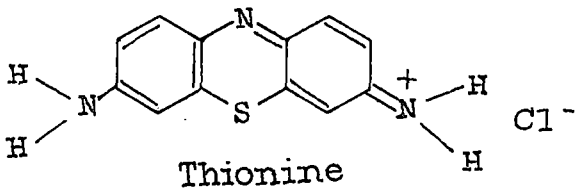
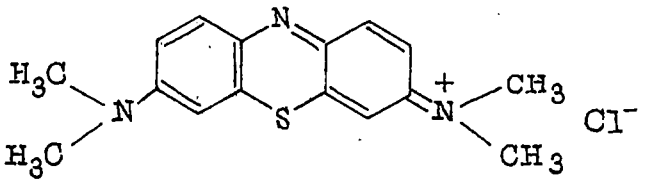
CHAPTER - III

A. Structure of the Dyes:

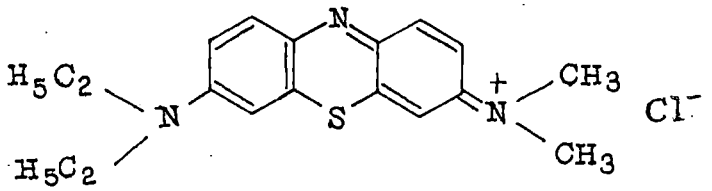
Four cationic dyes of phenothiazine class have been chosen in the present work. The structure of the dyes are as follows :



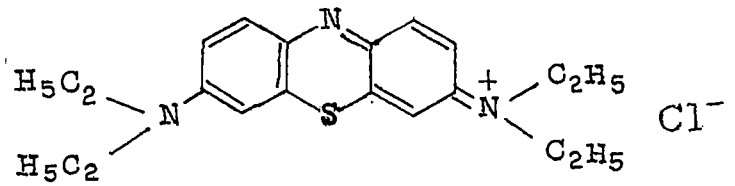
Thionine



Tetramethyl Thionine (MB)



Diethyl dimethyl Thionine (DEDMT)



Tetraethyl Thionine (TET)

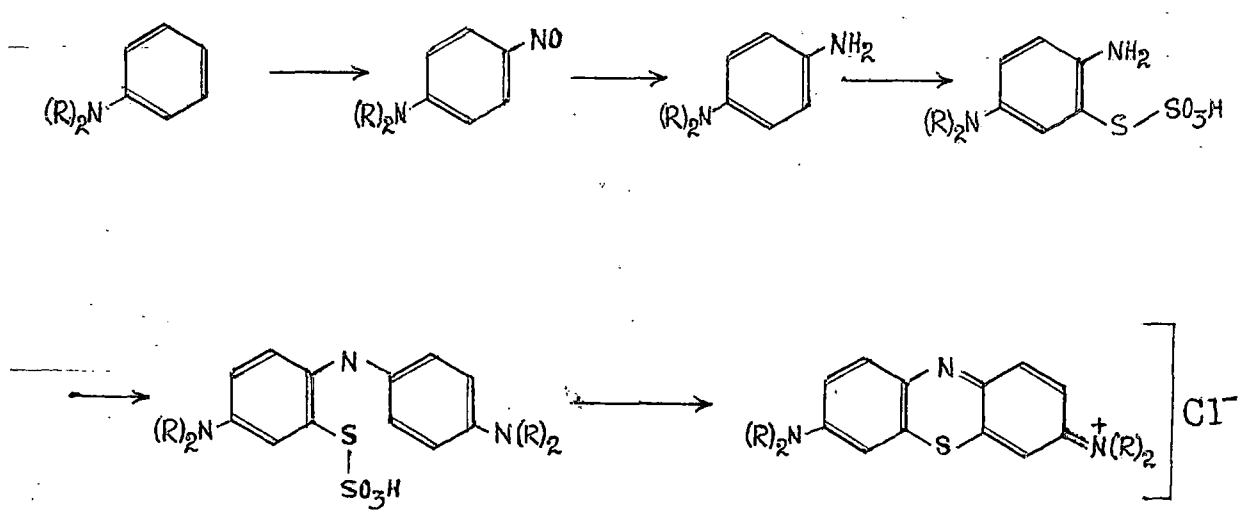
B. Synthesis of the Dyes:

The thiazine dyes may be synthesized by the following three steps (1).

