

CHAPTER - VI

Effect of Organic Solvent on Dye-DNA Interaction.

A. Introduction:

It is known from the works of Herkovits (133) that denaturation of DNA can be effected by increasing organic solvent concentration with melting profiles similar to those obtained on thermal denaturation. These experiments demonstrated the importance of hydrophobic forces for the stabilisation of the DNA double helix. The formation of the complexes of DNA with cationic dye by the stronger process, which corresponds to the monomer binding with the DNA bases is also strongly related to hydrophobic interactions. Organic solvents generally destabilise the complexes. Above a certain concentration, which is characteristic of a particular organic solvent, the binding decreases below the limits of spectroscopic detection. The decrease in the dye binding to DNA bases can be attributed to increase lyphilic interactions between the dye and the organic solvent molecules. It has been inferred by Lober et al. (134) that the organic solvent molecules compete with the DNA base pairs for the hydrophobic interactions with the dye molecules, and that the hydrophobic forces stabilizing the dye-DNA complexes are weaker than those stabilising the double helical conformation of the DNA.

It was found earlier that the weakly bound dye contributes significantly less to the stability of the DNA double helix than the strongly bound dye (135). The stabilization effect of pinacronol in aqueous medium is appreciably smaller than that of proflavine and can be considered as due mostly to electrostatic interaction of the dye with DNA phosphate groups (136).

It is, therefore, possible to elucidate the nature of the dye binding from the determination of melting profile of DNA-dye complex by the addition of organic solvent. The stabilization effect should be greater for the dye which binds with the bases than the dye which does not interact with the bases. In the present chapter the effect of various solvents on the dye-DNA complexes has been presented in order to understand the effect of shape and size of the dye molecules on dye-DNA interaction.

Materials and Methods:

Preparation, purification and estimation of dyes have been discussed in Chapter-III. Calf thymus DNA has been supplied by H/S Sigma Chemical Co., U.S.A. Phosphate in the DNA solution was estimated by the method of King (115). Methanol, ethanol n-propanol, isopropanol have been purified by fractional distillation.

Preparation of dye-DNA Complex in Different Solvent System:

To study the influence of solvent on the spectral properties of dye-DNA complexes, aqueous DNA solution was added into the dye solution, which contained the required amount of an organic solvent. The dye concentration was kept at 5.0×10^{-5} M and the DNA phosphate concentration at 5.0×10^{-6} M in the final volume of 10 ml.

B. Results and Discussion:

Addition of organic solvent to the solution of the dye-DNA complexes apparently decreases the dye binding and as a consequence the changes of absorbance become less expressive. Fig. 36 represents the influence of ethanol on the changes of the optical density in the visible absorption spectrum of Methylene Blue-DNA complex. It follows from the behaviour of Methylene Blue spectra in the presence of the organic solvent that the tendency for the complex formation is lowered. In the presence of about 50% ethanol the tendency vanishes completely when DNA phosphate concentration is 1.7×10^{-5} moles/l. Similar effects have also been found for methanol, n-propanol and iso-propanol. In order to demonstrate the decrease in the binding of dye more quantitatively, the relative absorbance of dye at 3×10^{-6} moles of DNA phosphate/l was plotted as a function of the volume percentage

