



Chapter II

EXPERIMENTAL RESULTS AND DISCUSSION

2.1 General Method for Preparation of the Azo Dye

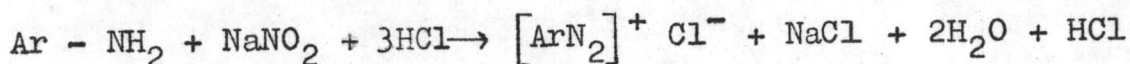
The azo compound is the compound in which two or more hydrocarbon nuclei are united by the azo group. One, two, three, or occasionally four of these groups may be present. The azo compound of simpler structure are usually yellow to red in color; those of more complex constitution, violet to blue and black. Amongst analogously constituted compounds with the same number of azo groups, those containing naphthalene nuclei have a darker color than those containing benzene nuclei. (54)

The reaction of an aromatic amine with nitrous acid to yield a diazo compound and the combination of the diazo compound with a suitable component to yield an azo derivative are two of the older reactions in organic chemistry. The former was discovered by Griess in 1858 (55), while the latter was discovered by Kekule and Hidegh in 1870 (56).

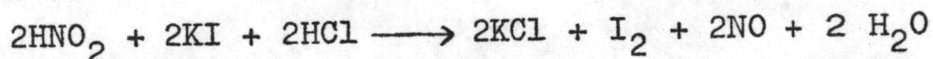
2.1.1 The diazotisation

There are many methods for the preparation of diazo compounds. But the important one is involving the reaction of an aromatic primary amine with nitrous acid in the presence of a mineral acid, usually in an aqueous medium. The diazotisation reaction proceeds

according to the equation:



The amine is dissolved in a suitable volume of water containing 2.5 - 3.0 equivalents of hydrochloric acid by the application of heat if necessary, and the solution is cooled in ice where the aminehydrochloride usually crystallises. The temperature is maintained at 0 - 5°C, an aqueous solution of sodium nitrite is added portion-wise until , after allowing 3 - 4 minutes for reaction, the solution gives immediate positive test for excess of nitrous acid with an external indicator; moist potassium iodide - starch paper.



The aminehydrochloride is dissolved during the diazotisation to give a solution of the highly soluble diazonium salt which is generally stable only at low temperature. Hence the reaction must be carried out at the temperature not more than 5°C and the solution is used immediately.

The amount of nitrite used in the reaction must be corresponded exactly to the quantity required theoretically if the coupling reaction is carried out in acid solution, or if the coupling is done in alkaline

solution and the mixture must be acidified subsequently in isolating the dye. Any excess of nitrite under these conditions would diazotise or nitrosate the coupler, or, in the latter case, would alter the finished dye. When both the coupling reaction and the isolation of the dye are carried out in alkaline solution, a small excess of nitrite does no harm. The use of too little nitrite is bad because it leads to the formation of diazoamino compounds or to coupling of the diazotised base itself.

While the nitrite must be used in exactly the amount theoretically required, an excess of acid is always used. The excess of acid (0.5 - 1.0 equivalents) maintains a proper condition of acidity required to stabilize the diazonium salt solution by reducing the secondary changes to a minimum, e.g., the interaction of some of the diazonium salt with unchanged amine to form a diazoamino compound. Hydrochloric acid is usually employed in diazotisation in aqueous solutions because it gives, in general, the most soluble salts with aromatic amine. But at the same time hydrochloric acid reacts with nitrite to give chlorine gas, which of course, leads to side reactions. For this reason, the diazotisation mixture should never contain more than

20 % of free hydrochloric acid.

2.1.2 The coupling reaction

Diazo compounds react with a large number of coupling components to form azo derivatives. These coupling components may be classified into 5 species:

- (1) Aromatic hydroxy compounds, e.g., phenol, naphthol, etc.
- (2) Aromatic amines
- (3) Substances containing reactive methylene group
- (4) Phenol and naphthol ether
- (5) Hydrocarbon

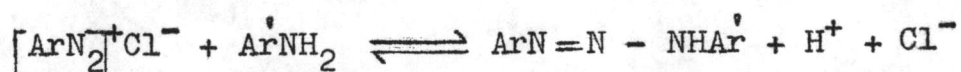
The rate at which an azo coupling proceeds in an aqueous medium at a given temperature is a function of the nature of the diazo compound, the nature of the coupling component, and the pH of the medium. The dye chemist has classified the diazo compounds as weak or strong (57) depending on the ease with which they react. Generally, the strong diazo components contain electron-donating substituents.

In the naphthalene series (58) α - naphthol and α - naphthylamine couple in the 4 position. If the 4 position is occupied and if a sulfonate group is in the 3 or 5 position, coupling takes place in the 2 position. β - naphthol

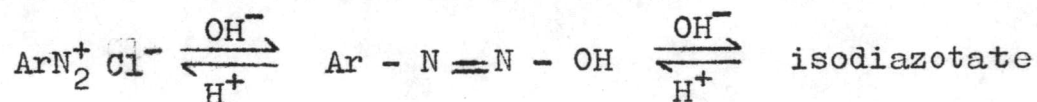
and β - naphthylamine couple only in the 1 position.

If both an amino group and a hydroxyl group are present, the amino group directs in weakly acid solution, and the hydroxyl group directs in alkaline solution.

The effect of the pH of the medium on the rate of coupling has been explained (59). The rate at which the coupling components undergo the coupling reaction increases directly with the pH of the medium until at a certain pH, the rate attains a maximum value. At pH value higher than this critical value, the rate decreases. This decrease in coupling rate is due to the conversion of the diazo compound to the non - coupling isodiazotates. Couplings of this type are usually carried out in weakly acid media, however to suppress the formation of triazene compounds. The equation of the coupling reaction is as follow:



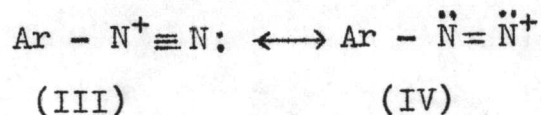
The diazo compounds exist in different forms in different pH ranges. In acid media they exist as diazonium salt (I). As the pH is raised to higher values,



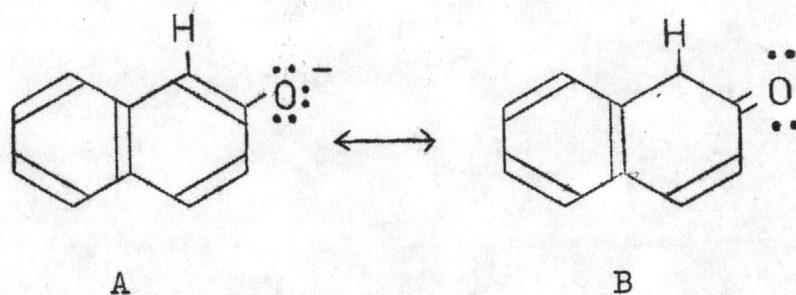
(I)

(II)

this form is converted successively to the diazohydroxide (II) and the non - coupling isodiazotate. It is shown that the rate of coupling of several naphtholsulfonic acids is proportional to the dissociation constants of the hydroxy group. In the coupling of dihydroxynaphthalenesulfonic acids the entering diazonium cation is oriented by the more acidic hydroxy group. It has been pointed out that the diazonium ion is a hybrid of the two limiting structures (III) and (IV) (60). Structure (III)



is the most important contributor to the hybrid, but the importance of (IV) is increased at the moment of coupling. The ease with which a coupling component undergoes reaction will be related directly to the electron density at the coupling position. This assumption explains why the naphtholate ions, rather than the corresponding unionized derivatives, are active in coupling. In the ions, the electron density at the coupling position is increased by the contributions of the resonance structures A and B respectively.

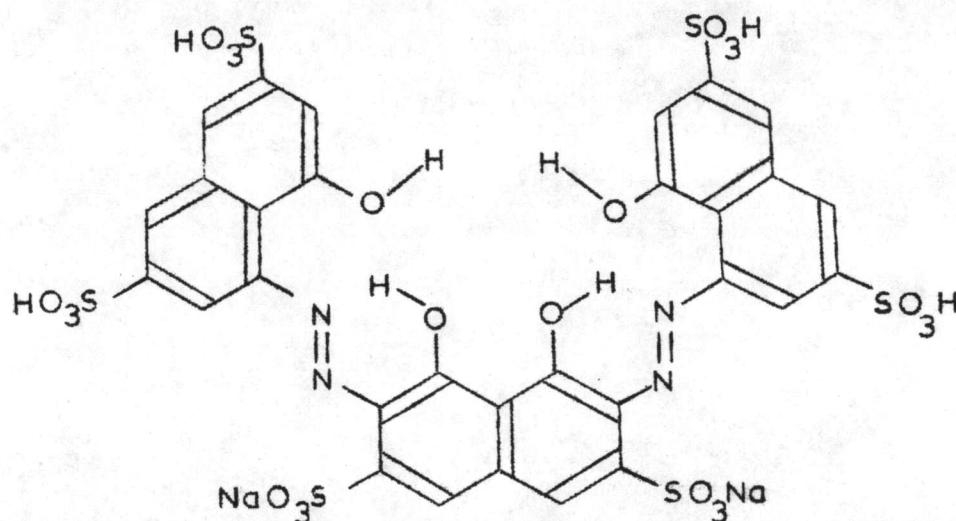


Analogous resonance structure for the unionized derivatives would require charge separation, would represent higher energy structures, and would be less effective in increasing the electron density at the coupling position.

2.2 Preparation , Isolation , and Purification of the Dye

2.2.1 Preparation of the 1,8-dihydroxy-2,7-bis(3,6-di- sulfo-8-hydroxy-1-naphthylazo)-naphthalene-3,6-disulfonic acid (dye)

The structure of the dye is proposed to be (65):



$C_{30}H_{18}N_4O_{22}S_6Na_2 \cdot 12H_2O$ molecular weight = 1240.969

Reagents

- (1) Chromotropic acid, sodium salt (BDH)
- (2) 8-Amino-1-naphthol-3,6-disulfonic acid, (H acid)
purified sodium hydrogen salt (BDH)
- (3) Calcium chloride, dihydrate
- (4) Sodium nitrite
- (5) Hydrochloric acid (Analar)
- (6) Sodium hydroxide (Analar)

Procedure

H acid 4.41996 g. was suspended in 4.8 ml. of concentrated hydrochloric acid in 100 ml. of water. Then a suspension was cooled in a mixture of crushed ice with sodium chloride to keep the temperature down at 0°C. A cold solution of 1.12 g. of sodium nitrite in 8 ml. of water was adding drop by drop, with constant stirring to cause diazotisation. A yellowish - green solution of the diazonium salt was obtained in about 1 hour.

1.45704 g. of chromotropic acid was dissolved in 12 ml. of water (warm for better dissolution) and mixed with a solution of calcium chloride prepared by dissolving calcium chloride 2.4 g. in 10 ml. of water. The whole was added to 20 ml. of 10 F. sodium hydroxide and cooled to 0°C.

The previously prepared diazonium salt was then added to this solution drop by drop with stirring while the temperature was kept below 5°C. Stirring was continued for 30 minutes after the addition had been completed. The solution was then added to 30 ml. of concentrated hydrochloric acid and stored in the refrigerator overnight. The precipitate was rinsed with 10 ml. of 6 F. hydrochloric acid after filtering

through a sintered - glass funnel. The dye was dried at 60°C for 24 hours. The crude yield was approximately 4.0 g.

2.2.2 Isolation and purification of the dye

The crude yield still had much of the impurities; excess of starting materials, and may be some of other derivatives of the dye with different in position of the azo group.

The crude dye was dissolved in a small amount of distilled water, and cooled in the ice bath, then dropped in a cold solution of about 20 ml. of 10 F. hydrochloric acid with constant stirring (by using a magnetic stirrer). The content was filtered through a sintered-glass funnel, and rinsed with some aliquots of 10 F. hydrochloric acid. The solid was dried at 60°C in the oven for 24 hours. The procedure was repeated six times until the dye was pure. The purity of the dye could be checked by applying a method of paper chromatography in a suitable solvent system which stated in the next section. The dye obtained was more than 90 % pure. About 0.6 g. of the pure dye was resulted.

The percentage yield calculated on the basis of the chromotropic acid used was 12.58.



2.3 Paper Chromatography Studies of the Dye

The method of paper chromatography could be used to state the purity of the dye. The impurity was separated from the pure dye by ascending development in a suitable solvent system. About ten mixed solvent systems of various composition were investigated. Table II shows the R_f values attained in the separation of the dye and its impurity.

Table II Solvent systems and the corresponding R_f values

System	Solvent	Composition	Rf value	
			impurity	dye
1	99%MeOH	-	0.15	0.15
2	80%EtOH	-	0.32	0.19
3	10%HCl	-	0.12	0.12
4	25%EtOH:5%NH ₄ OH	1:1	0.90	0.90
5	80%EtOH:10%HCl:H ₂ O	6:3:1	0.79	0.67
6	80%EtOH:10%HCl:H ₂ O	5:3:2	0.81	0.69
7	80%EtOH:10%HCl	8:2	0.83	0.66
8	80%EtOH:10%HCl	7:3	0.85	0.68
9	80%EtOH:10%HCl	6:4	0.76	0.60
10	80%EtOH:10%HCl:isoPrOH	6:4:5	0.83	0.66

It was found that the system number 10 was the most suitable one.

Procedure

A few milligrams of the dye were dissolved in a small amount of the mixed solvent system (6:4:5 of 80 % ethanol:10 % hydrochloric acid: isopropanol). The Whatman number 1 filter paper (15 x 10 cm.) was spotted with a small amount of the dye 2 cm. from the bottom and then dried in air. The paper was folded in a cylindrical shape, fastened with adhesive tape. About 20 ml. of the mixed solvent was placed in a chromatographic chamber (a gas collector was used as a chromatographic chamber) and the paper was inserted. The system was closed with a glass plate. The developing solvent was allowed to ascend to a premarked line (10 cm. above the starting point). Chromatogram was developed and completed in about half an hour at room temperature. The paper was then removed from the chamber and was dried in air.

After the sixth recrystallization of the dye, the paper chromatogram shows that the dye sample consists of one component with a purity of above 90 % and has an average Rf value of 0.66. The chromatogram obtained is shown in Fig. 1.

