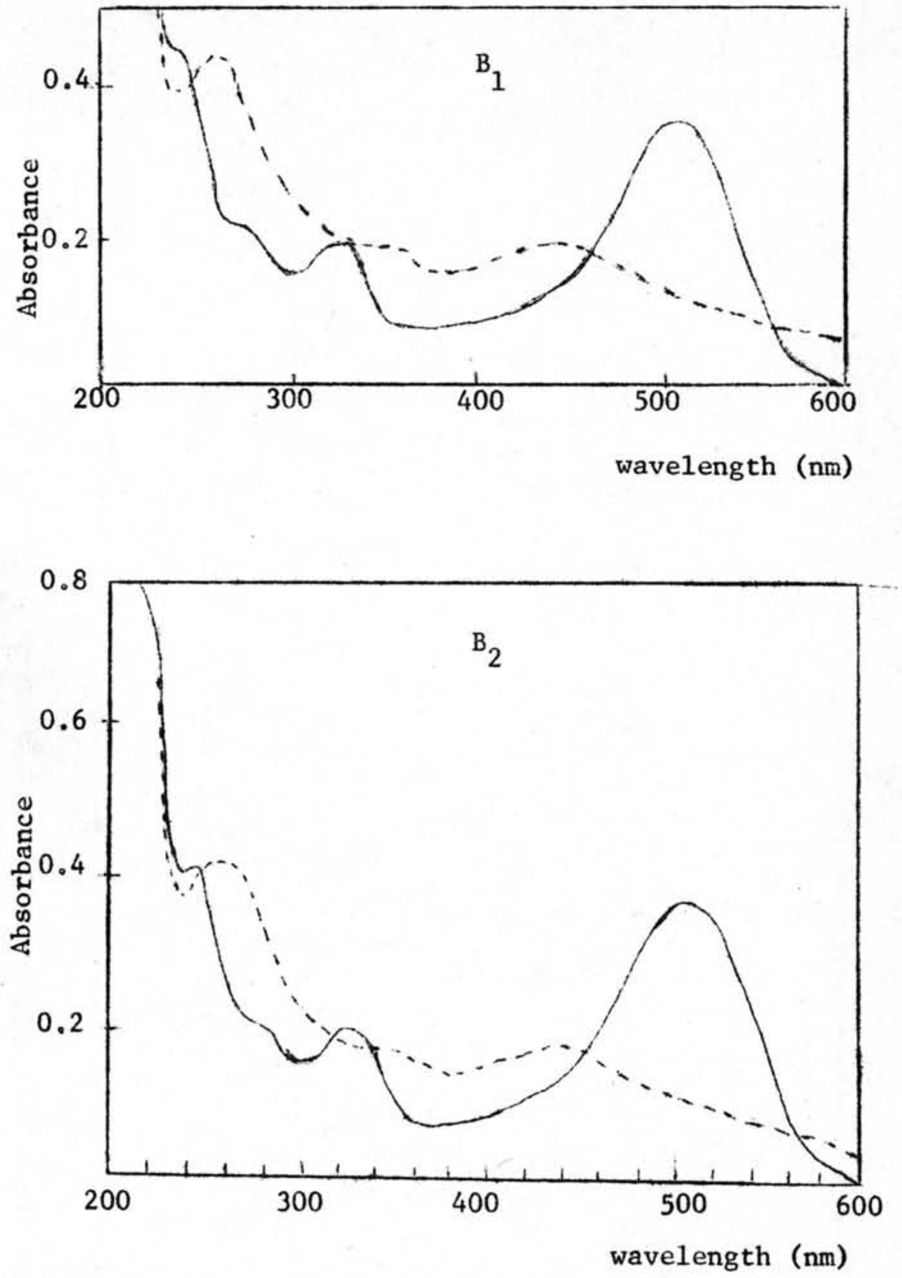

Figure 2B Comparision of ultraviolet-visible spectra of Ponceau 4R between B_1) experiment and B_2) literature(18); — in $\frac{N}{10}$ HCl and ..... in $\frac{N}{10}$ NaOH

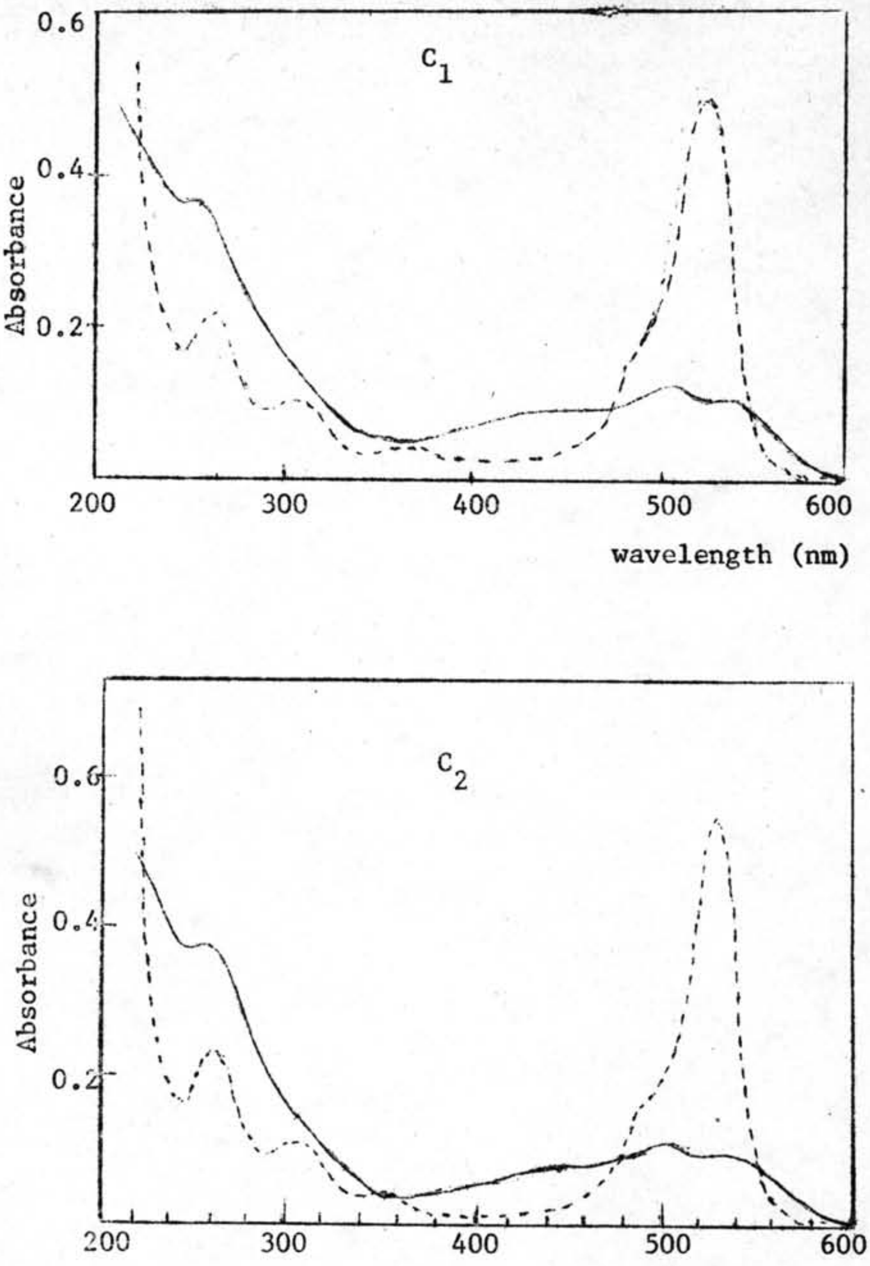

Figure 2C Comparision of ultraviolet-visible spectra of Erythrosine between C$_1$) experiment and C$_2$) literature$^{(18)}$; —$\frac{N}{10}$ HCl, ...$\frac{N}{10}$ NaOH

Table 1 Absorption characteristics of dyes in visible region

Dye	λ max, nm	molar absorptivity ε (calculated)	pK_a
Amaranth	521 in acid solution	29,420	10.0
Ponceau 4 R	505 in acid solution	11,700	11.0
Erythrosine	525 in alkali solution	76,500	4.2

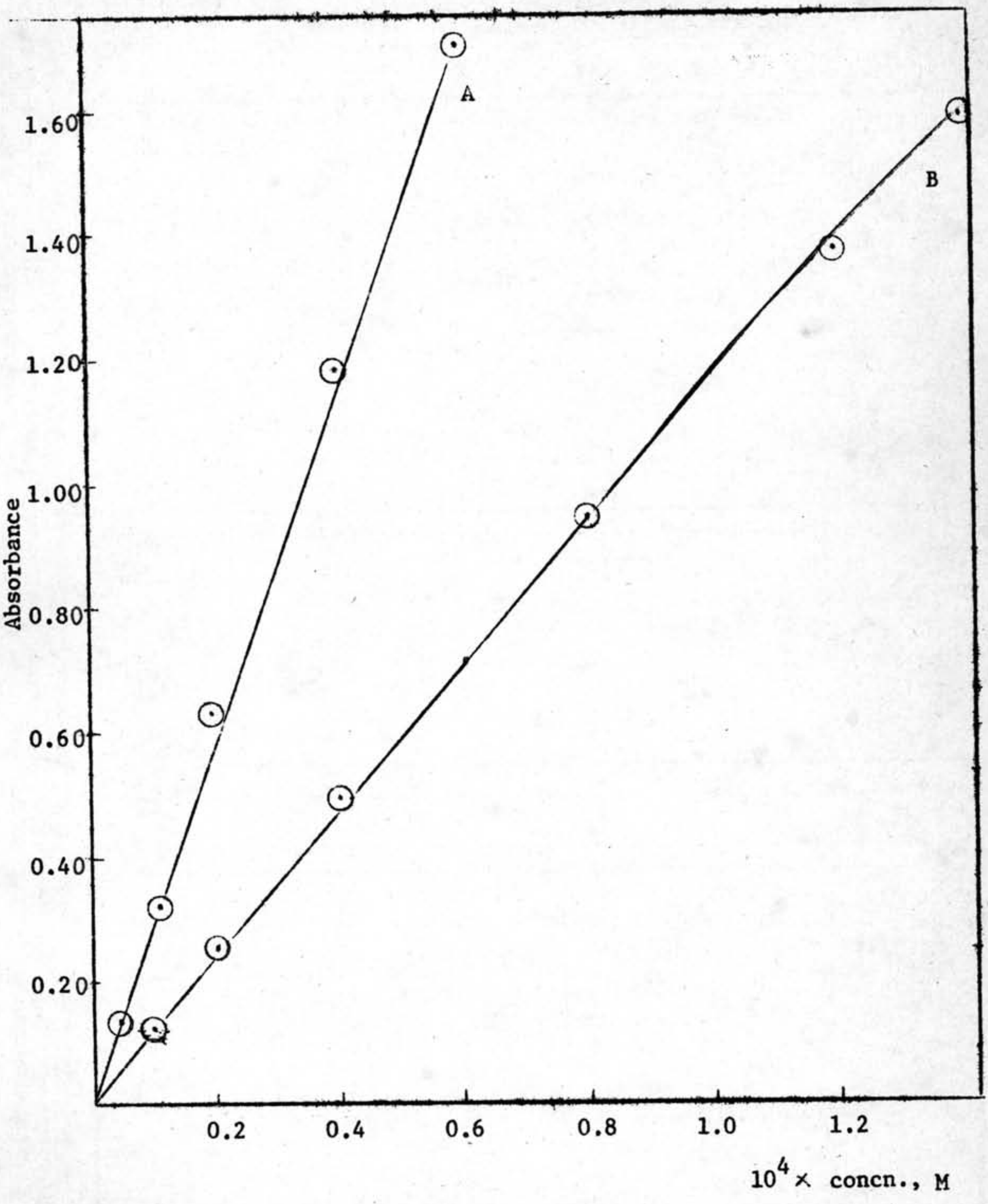

Figure 3 Dependences of absorbances on concentrations of dyes; A) Amaranth and B) Ponceau 4R

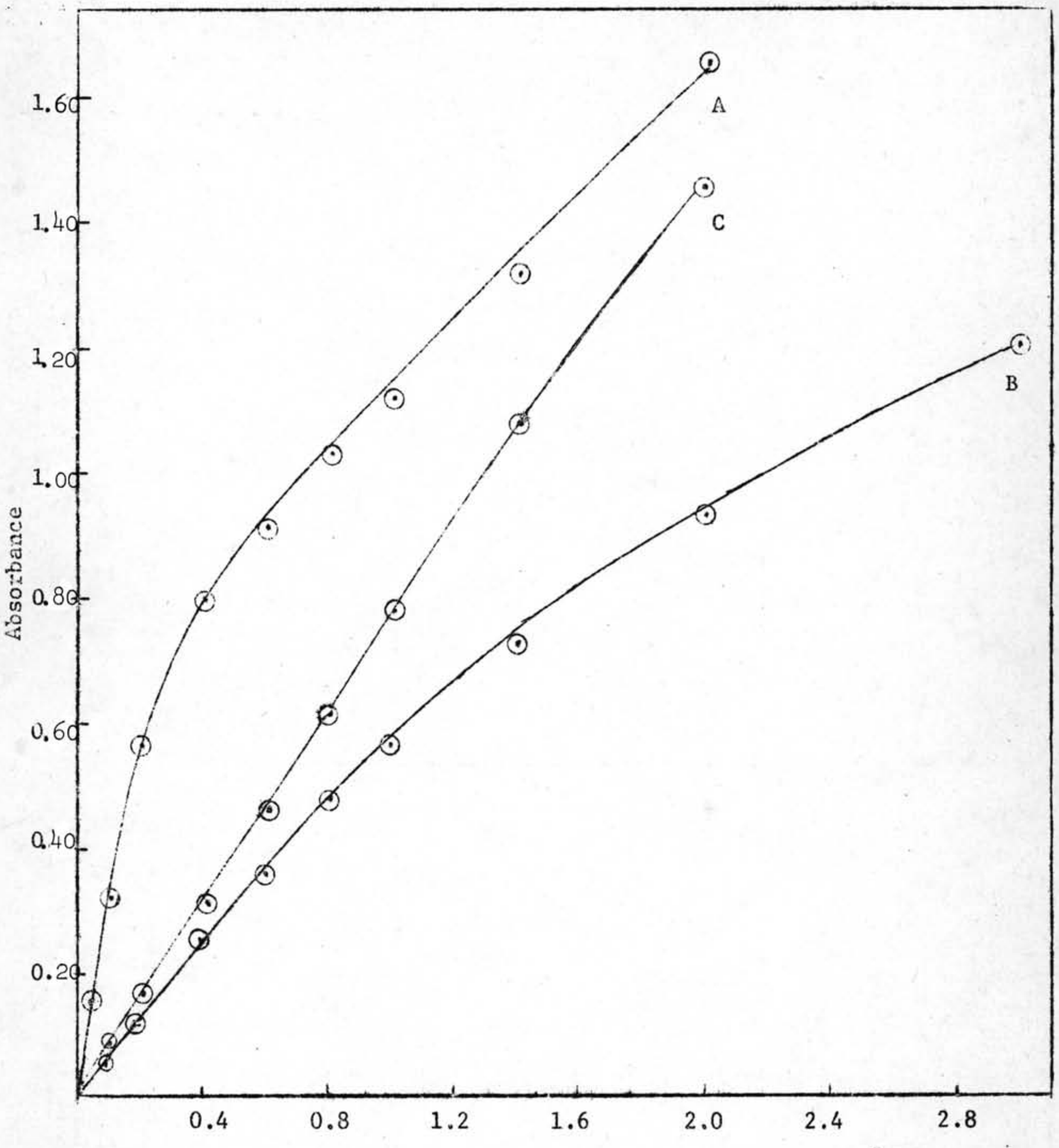

Figure 4 Dependences of absorbances on concentrations of Erythrosine at A) pH 3.3, B) pH 4.4 and C) pH 6.5

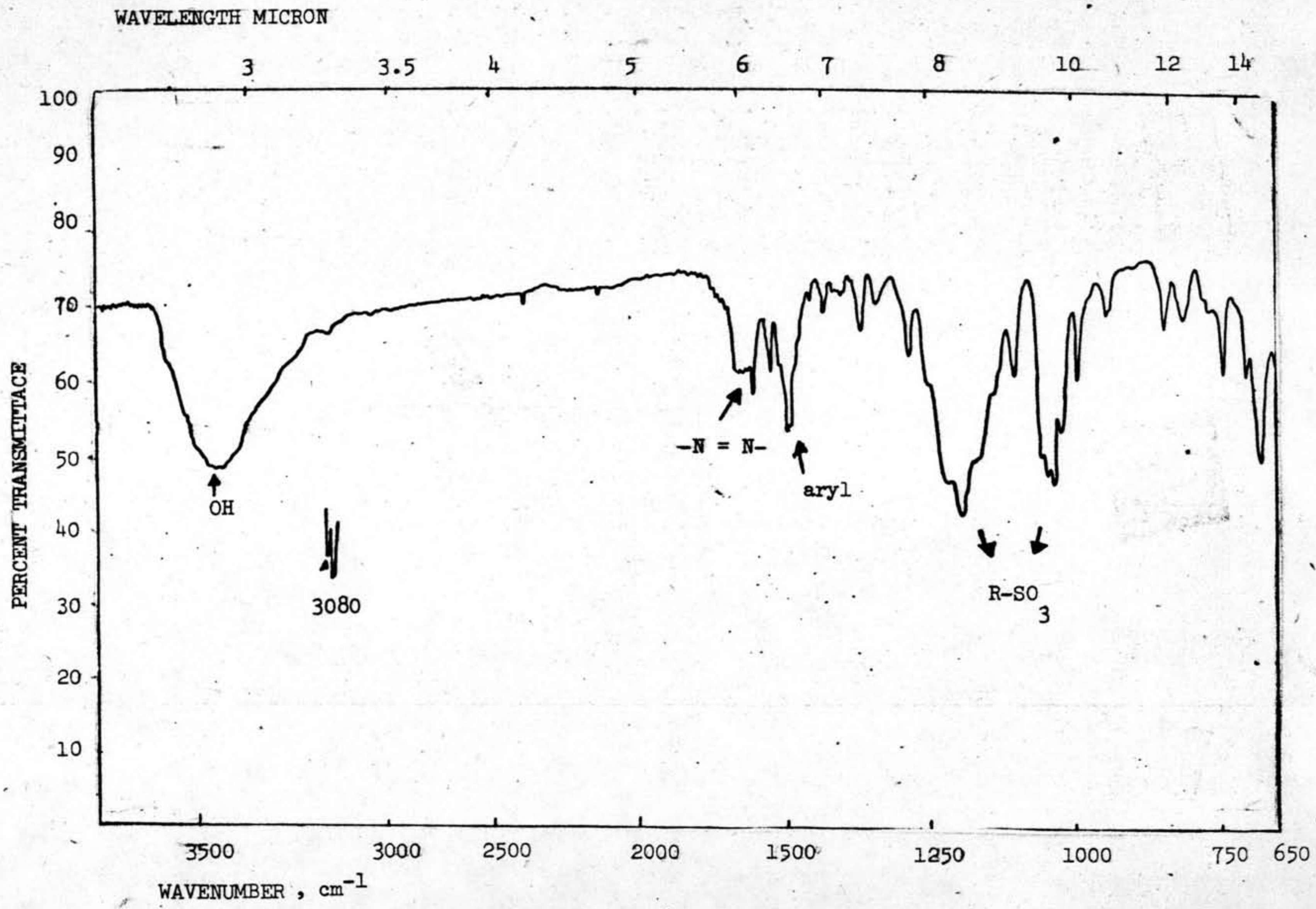

Figure 5A IR spectrum of Amaranth in solid KBr pellet.