(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-amino dimethyl aniline.

(b) The p-amino dimethyl aniline in an acid solution is oxidised together with additional molecule of dimethyl aniline and simultaneously the thiosulphuric acid is introduced into the molecule. This separates 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.



R = H , CH_3 , C_2H_5

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

0.2 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 formation of the nitroso compound is complete in 4 hours. 110 gm HCl (30%), 200 gm ice and at 15 minutes time 35 gm of good zinc dust is added at small instalments with constant stirring. The temperature may be raised to 25°C without any 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-amino dialkyl aniline which is made acidic with 4 gm. of conc. H_2SO_4 and mixed with 100 gm. 50% neutral $ZnCl_2$ solution.

Solution I = 38 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 = 57 gm. of sodium dichromate in 90 c.c. water.

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

The beaker containing p-amino 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 luster and the dye precipitates out of the concentrated zinc chloride solution. After half an 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. The crude blue matter is dissolved in one liter of 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. of common salt. After 24 hours the zinc chloride double salt is precipitated as a magnificient 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 8.0 and allowed to stand for half an hour when zinc hydroxide is precipitated from the solution and is filtered off.

Recrystallisation of Dyes:

The dyes are recrystallised thrice from water-ethanol mixture. After recrystallisation, zinc is separated by precipitation as zinc hydroxide at pH 8.0 and the zinc hydroxide is filtered off. After separation of zinc, the dyes are again recrystallised from water-alcohol mixture.

Identification of Dyes:

Formanek reported (2) the absorption peak of a number of substituted thionine dyes. The prepared dyes also give identical characteristic absorption band. Results are presented in Table - 1 . The analytical data are also shown below.

Table - 1

Compound	λ max	Solvent, buffered at pH 4.75	Extinction co-efficient
Thionine	595 nm	water	3.8×10^4
Methylene Blue	660 nm	water	8.4×10^4
Diethyl dimethyl Thionine	660 nm	water	9.4×10^4
Tetraethyl Thionine	665 nm	water	8.7×10^4

Analysis of the compound gave the following results:

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

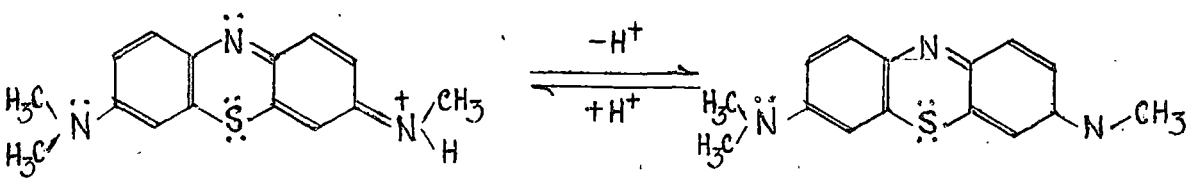
Methylene Blue : C = 60.00%; H = 5.69%; N = 13.16%;
S = 10.03%; Cl = 11.00%.

Diethyl dimethyl Thionine : C = 59.07%; H = 6.62%; N = 11.48%;
S = 8.75%; Cl = 9.68%.

Tetraethyl Thionine: C = 63.92%; H = 6.92%; N = 11.18%
S = 8.52%; Cl = 9.44%.

C. Purification of Methylene Blue:

It is known that commercial Methylene Blue contains the degradation product called trimethyl thionine or Methylene azure B. 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 is charged molecule, whereas at high pH it is apparently neutral. Solubility of the neutral molecule is higher in ethanol.

