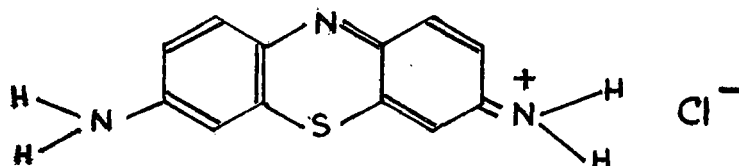


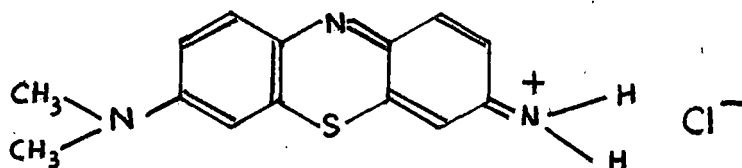
CHAPTER - III

1. Structure of the dyes:

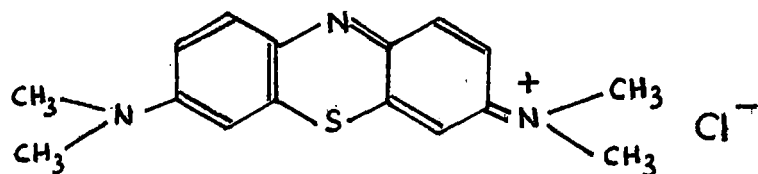
Four cationic dyes of phenothiazine class have been chosen in the present work. The structure of the dyes are as follow.



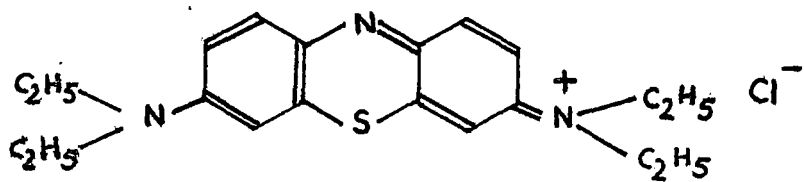
Thionine



Dimethyl Thionine (DMT)



Tetramethyl Thionine (MTB)



Tetraethyl Thionine (TET)

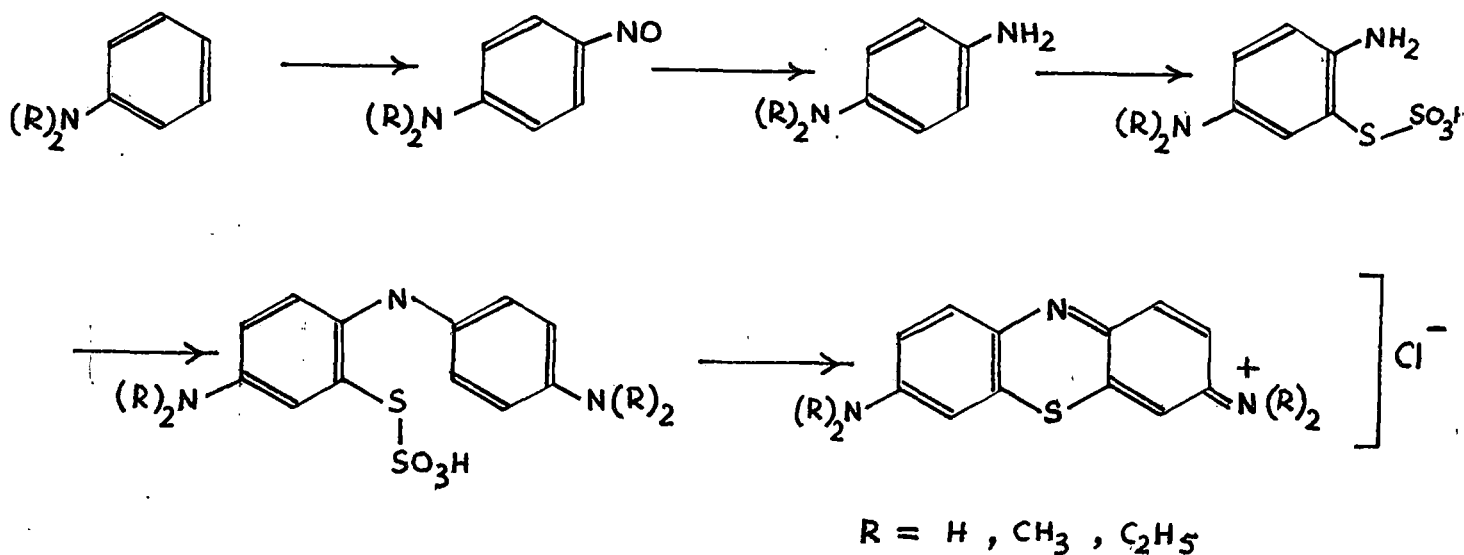
B. Synthesis of the dyes:

The Thiazine dyes may be synthesized by the following three steps (72).

(a) Nitrosodimethyl aniline is obtained from dimethyl aniline by treating it in hydrochloric acid solution with sodium nitrite. This nitroso compound is reduced obtaining thereby the p-aminodimethyl aniline.