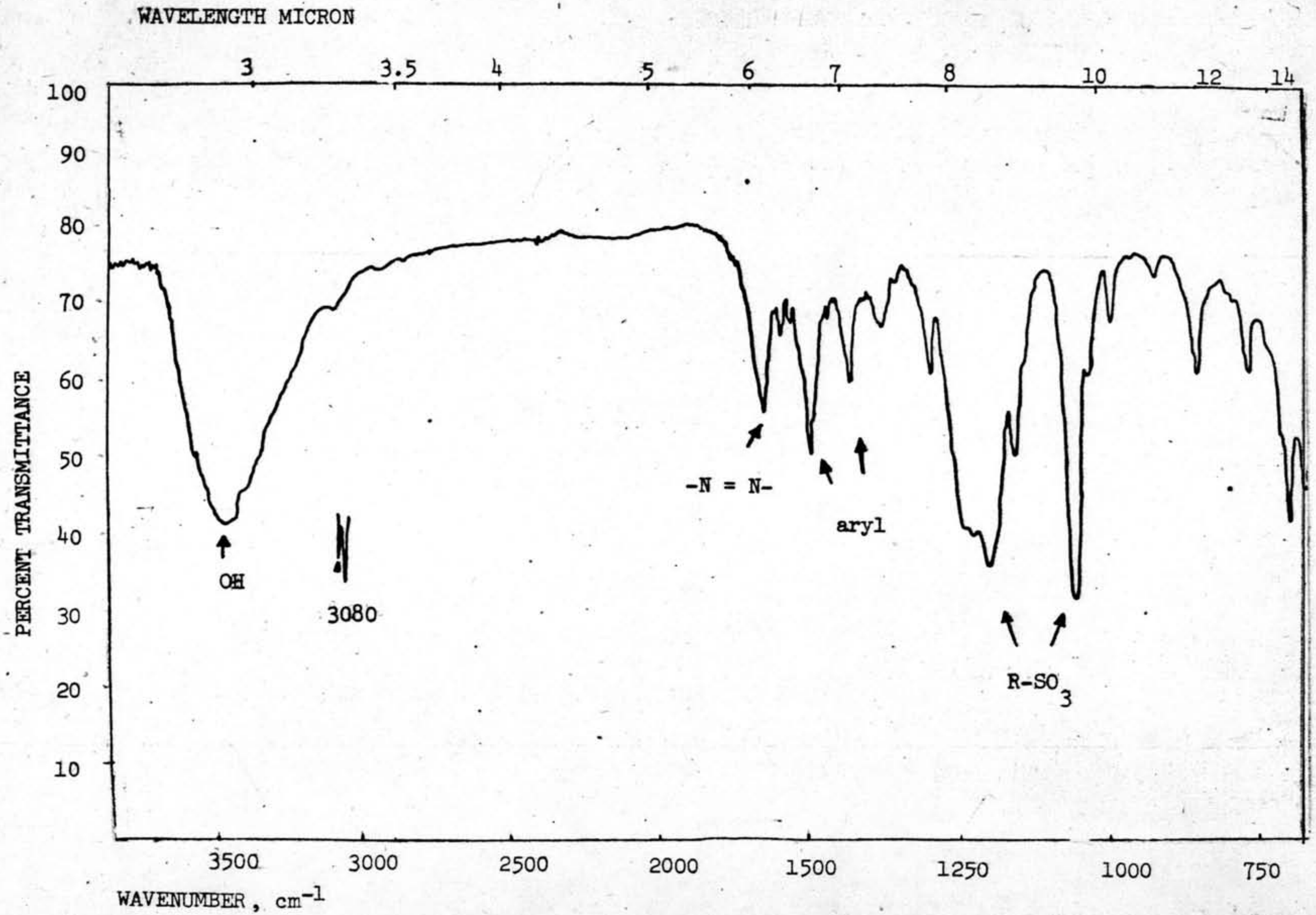

Figure 5B IR spectrum of Ponceau 4R in solid KBr pellet .

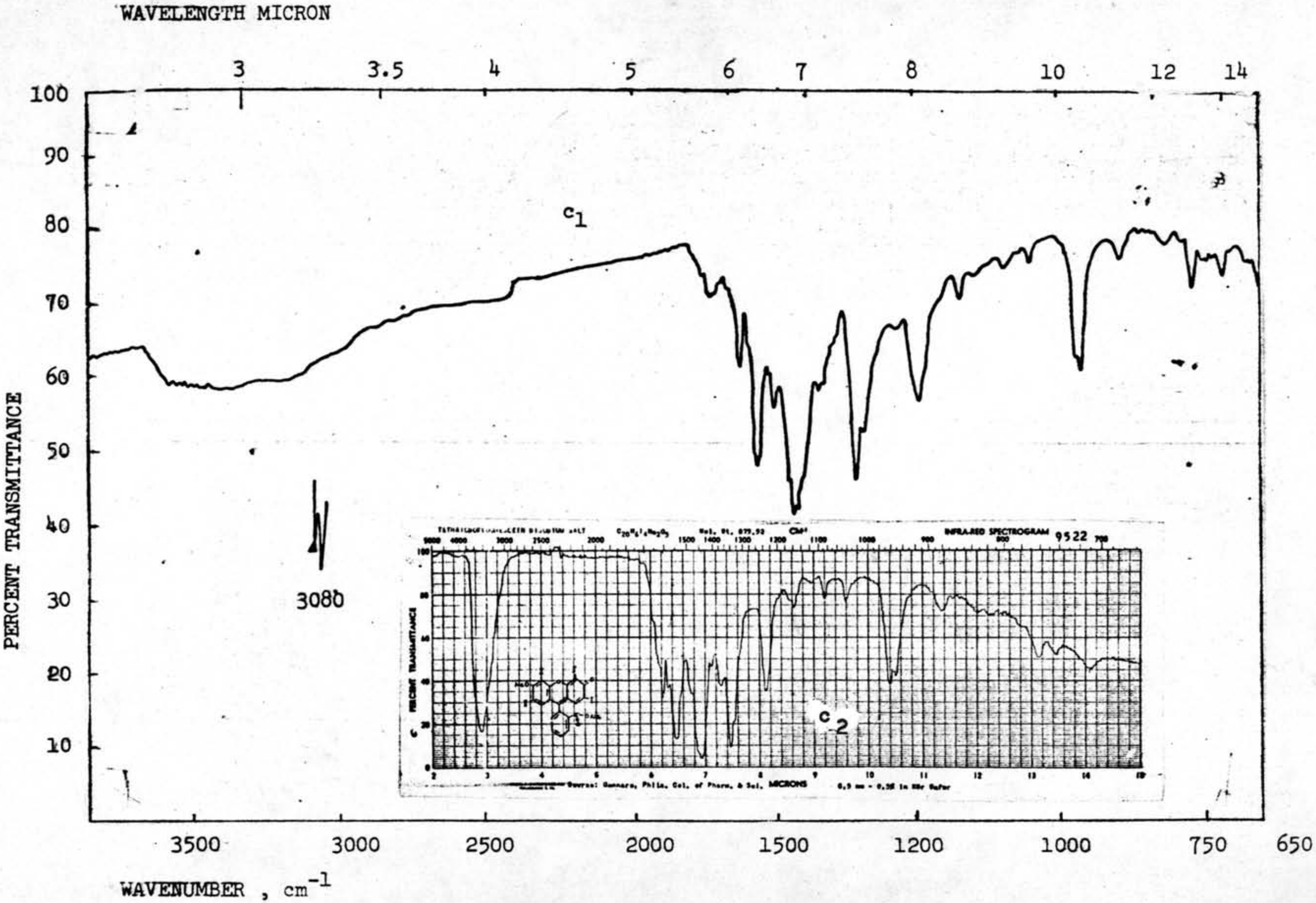

Figure 5C IR spectrum of Erythrosine in solid KBr pellet.
c_1) experiment c_2) literature(19)

Table 2 - a Dependences of absorbances on concentrations of dyes in aqueous solution.

$10^5 \times$ Concn, M	Absorbance
<u>Amaranth</u>	λmax = 521 nm
0.40	0.134
1.00	0.316
2.00	0.632
4.00	0.177
6.00	0.698
8.00	2.120
<u>Ponceau 4 R</u>	λmax = 505 nm
10.00	0.124
20.00	0.253
40.00	0.492
60.00	0.716
80.00	0.940
100.00	1.169
120.00	1.366
140.00	1.572
<u>Erythrosine</u>	λmax = 525 nm
0.10	0.079
0.20	0.164
0.40	0.313
0.60	0.459
0.80	0.608
1.00	0.778
1.40	1.074
2.00	1.453

Table 2 - b Dependences of absorbances on concentrations of Erythrosine in various buffer systems.

$10^5 \times$ Concn, M	Absorbance at at λmax = 525 nm
<u>in nitric acid pH 3.3</u>	
0.04	0.015
0.10	0.031
0.20	0.055
0.40	0.079
0.60	0.090
0.80	0.102
1.00	0.111
1.40	0.1303
2.00	0.165
3.00	
<u>in acetate buffer pH 4.4</u>	
0.10	0.059
0.20	0.130
0.40	0.252
0.60	0.363
0.80	0.481
1.00	0.561
1.40	0.726
2.00	0.975
3.00	1.200

Table 3 - a Dependence of absorbances on pH of Amaranth solutions.

pH	Absorbance at λ max = 521 nm	$\Delta A / \Delta pH$
12.5	0.390	
11.9	0.460	-0.116
11.4	0.540	-0.160
11.0	0.600	-0.150
10.0	0.840	-0.260
9.0	0.870	-0.030
8.0	0.870	0.000
7.0	0.870	0.000
6.0	0.870	0.000
5.0	0.870	0.000
4.0	0.870	0.000
3.0	0.870	0.000
2.0	0.870	0.000
1.0	0.870	0.000

Table 3 - b Dependence of absorbances on pH of Ponceau 4 R solutions.

pH	Absorbance at λ max = 505 nm	$\Delta A / \Delta pH$
12.0	0.250	
11.6	0.300	-0.120
11.0	0.500	-0.330
10.0	0.610	-0.110
9.0	0.630	-0.020
8.0	0.630	0.000
7.0	0.630	0.000
6.0	0.630	0.000
5.0	0.630	0.000
4.0	0.630	0.000
3.0	0.630	0.000
2.0	0.630	0.000
1.0	0.630	0.000

Table 3 - c Dependence of absorbances on pH of Erythrosine solutions.

pH	Absorbance at λmax = 525 nm	$\frac{\Delta A}{\Delta pH}$
Erythrosine		
10.0	1.540	
8.0	1.520	0.01
6.8	1.490	0.02
5.9	1.480	0.01
5.2	1.340	0.20
4.5	1.130	0.30
4.3	0.830	1.50
4.2	0.600	2.30
3.5	0.220	0.70
3.3	0.150	0.35
2.0	0.000	0.12
1.0	0.000	0.00

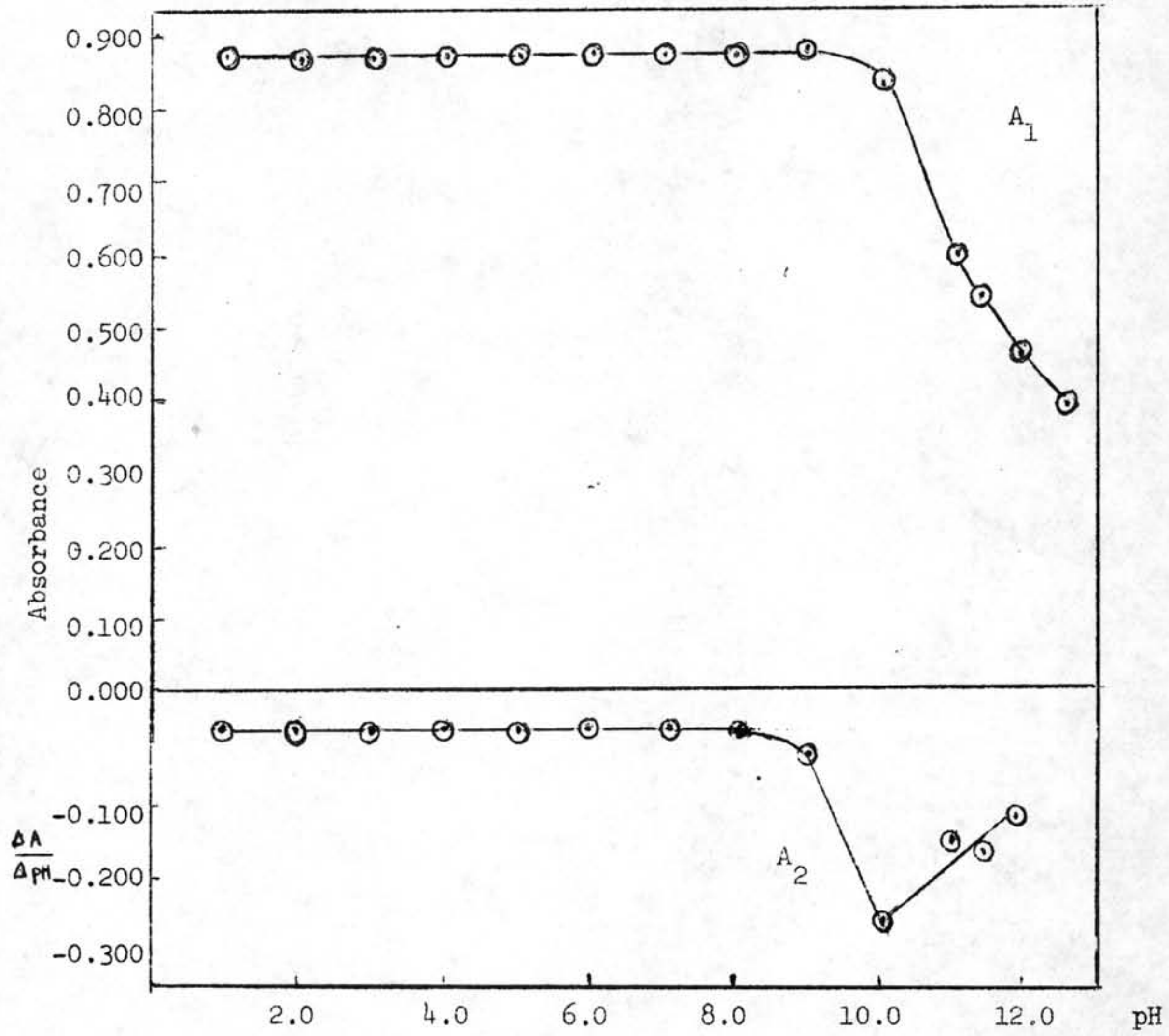

Figure 6A Determination of the pK_a value for Amaranth by visible spectrophotometric data; A_1) absorbance vs pH and A_2) $\Delta A/_{\Delta pH}$ vs pH

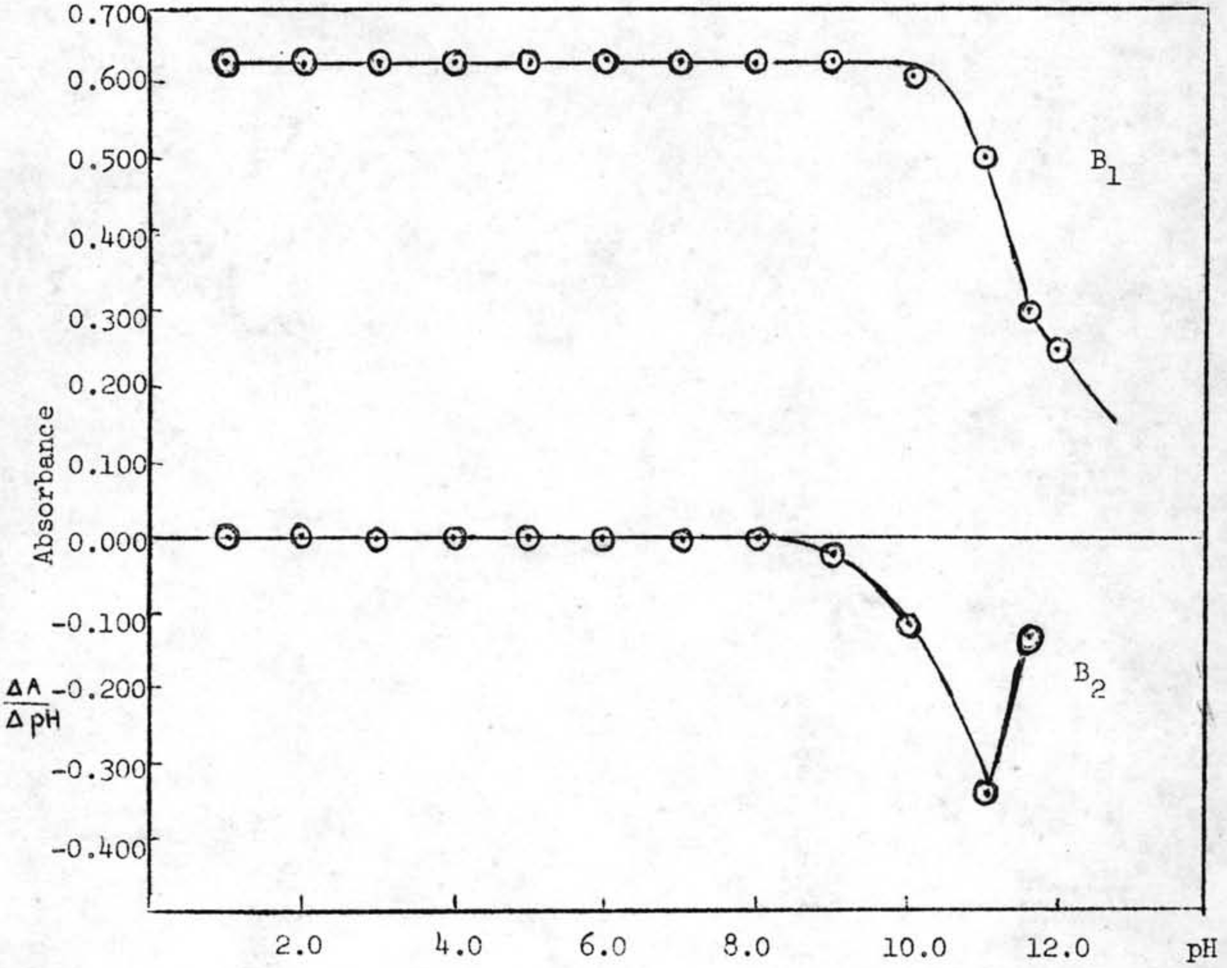

Figure 6B Determination of the pK_a value for Ponceau 4R by visible spectrophotometric data; A_1) absorbance vs pH and A_2) $\Delta A/_{\Delta pH}$ vs pH