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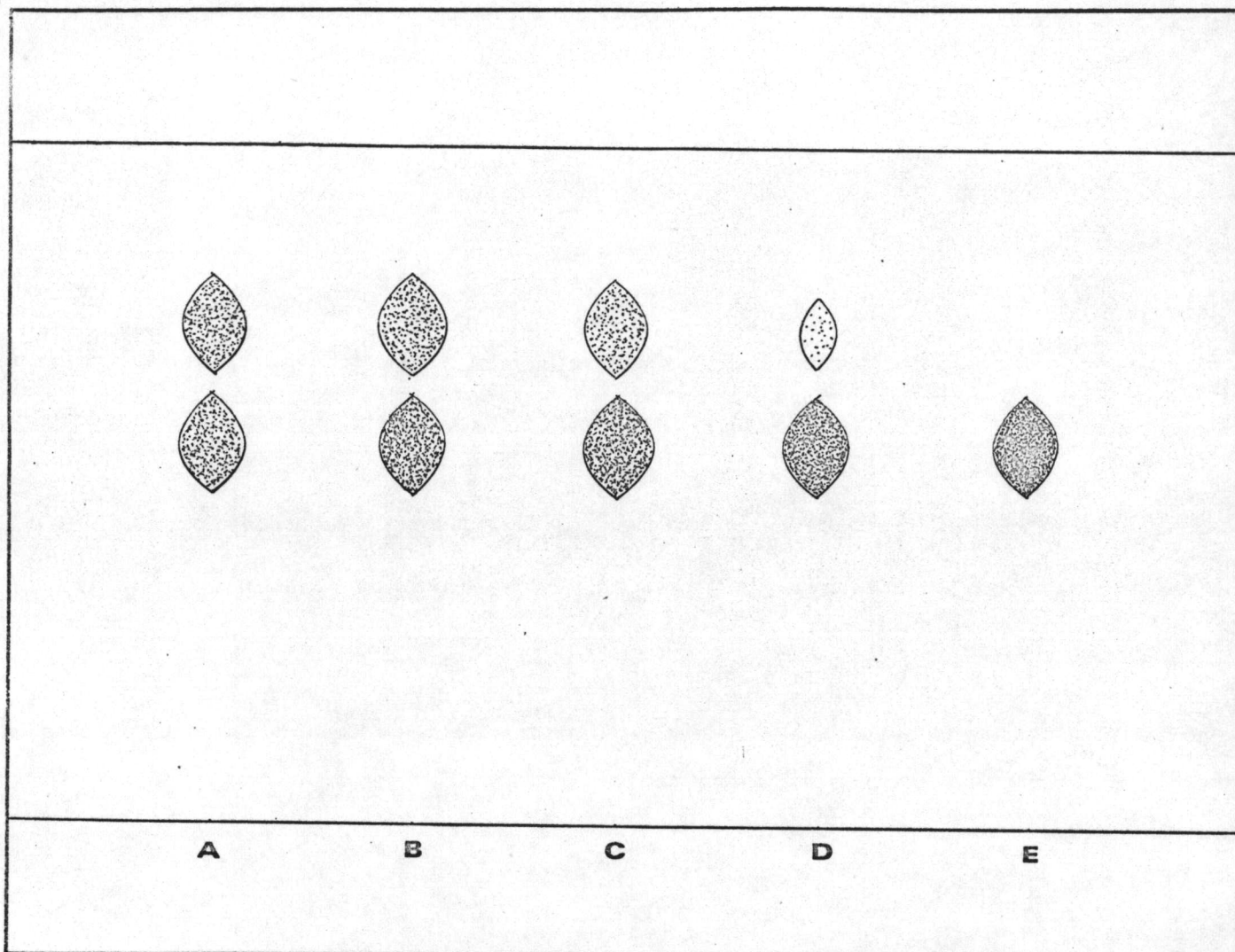


FIG. 1 Chromatogram of the dye at various stages of recrystallization.

- A** : Crude dye.
B : 1st recrystallization.
C : 3rd "
D : 5th "
E : 6th "

2.4 Preliminary Investigation of the Dye

Apparatus

Absorption spectra were made with a Perkin-Elmer recorder, model 56, and a Perkin-Elmer double beam spectrophotometer, model 124, with 1 cm. matched silica cells. Measurements of pH were made with a Radiometer Copenhagen, model 28, glass electrode pH meter. For all subsequent experiments, measurements were made by the use of the above apparatus.

Reagents

Standard beryllium solution. A standard beryllium solution was prepared by dissolving 1.7714 g. of beryllium sulfate tetrahydrate (BDH) in distilled water, then adding 1 ml. of concentrated hydrochloric acid to prevent possible hydrolysis and diluting the solution to 100 ml. with distilled water. The concentration of the solution was 0.1 F. of beryllium. This standard stock solution was then further diluted to the concentrations of 10^{-2} F. and 10^{-4} F. with distilled water. Dilution of a 10^{-4} F. solution was freshly prepared frequently to minimize the possibility of changes due to adsorption, base exchange with the glass container, etc.

Stock dye solution. A 10^{-3} F. dye solution was prepared by dissolving 0.1 g. of the purified dyestuff in 100 ml. of distilled water and diluted to the concentration of 10^{-4} F. when required.

Sodium hydroxide (10^{-2} F.), hydrochloric acid (10^{-2} F., 1.0F.) and the aqueous solution of hexamine (1%, 3%, 25%) were employed for pH adjustments of the solutions.

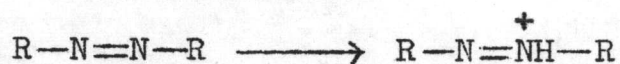
2.4.1 Qualitative color reaction of the beryllium dye complex

The qualitative color reaction of beryllium with the dye at pH 5-9 was carried out. The solution of 10^{-4} F. dye was adjusted to those pH values by using a 10^{-2} F. sodium hydroxide solution. Two series of the test tubes containing 2 ml. aliquots of the dye of pH 5,6,7,8,9 were placed on a test tube rack. A solution of 10^{-4} F. beryllium was neutralized to pH 7 by using also a 10^{-2} F. sodium hydroxide solution. To one series of the test tubes, was added 2 drops each, the neutral solution of 10^{-4} F. beryllium. Color changes due to the complex formation of beryllium with the dye were observed and compared with the other series as a blank. It was found that the color had been changed in every tube from wine-red to blue-mauve, when the system was set aside for an hour, the color in the test tube of pH 9 was faded. This indicated that the complex was not stable at this pH. The detailed studies of the complex formation should be varied only in the pH range 5 to 8.

2.4.2 Acid - base property of the dye

The color of the free dye was found to change with

pH. The change in color may be ascribed to changes in the structure caused by ionization. The change in color when the pH was lowered is due to the attachment of a proton to the auxochromic groups or on one of the nitrogen atoms of the azo group:



and by increasing the pH, the process is reversed.

Procedure

Twelve 10-ml. aliquots of 10^{-4} F. dye solution were pipetted into a series of 50-ml. beakers. The pH of the solution was adjusted to 1.0-12.0. For pH's below 4.0, hydrochloric acid (10^{-2} F., 1.0 F.) were used. 25 % aqueous solution of hexamine was used for pH range 4.0-10.0 and for pH's above 10.0, 1.0 F. sodium hydroxide was used as a pH adjusting reagent. Suitable amount of water was added. The pH was recorded by a pH meter. The solutions were then transferred quantitatively to a series of 25-ml. volumetric flasks and made up to the mark with distilled water to get the same final volume. The color changes were observed. The corresponding colors are reported in Table III.

The color changes resulted from Table III indicates that the dye has an acid base property.

Table III Colors observed at particular pH values

pH	Color observed
1	wine red
2	wine red
3	wine red
4	wine red
5	wine red
6	mauve red
7	purple
8	blue mauve
9	blue mauve
10	blue mauve
11	mauve blue
12	blue

2.4.3 Visible spectra of the dye solution at various pH

Procedure

The solutions prepared in 2.4.2 (preceding section) were used for the spectra study. The absorption spectra of the dye solutions at various pH values were recorded from 400-700 nm. using water as a reference. The gain of the spectrophotometer was set at 0-2, and the slit width was 0.25.

The absorption spectra obtained are shown in Fig.2. The maximum absorbance occurs at about 544 nm. over the pH range from 1.0-7.0, and it shows a bathochromic shift above pH 7.0.

2.4.4 Visible spectra of the complex at various pH

The result from the qualitative color reaction indicates that the suitable pH range for complex formation is from 5.0 to 8.0. It is necessary to determine first whether in a given pH range, beryllium and the dye form more than one complex.

Procedure

Seven 10-ml. aliquots of $10^{-4}F.$ beryllium were pipetted into a series of 50-ml. beakers containing accurately 10 ml. each of $10^{-4}F.$ dye solution. The pH of the solutions were adjusted to 5.0-8.0 with the interval of 0.5 by using 25 % aqueous solution of hexamine. The solutions were then transferred quantitatively into a series of 25-ml. volumetric flasks. Small amount of water was added to adjust the volume. The solutions were set aside for complete color development. The absorption spectra of the beryllium-dye complex at pH 5.0-8.0 measured against water as a reference were recorded from 400-700 nm. The absorbance of the solutions were also measured against a reagent blank treated in a

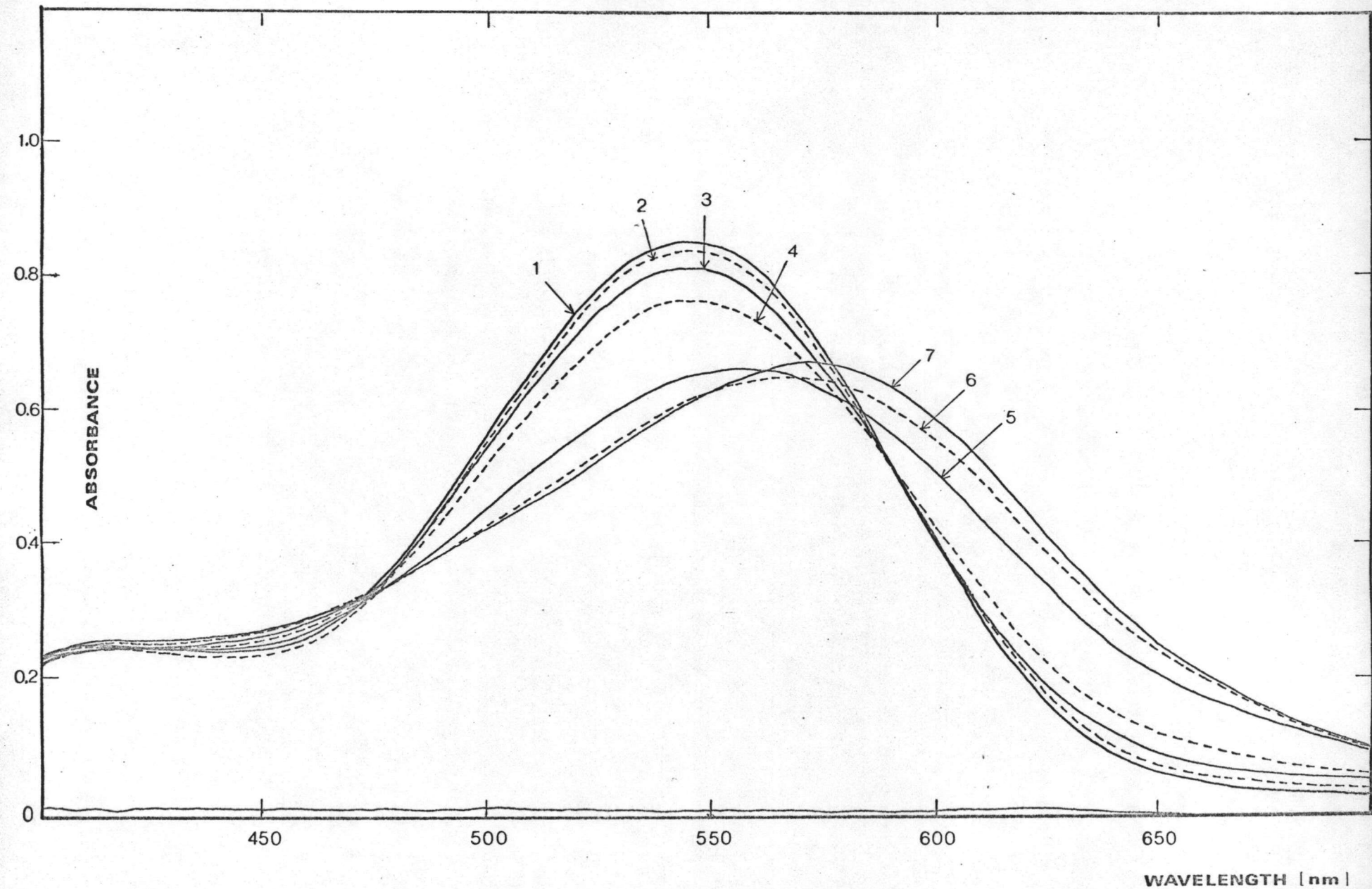


FIG. 2

Absorption spectra of the Dye at various pH.

Dye 10^{-4} F. 10 ml./ 25 ml. Reference : Water.

pH 1:4.0 , 2:5.0 , 3:6.0 , 4:7.0 , 5:8.0 , 6:9.0 , 7:10.0

similar manner as a reference. The gain of the spectrophotometer was set at 0-2, and the slit width was 0.25.

Fig.3 shows the absorption spectra of the beryllium-dye complex at various pH measured against water as a reference. The absorption spectra of the complex at various pH measured against a reagent blank are shown in Fig.4.

2.5 Spectrophotometric Determination of Beryllium Using the Dye as a Metallochromic Reagent

Apparatus

The same set of the instruments as previously described were used. The gain of the spectrophotometer was set at 0-2 when the spectra were recorded and 0-1 when the absorbance were recorded at a particular wavelength. The slit width was 0.25 in every case.

Reagents

Standard beryllium solution.(as described previously)

Stock dye solution.(as described previously)

1%,3%,25% aqueous solution of hexamine.

Diverse ions. Stock solutions were prepared from analytical grade of salts of nitrates, chlorides and, sulfates of various metal ions. Sodium salts were preferably employed in the case of anions. The concentrations were 0.1 F. , further dilutions were made when required.