From the different partition co-efficients of the ionised Methylene Blue and the neutral impurity between aqueous and organic phases, an extraction procedure for the purification of commercial Methylene Blue was suggested by Bergman and O'Konski (3). 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 that the extraction should be 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 is evidently base catalysed; therefore, the pH of the aqueous ammoniacal 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.

D. Estimation of Thiazine Dyes:

(a) Ascorbometric Titration (4)

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 4 N 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 calomel as reference. The potential became constant near the equivalence point, this being shown by arrow in the Figs. 1(a), 1(b), 1(c) and 1(d). At the equivalence point the dye loses its colour. The same method was followed for all the dyes.

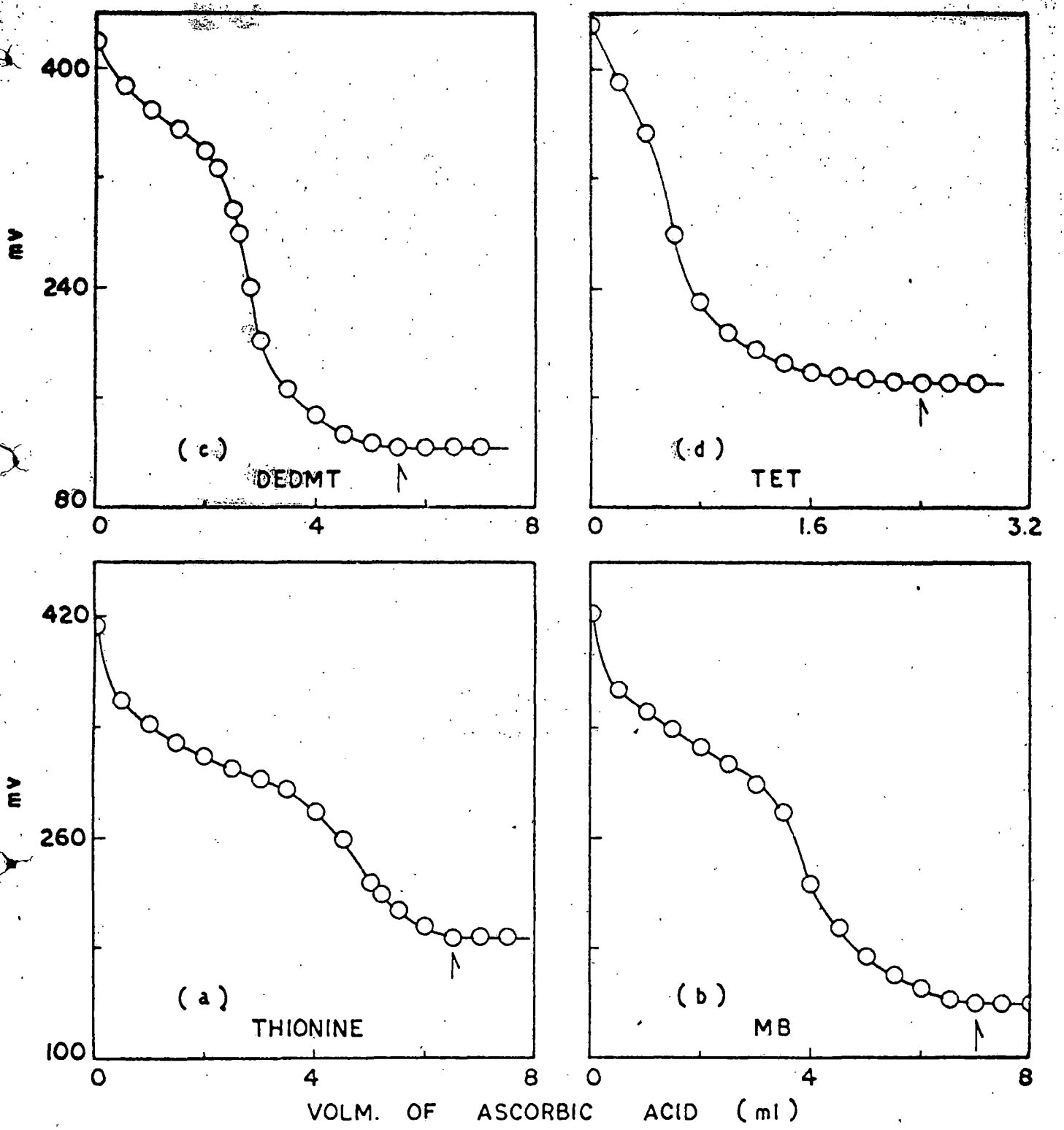
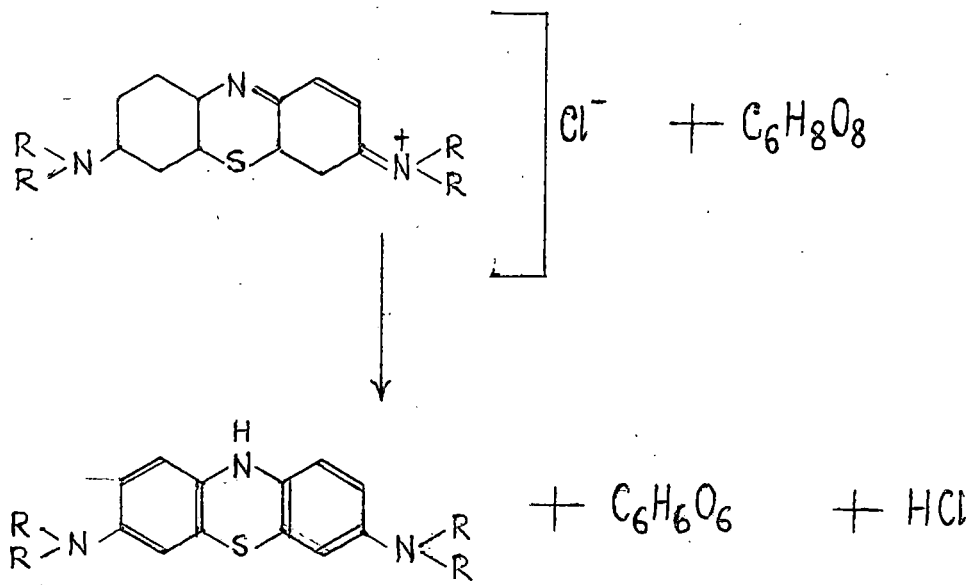