(b) The p-aminodimethyl aniline in an acid solution is oxidized together with additional molecule of dimethyl aniline and simultaneously the thiosulphuric acid is introduced into the molecule. This compound is in the nascent state when oxidation is done in the presence of thiosulphuric acid.

(c) This thiosulphuric acid is now transferred into Methylene Blue or other thiazine dyes by ring closure.



(a) p-aminodialkyl (or diethyl) aniline:

$\frac{2}{10}$ mol. of pure dialkyl aniline is taken in 75 gm. of conc. HCl (30% HCl) and allowed to cool. Afterwards it is treated with 150 gm. of ice and to this is dropped within one hour, 14.7 gm of 100% sodium nitrite (as 20% solution). The nitroaction is complete in 4 hours. Now one adds further 110 gm HCl (30%), 200 gm ice and during 1/4th hour under mechanical stirring 35 gm of good zinc dust. The temperature may be raised to 25°C without harm. The solution is now colourless and the mineral acid becomes neutralised; this is filtered and the zinc dust is washed well with little water.

(b) The following solutions are mixed with p-aminodialkyl aniline which is made acidic with 4 gm of conc. H_2SO_4 and mixed with 100 gm 50% neutral $SnCl_2$ solution.

Solution I = 30 gm of pure aluminium sulphate in 60 c.c. water.

Solution II = 52.5 gm sodium thiosulphate in 60 c.c. water.

Solution III = 1/6 mol. of dialkyl aniline in 27 gm conc. HCl.

Solution IV = 27 gm of sodium dichromate in 30 c.c. water

Solution V = 25 gm very finely ground pyrolusite in 30 c.c. water made to a homogeneous paste.

The beaker containing p-azino dialkyl aniline is covered and a jet of steam is introduced through a tube. Now under good stirring of the above at ordinary temperature, solution I is added, then solution II and immediately after two seconds 1/3rd of solution IV corresponding to 19 gm of sodium dichromate. Through introduction of steam, the temperature of the mixture is raised to 40°C, it is then treated with solution III and the remainder of solution IV and heated rapidly to 70°C. The solution becomes dark greenish blue. At 70°C one adds the paste of V and heats to 85°C.

(The purpose of the addition of pyrolusite is ring closure freeing sulphurous acid which is converted into unreactive dithionate. The place of pyrolusite could be taken by copper sulphate.)

At 85°C the solution assumes a beautiful bronze lustre and the dye precipitates out of the concentrated zinc chloride solution. After 1/2 hour, the solution is cooled to 50°C and 70 gm of conc. sulphuric acid is added, which dissolves the manganese salt, the aluminium hydroxide and chromium oxide. At 20°C it is filtered and washed with a little of 1% brine.

Crude blue is dissolved in 1 lit. water at 100°C, the undissolved material is filtered off and the clear filtrate precipitated with 50 gm ordinary 50% zinc chloride solution and 150 gm common salt. After 24 hours the zinc chloride double salt is precipitated as a magnificent bronze red deposit; this is filtered and washed with about 10% salt solution. The compound is dried below 50°C.

In order to separate zinc from the compound, the aqueous solution of the dye is raised to pH 9.0 and allowed to stand for half an hour when zinc hydroxide is precipitated from the solution and is filtered off.

Recrystallization of dyes:

The dyes are recrystallized thrice from water - ethanol mixture. After recrystallization, zinc is separated by precipitation as zinc hydroxide at pH 9.0 and zinc hydroxide is filtered off. After separation of zinc, the dyes are again recrystallized from water-alcohol mixture.

Identification of dyes:

Korunnik reported (73) the absorption peak of a number of substituted thionine dyes (Table-1). The prepared dyes also give the characteristic absorption band λ_{max} . 1(a), 1(b), 1(c) and 1(d). Tetraethyl thionine was not reported earlier.