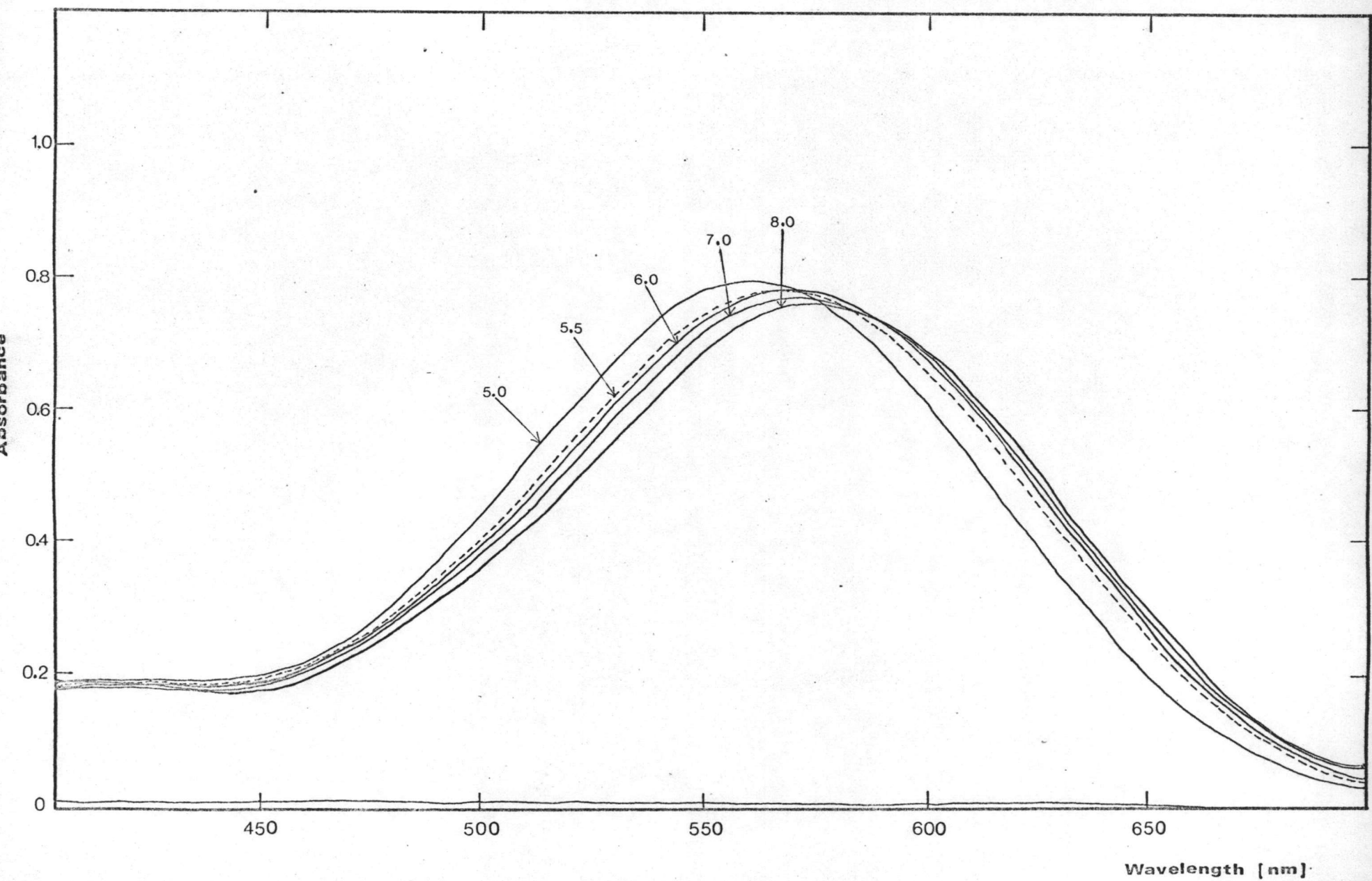


FIG. 3 Absorption spectra of beryllium-dye complex at various pH. Reference : Water .

Dye 10^{-4} F. 10 ml. / 25 ml.

Be 10^{-4} F. 10 ml. / 25 ml.

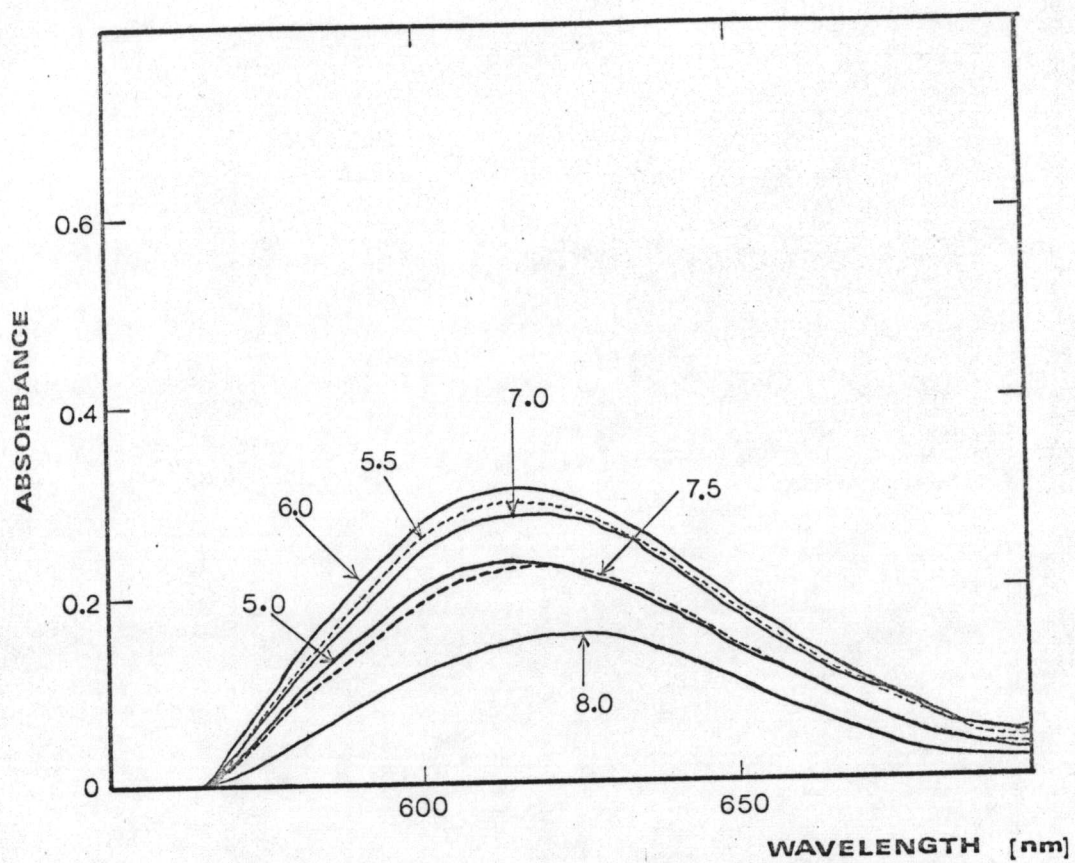


FIG. 4 Absorption spectra of Be-dye complex at various pH. Reference : reagent blank.

Be 10^{-4} F. 10 ml. / 25 ml.

Dye 10^{-4} F. 10 ml. / 25 ml.

EDTA solution. 0.9306 g. of EDTA (disodium salt) was dissolved in distilled water and then diluted to 250 ml. to obtain the solution of $10^{-2}F$. and stored in a polyethylene bottle. Further dilution to a concentration of $10^{-4}F$. was made when required.

2.5.1 Optimal conditions

Procedure

2.5.1.1 Optimal wavelength. The solution prepared in 2.4.4 was used for the investigation of the optimal analytical wavelength. The absorption spectra of the reagent and the complex at pH 6 were recorded from 400-700 nm. The free dye was measured against water as a reference. The complex was measured against both water and reagent blank. The absorption spectra of the reagent and the complex formed with beryllium are shown in Fig.5. Inspection of the curves shows that a wavelength of approximately 620 nm. gives the greatest difference in absorbance between the blank and the complex.

2.5.1.2 Optimal pH. The complex systems in the pH range from 5.0 to 7.0 were investigated. A series of solutions containing 3 ml. of $10^{-4}F$. beryllium, 10 ml. of $10^{-4}F$. dye solution, and appropriate amount of water was prepared. For pH's below 6.5, a 1 % aqueous solution

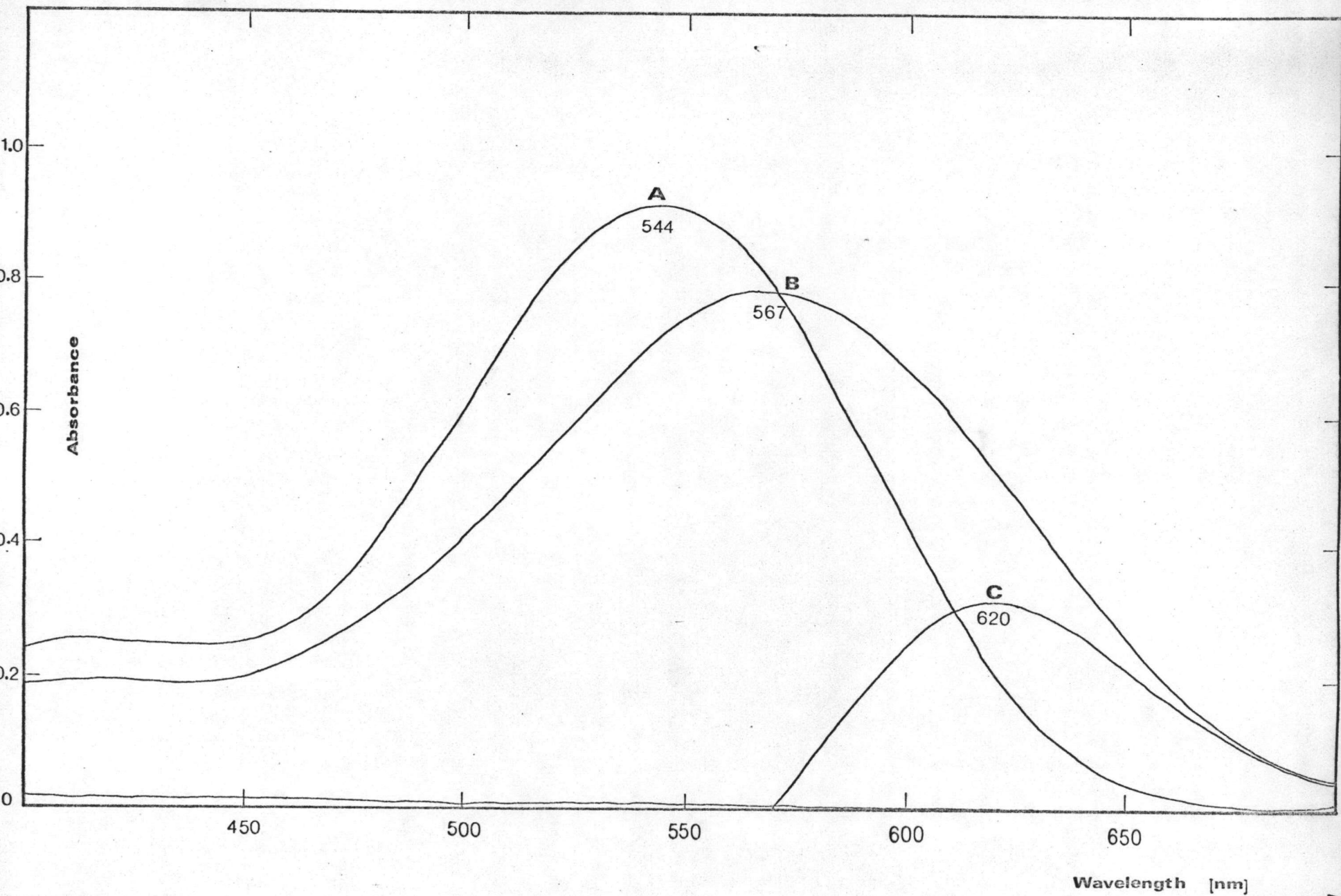


FIG. 5

Optimal wavelength of the dye and its beryllium complex at pH 6.0.

A : Dye 10^{-4} F. 10 ml. / 25 ml. Reference : Water .

B : Dye 10^{-4} F. 10 ml, beryllium 10^{-4} F. 10 ml. / 25 ml. Reference : Water .

C : Spectra of B against A .

of hexamine was employed and a 3 % solution was employed for pH 7.0. The final volume was 25 ml. After the color had been developed, the absorbances of solutions were measured at 620 nm. against a reagent blank, treated in a similar manner, as a reference. The results obtained are shown in Table IV. The plot of the absorbance versus pH is shown in Fig.6, from which it can be seen that the optimum pH range for analytical purposes lies between 5.5 and 6.4. But pH 6.0 is selected as the optimal pH.

Table IV Optimal pH

pH	Absorbance 620 nm.
5.0	0.072
5.5	0.091
6.0	0.092
6.5	0.091
7.0	0.083

2.5.1.3 Optimal amount of the dye. The effect of the dye concentration on the color development of the complex was examined by varying the concentration of the reagent at a constant beryllium concentration. A series of solutions containing 3 ml. of 10^{-4} F. beryllium, 10 ml. of distilled

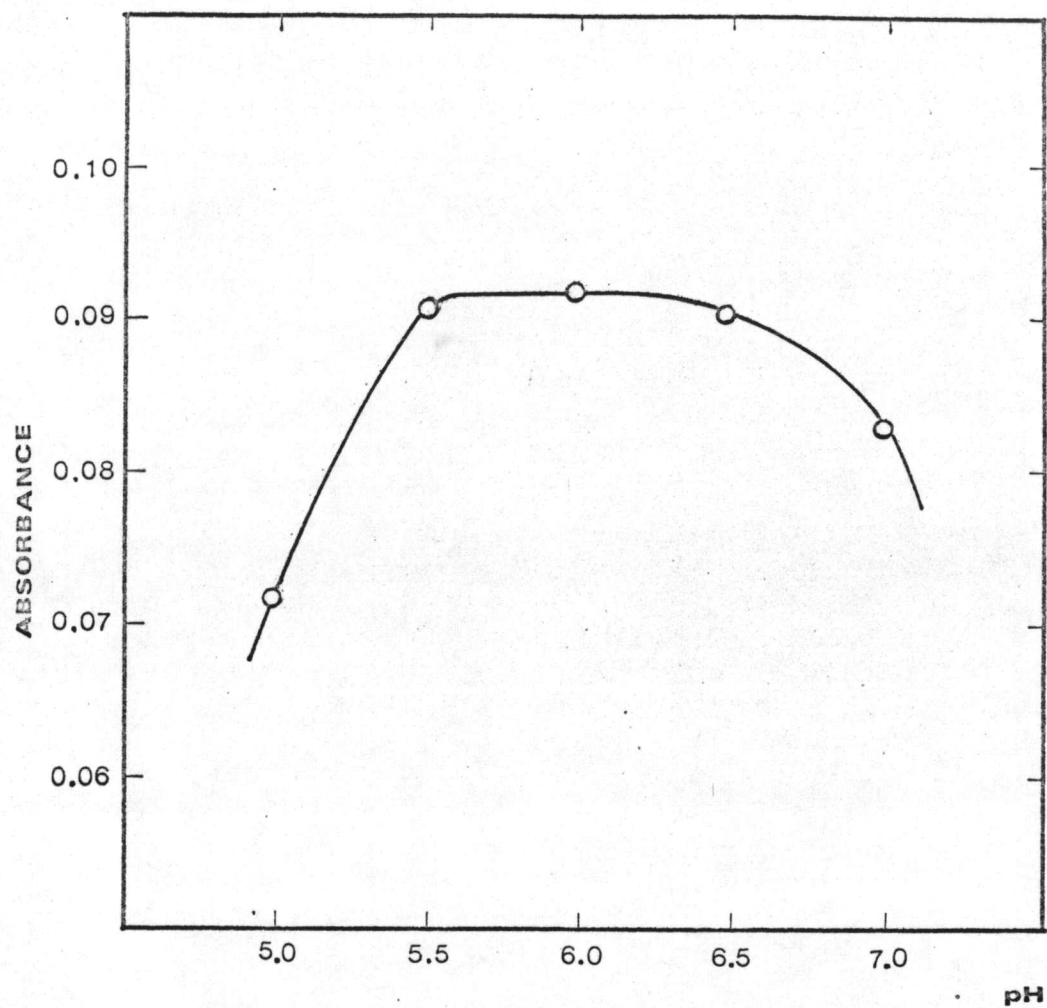


FIG. 6 Effect of pH on absorbance of Be-Dye complex measured against a reagent blank at 620 nm.