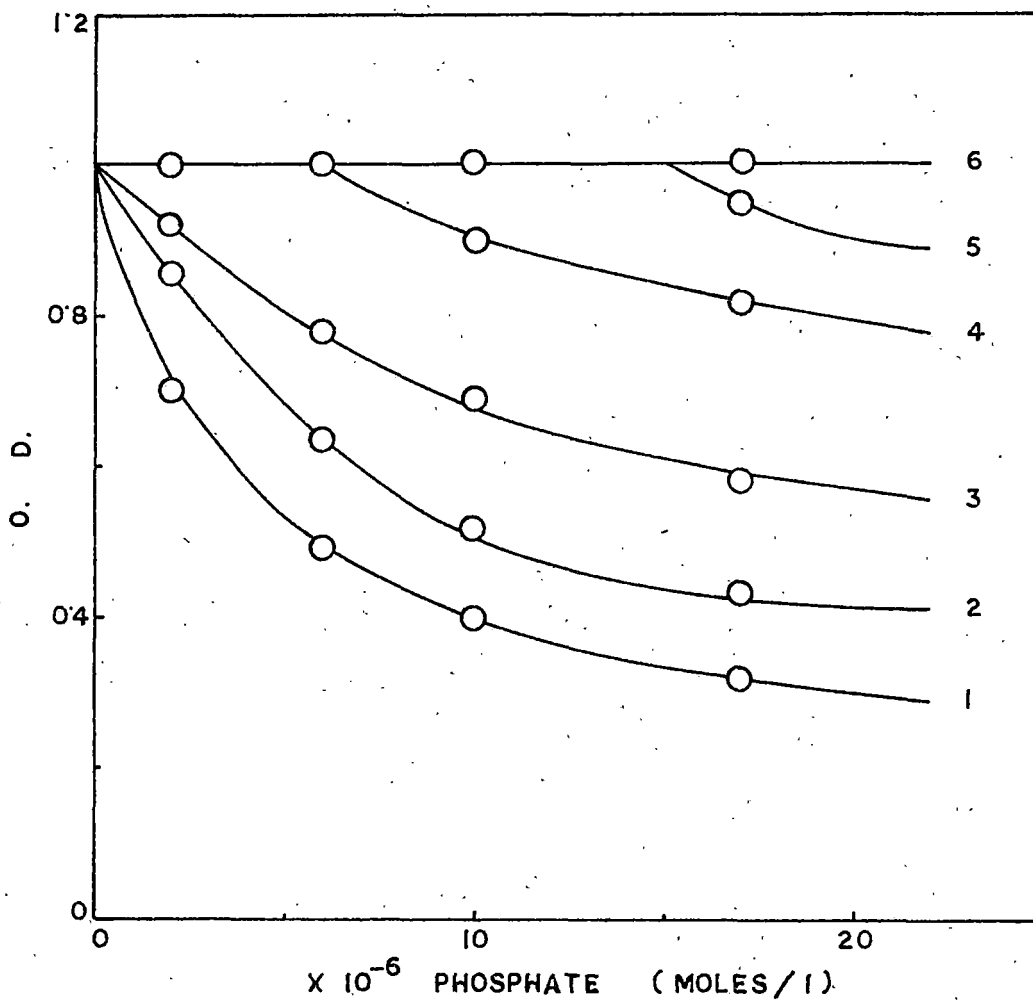
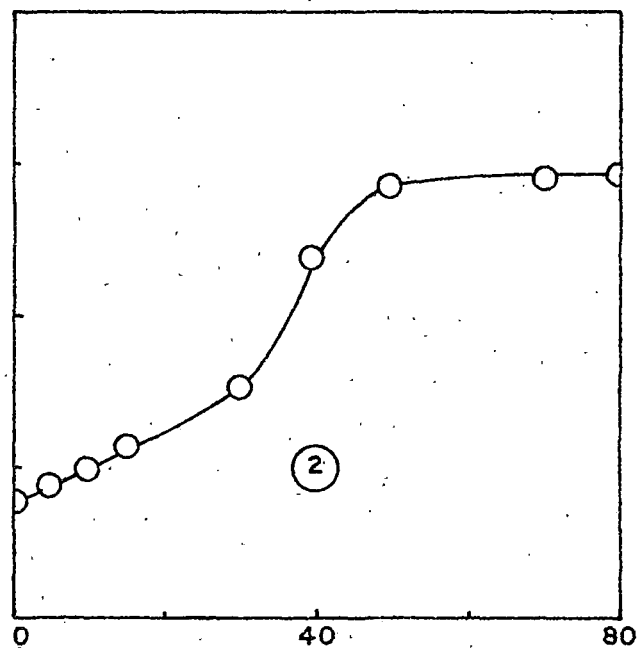
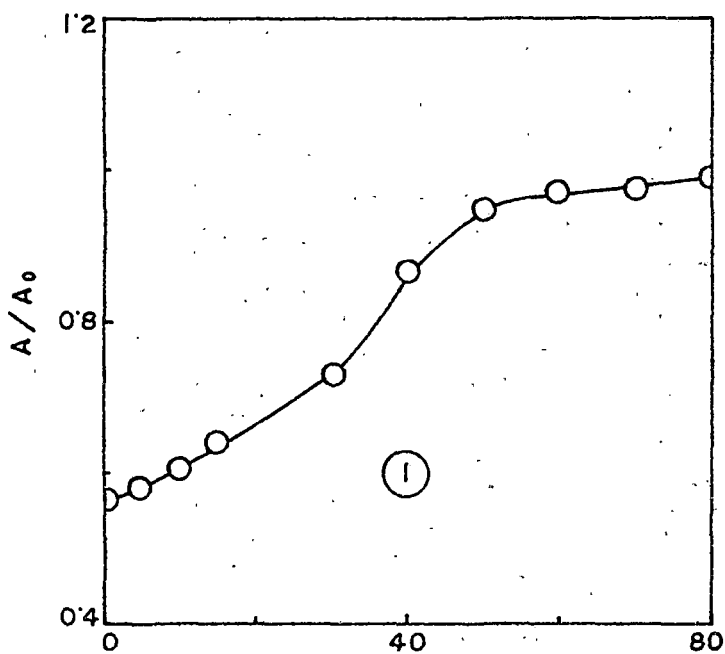