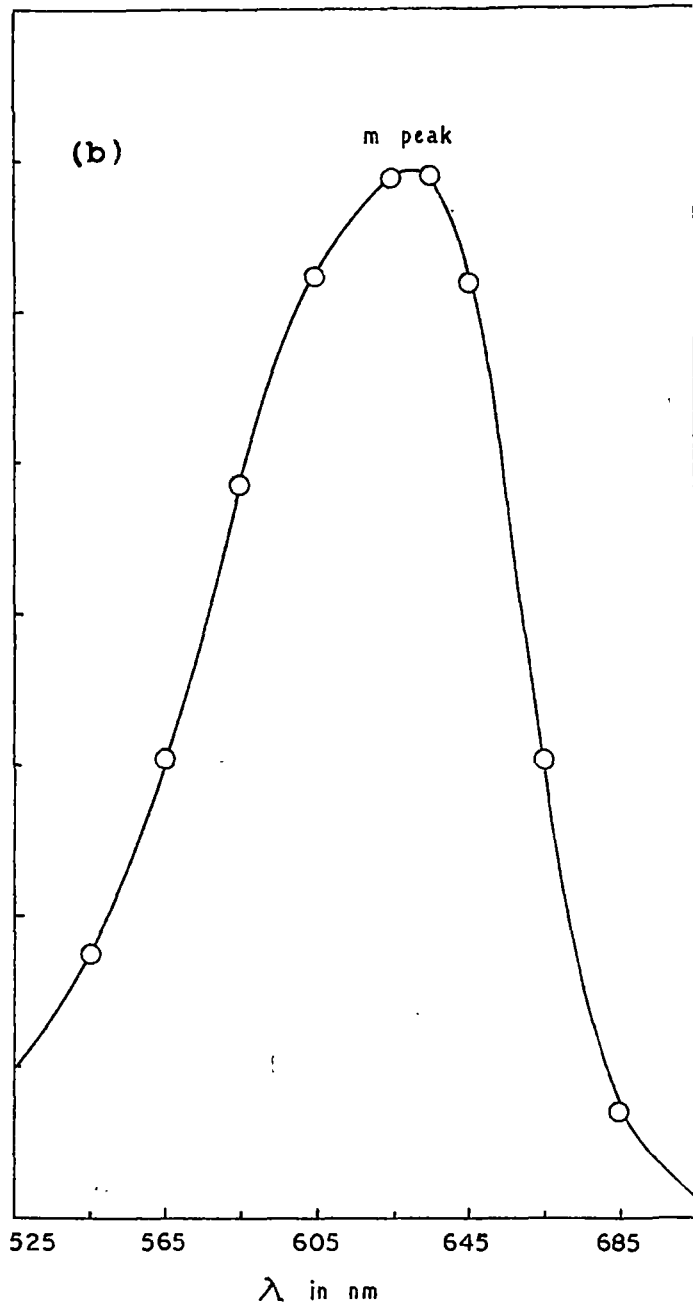
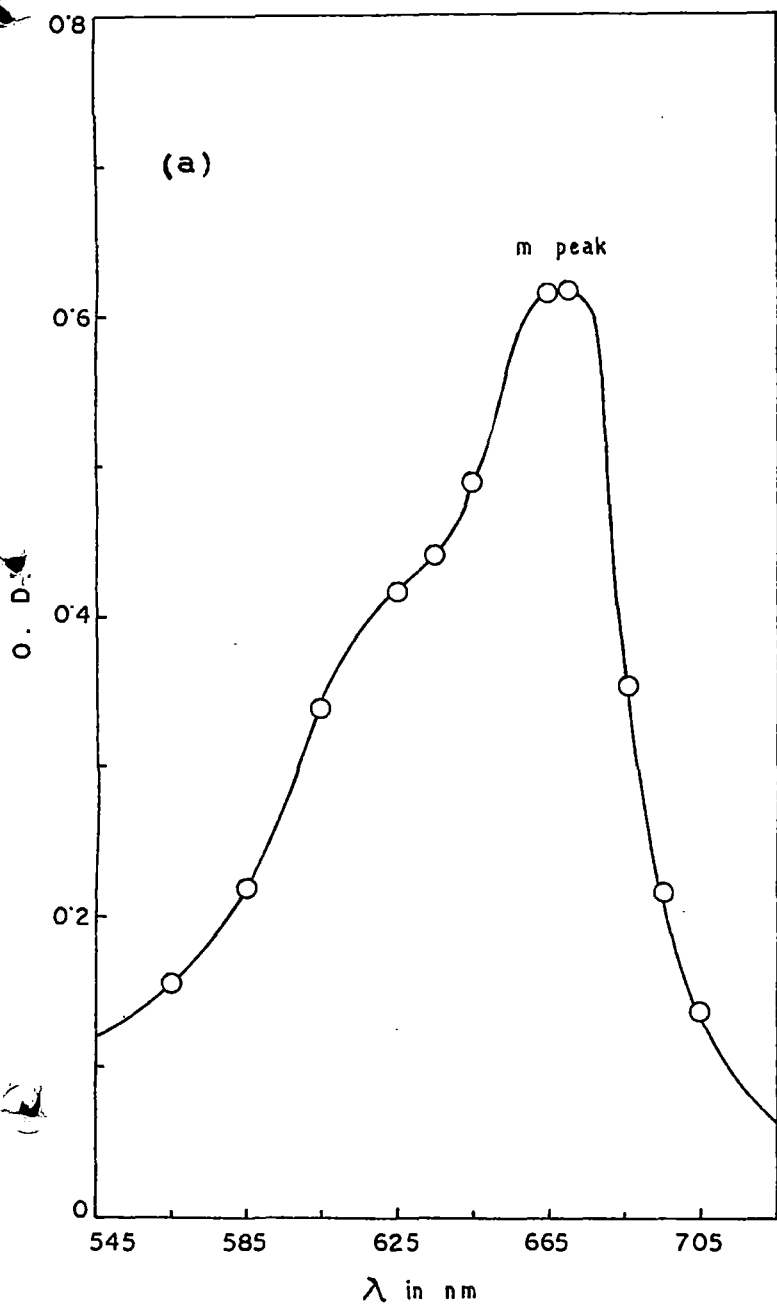