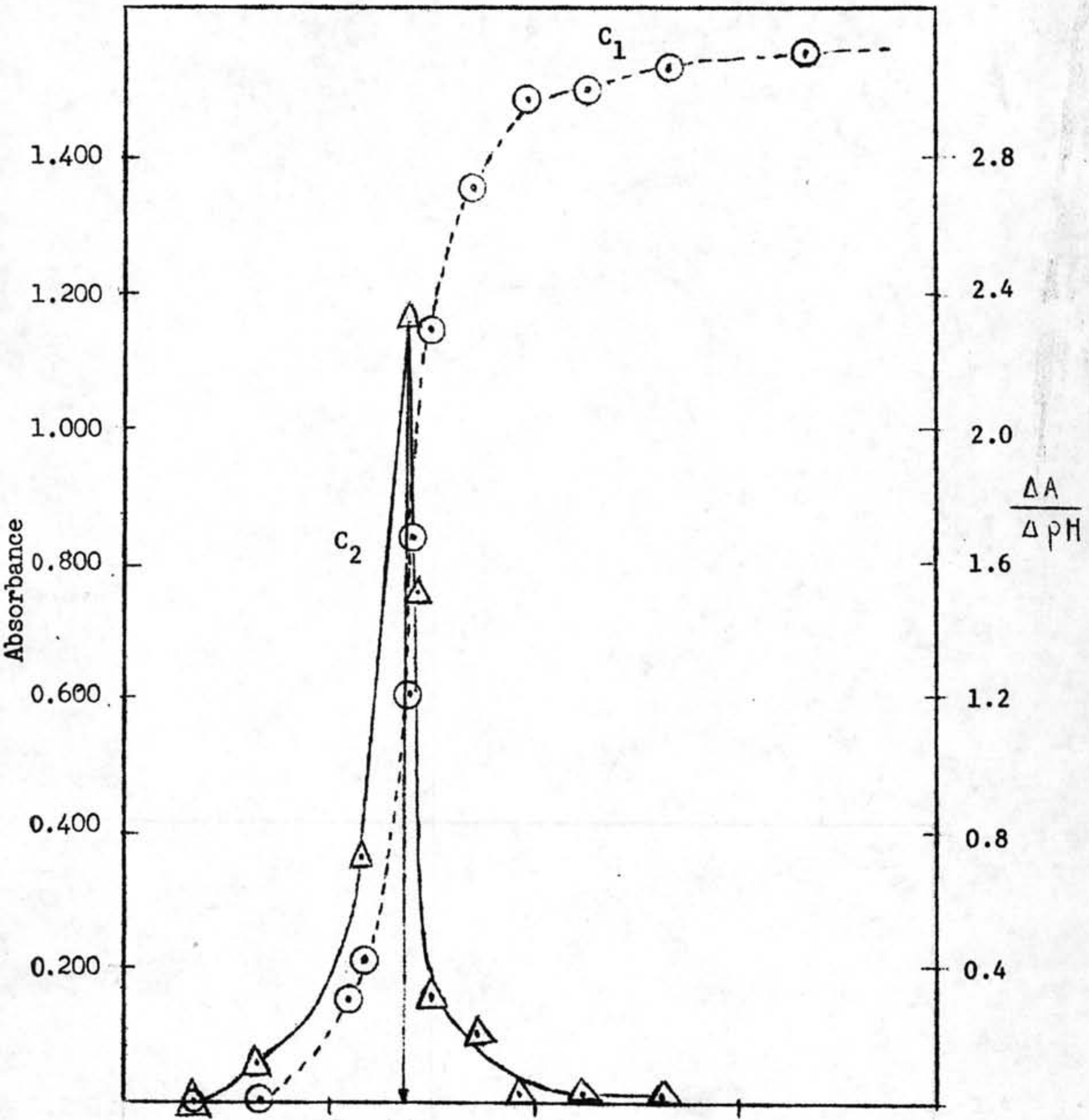

Figure 6C Determination of the pK_a value for Erythrosine by visible spectrophotometric data; C_1) absorbance vs pH and C_2) $\Delta A/\Delta pH$ vs pH

Table 4 - a Dependences of absorbances on concentrations of Hg (II) ion in nitric acid and the acetate buffer pH 4.4

concentration (ppm)	Absorbance at 253.7 nm in nitric acid	Absorbance at 253.7 nm in acetate buffer pH 4.4
30.0	0.020	0.020
50.0	0.040	0.040
80.0	0.050	0.050
100.0	0.070	0.070
120.0	0.080	0.080
140.0	0.090	0.090
160.0	0.100	0.100
180.0	0.130	0.130
200.0	0.130	0.130

Table 4 - b Dependence of absorbances on concentrations of Fe(III)ion in nitric acid.

concentration (ppm)	Absorbance at 248.3 nm
2.0	0.070
4.0	0.110
6.0	0.140
8.0	0.180
10.0	0.220
20.0	0.460

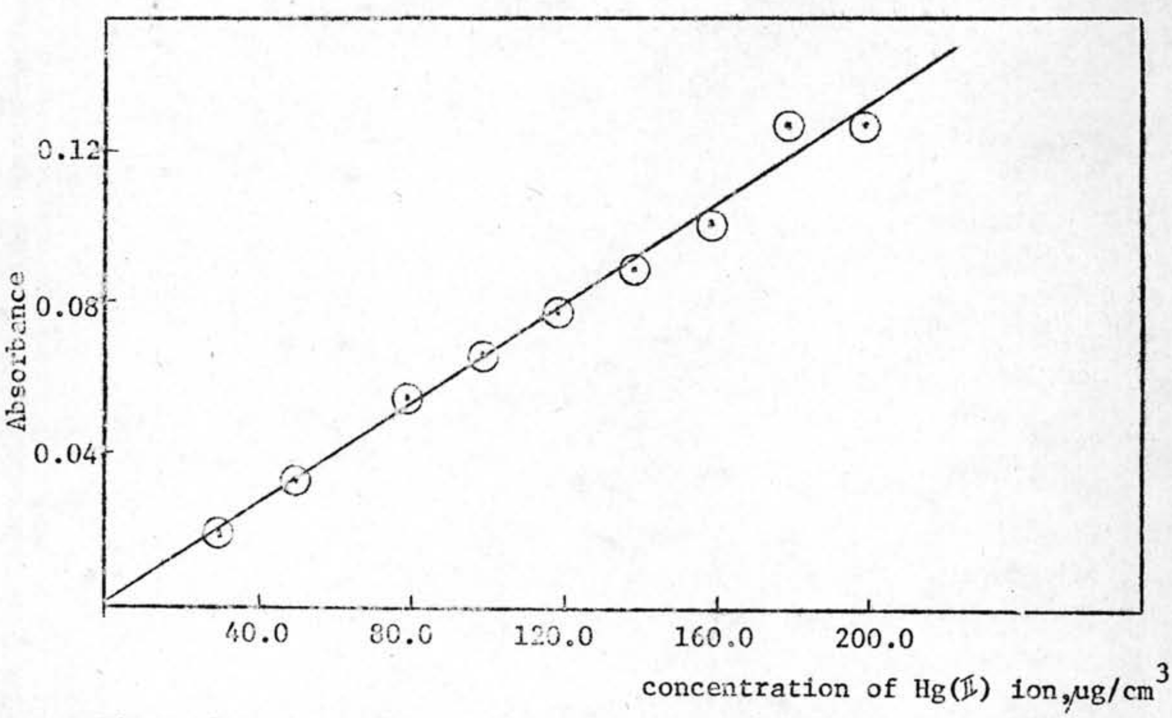

Figure 7A Dependence of absorbances on concentrations of Hg(II) ion.

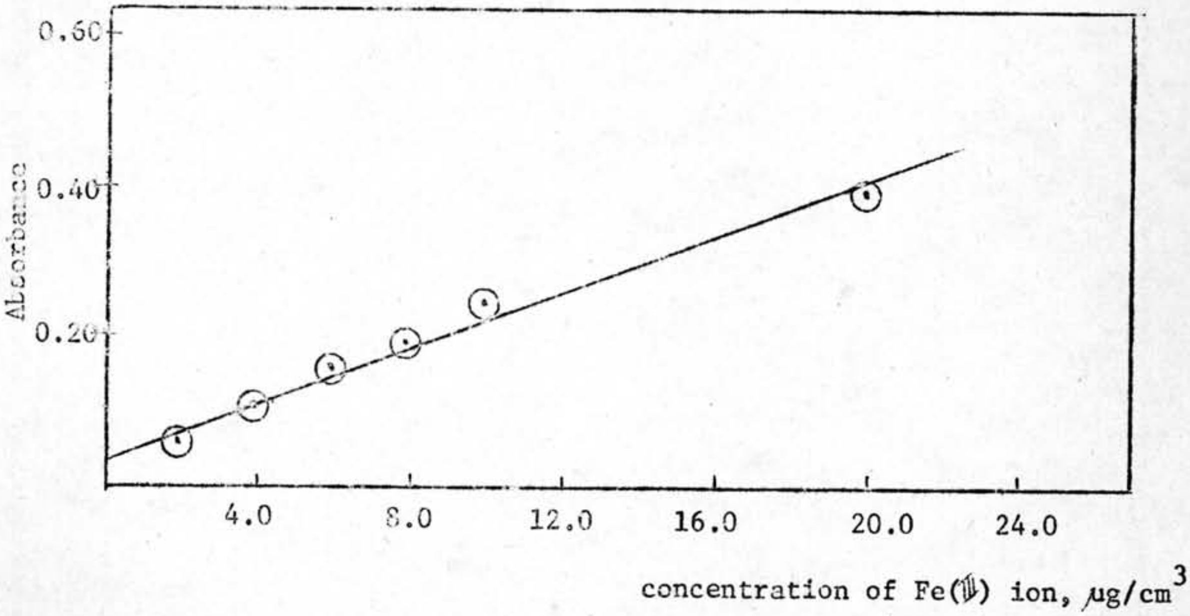

Figure 7B Dependence of absorbances on concentrations of Fe(III) ion.

Table 5 - a Molar ratio study of Amaranth and various metal ions in water by visible spectrophotometric technique

Molar ratio, Metal **ion** : Amaranth	Absorbance of the mixture at λ_{max} = 521 nm				
	Cd (II)	Hg (II)	Fe (II)	Fe (III)	Pb (II)
0.00	1.520	1.510	1.520	1.520	1.520
1.00	1.520	1.520	1.530	1.520	1.520
2.00	1.530	1.520	1.520	1.530	1.520
3.00	1.520	1.510	1.520	1.520	1.520
4.00	1.530	1.520	1.510	1.520	1.520
5.00	1.520	1.510	1.520	1.520	1.520
10.00	1.520	1.520	1.520	1.530	1.520

Table 5 - b Molar ratio study of Amaranth and various metal ions at pH 3.0 in acetic acid by visible spectrophotometric technique.

Molar ratio, Metal **ion** : Amaranth	Absorbance of the mixture at λ_{max} = 521 nm				
	Cd(II)	Hg (II)	Fe (II)	Fe (III)	Pb (II)
0.00	1.520	1.520	1.520	1.520	1.520
1.00	1.520	1.520	1.520	1.500	1 .20
2.00	1.520	1.520	1.520	1.500	1.520
3.00	1.520	1.530	1.520	1.510	1.520
4.00	1.520	1.510	1.520	1.500	1.520
5.00	1.520	1.520	1.520	1.510	1.510
10.00	1.520	1.500	1.530	1.500	1.520

Table 5 - c Molar ratio study of Amaranth and various metal ions in the phosphate buffer pH 6.4 by visible spectrophotometric technique

Molar ratio, Metal ion : Amaranth	Absorbance of the mixture at λ_{max} = 521 nm				
	Cd(II)	Hg (II)	Fe (II)	Fe (III)	Pb (II)
0.00	1.510	1.510	1.510	1.510	1.510
1.00	1.510	1.500	1.500	1.510	1.510
2.00	1.500	1.500	1.510	1.500	1.510
3.00	1.510	1.500	1.500	1.490	1.510
4.00	1.500	1.520	1.510	1.500	1.510
5.00	1.510	1.500	1.500	1.490	1.520
10.00	1.500	1.500	1.510	1.500	1.510

Table 5 - d Molar ratio study of Amaranth and various metal ions in the phosphate buffer pH 7.4 by visible spectrophotometric technique.

Molar ratio, Metal ion : Amaranth	Absorbance of the mixture at λ_{max} = 521 nm				
	Cd(II)	Hg (II)	Fe (II)	Fe (III)	Pb (II)
0.00	1.620	1.630	1.630	1.630	1.620
1.00	1.630	1.620	1.620	1.620	1.630
2.00	1.620	1.620	1.620	1.630	1.620
3.00	1.620	1.620	1.620	1.620	1.620
4.00	1.620	1.620	1.630	1.630	1.620
5.00	1.620	1.620	1.620	1.630	1.630
10.00	1.630	1.620	1.630	1.630	1.620

Table 5 - e Molar ratio study of Amaranth and various metal ions in diethylamine buffer pH . 12.5 by visible spectrophotometric technique

Molar ratio, Metal ion : Amaranth	Absorbance of the mixture at λ_{max} = 485 nm				
	Cd(II)	Hg (II)	Fe (II)	*Fe (III)	Pb (II)
0.00	0.850	0.860	0.890	0.890	0.890
1.00	0.850	0.850	0.890	0.890	0.890
2.00	0.860	0.860	0.880	0.890	0.890
3.00	0.850	0.850	0.890	0.890	0.890
4.00	0.850	0.860	0.880	0.890	0.890
5.00	0.850	0.860	0.880	0.880	0.890
10.00	0.860	0.860	0.880	0.890	0.890

* KCl + NaOH buffer was used instead since the mixture in the diethylamine buffer pH 12.5 had been done(7)

Table 5 - f Molar ratio study of Amaranth and various metal ions at pH 3.4 in McIlvaine buffer by visible spectrophotometric technique

Molar ratio, Metal ion : Amaranth	Absorbance of the mixture at λ_{max} = 521 nm				
	Cd (II)	Hg (II)	Fe (II)	Fe(III)	Pb (II)
0.00	0.920	0.910	0.910	0.930	0.930
1.00	0.940	0.910	0.910	0.920	0.920
2.00	0.930	0.910	0.900	0.930	0.920
3.00	0.930	0.910	0.910	0.920	0.920
4.00	0.930	0.920	0.900	0.910	0.920
5.00	0.930	0.910	0.910	0.920	0.920
10.00	0.930	0.910	0.900	0.930	0.92

Table 5 - g Molar ratio study of Amaranth and various metal ions in McIlvain buffer pH 6.4 by spectrophotometric technique

Molar ratio, Metal ion : Amaranth	Absorbance of the mixture at λ_{max} = 525 nm				
	Cd (II)	Hg (II)	Fe (II)	Fe (III)	Pb (II)
0.00	0.820	0.870	0.870	0.860	0.820
1.00	0.830	0.860	0.860	0.860	0.820
2.00	0.830	0.860	0.860	0.860	0.810
3.00	0.830	0.870	0.870	0.850	0.820
4.00	0.820	0.870	0.860	0.860	0.810
5.00	0.830	0.870	0.860	0.850	0.810
10.00	0.820	0.870	0.860	0.850	0.810

Table 5 - h Molar ratio study of Amaranth and various metal ions in McIlvain buffer pH 7.6 by visible **spectrophotometric technique**

Molar ratio, Metal ion : Amaranth	Absorbance of the mixture at λ_{max} = 525 nm				
	Cd (II)	Hg (II)	Fe (II)	Fe (III)	Pb (II)
0.00	0.870	0.880	0.670	0.670	0.670
1.00	0.870	0.880	0.660	0.670	0.670
2.00	0.860	0.880	0.670	0.660	0.680
3.00	0.850	0.880	0.660	0.670	0.680
4.00	0.860	0.880	0.670	0.660	0.670
5.00	0.860	0.880	0.670	0.650	0.680
10.00	0.860	0.880	0.660	0.660	0.670

Table 5 - 1 Molar ratio study of Amaranth and various metal ions in water by atomic absorption spectrophotometric technique.

Molar ratio, Amaranth : Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe (II)	Fe (III)	Pb(II)
0.00	0.850	0.330	0.900	0.890	0.620
0.10	0.860	0.340	0.900	0.890	0.620
0.20	0.850	0.350	0.900	0.900	0.620
0.30	0.860	0.340	0.900	0.890	0.620
0.50	0.850	0.350	0.900	0.890	0.620
1.00	0.860	0.340	0.900	0.900	0.620

Table 5 - 2 Molar ratio study of Amaranth and various metal ions in acetic acid at pH 3.0 by atomic absorption spectrophotometric technique.