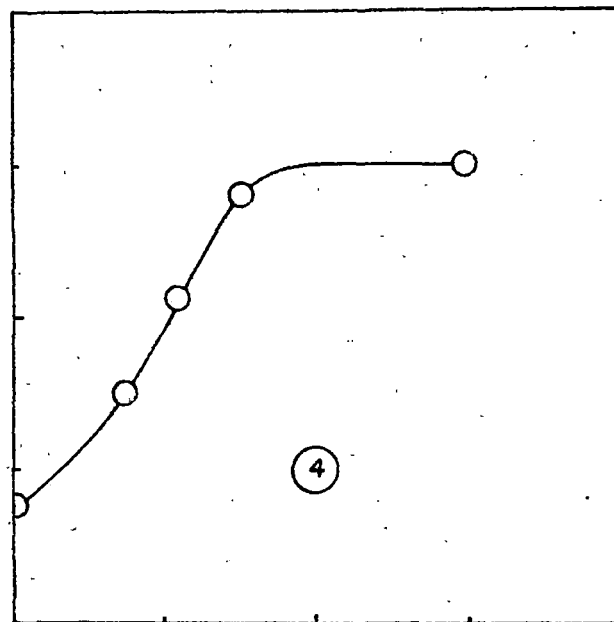
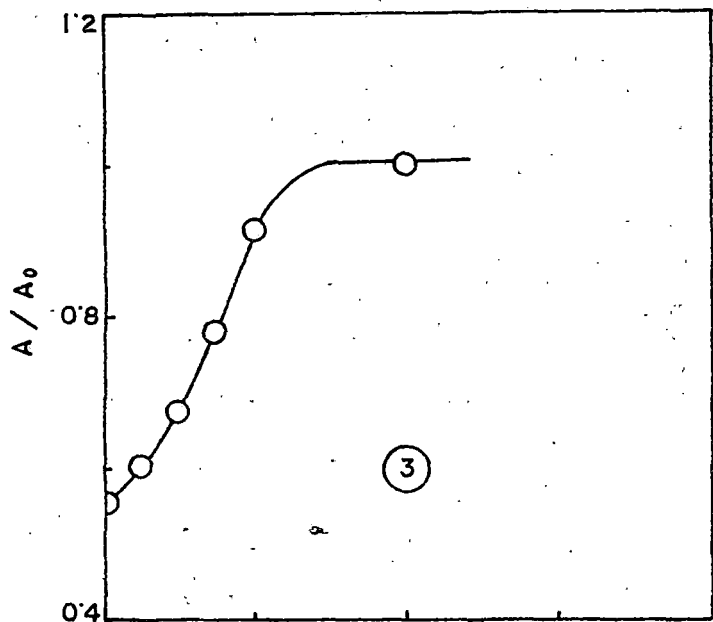