Dye 10^{-4} F 10 ml, Be 10^{-4} F, 3 ml./ 25 ml.

water, with various amount of 10^{-4} F. dye solution from 2 to 14 ml. was prepared. The pH of the solution was adjusted to 6.0 by using a 3 % aqueous solution of hexamine. All final volumes were 25 ml. Appropriate blanks (no beryllium) were also prepared. The solutions were set aside for color development. After that the absorbance of the complex was measured at 620 nm. against a reagent blank as a reference. The data obtained is shown in Table V. The plot of the absorbance versus amount of the dye is shown in Fig.7. Almost constant absorbance is obtained when a 6-7 ml. of the reagent is present, but a 10 ml. excess is recommended for practical purposes.

Table V Optimal amount of the dye

Dye 10^{-4} F. (ml)	Absorbance 620nm.
2	0.053
4	0.081
6	0.098
8	0.103
10	0.094
12	0.099
14	0.101

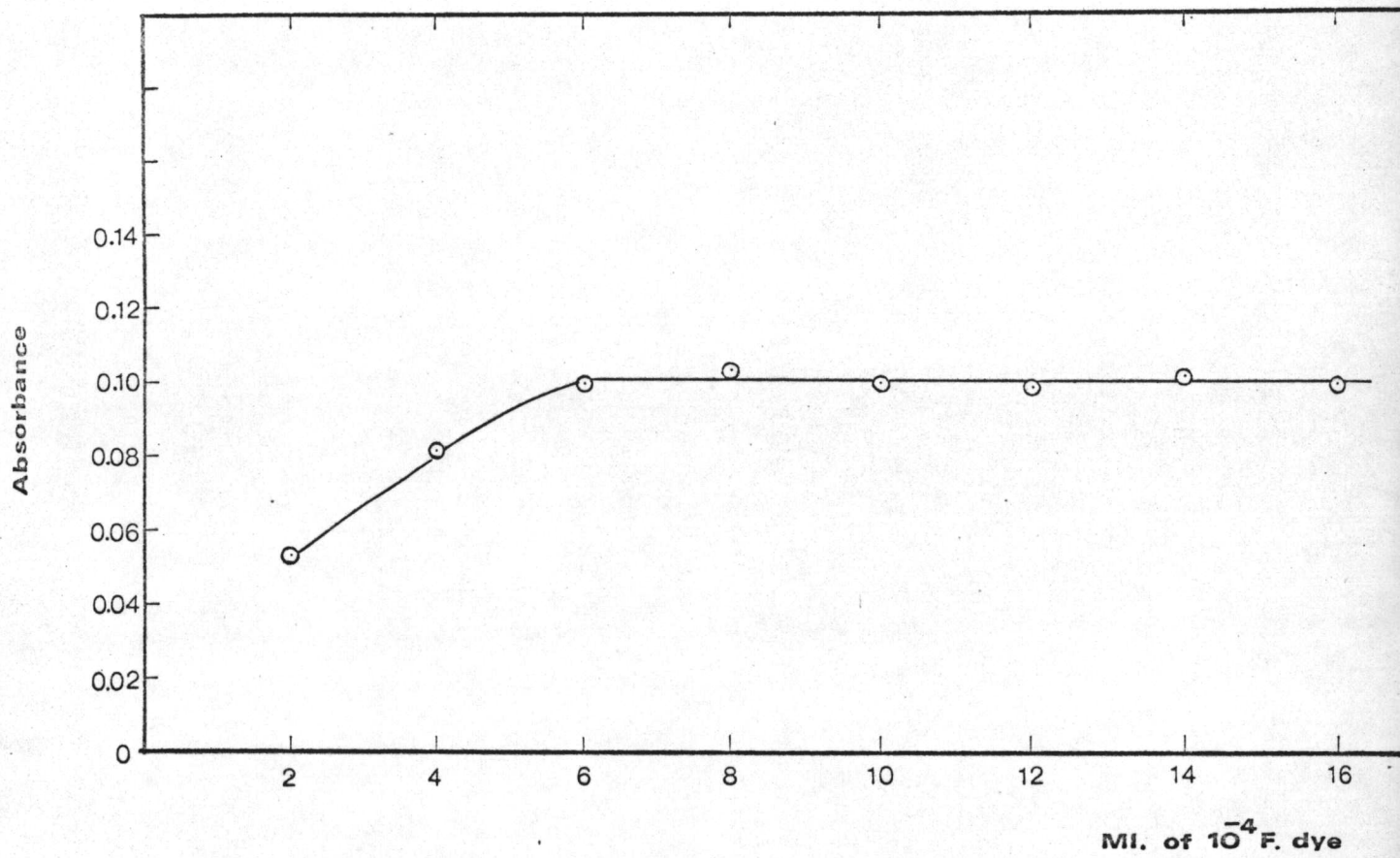


FIG. 7 Effect of reagent concentration.

Reference : Reagent blank ; Be 10^{-4} F. 3 ml. / 25 ml.

2.5.1.4 Effect of EDTA. As a dye might form colored compounds with other metallic ions at relatively low concentrations, a masking agent should be introduced into the solution to prevent the formation of interfering complexes and avoid chemical separation. The effect of EDTA on beryllium complex had been studied. A series of solutions containing various mole ratios of beryllium to EDTA was prepared. Five 3-ml. aliquots of $10^{-4}F.$ beryllium were pipetted into a series of 50-ml. beakers containing exactly 10 ml. of $10^{-4}F.$ dye solution and appropriate amount of water. The pH of the solution was adjusted to 6.0 by 3 % aqueous solution of hexamine. 3 ml. of $10^{-4}F.$ EDTA solution was pipetted into one of the beakers to make a mole ratio of 1:1. Into each of the beakers left, were pipetted 0.3 ml., 0.6 ml., 1.5 ml., 3.0 ml., and 6.0 ml. of $10^{-2}F.$ EDTA solution to make a mole ratio of 1:10; 1:20; 1:50; 1:100 and 1:200 beryllium to EDTA respectively. The pH of the solution was readjusted to 6.0 by using a 3 % aqueous solution of hexamine. The solutions were then transferred quantitatively to a series of 25-ml. volumetric flasks. The final volume was 25 ml. After the color had been developed, the absorbances of solutions were measured at 620 nm. against a reagent blank as a reference. The results obtained are shown in Table VI. The plot of the absorbance versus the mole ratio of beryllium

to EDTA is presented in Fig.8.

Table VI Effect of EDTA on beryllium complex

Mole ratio Be / EDTA	Absorbance 620nm.
1 : 1	0.092
1 : 10	0.084
1 : 20	0.073
1 : 50	0.054
1 : 100	0.048
1 : 200	0.048

2.5.1.5 Optimal time for color development.

A study was made of the influence of time on the color development of the three systems: (A) a system without EDTA; (B) a system with Be:EDTA = 1:33.3; and (C) a system with Be:EDTA = 1:100.

(A) system without EDTA : 3 ml. of 10^{-4} F. beryllium was pipetted into a 50-ml. beaker containing exactly 10 ml. of 10^{-4} F. dye solution, 10 ml. of distilled water. The pH of the solution was adjusted to 6.0 by 3 % aqueous solution of hexamine. The solution was then transferred quantitatively to a 25-ml. volumetric flask. Small amount

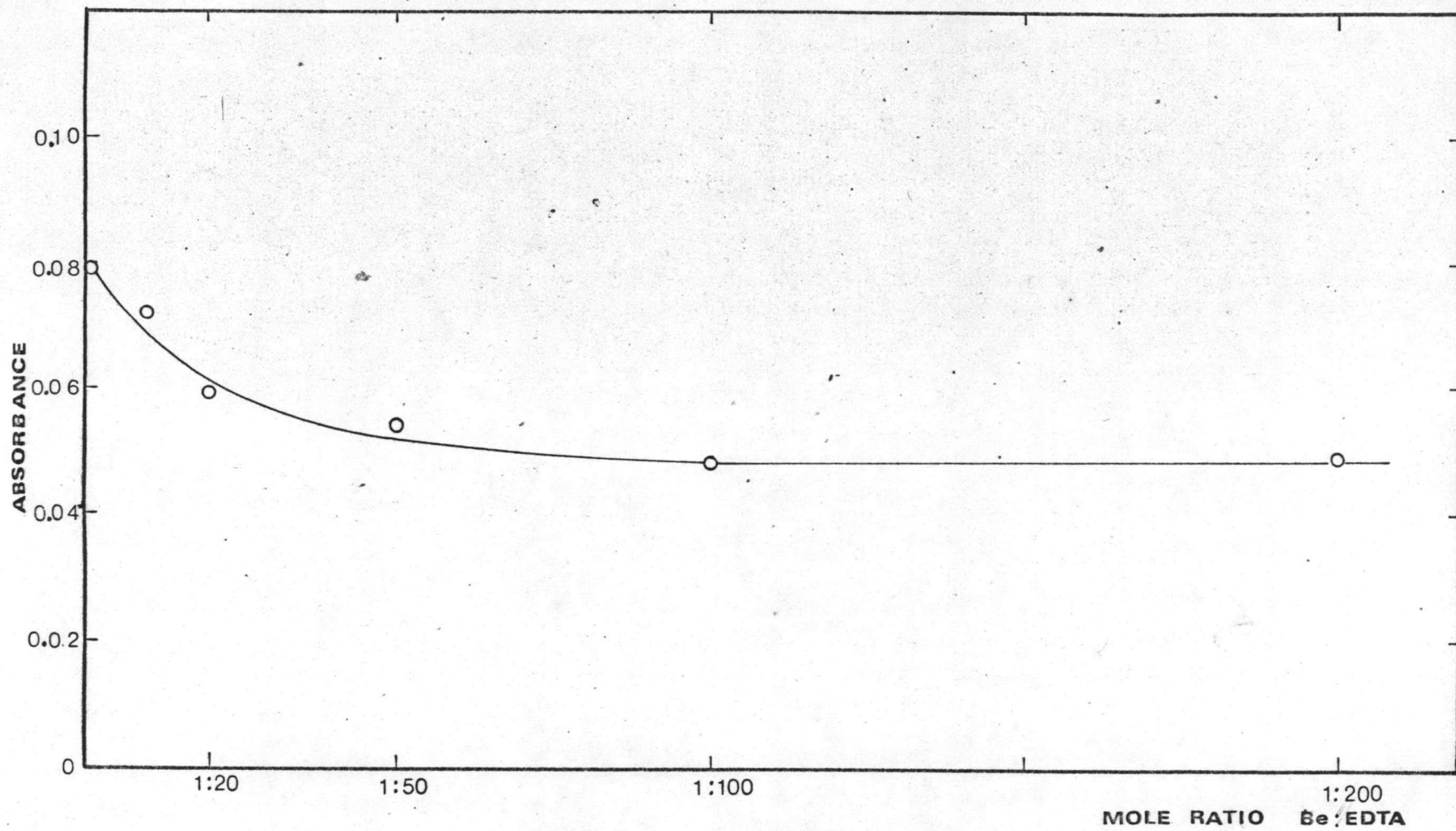


FIG. 8 Effect of EDTA on absorbance of beryllium dye complex.

of distilled water was added to adjust the volume. The absorbance of the solution was measured immediately after dilution against a reagent blank at 620 nm. After that the absorbance of the solution was measured in the time intervals of five minutes and one hour as indicated in Table VII.

(B) system with Be:EDTA = 1:33.3 : a solution containing 3 ml. of 10^{-4} F. beryllium, 10 ml. of 10^{-4} F. dye solution, 1 ml. of 10^{-2} F. EDTA solution and appropriate amount of water prepared as previously described in section 2.5.1.4 was used. The absorbance of the solution was measured at the time intervals as described in (A).

(C) system with Be:EDTA = 1:100 : a solution containing 3 ml. of 10^{-4} F. beryllium, 10 ml. of 10^{-4} F. dye solution, 3 ml. of 10^{-2} F. EDTA solution, appropriate amount of water prepared the same as (B) was used. The absorbance of the solution was measured at 620 nm. against a reagent blank as a reference in the time intervals as (A) and (B).

The results obtained are shown in Table VII. The plot of the absorbance versus time is shown in Fig.9. The color of the complex appears to be constant from 30 minutes after preparation up to at least 3 hours. The optimal times are 45 and 30 minutes for the system without EDTA and the system with EDTA respectively.