Molar ratio, Amaranth : Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.850	0.300	0.900	0.520	0.620
0.10	0.850	0.300	0.900	0.530	0.620
0.20	0.860	0.300	0.900	0.530	0.620
0.30	0.860	0.300	0.900	0.540	0.620
0.50	0.860	0.300	0.900	0.550	0.620
1.00	0.86	0.30	0.90	0.53	0.62

Table 5 - k Molar ratio study of Amaranth and various metal ions in the phosphate buffer pH 6.4 by atomic absorption spectrophotometric technique

Molar ratio, Amaranth: Metal ion	Absorptions of the mixture at the wavelength where the metal ion absorbed				
	Cd (II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.850	0.180	0.020	0.000	0.090
0.10	0.850	0.180	0.020	0.000	0.050
0.20	0.850	0.190	0.020	0.000	0.060
0.30	0.850	0.180	0.020	0.000	0.040
0.50	0.840	0.180	0.020	0.000	0.050
1.00	0.850	0.180	0.020	0.000	0.080

Table 5 - l Molar ratio study of Amaranth and various metal ions in the phosphate buffer pH 7.4 by atomic absorption spectrophotometric technique

Molar ratio, Amaranth: Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.810	0.100	0.110	0.050	0.010
0.10	0.810	0.100	0.110	0.030	0.010
0.20	0.810	0.100	0.110	0.040	0.010
0.30	0.810	0.100	0.095	0.030	0.010
0.50	0.810	0.110	0.100	0.030	0.010
1.00	0.800	0.100	0.100	0.030	0.010

Table 5 -- m Molar ratio study of Amaranth and various metal ions in diethylamine buffer at pH 12.5 by atomic absorption spectrophotometric technique

Molar ratio, Amaranth : Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed.				
	Cd(II)	Hg(II)	Fe(II)	Fe(II)	Pb(II)
0.00	0.470	0.200	0.010	0.010	0.540
0.10	0.470	0.200	0.010	0.010	0.550
0.20	0.470	0.210	0.010	0.010	0.540
0.30	0.480	0.220	0.010	0.010	0.490
0.50	0.480	0.200	0.010	0.010	0.530
1.00	0.480	0.200	0.010	0.010	0.540

Table 5 -- n Molar ratio study of Amaranth and various metal ions in McIlvaine buffer pH 3.4 by atomic absorption spectrophotometric technique

Molar ratio, Amaranth : Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(II)	Pb(II)
0.00	0.800	0.100	0.570	0.570	0.880
0.10	0.810	0.100	0.560	0.570	0.880
0.20	0.800	0.090	0.580	0.560	0.870
0.30	0.820	0.090	0.560	0.560	0.860
0.40	0.810	0.100	0.570	0.570	0.880
0.50	0.800	0.100	0.570	0.570	0.860
1.00	0.800	0.100	0.570	0.570	0.880

Table 5 - q Molar ratio study of Amaranth and various metal ions in McIlvain buffer pH 6.4 by atomic absorption spectrophotometric technique.

Molar ratio, Amaranth :	Absorption of the mixture at the wavelength where the metal ion absorbed				
Metal ion	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.810	0.080	0.580	0.590	0.020
0.10	0.810	0.080	0.580	0.590	0.020
0.20	0.810	0.080	0.570	0.580	0.020
0.30	0.800	0.080	0.580	0.590	0.020
0.40	0.810	0.070	0.570	0.580	0.020
0.50	0.810	0.080	0.570	0.590	0.020
1.00	0.800	0.080	0.570	0.580	0.020

Table 5 - p Molar ratio study of Amaranth and various metal ions in McIlvain buffer pH 7.6 by atomic absorption spectrophotometric technique.

Molar ratio, Amaranth :	Absorption of the mixture at the wavelength where the metal ion absorbed				
Metal ion	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.810	0.070	0.560	0.450	0.020
0.10	0.800	0.060	0.560	0.440	0.020
0.20	0.800	0.070	0.560	0.450	0.020
0.30	0.800	0.080	0.560	0.440	0.020
0.40	0.810	0.070	0.560	0.450	0.020
0.50	0.800	0.060	0.560	0.450	0.020
1.00	0.810	0.070	0.560	0.450	0.020

Table 6 - Molar ratio study of Ponceau 4 R and various metal ions in water by visible spectrophotometric technique

Molar ration; Metal ion : Ponceau 4 R	Absorbance of the mixture at λ_{max} 505 nm				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.690	0.630	0.680	0.680	0.690
1.00	0.690	0.680	0.690	0.690	0.680
2.00	0.680	0.680	0.680	0.680	0.680
3.00	0.690	0.680	0.680	0.690	0.690
4.00	0.690	0.630	0.690	0.680	0.680
5.00	0.680	0.680	0.680	0.680	0.690
10.00	0.690	0.680	0.690	0.680	0.680

Table 6 - b Molar ratio study of ponceau 4 R and various metal ions in the acetic acid pH 3.0 by visible spectrophotometric technique

Molar ratio, Metal ion : Ponceau 4 R	Absorbance of the mixture at λ_{max} = 505 nm				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.650	0.650	0.640	0.640	0.640
1.00	0.640	0.640	0.630	0.630	0.630
2.00	0.650	0.650	0.630	0.620	0.630
3.00	0.660	0.650	0.640	0.640	0.640
4.00	0.650	0.640	0.620	0.630	0.650
5.00	0.640	0.640	0.630	0.6[illegible]0	0.640
10.00	0.650	0.640	0.640	0.640	0.640

Table 6 - c Molar ratio study of Ponceau 4 R and various metal ions in the phosphate buffer pH 6.4 by visible spectrophotometric techique

Molar ratio, Metal ion : Ponceau 4 R	Absorbance of the mixture at λ_{max} = 505 nm				
	(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.65	0.650	0.650	0.660	0.650
1.00	0.640	0.640	0.650	0.650	0.660
2.00	0.650	0.650	0.640	0.650	0.650
3.00	0.650	0.650	0.650	0.660	0.650
4.00	0.640	0.640	0.640	0.650	0.650
5.00	0.660	0.640	0.650	0.650	0.660
10.00	0.650	0.640	0.640	0.660	0.650

Table 6 - d Molar ratio study of Ponceau 4 R and various metal ions in the phosphate buffer pH 7.4 by visible spectrophotometric technique

Molar ratio, Metal ion : Ponceau 4 R	Absorbance of the mixture at λ_{max} = 505 nm				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.680	0.690	0.680	0.690	0.680
1.00	0.670	0.680	0.680	0.680	0.680
2.00	0.680	0.690	0.670	0.680	0.670
3.00	0.680	0.670	0.690	0.670	0.680
4.00	0.680	0.680	0.680	0.680	0.690
5.00	0.680	0.670	0.680	0.690	0.680
10.00	0.680	0.670	0.670	0.680	0.670

Table 6 - e Molar ratio study of Ponceau 4 R and various metal ions in diethylamine buffer pH 12.5 by visible spectrophotometric technique

Molar ratio, Metal ion : Ponceau 4 R	Absorbance of the mixture at λ_{max} = 425 nm				
	Cd(II)	Hg(II)	*Fe(II)	Fe(III)	Pb(II)
0.00	0.690	0.690	0.690	0.690	0.690
1.00	0.680	0.680	0.680	0.690	0.680
2.00	0.670	0.690	0.680	0.680	0.690
3.00	0.680	0.690	0.690	0.680	0.680
4.00	0.690	0.680	0.690	0.690	0.690
5.00	0.680	0.690	0.680	0.690	0.690
10.00	0.680	0.690	0.680	0.680	0.680

*KCl + NaOH buffer was used instead since the mixture in the diethylamine buffer pH 12.5 had been done(7)

Table 6 - f Molar ratio study of Ponceau 4 R and various metal ions at pH 3.4 McIlvaine buffer by visible spectrophotometric techique

Molar ratio, Metal ion : Ponceau 4 R	Absorbance of the mixture at λ_{max} =505 nm				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.640	0.630	0.610	0.630	0.610
1.00	0.640	0.630	0.610	0.630	0.600
2.00	0.640	0.630	0.600	0.630	0.620
3.00	0.640	0.630	0.600	0.620	0.610
4.00	0.640	0.630	0.610	0.630	0.600
5.00	0.640	0.630	0.610	0.630	0.610
10.00	0.640	0.630	0.620	0.620	0.620

Table 6 - g Molar ratio study of Ponceau 4 R and various metal ion in McIlvaine buffer pH 6.4 by visible spectrophotrometric technique

Molar ratio, Metal ion: Ponceau 4 R	Absorbance of the mixture at λ_{max} = 505 nm				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.820	0.860	0.860	0.860	0.810
1.00	0.830	0.860	0.860	0.860	0.820
2.00	0.830	0.860	0.860	0.860	0.810
3.00	0.830	0.860	0.850	0.850	0.820
4.00	0.820	0.870	0.870	0.860	0.810
5.00	0.830	0.870	0.860	0.860	0.810
10.00	0.820	0.870	0.860	0.860	0.810

Table 6 - h Molar ratio study of Ponceau 4 R and various metal ions at in McIlvaine buffer pH 7.6 by visible spectrophotometric technique

Molar ratio, Metal ion: Ponceau 4 R	Absorbance of the mixture at λ_{max} = 505 nm				
	Cd (II)	Hg(II)	Fe(II)	**Fe(III)**	Pb(II)
0.00	0.570	0.630	0.630	0.570	0.570
1.00	0.570	0.630	0.620	0.560	0.580
2.00	0.580	0.630	0.620	0.560	0.570
3.00	0.580	0.640	0.620	0.570	0.560
4.00	0.580	0.640	0.630	0.560	0.570
5.00	0.580	0.630	0.620	0.560	0.590
10.00	0.580	0.620	0.630	0.560	0.570

Table 6 - i Molar ratio study of Ponceau 4 R and various metal ions in water by atomic absorption spectrophotometric technique

Molar ratio, Ponceau 4 R :Metal ion	Absorbance of the mixture at the wavelength Where the metal ion. absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.850	0.350	0.900	0.890	0.620
0.10	0.860	0.360	0.900	0.890	0.620
0.20	0.860	0.350	0.890	0.900	0.620
0.30	0.850	0.360	0.900	0.890	0.620
0.50	0.870	0.360	0.890	0.900	0.620
1.00	0.860	0.350	0.900	0.890	0.620

Table 6 - j Molar ratio study of Ponceau 4 R and various metal ions in acetic acid at pH 3.0 by atomic absoption spectrophotometric technique

Molar ratio, Ponceau 4 R :Metal ion	Absorption of the mixture at the wavelength Where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.870	0.300	0.840	0.500	0.620
0.10	0.870	0.300	0.840	0.500	0.630
0.20	0.870	0.300	0.850	0.500	0.520
0.30	0.870	0.300	0.840	0.500	0.630
0.50	0.870	0.300	0.840	0.510	0.630
1.00	0.870	0.300	0.840	0.500	0.620

Table 6 - k Molar ratio study of Ponceau 4 R and various metal ions in the phosphate buffer at pH 6.4 by atomic absorption spectrophotometric technique

Molar ratio, Ponceau 4 R : Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.840	0.230	0.020	0.090	0.070
0.10	0.840	0.230	0.020	0.090	0.070
0.20	0.850	0.230	0.020	0.090	0.070
0.30	0.850	0.230	0.020	0.090	0.070
0.50	0.850	0.240	0.020	0.090	0.070
1.0	0.840	0.230	0.020	0.090	0.070

Table 6 - 1 Molar ratio study of Ponceau 4 R and various metal ions in the phosphate buffer at pH 7.4 by atomic absorption spectrophotometric technique

Molar ratio, Ponceau 4 R : Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.820	0.110	0.020	0.040	0.004
0.10	0.810	0.110	0.020	0.040	0.004
0.20	0.810	0.110	0.020	0.040	0.004
0.30	0.820	0.100	0.020	0.040	0.004
0.50	0.810	0.110	0.020	0.040	0.004
1.0	0.820	0.110	0.020	0.040	0.004

Table 6 - m Molar ratio study of Ponceau 4 R and various metal ions in diethylamine buffer pH 12.5 by atomic absorption spectrophotometric technique

Molar ratio, Ponceau 4 R : Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	*Fe(II)	Fe(III)	Pb(II)
0.00	0.500	0.020	0.020	0.010	0.560
0.10	0.500	0.020	0.020	0.010	0.550
0.20	0.500	0.020	0.020	0.010	0.590
0.30	0.500	0.020	0.020	0.010	0.540
0.50	0.500	0.020	0.020	0.010	0.550
1.00	0.500	0.020	0.020	0.010	0.540

*KCl + NaOH buffer was used instead since the mixture in the diethylamine buffer pH 12.5 had been done(7)

Table 6 - n Molar ratio study of Ponceau 4 R and various metal ion in McIlvain buffer pH 3.4 by atomic absorption spectrophotometric technique

Molar ratio, Ponceau 4 R : Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.810	0.080	0.530	0.560	0.860
0.10	0.800	0.090	0.530	0.550	0.860
0.20	0.800	0.080	0.520	0.560	0.870
0.30	0.800	0.080	0.530	0.570	0.860
0.50	0.800	0.080	0.530	0.570	0.850
1.00	0.790	0.080	0.520	0.570	0.860

Table 6 - o Molar ratio study of Ponceau 4 R and various metal ions in McIlvaine buffer pH 6.4 by atomic absorption spectrophotometric technique

Molar ratio, Ponceau 4 R : Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed.				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.810	0.080	0.570	0.590	0.030
0.10	0.800	0.080	0.570	0.580	0.030
0.20	0.810	0.070	0.560	0.570	0.030
0.30	0.800	0.070	0.570	0.580	0.030
0.50	0.810	0.080	0.570	0.590	0.030
1.00	0.810	0.080	0.560	0.580	0.030

Table 6 - p Molar ratio study of Ponceau 4 R and various metal ions in the phosphate buffer at pH 7.6 by atomic absorption spectrophotometric technique

Molar ratio, Ponceau 4 R : Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.800	0.070	0.560	0.450	0.000
0.10	0.800	0.080	0.560	0.450	0.000
0.20	0.810	0.070	0.570	0.440	0.000
0.30	0.800	0.070	0.560	0.450	0.000
0.40	0.800	0.070	0.510	0.440	0.000
0.50	0.810	0.080	0.570	0.450	0.000
1.00	0.800	0.070	0.560	0.440	0.000

Table 7 - a Molar ratio study of Erythrosine and metal **ions** in acetic **acid pH** 3.0 by visible spectrophotometric technique

Molar ratio, Metal **ion** : Erythrosine	Absorbance of the mixture at λ_{max} = 525 nm				
	Cd (II)	Hg (II)	Fe (II)	Fe(III)	Pb (II)
0.00	0.170	0.160	0.170	0.150	0.160
1.00	0.170	0.170	0.180	0.140	0.170
2.00	0.170	0.160	0.180	0.150	0.160
3.00	0.170	0.170	0.170	0.150	0.160
4.00	0.170	0.160	0.170	0.150	0.170
5.00	0.170	0.160	0.130	0.140	0.160
10.00	0.170	0.170	0.170	0.150	0.160

Table 7 - b Molar ratio study of Erythrosine and various metal ions in the **phosphate** buffer pH 6.4 by visible spectrophotometric tecnique

Molar ratio, Metal **ion** : Erythrosine	Absorbance of the mixture at λ_{max} = 525 nm				
	Cd (II)	Hg (II)	Fe (II)	Fe(III)	Pb (II)
0.00	1.540	1.550	1.540	1.550	1.550
1.00	1.540	1.530	1.530	1.520	1.540
2.00	1.530	1.530	1.530	1.540	1.550
3.00	1.540	1.550	1.520	1.530	1.550
4.00	1.540	1.550	1.510	1.530	1.540
5.00	1.540	1.540	1.520	1.520	1.550
10.00	1.540	1.530	1.510	1.530	1.530

Table 7 - c Molar ratio study of Erythrosine and various metal ions in the phosphate buffer pH 7,4 by visible spectrophotometric technique

Molar ratio, Metal ion : Erythrosine	Absorbance of the mixture at λ_{max} = 525 nm				
	Cd (II)	Hg (II)	Fe (II)	Fe (III)	Pb (II)
0.00	1.520	1.510	1.510	1.540	1.540
1.00	1.520	1.500	1.500	1.540	1.550
2.00	1.520	1.510	1.500	1.540	1.530
3.00	1.510	1.520	1.510	1.520	1.530
4.00	1.510	1.510	1.500	1.540	1.540
5.00	1.520	1.500	1.470	1.520	1.500
10.00	1.510	1.500	1.500	1.530	1.520

Table 7 - d Molar ratio study of Erythrosine and various metal ions in KCl + NaOH buffer pH 12.5 by visible spectrophotometric technique

Molar ratio, Metal ion : Erythrosine	Absorbance of the mixture at λ_{max} = 525 nm				
	Cd (II)	Hg (II)	Fe (II)	Fe(III)	Pb (II)
0.00	1.240	1.250	1.260	1.240	1.260
1.00	1.230	1.260	1.270	1.240	1.250
2.00	1.240	1.250	1.270	1.250	1.260
3.00	1.230	1.260	1.260	1.250	1.250
4.00	1.220	1.240	1.270	1.250	1.250
5.00	1.240	1.250	1.260	1.240	1.260
10.00	1.230	1.250	1.270	1.260	1.250

Table 7 - e Molar ratio study of Erythrosine and various metal ions in McIlvain buffer pH 3.4 by visible spectrophotometric technique

Molar ratio, Metal ion : Erythrosine	Absorbance of the mixture at λ_{max} = 525 nm				
	Cd (II)	Hg(II)	Fe (II)	Fe(III)	Pb (II)
0.00	0.020	0.020	0.019	0.020	0.020
1.00	0.020	0.020	0.013	0.020	0.020
2.00	0.019	0.019	0.017	0.019	0.020
3.00	0.020	0.020	0.011	0.020	0.020
4.00	0.020	0.020	0.011	0.019	0.020
5.00	0.020	0.020	0.011	0.020	0.020
10.00	0.020	0.020	0.011	0.020	0.020

Table 7 - f Molar ratio study of Erythrosine and various metal ions in McIlvain buffer pH 6.4 by visible spectrophotometric technique

Molar ratio, Metal ion : Erythrosine	Absorbance of the mixture at λ_{max} = 525 nm				
	Cd (II)	Hg (II)	Fe (II)	Fe(III)	Pb (II)
0.00	1.440	1.420	1.410	1.450	0.980
1.00	1.430	1.420	1.410	1.450	0.980
2.00	1.440	1.420	1.410	1.450	0.980
3.00	1.440	1.420	1.410	1.440	0.960
4.00	1.440	1.410	1.400	1.460	0.980
5.00	1.450	1.420	1.400	1.450	0.970
10.00	1.440	1.420	1.410	1.450	0.970

Table 7 - g Molar ratio study of Erythrosine and various metal ions in McIlvain buffer pH 7.6 by visible spectrophotometric technique

Molar ratio, Metal ion : Erythrosine	Absorbance of the mixture at λ_{max} = 525 nm				
	Cd (II)	Hg (II)	Fe (II)	Fe(III)	Pb (II)
0.00	0.340	0.340	0.340	0.340	0.340
1.00	0.350	0.340	0.350	0.330	0.340
2.00	0.340	0.350	0.340	0.340	0.350
3.00	0.340	0.340	0.340	0.330	0.340
4.00	0.350	0.350	0.340	0.340	0.340
5.00	0.340	0.340	0.350	0.330	0.340
10.00	0.340	0.350	0.340	0.320	0.350

Table 7 - h Molar ratio study of Erythrosine and various metal ions in acetic acid at pH 3.0 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine: Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.840	0.220	0.930	0.520	0.620
0.10	0.850	0.220	0.930	0.510	0.630
0.20	0.850	0.230	0.930	0.510	0.600
0.30	0.850	0.240	0.930	0.520	0.610
0.50	0.850	0.230	0.930	0.520	0.600
1.00	0.850	0.220	0.930	0.510	0.600

Table 7 - i Molar ratio study of Erythrosine and various metal ion in the phosphate buffer pH 6.4 by atomic absorption spectrephotometric technique