FIG. 1(a) ABSORPTION SPECTRUM OF THIONINE, $1.6 \times 10^{-5} M$, IN Na - ACETATE - ACETIC ACID BUFFER PH 5.7

FIG. 1(b) ABSORPTION SPECTRUM OF DMT, $1.62 \times 10^{-5} M$, IN Na - ACETATE - ACETIC ACID BUFFER, PH 5.7

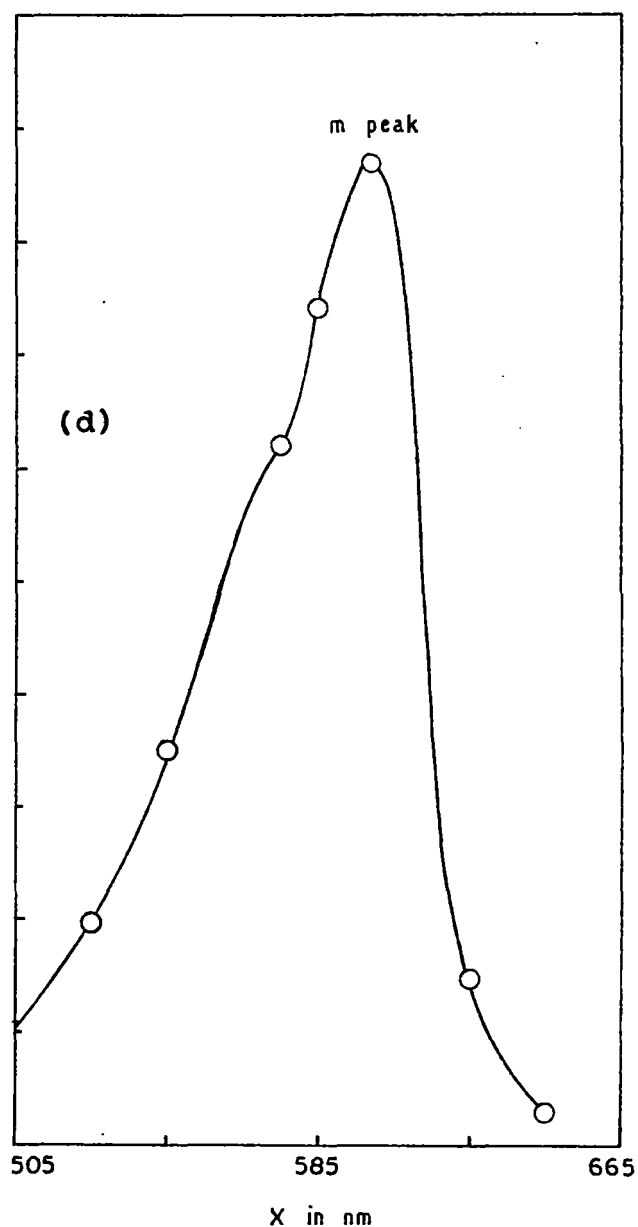
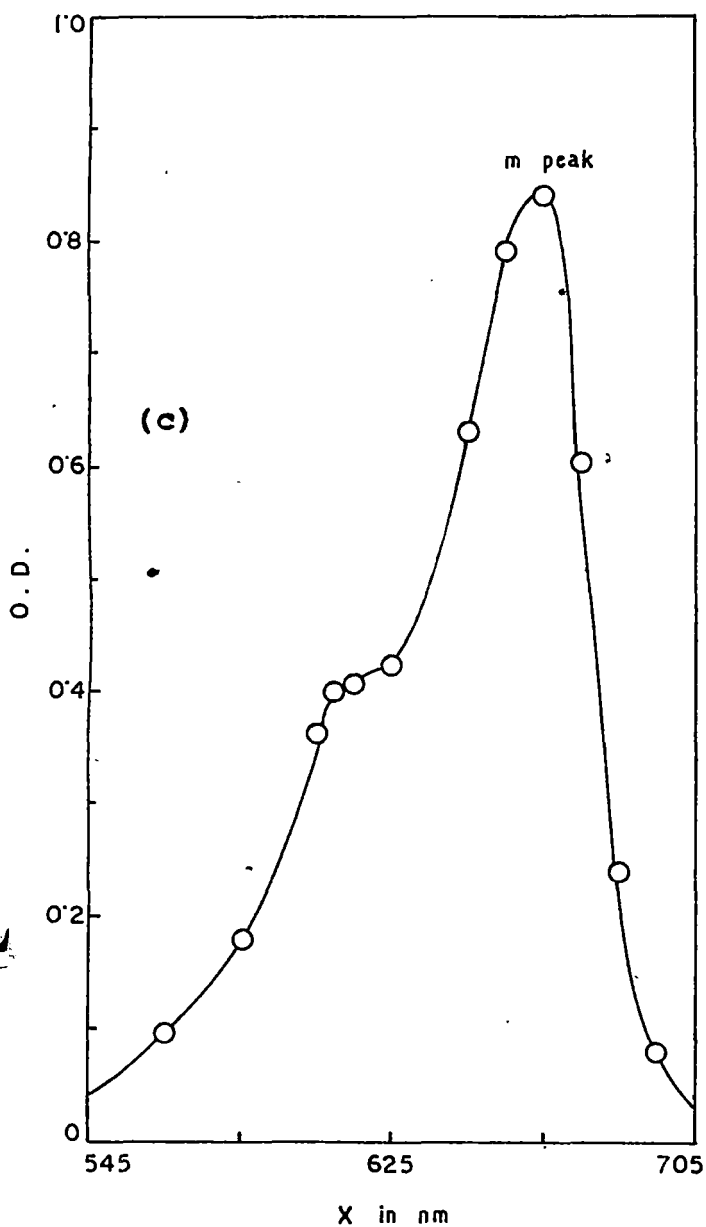