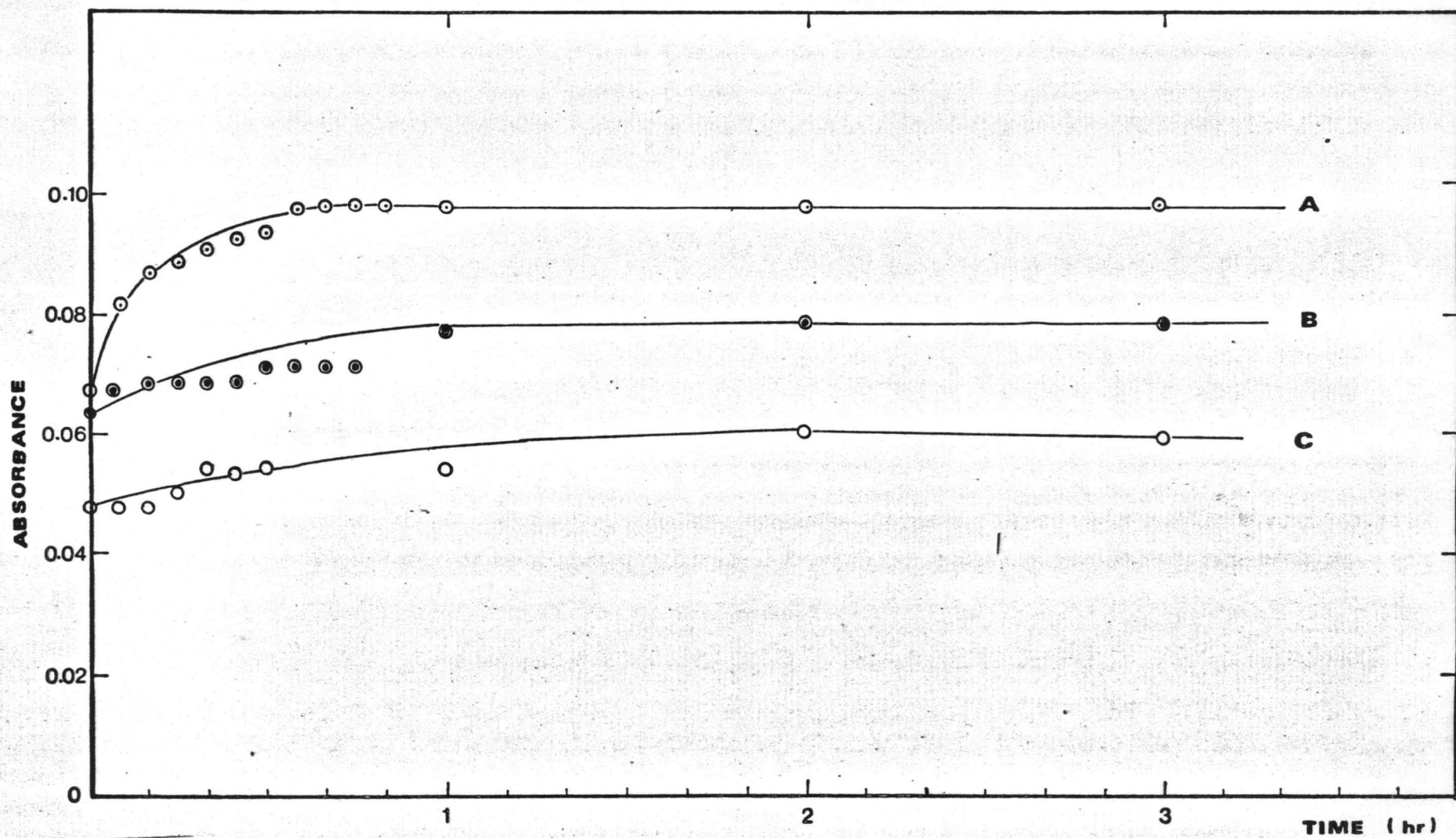


FIG. 9 Influence of time on color development of the complex.

- A** : Without EDTA
- B** : beryllium : EDTA = 1:33.3
- C** : beryllium : EDTA = 1:100

Table VII Effect of time on color development of the complex

Time	Absorbance 620 nm.		
	(A)	(B)	(C)
0 minute	0.064	0.063	0.048
5	0.082	0.068	0.048
10	0.087	0.069	0.048
15	0.089	0.069	0.051
20	0.091	0.069	0.055
25	0.093	0.069	0.054
30	0.094	0.072	0.055
35	0.098	0.072	-
40	0.098	0.072	-
45	0.098	0.072	-
50	0.098	-	-
1 hour	0.098	0.078	0.055
2	0.098	0.079	0.061
3	0.098	0.079	0.058

2.5.2 Adherence to Lambert - Beer law

2.5.2.1 Calibration curves. The linearity between the absorbance of the beryllium dye complex and the beryllium concentration was examined both with the presence of EDTA

and without.

(A) calibration curve without EDTA : aliquots of 1 to 10 ml. of 10^{-4} F. beryllium were pipetted into a series of 50-ml. beakers containing pipetted 10 ml. of 10^{-4} F. dye solution and appropriate amount of water. The pH of the solution was adjusted to 6.0 by using a 3 % aqueous solution of hexamine. The solutions were then transferred quantitatively to a series of 25-ml. volumetric flasks. Small amount of distilled water was added to adjust the final volume. After 45 minutes, the absorbances of solutions were measured at 620 nm. against a reagent blank as a reference. The data obtained is shown in Table VIII. The plot of the curve is shown in Fig.10, from which it can be seen that the beryllium complex obeys Lambert - Beer law over the concentration range up to about 6 μ g. of beryllium per 25 ml.

(B) calibration curve with Be:EDTA = 1:33.3 : working individually, aliquots containing 1 to 9 ml. of 10^{-4} F. beryllium were pipetted into a series of 50-ml. beakers containing accurately 10 ml. of 10^{-4} F. dye solution. Appropriate amount of distilled water was added to bring the volume about 20 ml. The pH of the solution was adjusted to 6.0 by using a 3 % aqueous solution of hexamine. Then 1 ml. aliquots of 10^{-2} F. EDTA was added. The pH of the solution was readjusted to 6.0. The solutions were

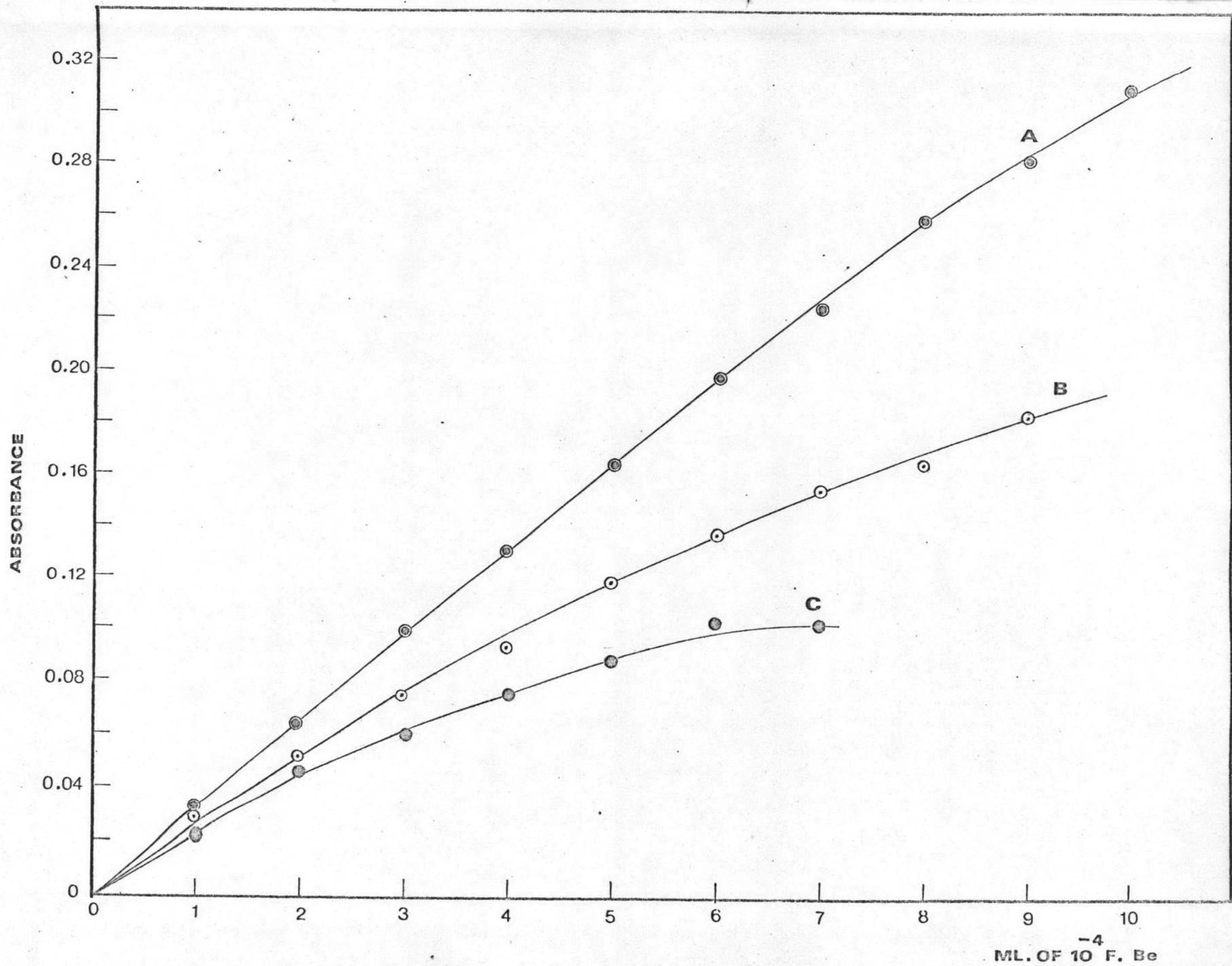


FIG. 10 Calibration curves

A : Without EDTA

B : Be : EDTA = 1 : 33.3

Table VIII Calibration curve without EDTA

Dye 10^{-4} F. (ml)	Be 10^{-4} F.		Absorbance 620nm.
	(ml)	$\mu\text{g}/25\text{ml.}$	
10	0	0 -	-
10	1	0.9013	0.033
10	2	1.8026	0.062
10	3	2.7039	0.099
10	4	3.6052	0.129
10	5	4.5065	0.164
10	6	5.4078	0.198
10	7	6.3091	0.225
10	8	7.2104	0.258
10	9	8.1117	0.282
10	10	9.0130	0.311

then transferred quantitatively to a series of 25-ml. volumetric flasks. Distilled water was used to dilute the solutions to volume and allowed to stand 30 minutes for color development. Then the absorbances of solutions were measured at 620 nm. against a reagent blank as a reference. The results obtained are shown in Table IX. The plot of the curve is also shown in Fig.10. Lambert - Beer law was obeyed for solutions containing beryllium up to 3 $\mu\text{g.}$ per 25 ml.

Table IX Calibration curve with Be:EDTA = 1:33.3

Dye 10^{-4} F. (ml)	Be 10^{-4} F.		EDTA 10^{-2} F. (ml)	Absorbance 620nm.
	(ml)	$\mu\text{g}/25\text{ml}$		
10	0	-	1	-
10	1	0.9013	1	0.028
10	2	1.8026	1	0.052
10	3	2.7039	1	0.075
10	4	3.6052	1	0.093
10	5	4.5065	1	0.118
10	6	5.4078	1	0.138
10	7	6.3091	1	0.154
10	8	7.2104	1	0.164
10	9	8.1117	1	0.185

(C) calibration curve with Be:EDTA = 1:100 : a series of solutions containing aliquots of 1 to 7 ml. of 10^{-4} F. beryllium, 10 ml. of 10^{-4} F. dye solution, appropriate amount of water and 3 ml. of 10^{-2} F. EDTA solution was prepared in the same way as (B). The data obtained is shown in Fig.10. The beryllium complex in this system is found to follow Lambert - Beer law up to the concentration of 2 μg . beryllium per 25 ml. The detailed data is shown in Table X.



Table X Calibration curve with Be:EDTA = 1:100

Dye 10^{-4} F. (ml)	Be 10^{-4} F.		EDTA 10^{-2} F. (ml)	Absorbance 620nm.
	(ml)	$\mu\text{g}/25\text{ml}$.		
10	0	-	3	-
10	1	0.9013	3	0.023
10	2	1.8026	3	0.046
10	3	2.7039	3	0.061
10	4	3.6052	3	0.076
10	5	4.5065	3	0.087
10	6	5.4078	3	0.102
10	7	6.3091	3	0.102

2.5.2.2 Apparent absorptivity. With the standard beryllium solution, the apparent absorptivity, a_m , is the slope of a plot of absorbance of the complex versus beryllium concentration. It should be used in calculations only over the absorbance range that is linear. The absorptivities in this experiment were found to be 8.3×10^3 ; 6.4×10^3 ; 5.5×10^3 for the system without EDTA, the system with Be:EDTA = 1:33.3, the system with Be:EDTA = 1:100 respectively.

2.5.2.3 Sensitivity and precision. If the molar absorptivity of the colored complex is known, the sen-

sitivity as here defined (γ / cm^2 for $\log I_0/I = 0.001$) is given by M / ϵ ; where M is the molecular weight (in grams) of the compound. Expressed in terms of element, the sensitivity is nM / ϵ ; where n is the number of atoms of the element in a molecule of a compound. (66). The sensitivity of the beryllium - dye complex is estimated to be $0.0011 \mu\text{g.}$ of beryllium per cm^2 , corresponding to $\log I_0/I = 0.001$ for the system without EDTA. For the system with $\text{Be:EDTA} = 1:33.3$ and the system with $\text{Be:EDTA} = 1:100$, the sensitivities were estimated to be 0.0014 and $0.0016 \mu\text{g}$ of beryllium per cm^2 respectively.

Precision of method. These were obtained by multiple analyses of a series of solutions containing 3 ml. of 10^{-4}F. beryllium, 10 ml. of 10^{-4}F. dye solution and appropriate amount of water under the optimal conditions. The data obtained is reported in Table XI.

The standard deviation, δ , was calculated using the formula:

$$\delta = \pm \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

where x = individual value
 \bar{x} = mean value
 n = number of value

Table XI The detailed data on standard deviation calculation

Dye 10^{-4} F. (ml)	Be 10^{-4} F. (ml)	Absorbance 620nm.
10	3	0.090
10	3	0.094
10	3	0.099
10	3	0.095
10	3	0.099
10	3	0.094
10	3	0.091
10	3	0.095
10	3	0.092
10	3	0.091

average value = 0.094

 \bar{x} = 0.094

n = 10

Calculation of standard deviation.

x	(x - \bar{x})	(x - \bar{x}) ²
0.090	- 0.004	0.000016
0.094	0.000	0.000000
0.099	+ 0.005	0.000025
0.095	+ 0.001	0.000001
0.099	+ 0.005	0.000025
0.094	0.000	0.000000
0.091	- 0.003	0.000009
0.095	+ 0.001	0.000001
0.092	- 0.002	0.000004
0.091	- 0.003	0.000009

$$\delta = \pm \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

$$\delta = \pm \sqrt{\frac{0.000090}{9}}$$

$$\delta = \pm 0.003$$

∴ Percent standard deviation = ± 0.3

2.5.3 Effect of diverse ions

The effect of 20 cations and 8 anions on the determination of beryllium was examined in various concentrations both in the absence and the presence of EDTA.

2.5.3.1 Without EDTA.

Procedure

A series of solutions containing 3 ml. of 10^{-4} F. beryllium, 10 ml. of 10^{-4} F. dye solution with various mole ratios of the diverse ions to beryllium from 100:1; 50:1; 10:1; and 1:1 was prepared. 3ml., 1.5 ml., 0.3 ml., of 10^{-2} F. diverse ions solutions were used to make a mole ratio of diverse ions to beryllium of 100:1; 50:1; and 10:1 respectively. In the case of 1:1 mole ratio, 3 ml. of 10^{-4} F. diverse ion solution was used.

These diverse ions were added individually to a beryllium - dye solution. The pH of the solutions were adjusted to 6.0 by 3 % or 25 % aqueous solution of hexamine. The final volumes were 25 ml.

The absorbance of the solution was measured at 620 nm. after 45 minutes of preparation against a reagent blank. The gain of the spectrophotometer was set at 0-1 and the slit width was 0.25. The results obtained are summarized in Table XII.