Molar ratio, Erythrosine: Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.850	0.280	0.030	0.000	0.070
0.10	0.840	0.280	0.030	0.000	0.050
0.20	0.840	0.280	0.030	0.000	0.050
0.30	0.840	0.280	0.030	0.000	0.070
0.50	0.850	0.280	0.030	0.000	0.050
1.00	0.840	0.280	0.030	0.000	0.050

Table 7 - j Molar ratio study of Erythrosine and various metal ions in the phosphate buffer pH 7.4 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine: Metal ion	Absorption of the mixture at the wavelength where the metal ion absorbed.				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.820	0.110	0.100	0.030	0.010
0.10	0.820	0.110	0.100	0.030	0.010
0.20	0.830	0.110	0.090	0.030	0.010
0.30	0.820	0.110	0.090	0.030	0.010
0.50	0.830	0.110	0.090	0.030	0.010
1.00	0.830	0.110	0.090	0.030	0.010

Table 7 - k Molar ratio study of Erythrosine and various metal ions in KCl + NaOH buffer at pH 12.5 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine : Metal ion	Absorption of the mixture at wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.490	0.020	0.020	0.010	0.560
0.10	0.490	0.020	0.020	0.010	0.560
0.20	0.480	0.020	0.020	0.010	0.560
0.30	0.490	0.020	0.020	0.010	0.560
0.50	0.480	0.020	0.020	0.010	0.560
1.00	0.480	0.020	0.020	0.010	0.560

Table 7 - 1 Molar ratio study of Erythrosine and various metal ions in McIlvaine buffer pH 3.4 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine : Metal ion	Absorption of the mixture at wavelength where the metal ion absorbed.				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.790	0.100	0.570	0.570	0.870
0.10	0.790	0.100	0.570	0.560	0.870
0.20	0.800	0.100	0.570	0.570	0.870
0.30	0.790	0.100	0.560	0.560	0.870
0.50	0.800	0.100	0.560	0.560	0.860
1.00	0.800	0.100	0.560	0.570	0.860

Table 7 - m Molar ratio study of Erythrosine and various metal ions in McIlvaine buffer pH 6.4 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine : Metal ion	Absorption of the mixture at wavelength where the metal ion absorbed				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.810	0.080	0.580	0.590	0.020
0.10	0.810	0.080	0.580	0.580	0.020
0.20	0.810	0.080	0.580	0.590	0.020
0.30	0.800	0.070	0.580	0.580	0.020
0.50	0.800	0.070	0.570	0.580	0.020
1.00	0.800	0.070	0.570	0.590	0.020

Table 7 - n Molar ratio study of Erythrosine and various metal ions in McIlvaine buffer pH 7.6 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine : Metal ion	Absorption of the mixture at wavelength where the metal ion absorbed.				
	Cd(II)	Hg(II)	Fe(II)	Fe(III)	Pb(II)
0.00	0.800	0.020	0.450	0.560	0.050
0.10	0.810	0.020	0.460	0.570	0.050
0.20	0.800	0.020	0.480	0.560	0.050
0.30	0.810	0.020	0.450	0.560	0.060
0.50	0.800	0.020	0.450	0.570	0.070
1.00	0.800	0.020	0.050	0.560	0.050

Table 8 Molar ratio study of Erythrosine and Cd (II) ion in water by visible spectrophotometric method

Molar ratio, Cd(II) : Erythrosine	Absorbance of the mixture at λ_{max} 525 nm
0.00	1.510
0.50	1.510
1.00	1.500
1.50	1.510
2.00	1.500
2.50	1.510
3.00	1.500
4.00	1.510
5.00	1.520
6.00	1.510
10.00	1.500

Table 9-a Molar ratio study of Erythrosine and Hg(II) ion in nitric acid (pH 6.8-3.1) by visible spectrophotometric technique

Mole ratio, Hg(II) : Erythrosine	Absorbance at 525 nm
0.00	1.510
0.20	1.390
0.40	1.100
0.60	0.740
0.80	0.260
1.00	0.060
1.20	0.010
1.40	0.010
1.60	0.010
1.80	0.010
2.00	0.000
3.00	0.000
4.00	0.000
5.00	0.000
6.00	0.000

Table 9-b Molar ratio study of Erythrosine and Hg (II) ion in nitric acid (pH 6.2-4.2) by atomic absorption spectrophotometric technique

Mole ratio, Erythrosine : Hg(II)	Absorbance at 253.7 nm
0.12	0.100
0.24	0.093
0.36	0.086
0.48	0.078
0.60	0.069
0.72	0.065
0.84	0.056
0.96	0.047
1.08	0.053
1.20	0.064
1.30	0.073
1.40	0.081

Table 9-c Molar ratio study of Erythrosine and Hg (II) ion in the nitric acid pH 4.3 by visible spectrophotometric technique

Molar ratio, Hg(II) : Erythrosine	Absorbance at 525 nm
0.00	0.797
0.20	0.738
0.40	0.537
0.60	0.363
0.80	0.269
1.00	0.256
1.50	0.260
2.00	0.043
2.50	0.017
3.00	0.007
3.50	0.005
4.00	0.003
4.50	0.003
5.00	0.000
6.00	0.000
7.00	0.000
8.00	0.000

Table 9-d Molar ratio study of Erythrosine and Hg (II) ion in the nitric acid pH 4.3 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine : Hg(II)	Absorbance at 253.7 nm
0.00	0.137
0.24	0.120
0.48	0.103
0.60	0.098
0.84	0.080
0.96	0.071
1.08	0.073
1.20	0.088
1.40	0.082
1.50	0.084
1.60	0.077
1.70	0.087
1.80	0.089
2.00	0.094
2.20	0.094
2.40	0.100

Table 9-e Molar ratio study of Erythrosine and Hg (II) ion in the acetate buffer pH 4.4 by visible spectrophotometric technique

Molar ratio, Hg(II) : Erythrosine	Absorbance at 525 nm
0.00	0.820
0.20	0.667
0.40	0.654
0.60	0.533
0.80	0.351
1.00	0.335
1.20	0.323
1.40	0.308
1.60	0.308
1.80	0.320
2.00	0.280
3.00	0.210
4.00	0.120
5.00	0.098
6.00	0.086

Table 9-f Molar ratio study of Erythrosine and Hg (II) ion in the acetate buffer pH 4.4 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine : Hg(II)	Absorbance at 253.7 nm
0.10	0.066
0.20	0.062
0.30	0.059
0.40	0.055
0.50	0.052
0.60	0.048
0.70	0.045
0.80	0.042
0.90	0.039
1.00	0.035
1.20	0.036
1.50	0.038
2.00	0.040

Table 9-g Molar ratio study of Erythrosine and Hg (II) ion in the acetate buffer pH 6.0 by visible spectrophotometric technique

Molar ratio, Hg(II) : Erythrosine	Absorbance at 525 nm
0.00	1.027
0.20	0.971
0.60	0.950
0.80	0.949
1.00	0.884
1.50	0.869
2.00	0.861
2.50	0.837
3.00	0.821
3.50	0.782
4.00	0.750
4.50	0.711
5.00	0.711
6.00	0.695

Table 9-h Molar ratio study of Erythrosine and Hg (II) ion in the acetate buffer pH 6.0 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine : Hg(II)	Absorbance at 253.7 nm
0.00	0.190
0.35	0.130
0.48	0.115
0.70	0.085
0.84	0.065
1.20	0.048
1.40	0.056
1.80	0.080
2.00	0.090

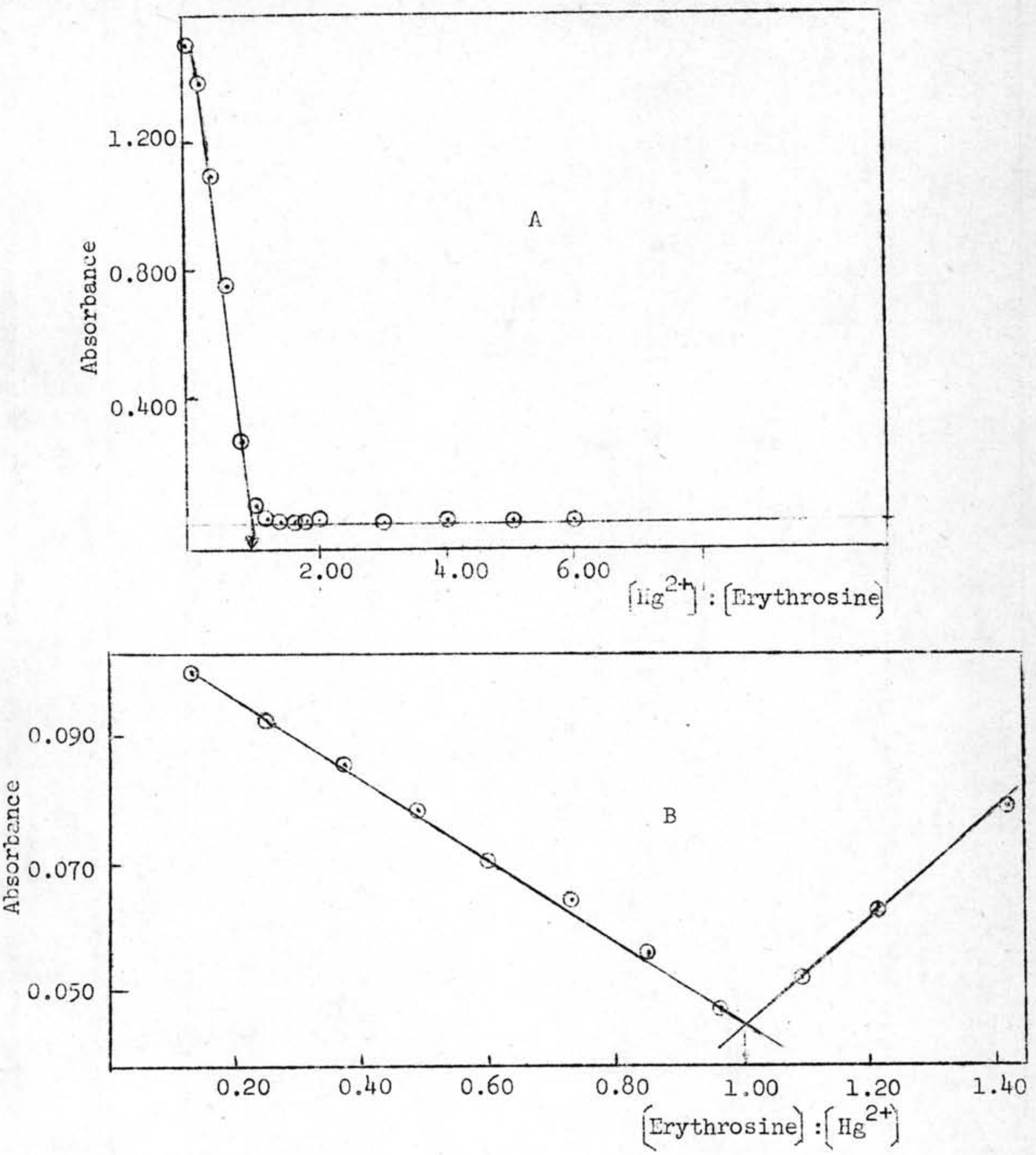

Figure 8A Molar ratio plots for systems in nitric acid; (pH 6.8-3.1)
A) 2.00×10^{-5}M Erythrosine and various concentrations of Hg(II) ion by visible spectrophotometric method and
B) 1.00×10^{-3}M Hg(II) ion and various concentrations of Erythrosine by atomic absorption spectrophotometric method

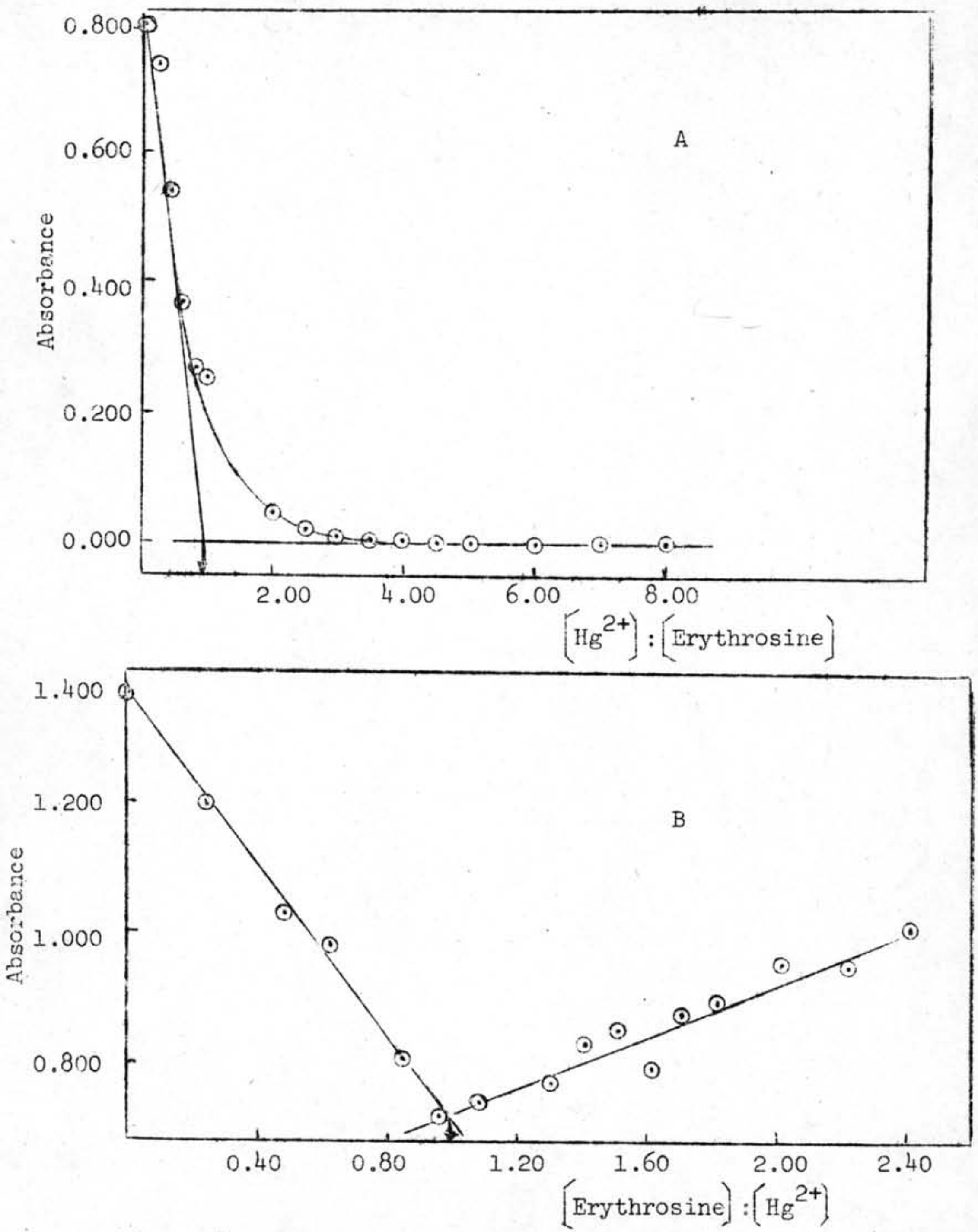

Figure 8B Molar ratio plots for systems in the nitric acid pH 4.3; A) 2.00 × 10^{-5}M Erythrosine and various concentrations of Hg(II) ion by visible spectrophotometric method and B) 1.00 × 10^{-3}M Hg(II) ion and various concentrations of Erythrosine by atomic absorption spectrophotometric method.

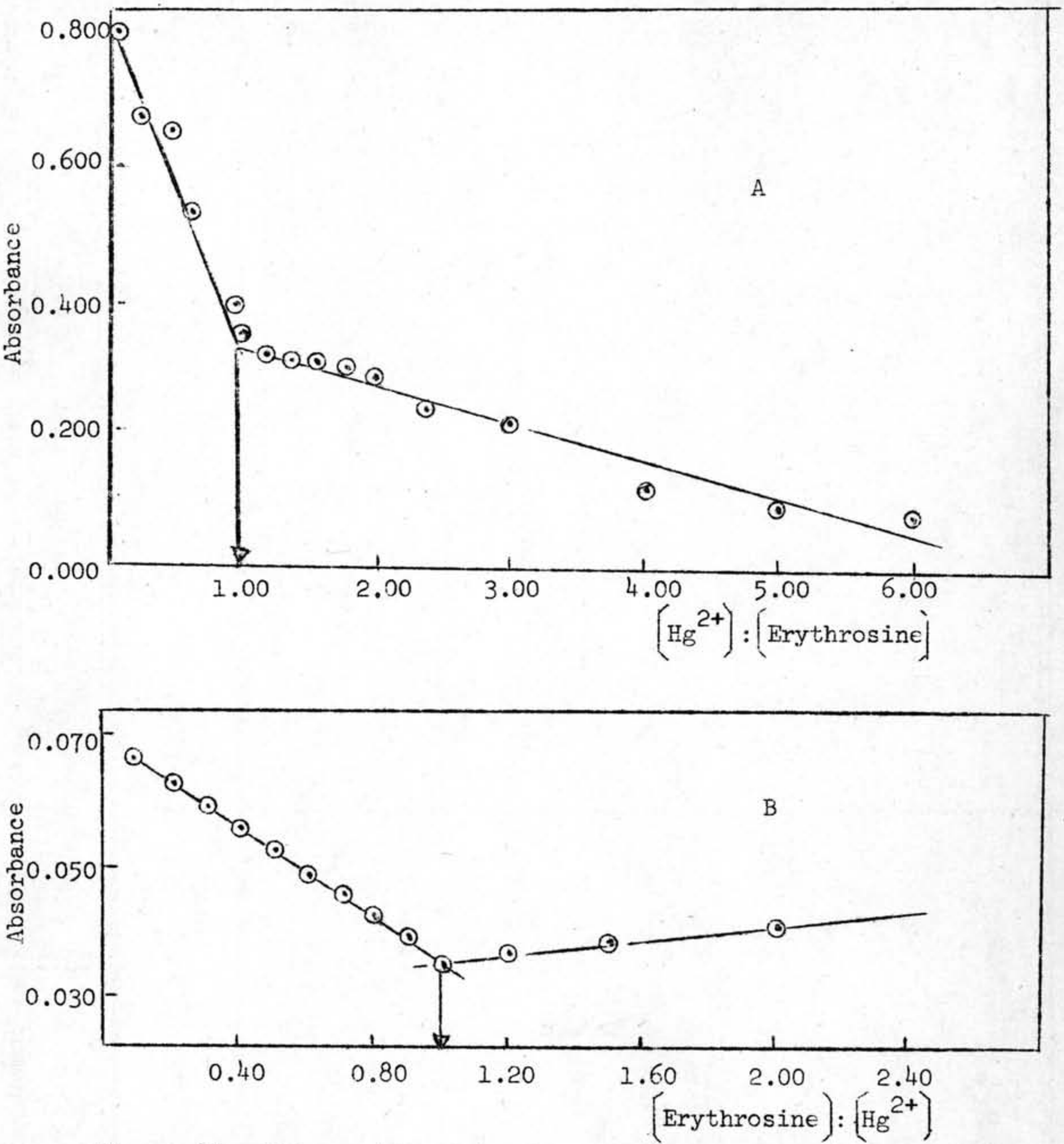

Figure 8C Molar ratio plots for systems in the acetate buffer pH 4.4; A) 2.00×10^{-5}M Erythrosine and various concentrations of Hg(II) ion by visible spectrophotometric method and B) 1.00×10^{-3}M Hg(II) ion and various concentrations of Erythrosine by atomic absorption spectrophotometric method