FIG. 1(c) ABSORPTION SPECTRUM OF METHYLENE BLUE, $1.0 \times 10^{-5} \text{ M}$ IN Na - ACETATE - ACETIC ACID BUFFER PH 5.7

FIG. 1(d) ABSORPTION SPECTRUM OF TET, $1.0 \times 10^{-5} \text{ M}$ IN Na-ACETATE - ACETIC ACID BUFFER PH 5.7

Table - 1

Compound	λ Max	Solvent, buffered at pH 4.75	Extinction co-efficient
Methylene Blue	665 nm	water	8.6×10^4
Thionine	691 nm	water	4.2×10^4
Diethyl Thionine	685 nm	water	5.0×10^4
Tetraethyl Thionine	670 nm	water	3.3×10^4

Analysis of the compounds gave the following results:

Thionine C = 54.70%; H = 3.78%; N = 13.28%
S = 12.12%; Cl = 16.00%

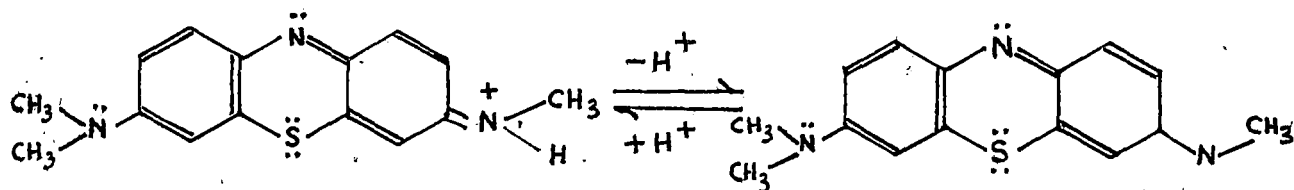
Methylene Blue C = 63.00%; H = 5.69%; N = 13.16%
S = 10.03%; Cl = 11.00%

Diethyl Thionine C = 67.25%; H = 5.43%; N = 14.31%
S = 10.90%; Cl = 13.03%

Diethyl Thionine C = 63.98%; H = 6.92%; N = 11.13%
S = 8.52%; Cl = 9.44%

ii. Purification of Methylene Blue:

It is known that commercial Methylene Blue compound contains the degradation product called trimethyl thionine or Methylene Azure D. In acid solution the impurity has got blue colour and in alkaline medium, around pH 10.00, the colour of the compound is pink. The compound can therefore, act as an acid-base indicator. The acid - base equilibrium may be expressed as



At low pH the impurity molecule is a charged molecule, whereas at high pH it apparently is neutral. Solubility of the neutral molecule is higher in ethanol.

From the different partition co-efficients of the ionized Methylene Blue and the neutral impurity between aqueous and organic phases, an extraction procedure for the purification of commercial Methylene Blue was suggested by Bergson and O'Keefe (74). Following the same method the

solution containing about 0.1 gm of Methylene Blue in 100 c.c. water was raised to a pH of 10.5 by NH_4OH and it was then extracted 10 times with 100 ml of Thiophene free Benzene. It was essential to do the extraction as fast as possible since in basic solution MB would be converted into the impurity. During the extraction, the red colour of the benzene phase became almost colourless. The demethylation reaction evidently is base catalysed; therefore the pH of the aqueous ammonical phase was lowered after the extraction from 10.5 to 8.0 in order to prevent further reaction of the MB. This was achieved conveniently during evaporation in a vacuum - drying apparatus at room temperature.

B. Estimation of Thiazine Dyes:

(a) Ascorbic Titration (75).

Stock solution of thiazine dyes of the order of 6×10^{-3} M was used for the estimation of dyes. 2 c.c. of dye solution was mixed with 2 c.c. of 4N HCl in a glass vessel, which was kept in water bath at 70°C . Carbon dioxide gas was passed in the solution to prevent aerial oxidation. The dye solution was treated with ascorbic acid solution of the order of 10^{-2} M. The course of titration was followed potentiometrically with a platinum indicator electrode with