Table XII Effect of diverse ions on the determination of beryllium without EDTA

Diverse ions	Ion:Be = 100:1		Ion:Be = 50:1		Ion:Be = 10:1		Ion:Be = 1:1	
	Absorbance 620nm.	Be found $\mu\text{g}/25\text{ml.}$	Absorbance 620nm.	Be found $\mu\text{g}/25\text{ml.}$	Absorbance 620nm.	Be found $\mu\text{g}/25\text{ml.}$	Absorbance 620nm.	Be found $\mu\text{g}/25\text{ml.}$
-	0.094	2.58	0.094	2.58	0.094	2.58	0.094	2.58
Th ⁴⁺	<< Be	<< Be	<< Be	<< Be	0.108	2.97	0.061	1.69
Ni ²⁺	0.137	3.76	0.148	4.06	0.165	4.51	0.115	3.18
Co ²⁺	0.119	3.29	0.121	3.35	0.115	3.18	0.092	2.54
Ca ²⁺	0.102	2.80	0.101	2.78	0.091	2.52	0.090	2.48
Mn ²⁺	0.102	2.80	0.100	2.75	0.092	2.54	0.083	2.28
Sr ³⁺	0.108	2.97	0.100	2.75	0.092	2.54	0.090	2.48
Cu ²⁺	0.085	2.34	0.100	2.75	0.102	2.80	0.122	3.37
Cd ²⁺	0.120	3.31	0.120	3.31	0.089	2.46	0.092	2.54
Fe ²⁺	0.118	3.27	0.122	3.38	0.127	3.49	0.123	3.39
Mg ²⁺	0.098	2.70	0.100	2.75	0.087	2.41	0.092	2.54
UO ₂ ²⁺	0.078	2.14	0.073	2.01	0.080	2.21	0.112	3.10
Mo ⁶⁺	0.094	2.58	0.089	2.46	0.082	2.25	0.089	2.46
Hg ²⁺	0.142	3.90	0.141	3.88	0.129	3.58	0.105	2.88
NO ₃ ⁻	0.092	2.54	0.089	2.46	0.082	2.25	0.082	2.25

Table XII Continued

Diverse ions	Ion:Be = 100:1		Ion:Be = 50:1		Ion:Be = 10:1		Ion:Be = 1:1	
	Absorbance 620nm.	Be found $\mu\text{g}/25\text{ml.}$	Absorbance 620nm.	Be found $\mu\text{g}/25\text{ml.}$	Absorbance 620nm.	Be found $\mu\text{g}/25\text{ml.}$	Absorbance 620nm.	Be found $\mu\text{g}/25\text{ml.}$
-	0.094	2.58	0.094	2.58	0.094	2.58	0.094	2.58
$\text{C}_6\text{H}_5\text{O}_7^{3-}$	0.019	0.518	0.029	0.721	0.049	1.35	0.083	2.28
F^-	0.009	0.252	0.012	0.333	0.062	1.71	0.093	2.56
PO_4^{3-}	0.072	1.98	0.081	2.23	0.079	2.18	0.088	2.44
OAc^-	0.083	2.28	0.083	2.28	0.078	2.14	0.089	2.46
MoO_4^{2-}	0.091	2.52	0.086	2.39	0.078	2.14	0.088	2.44
SO_4^{2-}	0.086	2.39	0.088	2.44	0.075	2.07	0.092	2.54
Cl^-	0.089	2.46	0.083	2.28	0.079	2.16	0.097	2.68

2.5.3.2 With EDTA. The effect of EDTA as a mass masking agent for the diverse ions was studied in two ways: (A) the concentration of EDTA was varied according to the concentration of diverse ions used and (B) the concentration of EDTA was fixed at 100 fold excess over beryllium.

Procedure

(A) The solutions prepared the same as 2.5.3.1 (preceding section) were used. After the diverse ion had been added and the pH of the solution was adjusted to 6.0, an aliquot of EDTA of the same amount and concentration as diverse ion was added, i.e., 3 ml., 1.5 ml., 0.3 ml., of $10^{-2}F.$ and 3 ml. of $10^{-4}F.$ EDTA solution. The pH of the solution was readjusted to 6.0. The absorbance of the solution was measured under the same conditions as 2.5.3.1. The results obtained are shown in Table XIII.

(B) The solutions prepared the same as (A) were used but the concentration of EDTA was fixed at 100 fold excess over beryllium (3 ml. of $10^{-2}F.$ EDTA solution). All of the conditions were the same as (A). Results from the absorbance measurement are shown in Table XIV.

It was found that some cations precipitated with the dye at pH 6.0 , they were Ba^{2+} , Sn^{2+} , Pb^{2+} , La^{3+} , Cr^{3+} , Sc^{3+} , and Al^{3+} .

Table XIII Effect of diverse ions on the determination of beryllium with EDTA (system A)

Diverse ions	Ion:Be = 100:1 Absorbance 620nm.	Ion:Be = 50:1 Absorbance 620nm.	Ion:Be = 10:1 Absorbance 620nm.	Ion:Be = 1:1 Absorbance 620nm.
Th ⁴⁺	0.082	0.088	0.093	0.098
Ni ²⁺	0.100	0.092	0.100	0.092
Co ²⁺	0.100	0.100	0.098	0.098
Ca ²⁺	0.077	0.082	0.098	0.097
Mn ²⁺	0.100	0.093	0.089	0.098
Sr ³⁺	0.072	0.086	0.092	0.098
Cu ²⁺	0.134	0.112	0.098	0.101
Cd ²⁺	0.099	0.098	0.092	0.097
Fe ²⁺	0.132	0.117	0.090	0.097
Mg ²⁺	0.071	0.072	0.098	0.098
UO ₂ ²⁺	0.098	0.078	0.080	0.120
Mo ⁶⁺	0.052	0.057	0.076	0.096
Hg ²⁺	0.075	0.089	0.091	0.096
-	0.048	0.054	0.084	0.092

Table XIV Effect of diverse ions on the determination of beryllium with EDTA (system B)

Diverse ions	Ion:Be = 100:1 Absorbance 620nm.	Ion:Be = 50:1 Absorbance 620nm.	Ion:Be = 10:1 Absorbance 620 nm.	Ion:Be = 1:1 Absorbance 620nm.
Th ⁴⁺	0.082	0.039	0.023	0.050
Ni ²⁺	0.100	0.033	0.028	0.042
Co ²⁺	0.100	0.032	0.040	0.039
Ca ²⁺	0.077	0.050	0.046	0.041
Mn ²⁺	0.100	0.060	0.058	0.050
Sr ³⁺	0.072	0.042	0.059	0.052
Cu ²⁺	0.134	0.050	0.022	0.037
Cd ²⁺	0.099	0.050	0.075	0.038
Fe ²⁺	0.132	0.033	0.039	0.041
Mg ²⁺	0.071	0.055	0.048	0.038
UO ₂ ²⁺	0.098	0.072	0.062	0.047
Mo ⁶⁺	0.052	0.048	0.048	0.056
Hg ²⁺	0.075	0.070	0.052	0.045
-	0.048	0.048	0.048	0.048

2.6 Determination of Empirical Formula of the Complex

2.6.1 Method of continuous variation (Job's Plot)

Numerous techniques have been devised for identifying complexes in solution by spectrophotometric measurements. The method of continuous variation is one of the most widely used, first described by Job (67) and later extended in applicability to include successive complex formation by Vosburgh and Cooper (68). To determine the value of n in a complex system AB_n , it is convenient to start with solutions of A and B of equal concentration. A series of solutions with different ratios of A to B but for which the sum of the concentration of A and B in each solution is a constant, is prepared by adding x mole per liter of B to $(1-x)$ mole per liter of A. The solutions are then examined spectrophotometrically. Absorbance at a given wavelength, e.g., at which AB_n absorbs but not A or B, are plotted versus x . From the value of x at the maximum absorbance and the relationship $n = x / 1-x$, the value of n is obtained. In a weak complex system, a curvature of the graph is established. It is customary in such a case to extrapolate the two straight-line portions on either side of the maximum in order to use their point of intersection as a more precise estimation

for the position of maximum absorbance.

Procedure

A series of solutions of beryllium-dye mixtures containing various mole fractions of beryllium from 0 to 1 was prepared. The sum of the concentrations of beryllium and the dye was kept constant at 0.2×10^{-4} mole per liter. The pH of the solutions was adjusted to 6.0 by 1 % aqueous solution of hexamine. The final volume was 25 ml. After 45 minutes, the absorbance of each solution was measured at 620 nm. and 525 nm. against water as the reference. The gain of the spectrophotometer was set at 0-1 and the slit width was 0.25. The data is shown in Table XV and the corresponding Job's plot is shown in Fig. 11.

Fig. 11 shows that a 1:1 complex is formed between beryllium and the dye.

2.6.2 The mole ratio method

Yoe and Jones (69) found that for a very stable complex, a plot of absorbance against molar ratio of component B to component A, with A constant, rose from the origin as a straight line and deflect sharply to constant absorbance at the molar ratio of the components in the complex.

Table XV The detailed data on method of
continuous variation

Dye 10^{-4} F. (ml)	Be 10^{-4} F. (ml)	$\frac{[\text{Be}]}{[\text{Be}] + [\text{Dye}]}$	Absorbance	
			620nm.	525nm.
5.0	0.0	0.0	0.116	0.392
4.0	1.0	0.2	0.121	0.288
3.0	2.0	0.4	0.122	0.190
2.5	2.5	0.5	0.115	0.150
2.0	3.0	0.6	0.100	0.115
1.5	3.5	0.7	0.075	0.083
1.0	4.0	0.8	0.049	0.058
0.0	5.0	1.0	0.002	0.002

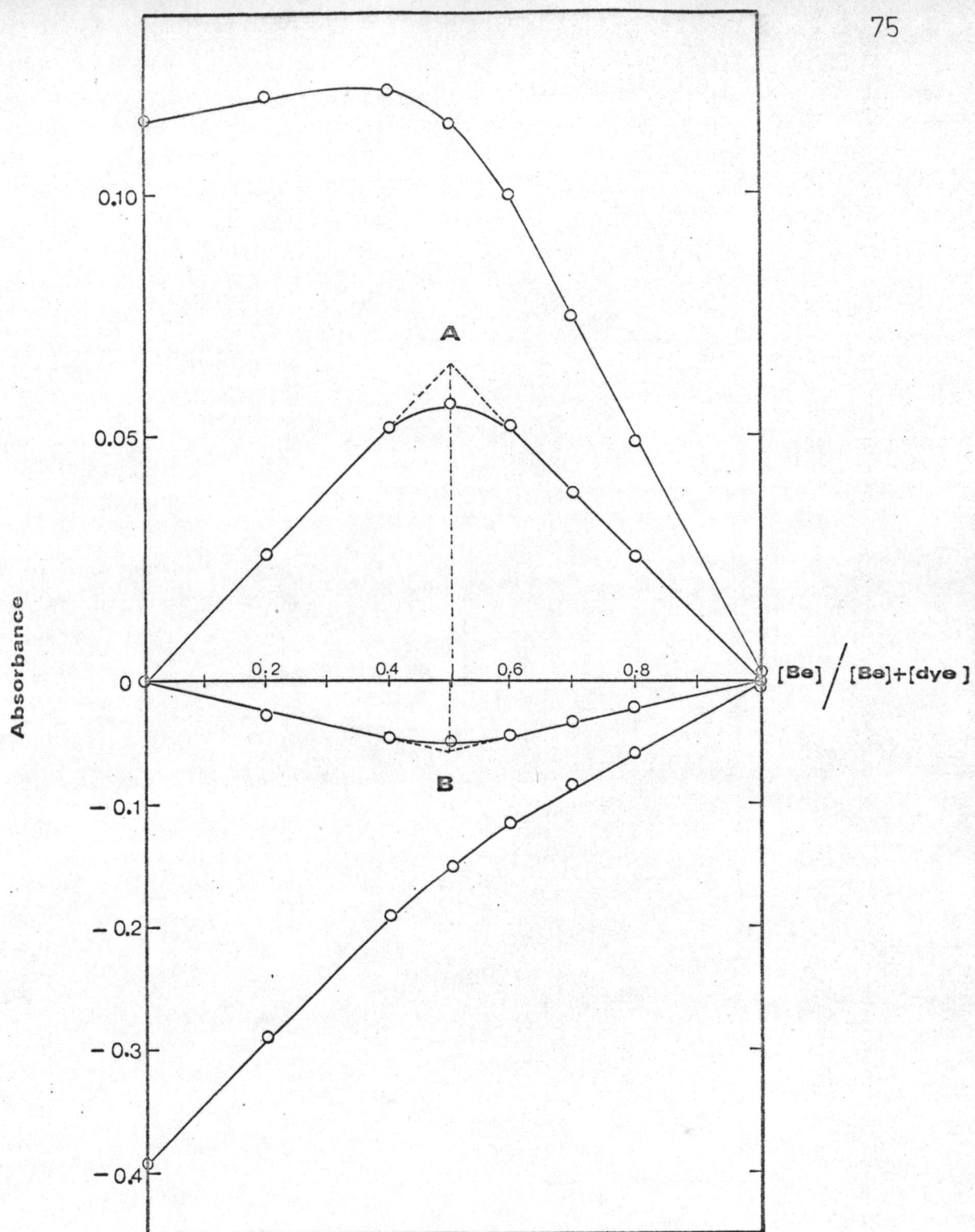


FIG. 11 Continuous variation method.

A : 620 nm.

B : 525 nm.

A complex that undergoes appreciable dissociation in solution gives, however, a continuous curve which becomes approximately parallel to the molar ratio axis only when an excess of the variable component is added. Results obtained by extrapolation of this curve are uncertain.

Procedure

A series of solutions containing equal formal concentrations of the dye but different formal concentrations of beryllium was prepared. The concentration of the dye was fixed at 0.16×10^{-4} mole per liter (4 ml. of 10^{-4} F. dye solution per 25 ml.) with variation of 10^{-4} F. beryllium from 0 - 8 ml. The pH of the solutions were adjusted to 6.0 by 1 % aqueous solution of hexamine. The final volumes were 25 ml. After 45 minutes of preparation, the absorbance of the solution was measured against a reagent at 620 nm. The gain of the spectrophotometer was set at 0-1 and the slit width was 0.25. The detailed data is shown in Table XVI . The mole ratio plot is shown in Fig.12.

It is seen from Fig.12 that beryllium forms a 1:1 metal - ligand complex with the dye.

2.6.3 Slope ratio method

A so-called slope ratio method was proposed by Aubrey E. Harvey, Jr., and Delmer L. Manning in 1950 (70).