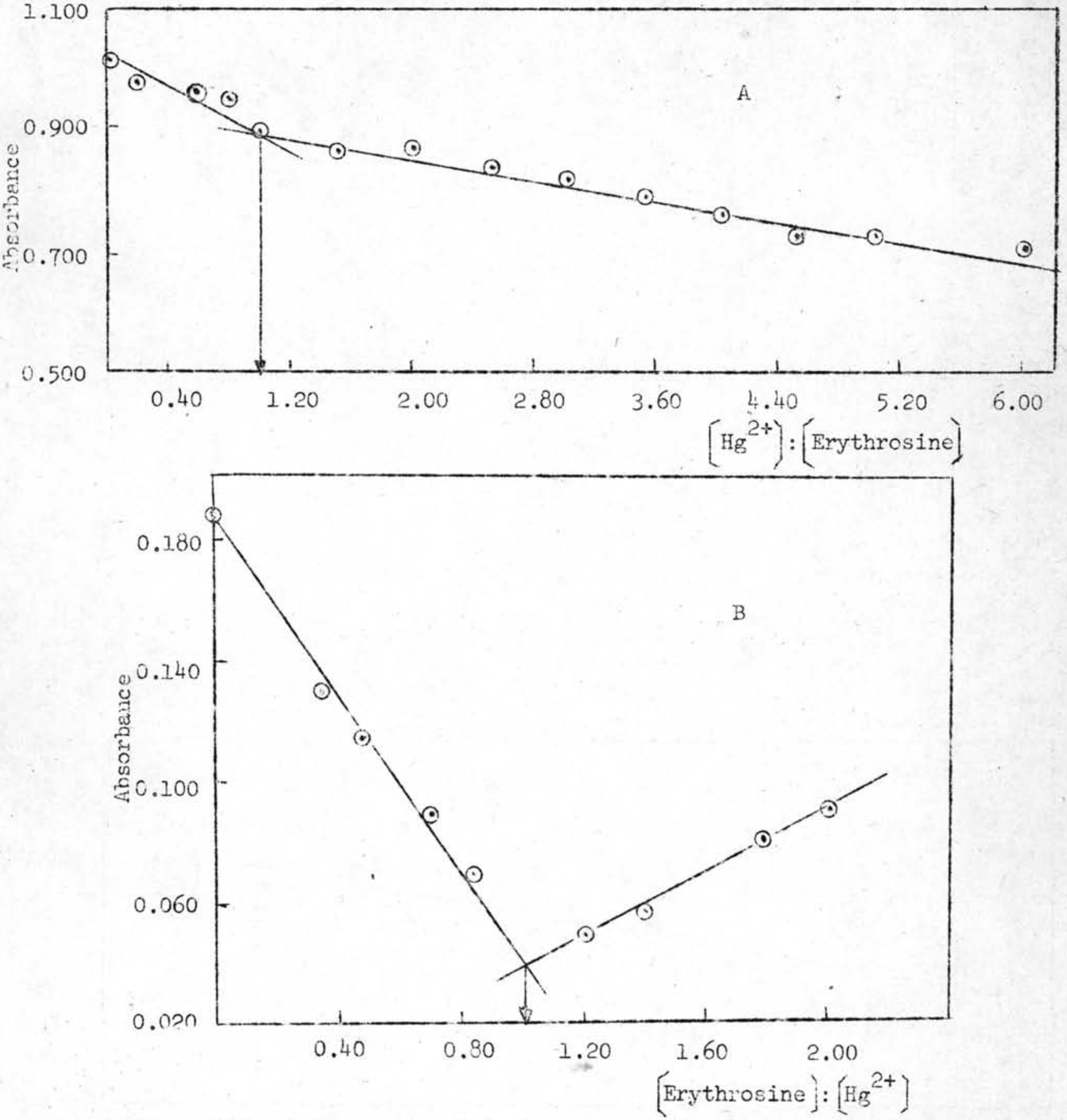

Figure 8D Molar ratio plots for systems in the acetate buffer pH 6.0; A) 2.00×10^{-5}M Erythrosine and various concentration of Hg(II) ion by visible spectrophotometric method and B) 1.0×10^{-3}M Hg(II) ion and various concentrations of Erythrosine by atomic absorption spectrophotometric method

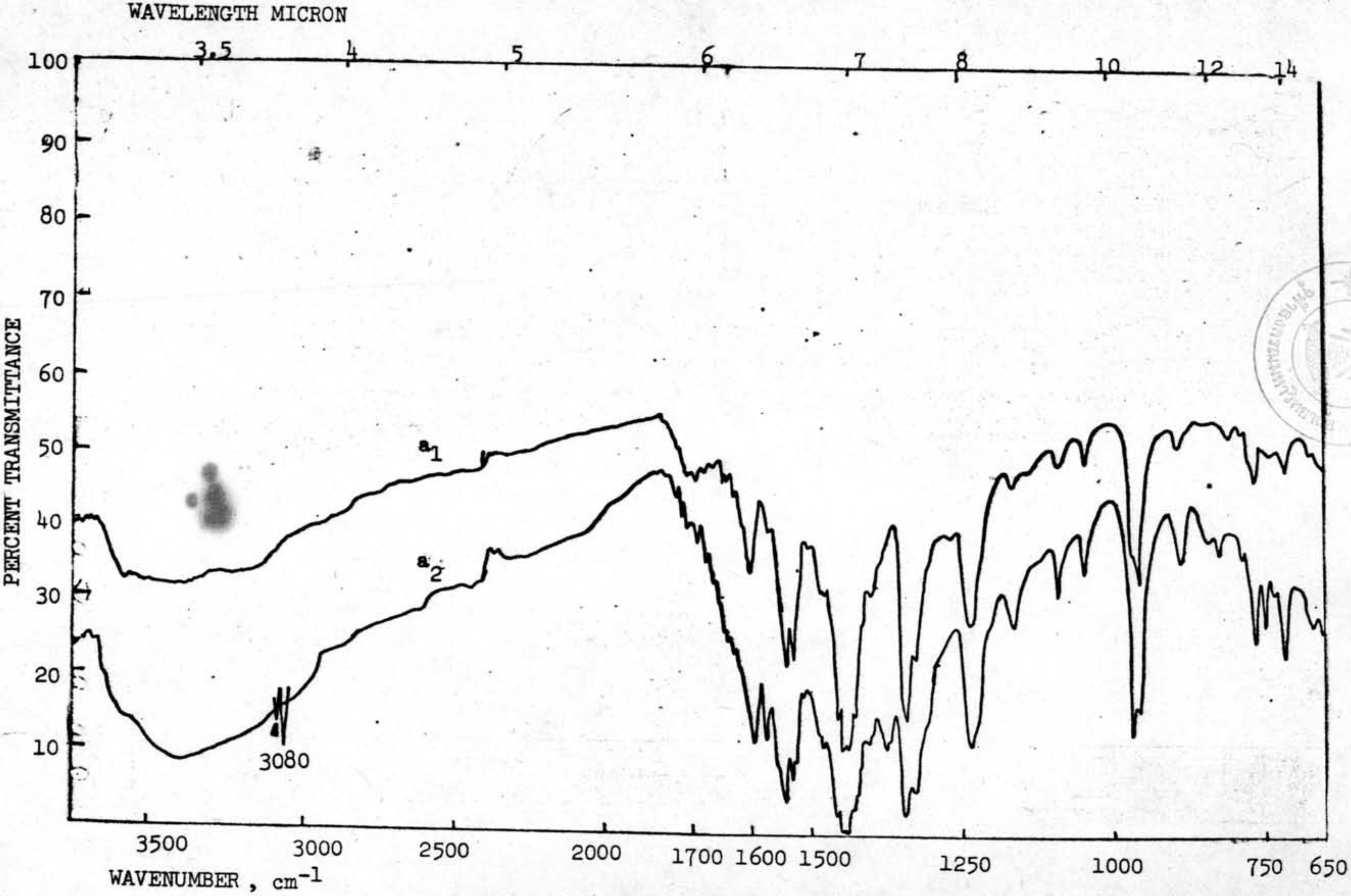

Figure 8E Comparisidon of IR spectra between a_1) Erythrosine a_2) Hg(II) Erythrosinate

Table 10-a Molar ratio study of Erythrosine and Fe (III) ion in sulphuric acid (pH 6.8-3.3) by visible spectrophotometric technique

Molar ratio, Fe(III) : Erythrosine	Absorbance at 525 nm
0.00	1.540
0.20	1.500
0.40	1.300
0.60	0.720
0.80	0.420
1.00	0.160
1.50	0.120
2.00	0.080
3.00	0.040
4.00	0.020
5.00	0.020
8.00	0.020

Table 10-b Molar ratio study of Erythrosine and Fe (III) ion in the sulphuric acid pH 3.3 by visible spectrophotometric technique

Molar ratio, Fe(III) : Erythrosine	Absorbance at 525 nm
0.00	0.100
0.20	0.096
0.40	0.094
0.60	0.075
0.80	0.031
1.00	0.015
1.50	0.015
2.00	0.015
2.50	0.015
3.00	0.011
3.50	0.011
4.00	0.011
5.00	0.010
6.00	0.010

Table 10-c Molar ratio study of Erythrosine and Fe (III) ion in the sulphuric acid pH 3.3 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine : Fe(III)	Absorbance at 248.3 nm
0.00	0.061
0.40	0.420
0.80	0.330
1.00	0.320
1.40	0.300
1.60	0.260
1.80	0.240
2.00	0.190
3.00	0.140
4.00	0.200
5.00	0.140
7.00	0.110

Table 10-d Molar ratio study of Erythrosine and Fe(III) ion in sulphuric acid pH 5.5 - 3.3 by visible spectrophotometric technique

Molar ratio, Fe (III) : Erythrosine	Absorbance at 525 nm
0.00	1.260
0.20	1.058
0.40	0.630
0.60	0.510
0.80	0.180
1.00	0.120
1.50	0.094
2.00	0.050
3.00	0.030
4.00	0.012
5.00	0.012

Table 10-e Molar ratio study of Erythrosine and Fe (III) ion in the nitric acid pH 3.3 by visible spectrophotometric technique

Molar ratio Fe(III) : Erythrosine	Absorbance at 525 nm
0.00	0.123
0.20	0.123
0.40	0.111
0.60	0.091
0.80	0.083
1.00	0.039
1.50	0.028
2.00	0.024
3.00	0.008

Table 10-f Molar ratio study of Erythrosine and Fe (III) ion at in the nitric acid pH 3.3 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine : Fe(III)	Absorbance at 248.3 nm
0.00	0.790
0.20	0.600
0.40	0.650
0.60	0.490
0.80	0.380
1.00	0.330
1.20	0.260
1.40	0.220
1.60	0.200
1.80	0.180
2.00	0.140

Table 10-g Molar ratio study of Erythrosine and Fe (III) ion in the acetate buffer pH 4.4 by visible spectrophotometric technique

Molar ratio, Fe(III) : Erythrosine	Absorbance at 525 nm
0.00	0.841
0.30	0.746
0.60	0.695
0.90	0.639
1.50	0.564
2.50	0.505
3.00	0.580
3.50	0.517
4.00	0.560
5.00	0.525
6.00	0.517

Table 10-h Molar ratio study of Erythrosine and Fe (III) ion in the acetate buffer pH 4.4 by visible spectrophotometric technique

Molar ratio, Fe(III) : Erythrosine	Absorbance at 525 nm
0.10	1.700
0.20	1.524
0.40	0.718
0.80	0.521
1.00	0.450
1.50	0.268
2.00	0.244
2.50	0.205
3.00	0.150
4.00	0.110

Table 10-i Molar ratio study of Erythrosine and Fe (III) ion in the acetate buffer pH 6.0 by visible spectrophotometric technique

Molar ratio, Fe(III) : Erythrosine	Absorbance at 525 nm
0.00	1.040
0.30	1.010
0.60	0.980
1.00	0.960
1.20	0.910
1.50	0.905
2.00	0.875
2.50	0.870
3.00	0.900
3.50	0.880
4.00	0.900
5.00	0.840
6.00	0.800

Table 10-j Molar ratio study of Erythrosine and Fe (III) ion in the acetate buffer pH 6.0 by visible spectrophotometric technique

Molar ratio, Fe(III) : Erythrosine	Absorbance at 525 nm
0.10	2.300
0.20	2.200
0.60	1.510
0.80	0.750
1.00	0.630
1.50	0.390
2.00	0.280
2.50	0.250
3.00	0.190
4.00	0.130

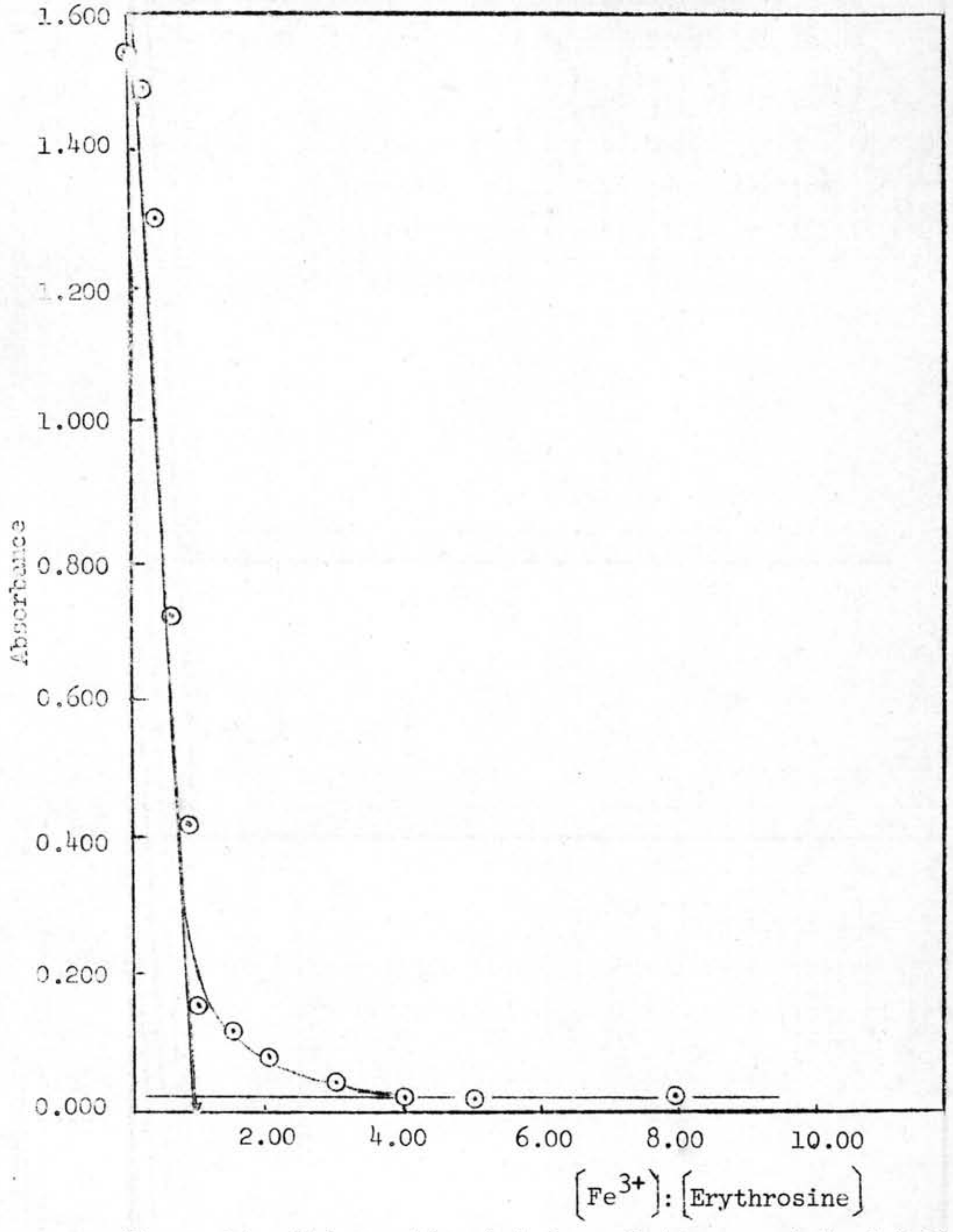

Figure 9A Molar ratio plot for solutions contained 2.00 × 10^{-5}M Erythrosine and various concentrations of Fe(III) ion in sulfuric acid (pH 6.8-3.3) by visible spectrophotometric method