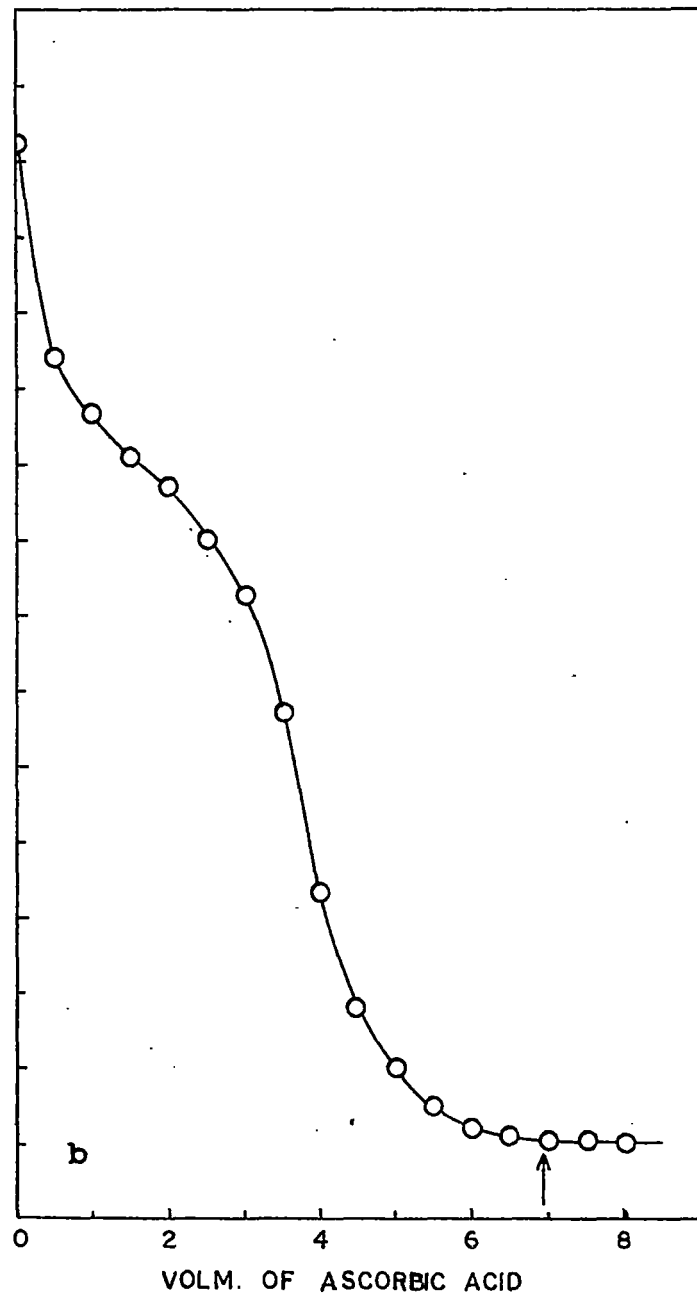
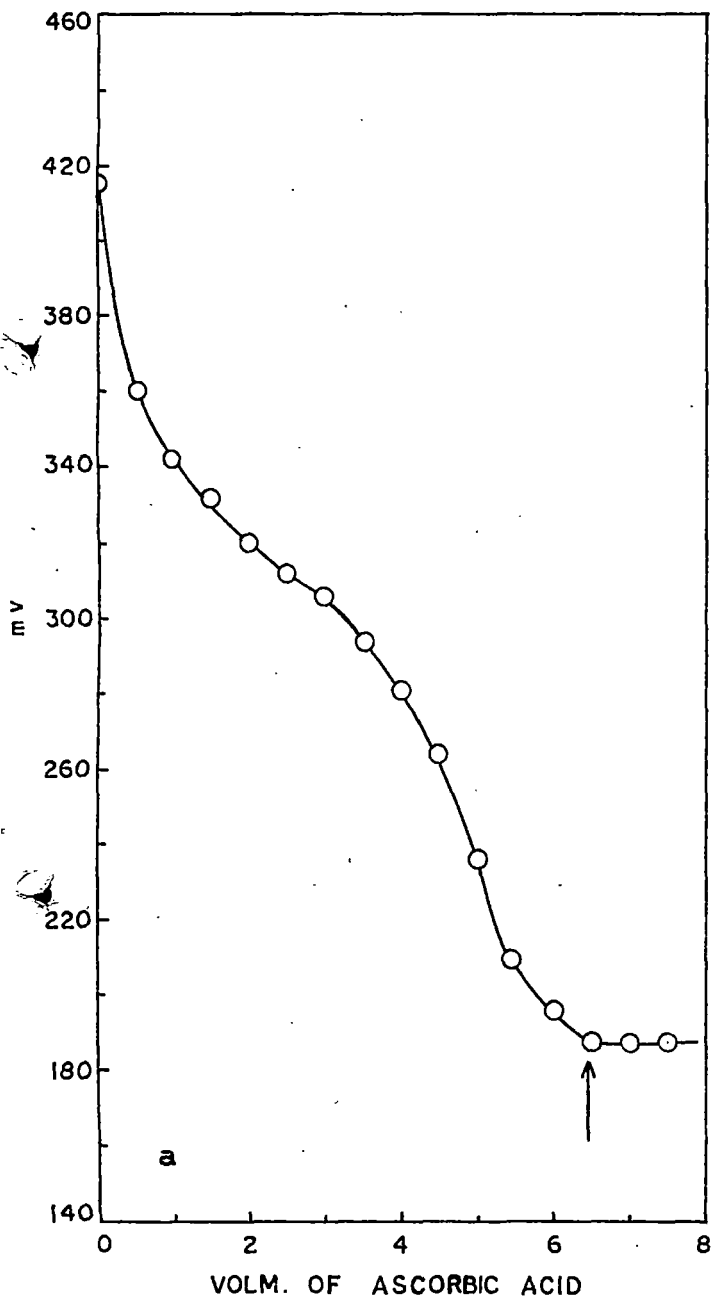