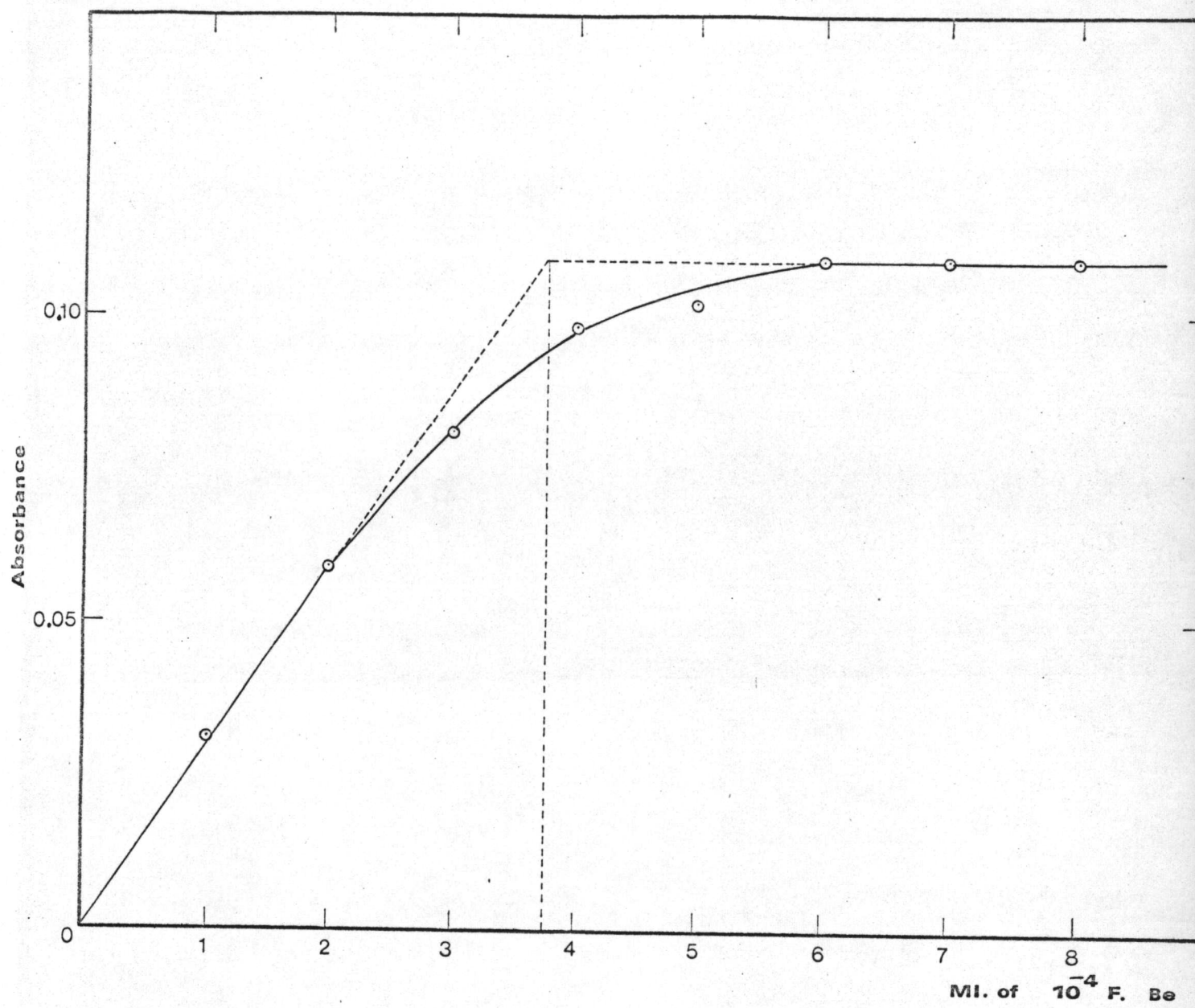


FIG. 12

Mole ratio method.

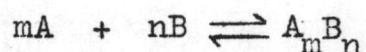
Fixed dye 4 ml.

Table XVI The detailed data on molar ratio method

Dye 10^{-4} F. (ml)	Be 10^{-4} F. (ml)	Absorbance 620nm.
4	0	-
4	1	0.031
4	2	0.059
4	3	0.081
4	4	0.098
4	5	0.102
4	6	0.109
4	7	0.109
4	8	0.109

The method is based upon absorbance measurements of solutions in which dissociation of the complex is repressed by a large excess of one of the reactants. The slope ratio method, however can be applied in relatively few cases. To be applicable, the system under investigation

must not give rise to more than one complex, the complex must exhibit characteristic absorption, different from its progenitors, and Beer's law should be followed. If the complex formed in the reaction



is to be identified, small amounts of A are added to a solution containing a large excess of B. The slope S_A , of a plot of absorbance versus formal concentration of A is determined. Similarly, small amounts of B are added to a solution containing a large excess of A and the slope, S_B , of a plot of absorbance (at the same wavelength as before) versus formal concentration of B is determined. A simple derivation, based upon Beer's law and the assumption that the small quantities added in each case are quantitatively converted to equivalent amounts of $A_m B_n$, gives the relationship :

$$\frac{S_A}{S_B} = \frac{m}{n}$$

from which the empirical formula is easily deduced.

Procedure

For the study by this method, two series of solutions were prepared. In one series the concentration

of beryllium was varied from 0-0.16 x 10⁻⁴ mole per liter in the presence of a constant 10 ml. of 10⁻⁴F. dye solution; in the other, the amount of the dye was varied and beryllium fixed (the same concentration as before). The pH of the solution was adjusted to 6.0 by 3 % aqueous solution of hexamine in the constant dye system (B) and 1 % aqueous solution of hexamine in the constant beryllium system (A). Final volume of all solutions were 25 ml. The absorbance was measured at 620 nm. within 45 minutes after preparing the solutions against dye in (B) and water in (A). The data obtained is shown in Table XVII , and the slope ratio plot is shown in Fig.13.

Table XVII The detailed data on slope ratio method
(A) Beryllium >> Dye

Be10 ⁻⁴ F. (ml)	Dye10 ⁻⁴ F. (ml)	Absorbance 620nm.
10	0	-
10	1	0.052
10	2	0.103
10	3	0.153
10	4	0.198

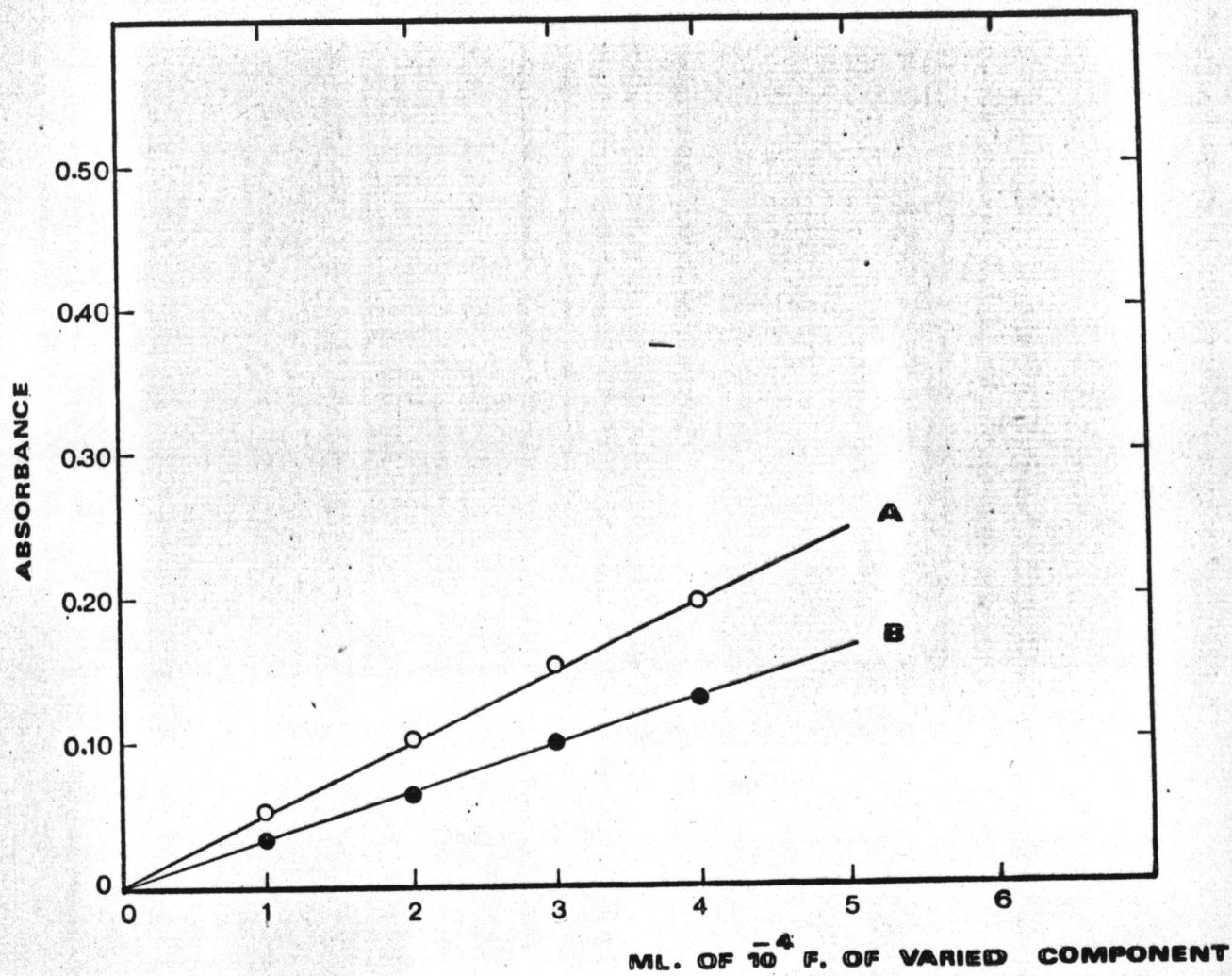


FIG. 13

Slope ratio method.

A : Fixed Bc 10 ml.

B : Fixed dye 10 ml.



(B) Dye >> Beryllium

Dye 10^{-4} F. (ml)	Be 10^{-4} F. (ml)	Absorbance 620nm.
10	0	-
10	1	0.033
10	2	0.062
10	3	0.099
10	4	0.129

From Fig.13, the slope ratio of A/B is approximately 1.0. This is in accordance with the result obtained by either of the preceding methods that beryllium forms a 1:1 metal - ligand complex with the dye.

2.6.4 Absorption spectra studies of beryllium - dye complex

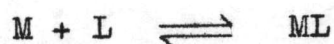
Procedure

A series of solutions containing various molar ratios of dye to beryllium was prepared. 10 ml. of 10^{-4} F. dye solution was used in each case with 5 ml., 10 ml. of 10^{-4} F. beryllium, and 0.20 ml. of 10^{-2} F. beryllium to make a molar ratio of dye to beryllium of 2:1, 1:1, and 1:2 respectively. The pH of the solutions were adjusted to 6.0 by using a 3 % aqueous solution of hexamine. The final

volumes were 25 ml. The spectra of the three systems were recorded from 400-700 nm. using water as a reference. The spectra of the dye was also recorded. The gain of the spectrophotometer was set at 0-2 and the slit width was 0.25. The results obtained are shown in Fig. 14.

2.6.5 Apparent stability constant of complex

The apparent stability constant of the beryllium-dye complex under the conditions of this experiment was evaluated. On the basis of the previous experiments, a 1:1 metal-ligand complex is assumed,



it is possible to write the stability constant as;

$$K = \frac{(1 - \alpha)}{\alpha^2 C}$$

where α , the degree of dissociation, was established from the relationship:

$$\alpha = \frac{A_m - A_s}{A_m}$$

and C is the concentration of the complex in mole per liter.

The continuous variation curve (Fig. 11) may be used to obtain the values of A_m and A_s . From Fig. 11, it was found that A_m is equal to 0.065 and A_s is equal

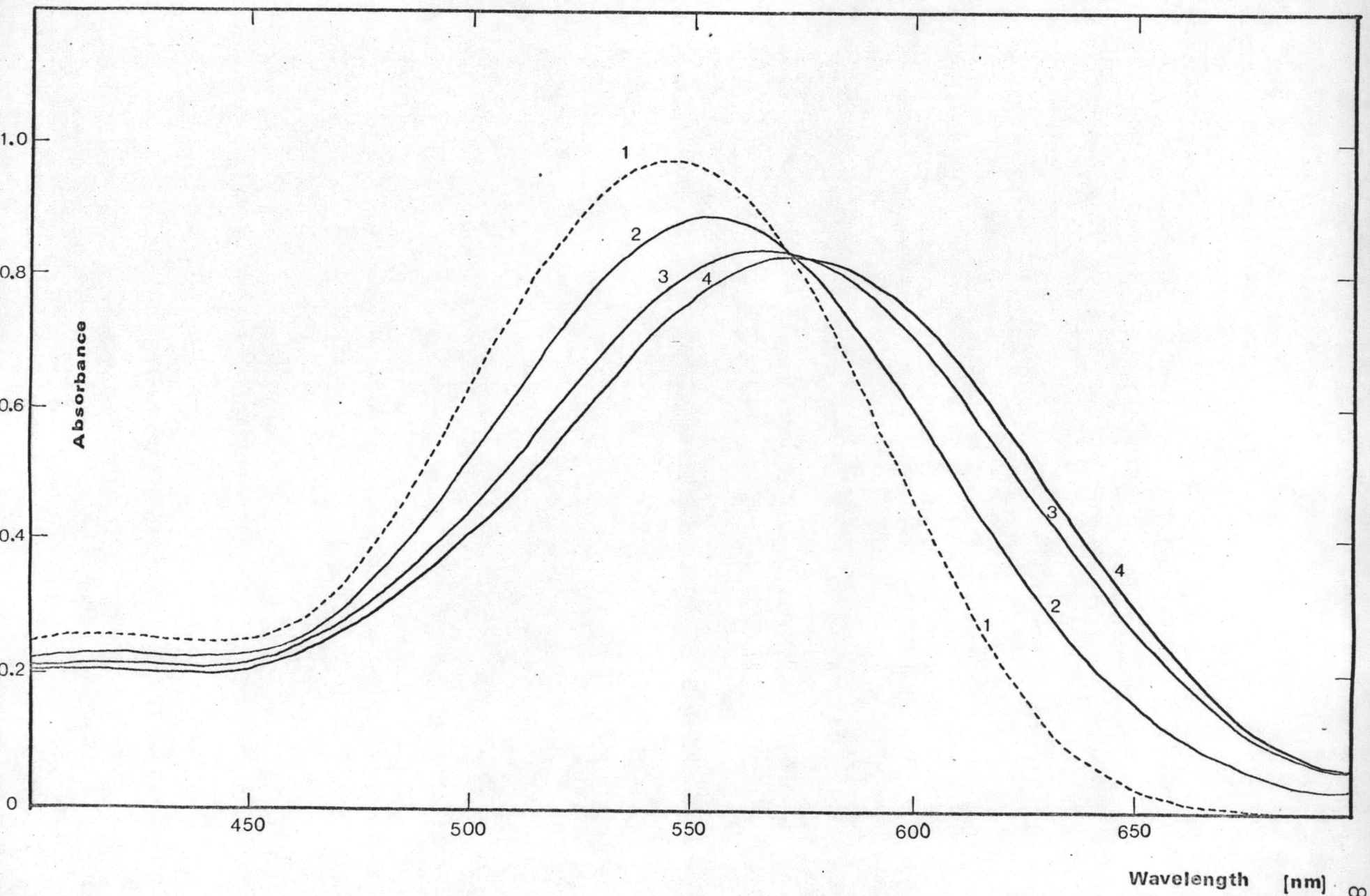


FIG. 14 Absorption spectra of variation of dye / Be ratio at pH 6. Reference : Water.
 1 : Free dye ; 2 : 1:0.5 ; 3 : 1:1 ; 4 : 1:2

to 0.056 for a solution that was $10^{-5}M.$ in beryllium. The degree of dissociation constant was calculated to be 0.138. The value of the apparent stability constant, K , under these particular conditions was found to be 4.5×10^6 with the pK value of 6.65.