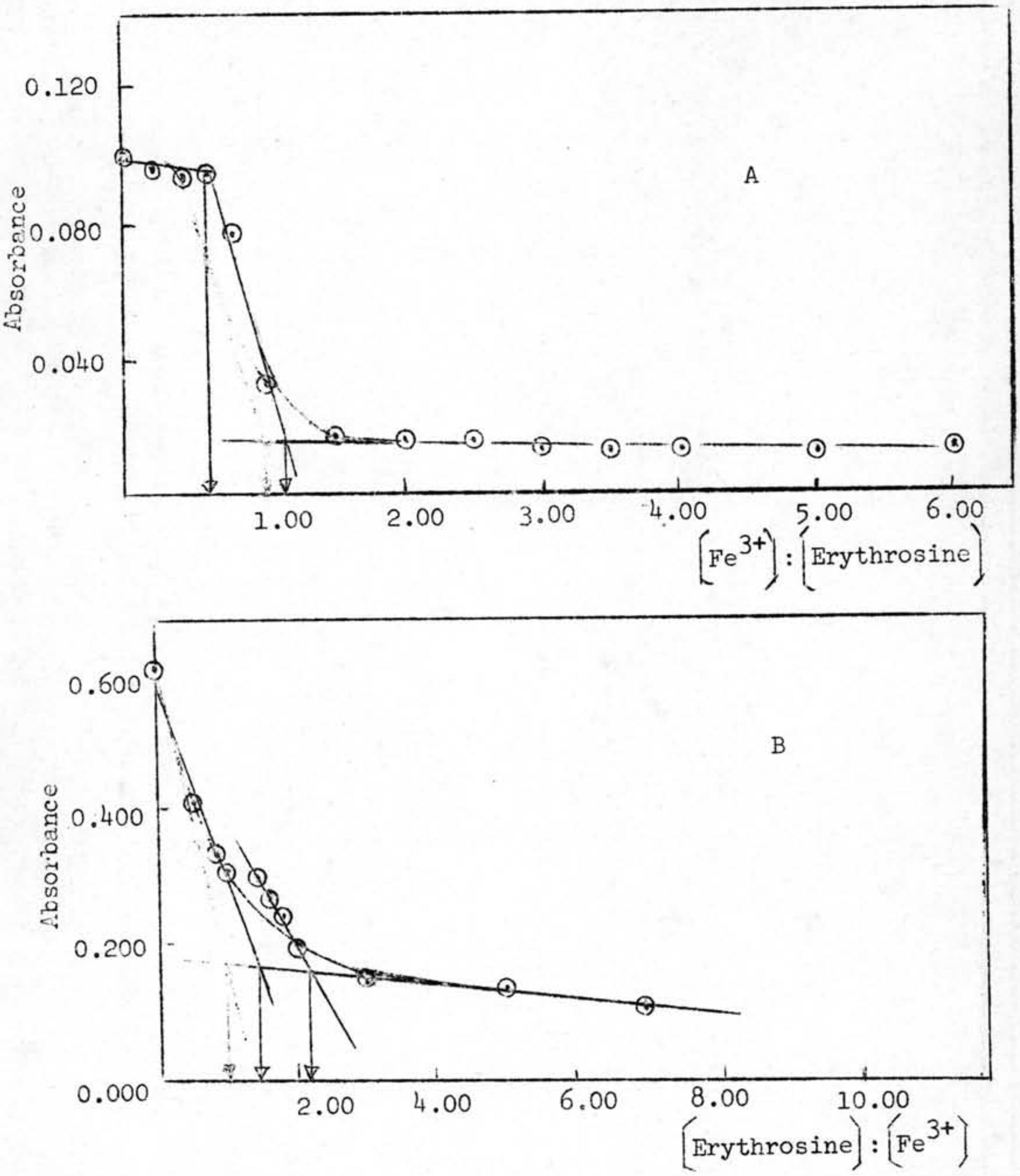

Figure 9B Molar ratio plots for systems in the sulfuric acid pH 3.3; A) 2.00×10^{-5}M Erythrosine and various concentrations of Fe(III) ion by visible spectrophotometric method and B) 6.00×10^{-4}M Fe(III) ion and various concentrations of Erythrosine by atomic absorption spectrophotometric method

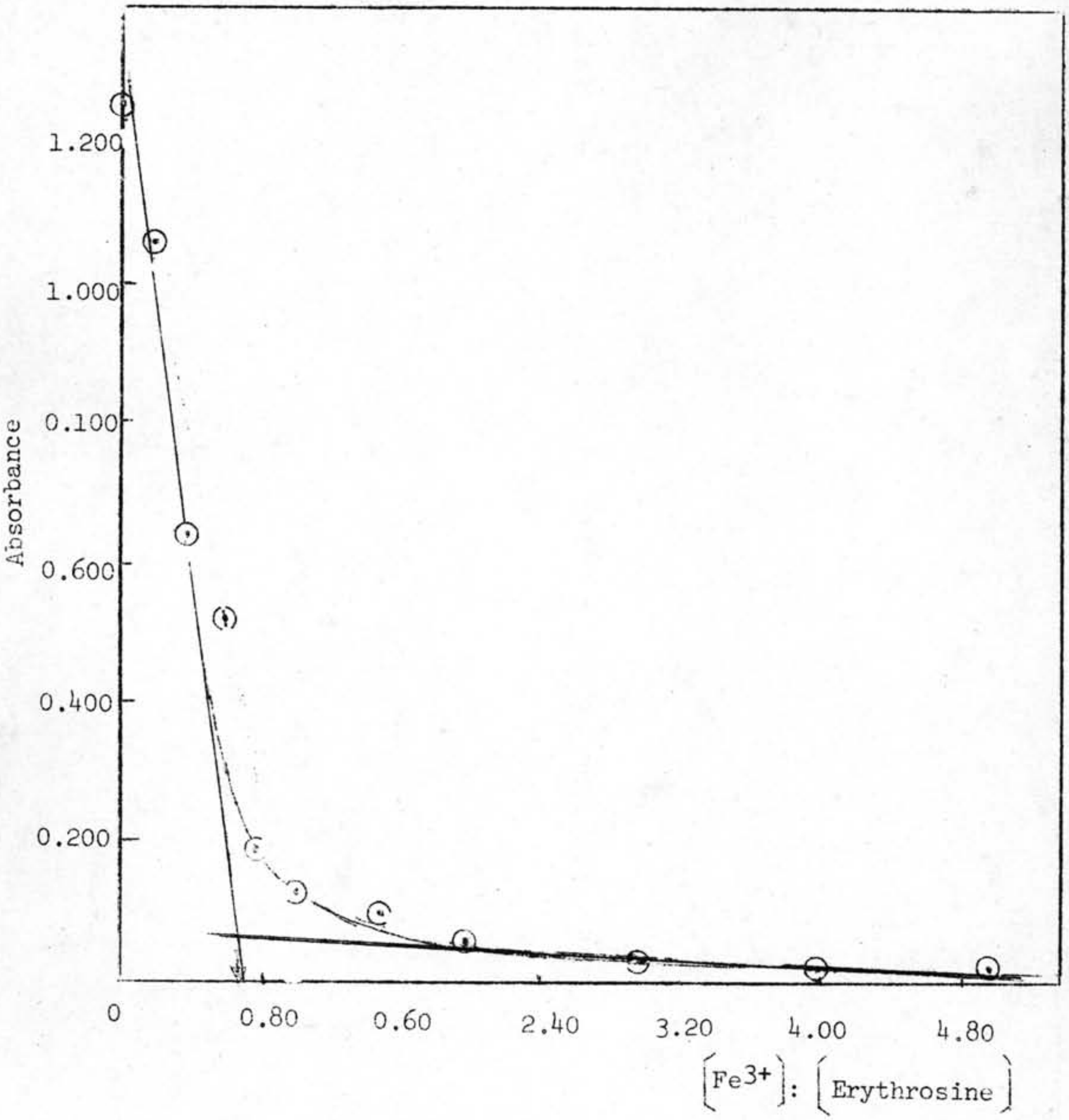

Figure 9C Molar ratio plot for solutions contained 2.00×10^{-5}M Erythrosine and various concentrations of Fe(III) ion in nitric acid (pH 3.5-5.5) by visible spectrophotometric method

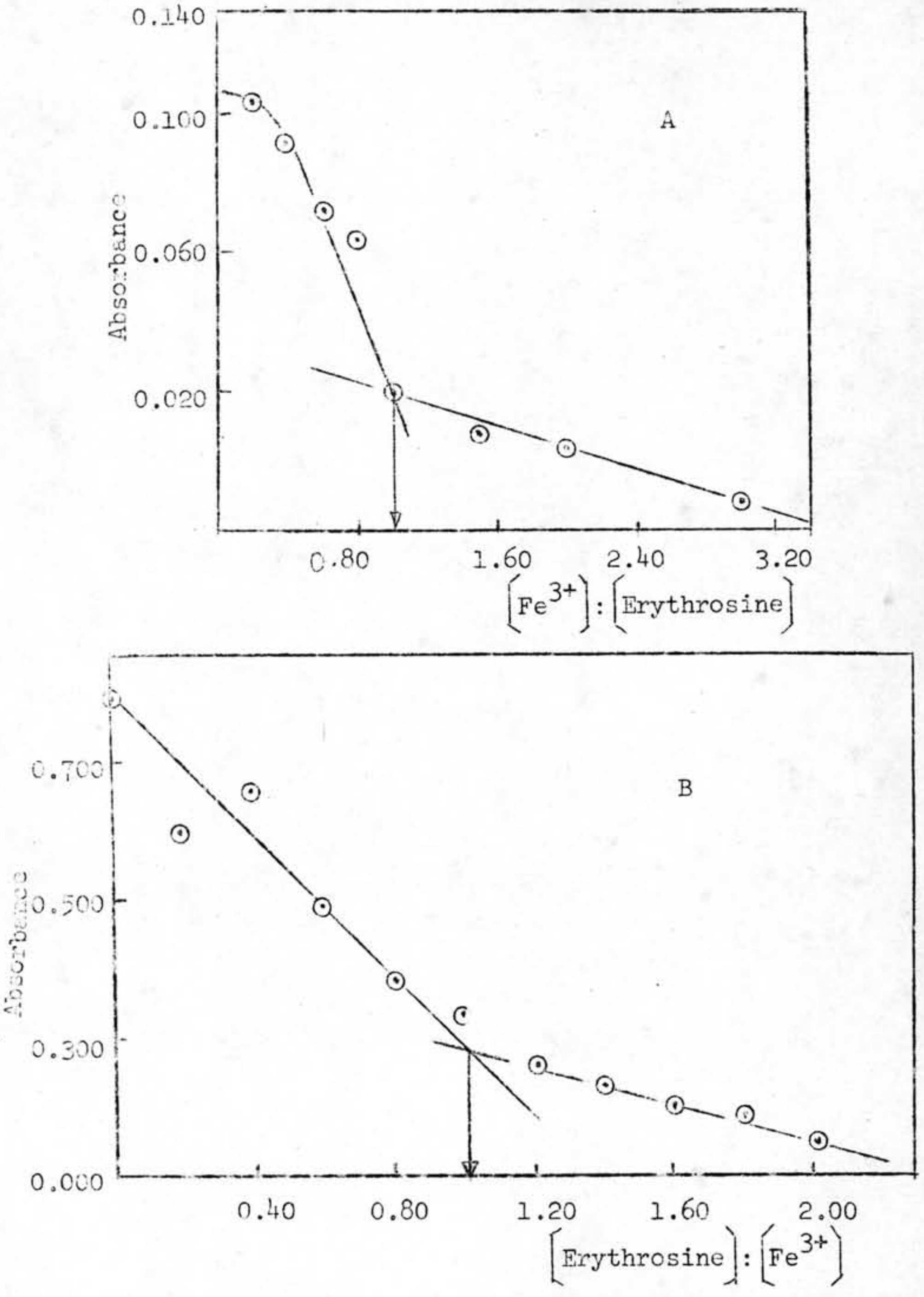

Figure 9D Molar ratio plots for systems in the nitric acid pH 3.3; A) 2.0×10^{-5}M Erythrosine and various concentration of Fe(III) ion by visible spectrophotometric method and B) 6.0×10^{-4}M Fe(III) ion and varioous concentrations of Erythrosine by atomic absorption spectrophotometric method

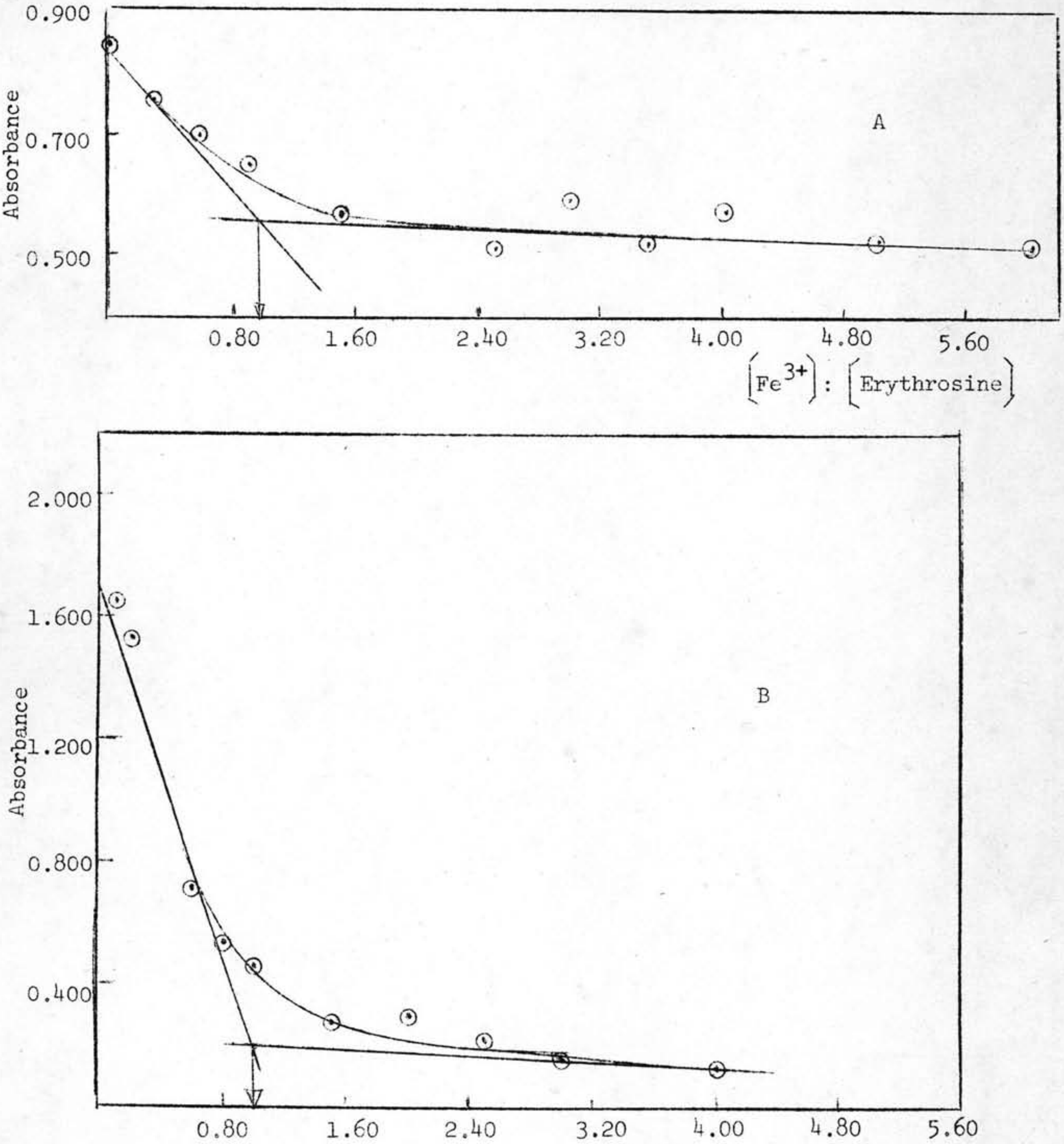

Figure 9E Molar ratio plots for systems in the acetate buffer pH 4.4; A) 2.0×10^{-5}M Erythrosine and various concentrations of Fe(III) ion and B) 6.0×10^{-4}M Fe(III) ion and various concentrations of Erythrosine; by visible spectrophotometric method.

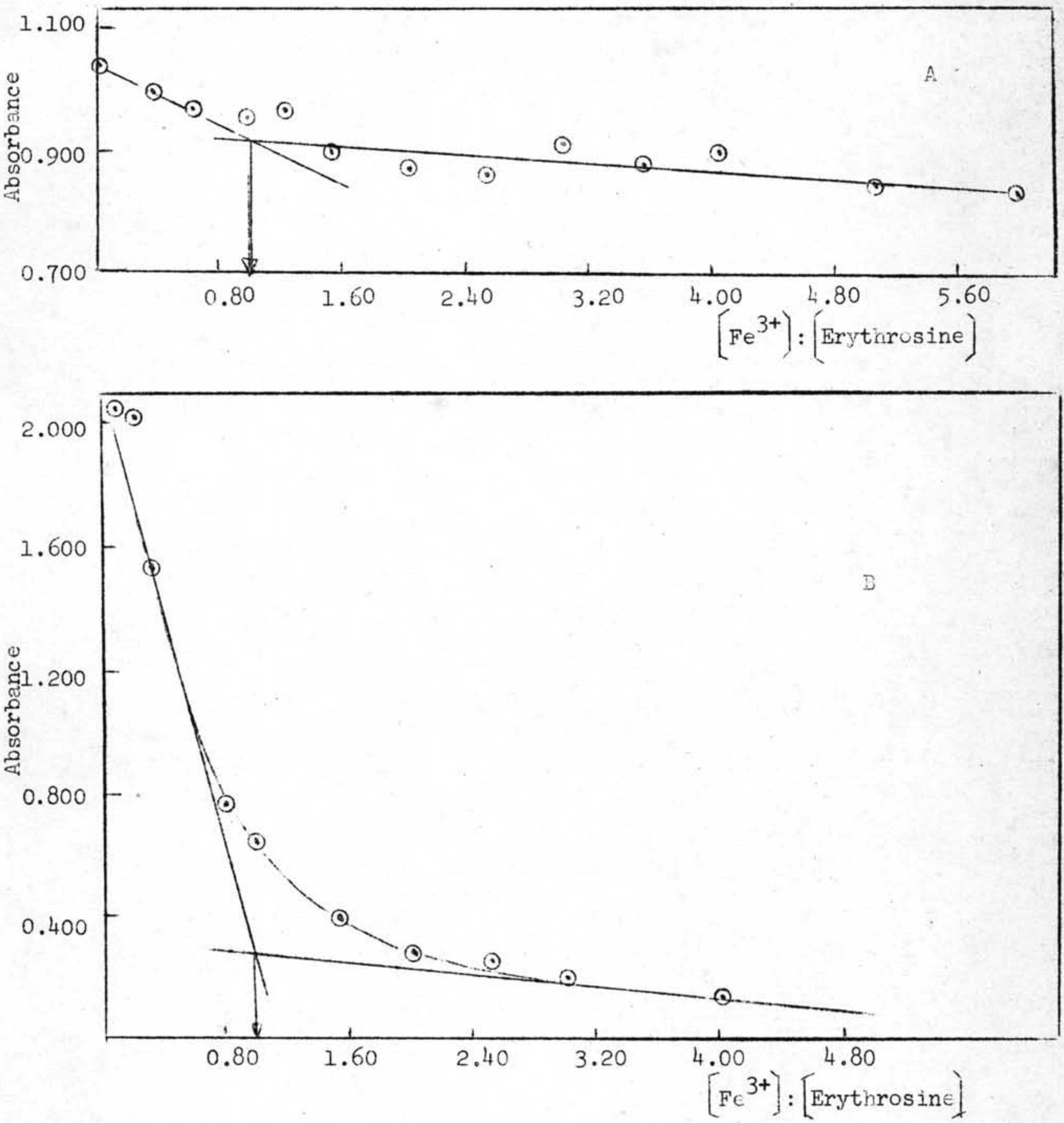

Figure 9F Molar ratio plots for systems in the acetate buffer pH 6.0; A) 2.0×10^{-5}M Erythrosine and various concentrations of Fe(III) ion and B) 6.0×10^{-4}M Fe(III) ion and various concentrations of Erythrosine, by visible spectrophotometric method