FIG. 2(a) POTENTIOMETRIC TITRATION OF MB, 2ml. BY ASCORBIC ACID, 4.89×10^{-3} M

FIG. 2(b) POTENTIOMETRIC TITRATION OF THIONINE, 2ml. BY ASCORBIC ACID,

4.89×10^{-3} M.

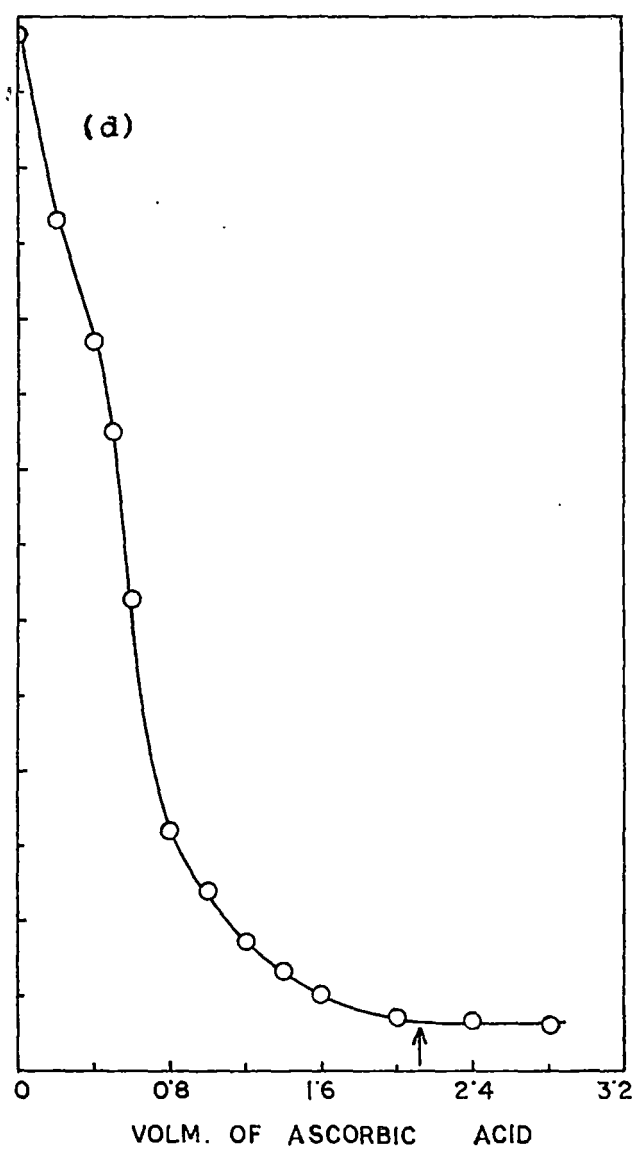
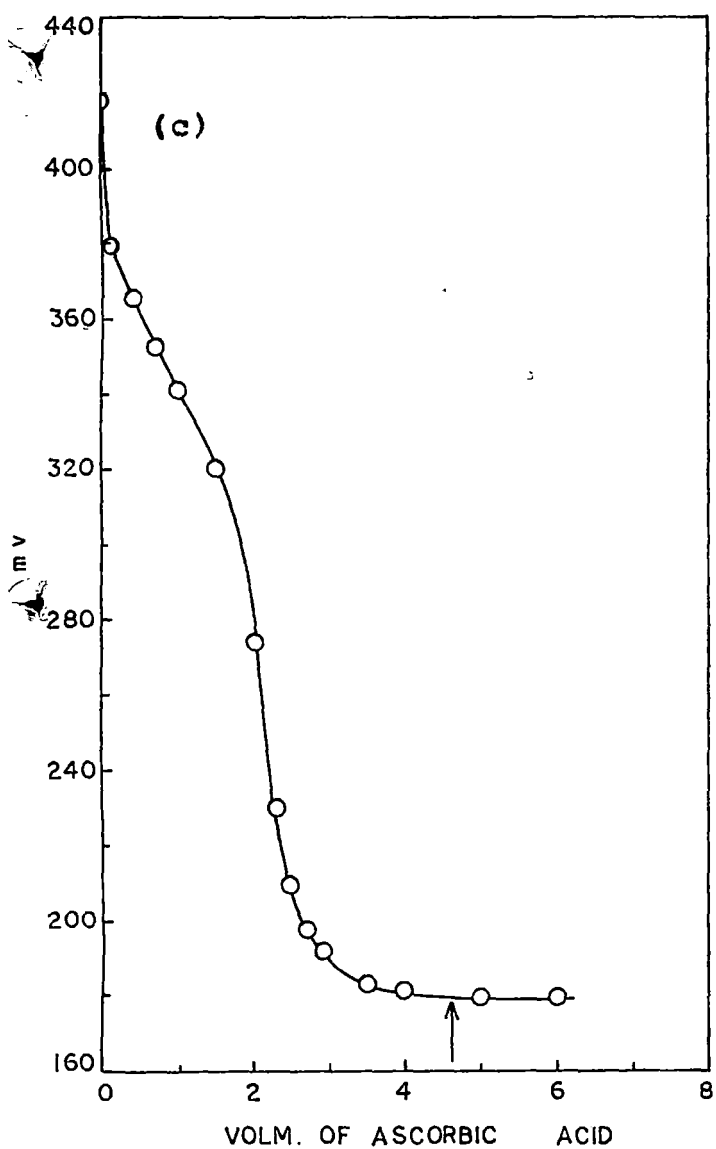
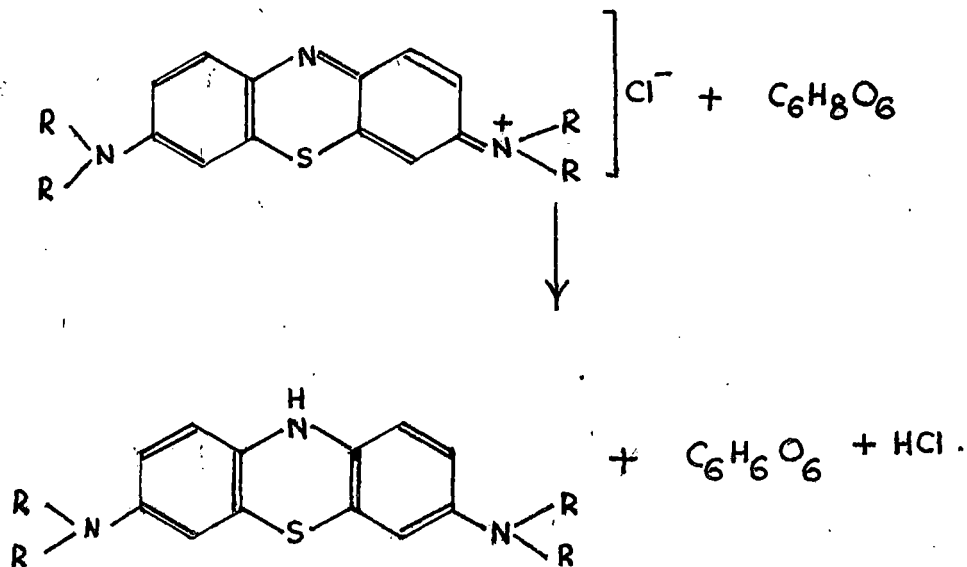