FIG. 1 . POTENTIOMETRIC TITRATION OF (a) THIONINE - 2ml, b) M.B. - 2ml, (c) DEDMT - 2 ml AND (d) TET - 2ml BY ASCORBIC ACID. CONC. 4.92×10^{-3} M.

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



(b) Ferry's Method (5) :

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 by 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. iodide 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.

Volumetric:

To 4 c.c. of solution containing about 20 mg. of dye 50 ml. of 0.1 N 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^{0}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 ascorbometric method are compared. The results show that the two methods are in excellent agreement.

Table - 2

Dye	Ascorbometric method	Ferry's method
Thionine	1.806×10^{-2} M	1.804×10^{-2}M
Methylene Blue	1.6237×10^{-2}M	1.6225×10^{-2}M
Diethyl dimethyl Thionine	1.7267×10^{-2}M	1.7277×10^{-2}M
Tetramethyl Thionine	5.916×10^{-2}M	5.919×10^{-2}M

R E F E R E N C E S

1. Fiery David and Blangey, Grundlege vde operationer der Farben Chemie P. 294.

2. J. Formanek,"Untersuchung and Nachweis Organischer Farbstoffe Spectroskom Schem Wege " 2 Auflage, 1 Teil, Berlin, 1906, P. 142-44.

3. K. Bergmen and C. T. O'Konski, J. Phys. Chem., 67, 2169, 1963.

4. E. Ruzicka and M. Kotoucek, Z. anal. Chem., 180, (1961), 429, 33.

5. G. J. W. Ferry, Analyst, 69 (1944) 54.