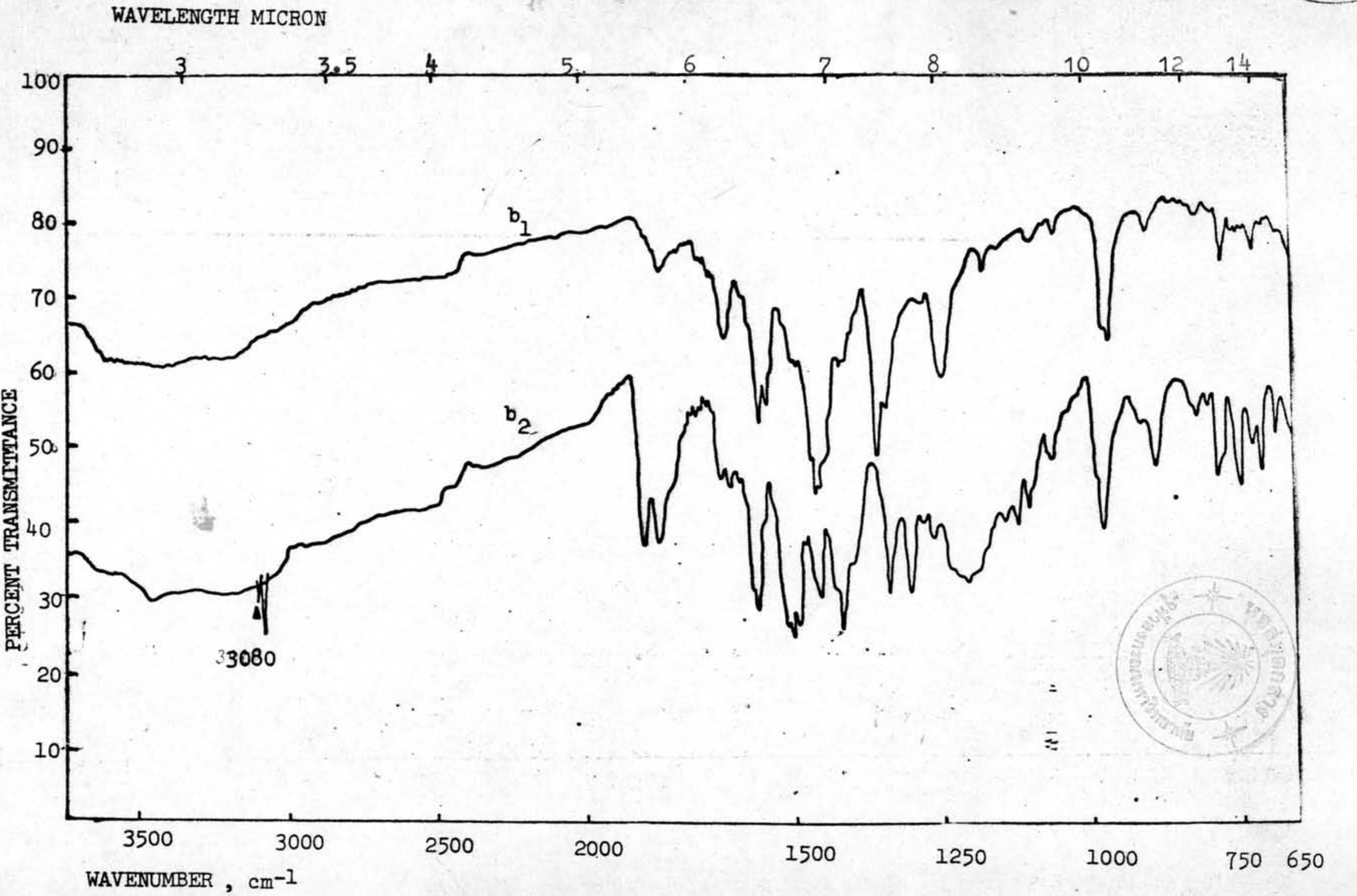

Figure 9G Comparision of IR spectra between b$_1$) Erythrosine b$_2$) Fe(III)Erythrosinate

Table 11-a Molar ratio study of Erythrosine and Pb(II)ion in water (pH 5.5-4.6) and in nitric acid pH 5.5 by visible spectrophotometric technique

Molar ratio, Pb(II): Erythrosine	Absorbance at λ_{max} = 525 nm in aqueous solution	Absorbance at λ_{max} = 525 nm in nitric acid pH 5.5
0.00	1.343	1.180
0.50	1.358	1.120
1.00	1.295	0.992
1.50	1.240	1.010
2.00	1.098	0.900
2.50	0.940	0.730
3.00	0.853	0.640
3.50	0.750	0.620
4.00	0.718	0.540
5.00	0.568	0.475
6.00	0.537	0.430

Table 11-b Molar ratio study of Erythrosine and Pb(II) ion in water pH 6.0 by visible spectrophotometric technique

Molar ratio, Pb(II):Erythrosine	Absorbance at λ_{max} = 525 nm
0.50	1.450
0.60	1.195
0.70	0.925
1.00	0.660
1.50	0.411
2.00	0.315
2.50	0.265
3.00	0.205
3.50	0.170
4.00	0.150
5.00	0.110
6.00	0.090

Table 11-c Molar ratio study of Erythrosine and Pb(II) ion in water pH 7.0-5.8 and in nitric acid pH 5.5 by atomic absorption spectrophotometric technique

Molar ratio, Erythrosine:Pb(II)	Absorbance at λ_{max} = 217.0 nm either in water or in HNO_3 pH 5.5
0.00	0.510
0.20	0.338
0.40	0.225
0.50	0.177
0.60	0.135
0.70	0.140
0.90	0.165
1.00	0.185
1.50	0.345
2.00	0.370
3.00	0.370
4.00	0.355
5.00	0.370
6.00	0.360

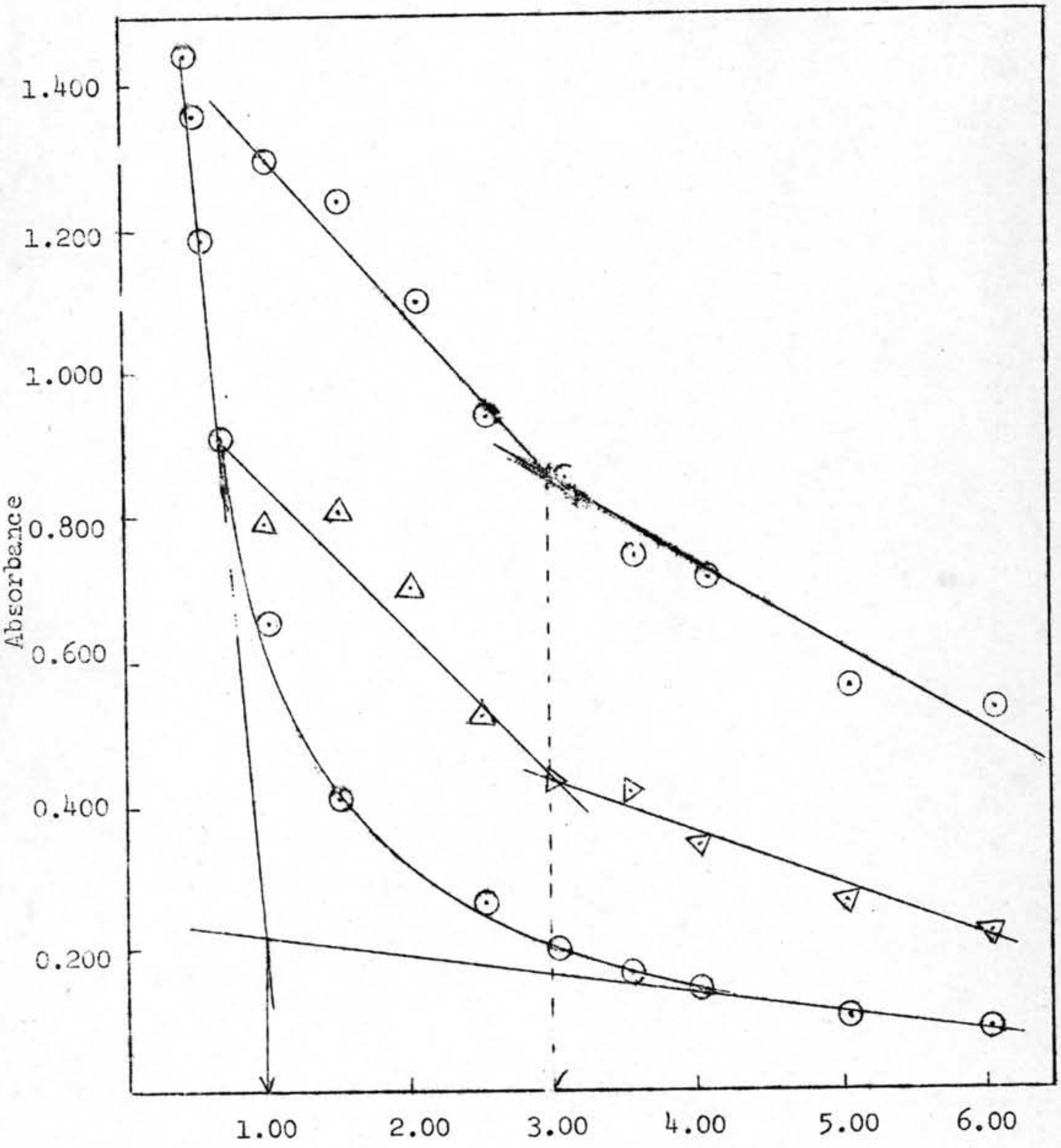

Figure 10A Molar ratio plots for systems; A) 1.20×10^{-5}M Pb(II) ion and various concentrations of Erythrosine in water (pH 6.0) B) and C) 2.00×10^{-5}M and various concentrations of Pb(II) ion in water (pH 5.5-4.6) and in nitric acid (pH 5.5), respectively by visible spectrophotometric method

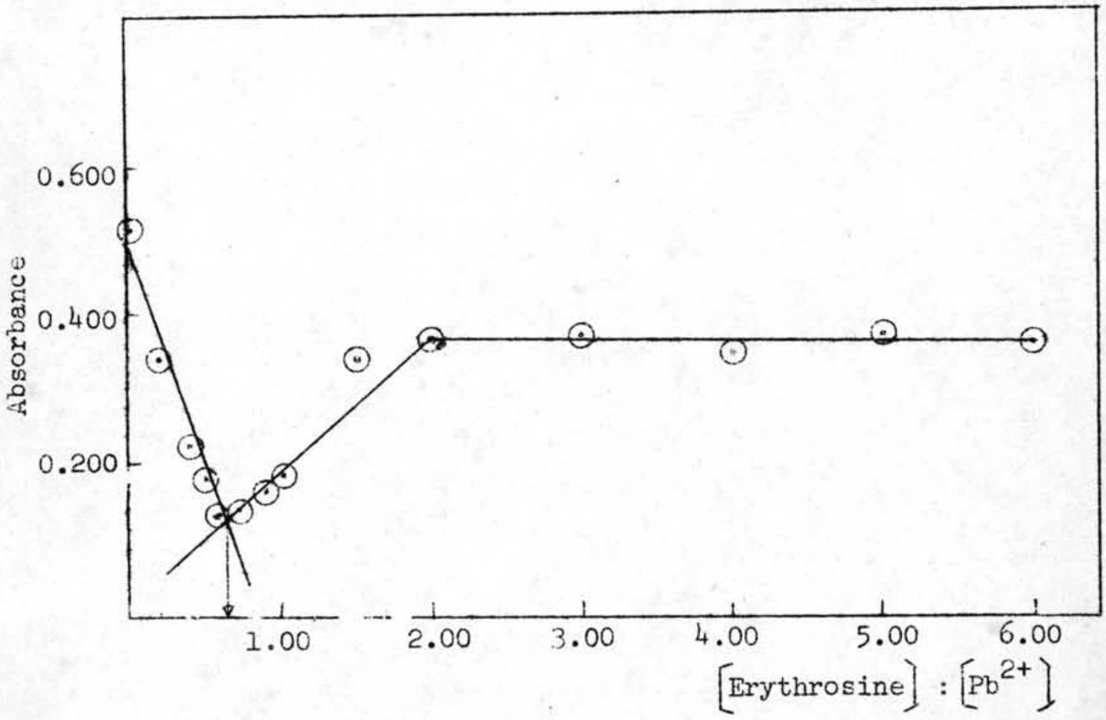

Figure 10B Molar ratio plot for solutions contained 2.00 × 10^{-4} M Pb(II) ion and various concentrations of Erythrosine either in **water** . (pH 7.0-5.8) or in the nitric nitric acid pH 5.5 by atomic absorption spectrophotometric method

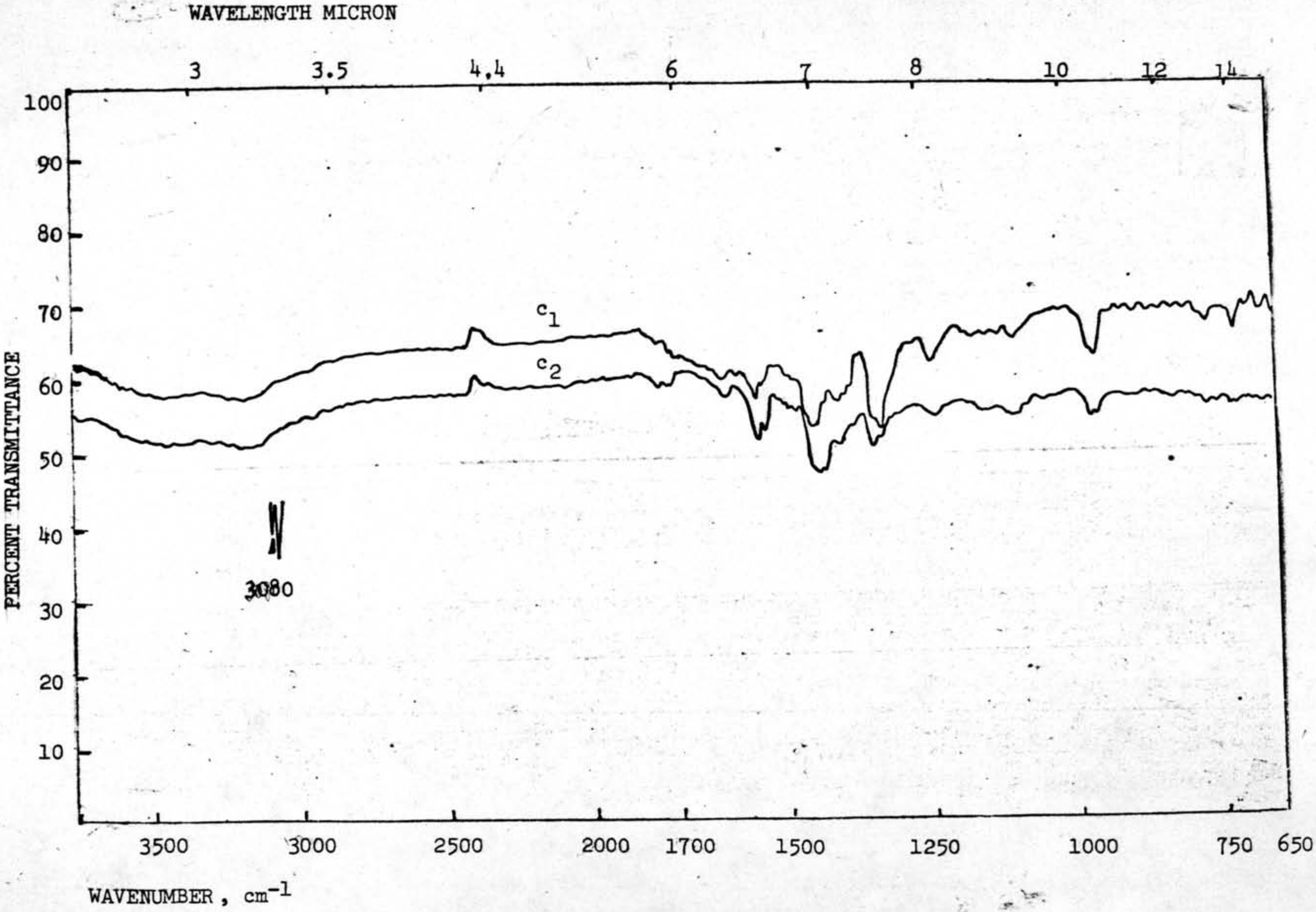

Figure 10C Comparision of IR spectra between c_1) Erythrosine c_2)Pb(II)Erythrosinate

Table 12-a Stability constants and solubility products of Hg(II) Erythrosinate in various buffers

pH and buffer used	Method	Stability constant[a]		Solubility product[a]	
		Hg(Eryth)	$Hg_2(Eryth)_2$	Hg(Eryth)	$Hg_2(Eryth)_2$
6.8-3.1; HNO_3	Visible	7.5×10^{9}	5.6×10^{19}	1.3×10^{-10}	1.8×10^{-20}
4.3 ; HNO_3	Visible	6.0×10^{9}	3.6×10^{19}	1.7×10^{-10}	2.8×10^{-20}
4.4 ; acetate	Visible	5.4×10^{9}	2.9×10^{19}	1.9×10^{-10}	3.4×10^{-20}
6.0 ; acetate	Visible	3.0×10^{9}	9.2×10^{18}	3.3×10^{-10}	1.1×10^{-19}
6.2-4.2; HNO_3	AAS	9.2×10^{6}	8.0×10^{13}	1.1×10^{-7}	1.3×10^{-14}
4.3 ; HNO_3	AAS	4.1×10^{6}	1.7×10^{13}	2.4×10^{-7}	5.9×10^{-14}
4.4 ; acetate	AAS	1.5×10^{7}	2.1×10^{14}	6.7×10^{-8}	4.8×10^{-15}
6.0 ; acetate	AAS	6.1×10^{6}	4.0×10^{13}	1.6×10^{-7}	2.5×10^{-14}

[a] Average of at least 3 mixture solutions by visible absorption and atomic absorption spectrophotometric methods.

Table 12-b Stability constant and Solubility product of Fe(III)Erythrosinate

pH and buffer used	Method	Stability constant		Solubility product	
		$Fe_2(Eryth)_3$	$Fe_4(Eryth)_6$	$Fe_2(Eryth)_3$	$Fe_4(Eryth)_6$
3.3 ; H_2SO_4	Visible	3.9×10^{23}	1.5×10^{47}	2.6×10^{-24}	6.7×10^{-48}
3.3 ; H_2SO_4	AAS	6.0×10^{17}	3.6×10^{35}	1.7×10^{-18}	2.8×10^{-36}

Table 12-c Stability constant and Solubility product of Pb(II)Erythrosinate

pH and buffer used	Method	Stability constant		Solubility product	
		Pb(Eryth)	$Pb_2(Eryth)_2$	Pb(Eryth)	$Pb_2(Eryth)_2$
6.0 ; aqueous solution	Visible	7.5×10^{8}	1.5×10^{17}	1.3×10^{-9}	6.7×10^{-18}