FIG. 2(c) POTENTIOMETRIC TITRATION OF DMT, 2ml. BY ASCORBIC ACID, $4.7 \times 10^{-3} M$
 FIG. 2(d) POTENTIOMETRIC TITRATION OF TET, 2ml. BY ASCORBIC ACID, $6.41 \times 10^{-3} M$.

calomet as reference. The potential becomes constant near the equivalence point, this being shown by arrow in the Figs. 2(a), 2(b), 2(c) and 2(d). At the equivalence point the dye loses its colour. The same method was followed for all the dyes.

The reducing property of ascorbic acid is well known. The reduction of thiazine dyes takes place according to the following mechanism,



Serry's Method (70)

The method is based on the precipitation of thiazine dyes with potassium dichromate. The complex is formed on replacement of chloride ion of the dye dichromate ion. The complex is very nearly insoluble in water or dilute acetic

acid containing a moderate excess of dichromate but is slightly soluble in presence of sodium acetate. Unlike the precipitate in the A.O.A.C. iodine method, the precipitate has a composition independent within wide limits of the excess of the reagent and since a stable reagent is used, the procedure is more convenient than the titanous chloride method.

Voluntario:

To 4 c.c. of solution containing about 20 mg of dye 50 ml of 0.1 M potassium dichromate is added. The mixture is left for 5 minutes with occasional shaking and filtered through No. 3 Gooch crucible and the precipitate is washed with 2 c.c. of water. A tendency to clog the filter can be prevented by heating the reaction mixture to 75°C for 5 minutes and cooling to room temperature before filtering. No decomposition occurs during this treatment. In the Table-2, the results of Ferry's method and those obtained by ascorbenetric method are compared. The results show that the two methods are in excellent agreement.

Table-2

Dye	Absorbometric method	Derry's method
Methylene Blue	$1.5773 \times 10^{-2} (\text{M})$	$1.5757 \times 10^{-2} (\text{M})$
Thionine	$1.701 \times 10^{-2} (\text{M})$	$1.633 \times 10^{-2} (\text{M})$
Dimethyl Thionine	$1.0319 \times 10^{-2} (\text{M})$	$1.11 \times 10^{-2} (\text{M})$
Tetraethyl Thionine	$6.501 \times 10^{-3} (\text{M})$	$6.795 \times 10^{-3} (\text{M})$