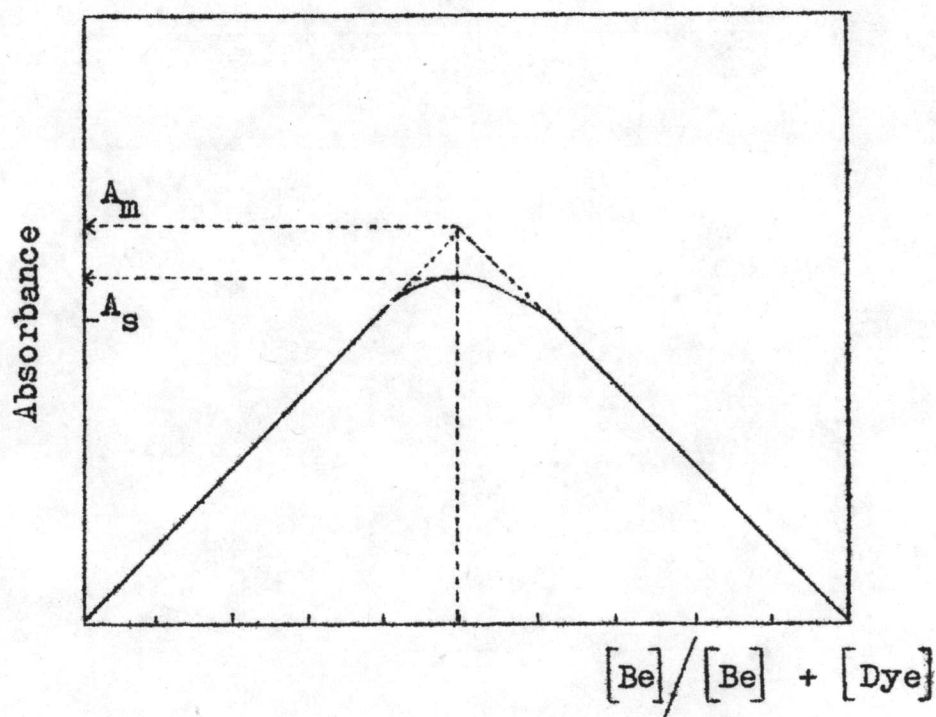


Fig.11 Continuous variation method.

2.7 Discussion and Conclusion

In the present investigation, the 1,8-dihydroxy-2,7-bis(3,6-disulfo-8-hydroxy-1-naphthylazo)-naphthalene-3,6-disulfonic acid (dye) has been synthesized and used as a metallochromic reagent for beryllium. The dye was found to have an acid-base property. The color changes from wine-red in acid solution to purple, blue-mauve and finally blue as the pH is increased.

Qualitative color reaction of beryllium with the dye solution has shown that beryllium can form complex with the dye but the complex is stable in limited pH range.

The absorption spectra of the dye solution at various pH values was studied as shown in Fig.2 (page 42). The maximum absorbance occurs at about 544 nm. over the pH range from 1.0 to 7.0. The absorbance decreases above pH 6, and the maximum absorbance shows a bathochromic shift above pH 7.0.

The absorption spectra of the beryllium-dye complex was obtained in the pH range from 5.0 to 8.0 as shown in Fig.3 (page 44). The spectra is shifted by changing the pH since there are some ionizable hydroxy groups left after the complex has been formed. (The structure of the beryllium-dye complex is shown on page 91). The maximum absorbance also shows a bathochromic shift

but in a lesser extent when compared with the free dye. Fig.4 (page 45) shows the spectra of the complex measured against a reagent blank. The beryllium-dye complex exhibits only one absorption peak at each investigated pH values. This suggests that there is only one species of the beryllium-dye complex exists.

In the spectrophotometric determination of beryllium with the dye as a metallochromic reagent, the optimal conditions must be investigated.

The absorption curve of the beryllium-dye complex with a reagent blank as a reference has an absorption maximum at about 620 nm., which is the optimal wavelength, as shown in Fig.5 (page 47). The effect of pH on the absorbance of the complex was examined as shown in Fig.6 (page 49). It can be seen that the optimum pH range for analytical purposes lies between 5.5 and 6.4 at 620 nm. The effect of the dye concentration on the color development of the complex at pH 6.0 was examined. The absorbance of the complex was found to increase gradually at higher reagent concentrations (Fig.7 page 51). However, above 6 ml. of 10^{-4} F. dye solution in final volume of 25 ml., the rate of the increase of the absorbance is relatively small. On the basis of this information, 10 ml. of 10^{-4} F. dye solution was chosen as the fixed quantity to be added.

EDTA was tried to use as a mass masking agent. The effect of EDTA on the absorbance of the complex was studied. It was found that EDTA has no effect on the absorbance of the dye but has some effects on the complex. From Fig.8 (page 54), it can be seen that the absorbance of the complex decreases gradually as the mole ratio of EDTA to beryllium increases. The linearity is obtained when the mole ratio of EDTA to beryllium reaches 100:1. At the mole ratio of EDTA to beryllium higher than this, the absorbance of the complex is almost constant. The influence of time on color development was also studied both with the presence of EDTA and without. It can be seen from Fig.9 (page 56) that the color of the beryllium-dye complex developed gradually; maximum color development was attained in about 45 minutes after preparation for the system without EDTA and 30 minutes with the presence of EDTA. The absorbance then remained constant for at least 3 hours.

The calibration curves were established under the optimal conditions as shown in Fig.10 (page 59). Lambert-Beer law is obeyed up to a beryllium concentration of 0.24 ppm. (6 μg .of beryllium per 25 ml.) for the system without EDTA; 0.12 ppm. (3 μg .of beryllium per 25 ml.)for the system with Be:EDTA = 1:33.3 .

and 0.08 ppm. (2 $\mu\text{g.}$ of beryllium per 25 ml.) for the system with Be:EDTA = 1:100, and the molar absorptivities are 8.3×10^3 ; 6.4×10^3 and 5.5×10^3 respectively. The sensitivity of the reaction as expressed by Sandell's notation is 0.0011 $\mu\text{g.}$ of beryllium per cm.^2 for $\log I_0/I = 0.001$ for the system without EDTA. For the system with Be:EDTA = 1:33.3 and the system with Be:EDTA = 1:100, the sensitivities are 0.0014 $\mu\text{g.}$ of beryllium per cm.^2 and 0.0016 $\mu\text{g.}$ of beryllium per cm.^2 for $\log I_0/I = 0.001$ respectively. The percent standard deviation for the determination of beryllium is ± 0.3 for 10 samples containing 2.70 $\mu\text{g.}$ of beryllium per 25 ml.

The spectrophotometric determination of beryllium in the presence of diverse ions was subsequently studied. The effects of diverse ions on the determination of beryllium were investigated both with EDTA as a mass masking agent and without. The results are summarized in Table XII (page 67-68), Table XIII (page 70) and Table XIV (page 71). It can be concluded from Table XII (page 67-68), based on the deviation of $\pm 5\%$ from the standard sample (without diverse ions), that the following ions (the ratio of ion to beryllium is indicated in parentheses) do not interfere with the determination of beryllium without EDTA as a mass masking agent: Mg(II)(100), Mo(VI)(100); Ca(II)(10),

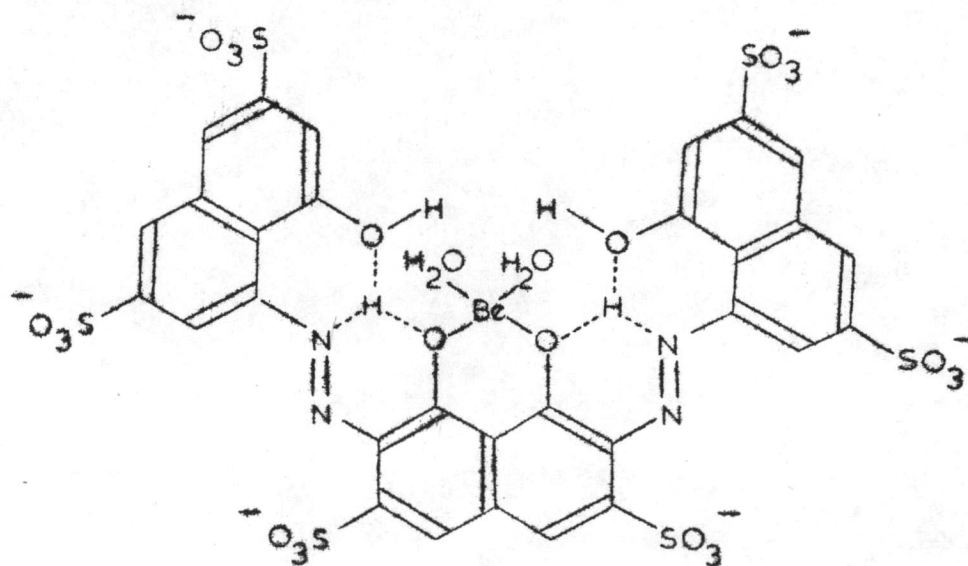
Mn(II)(10), Sr(III)(10), Cd(II)(10); Co(II)(1); NO_3^- (100), MoO_4^{2-} (100), Cl^- (100); F^- (1), OAc^- (1) and SO_4^{2-} (1) whereas Th(IV), Ni(II), Cu(II), Fe(II), UO_2 (II), Hg(II), and citrate interfere in all concentrations. Ba(II), Sn(II), Pb(II), La(III), Cr(III), Sc(III), and Al(III) were precipitated with the dye.

EDTA was then tried to use as a mass masking agent. First the quantities of EDTA added were varied according to the amount of diverse ions present. The results obtained are summarized in Table XIII (page 70). It seems that EDTA can be used as a mass masking agent but it is not practical. It is not reasonable to state which ion is interfered with the determination of beryllium by comparing the measured absorbance with the standard sample as a reference because there is no relation in the calibration curve between them. In fact the quantities of the diverse ions in the actual sample are unknown.

According to the effect of EDTA on the absorbance of beryllium-dye complex as previously studied, 100 mole excess of EDTA over beryllium concentration was selected as the quantity of the masking mass added. The results obtained are summarized in Table XIV (page 71). But again the results obtained are not satisfactory. Although there is a calibration curve for the system of beryllium

complex in the presence of 100 fold excess of EDTA, it is not reasonable to use it in practice. This is because of the quantities of EDTA left after masking the diverse ions are not in the linear range of the curve as in Fig.8 (page 54). It is suggested that the amount of EDTA added as a mass masking agent should be 200-300 fold molar excess over beryllium concentration, rather than 100 fold molar excess as investigated. The alternative for improving the selectivity of the method can be achieved by eliminating the diverse ions before the determination of beryllium either by physical separation or chemical separation for instance: ion-exchange, solvent extraction, precipitation, etc.,.

It was found that beryllium reacts with the dye at pH 6.0 to form a 1:1 metal-ligand complex. The following possible structure may be assigned:



The proposed structure is based on maximum coordination number 4 of beryllium. The evidence for the structure of the complex was obtained from spectral studies, from the method of continuous variation, the mole ratio method and the slope ratio method.

The curves shown in Fig.14 (page 84) indicate the changes in the dye spectrum when beryllium is added in various mole ratios with the same quantity of dye being present in each case. When beryllium and dye are present in equimolar quantities, an absorption peak appears at about 567 nm. Where there is a twofold molar excess of dye, it appears that only a mixture of 1:1 complex and free dye exists, as shown by the fact that the 2:1 curve passes through the intersection of the curve for 1:1 complex and free dye. When beryllium is increased beyond 1:1 ratio, the peak at 567 nm. persists and the height of the 620 nm. peak (which was measured against a reagent blank and did not show in that Figure) increases. This suggests that there is no complex containing beryllium than 1:1 complex but the excess of beryllium serves to force all the dye into the 1:1 form.

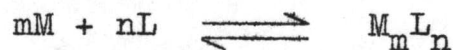
In the method of continuous variation, the absorbance of beryllium-dye solution was measured at 620 nm. and 525 nm. These two wavelengths were selected because they both give the greatest difference in absorbance



between the dye and beryllium complex. At 620 nm., the absorbance of the complex is greater than the dye and vice versa at 525 nm. The method establishes that the beryllium-dye ratio is 1:1 as shown in Fig.11 (page 75).

The curve in Fig.12 (page 77) was obtained by the molar ratio method. The curve shows no sharp break at the point of equimolar amount of the dye and beryllium, but rather gradual sloping off to become parallel to the Y axis at a higher amount of beryllium. The sloping portion of the curve indicates that appreciable dissociation of the complex occurs. However, result obtained by extrapolation shows that beryllium forms 1:1 metal-ligand complex with the dye.

Further evidence was made by the slope ratio method. The results obtained are shown in Fig.13 (page 81), from which the $\text{slope A} / \text{slope B}$ gives the ratio of n to m of the reaction,



From Fig.13 ; slope A = 0.049

 slope B = 0.040

$$\text{slope A} / \text{slope B} = \frac{0.049}{0.040} = 1.22 \approx 1$$

The ratio of beryllium to dye is agreed with the formula obtained by the preceding studies.

The probable site of coordination involves the 1,8-hydroxy groups in the chromotropic acid residue in the center of the dye molecule because beryllium is so small (ionic radius of 0.30A) and it is well known to react with 1,8-dihydroxy naphthalene. The apparent stability constant for the complex at pH 6.0 was calculated from the curve of the continuous variation (Fig.11 page 75), to be 4.5×10^6 with the pK value of 6.65.

As a conclusion, the spectrophotometric determination of beryllium using this dye , 1,8-dihydroxy-2,7-bis(3,6-disulfo-8-hydroxy-1-naphthylazo)-naphthalene-3,6-disulfonic acid, as a metallochromic reagent requires more detailed improvements. Although the molar absorptivity is not high when compared with the other reagents, the method is simple and the complex is reasonably stable. However, the determination of beryllium in the absence of diverse ions gives satisfactory results because the calibration curve obeys Lambert-Beer law reasonably well.

Further work is necessary to evaluate fully the applicability of the dye to the determination of trace amounts of beryllium in the presence of other ions. However, this reagent would appear to be a potentially useful reagent.