FIG. 36. DNA INDUCED CHANGES IN THE ABSORBANCE OF METHYLENE BLUE IN THE PRESENCE OF ETHANOL. A_0 = ABSORBANCE WITHOUT DNA; 1 : 0 %, 2 : 10 %, 3 : 20 %, 4 : 30 %, 5 : 40 %, 6 : 50 %.



V % OF ORGANIC SOLVENT

FIG.37a. INFLUENCE OF VARIOUS ORGANIC SOLVENTS ON DYE - DNA COMPLEX. 5×10^{-6} M THIONINE. 8×10^{-6} M DNA - PHOSPHATE. 1: METHANOL 2: ETHANOL 3: n-PROPANOL 4: ISO-PROPANOL. A_0 IS THE ABSORBANCE OF DYE IN THE ABSENCE OF DNA.

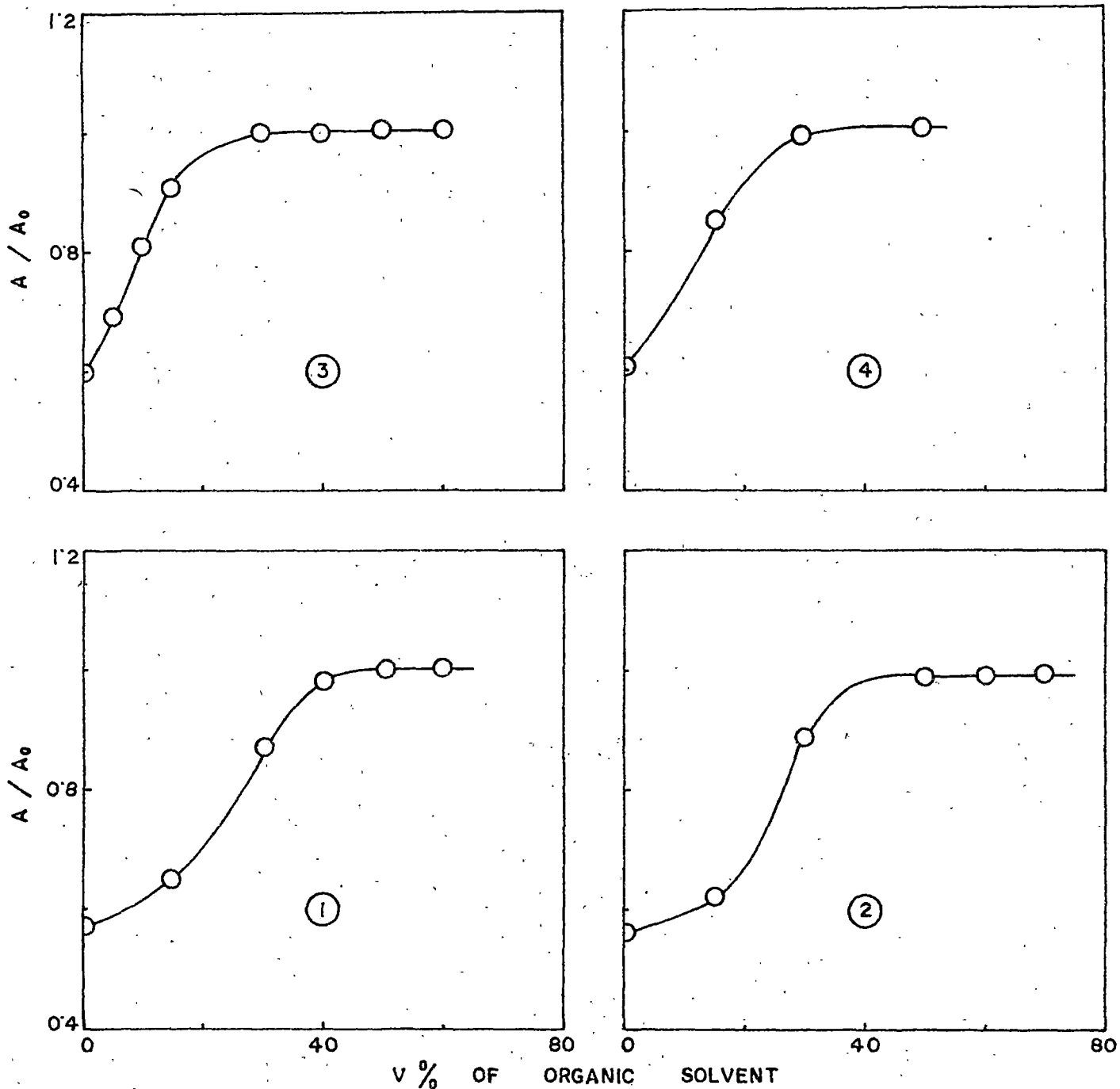
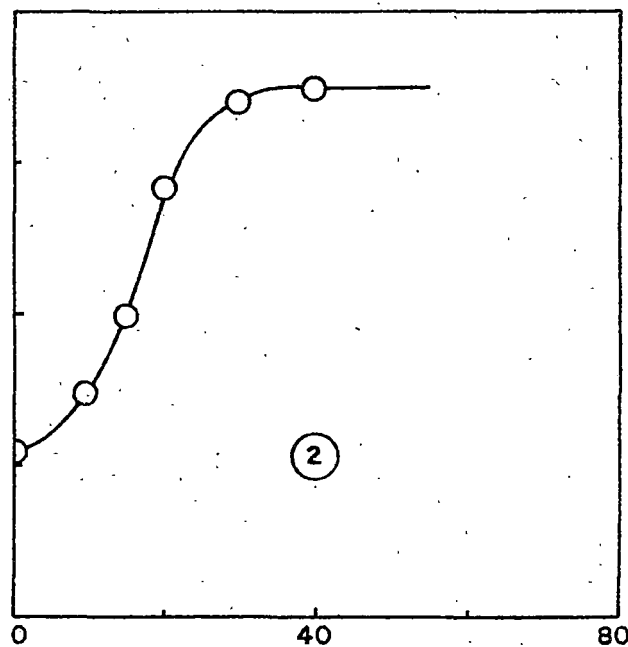
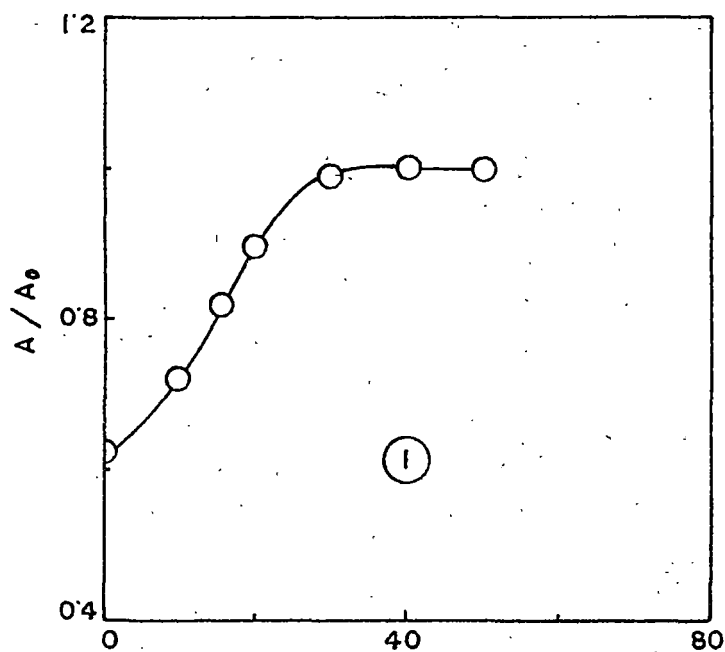
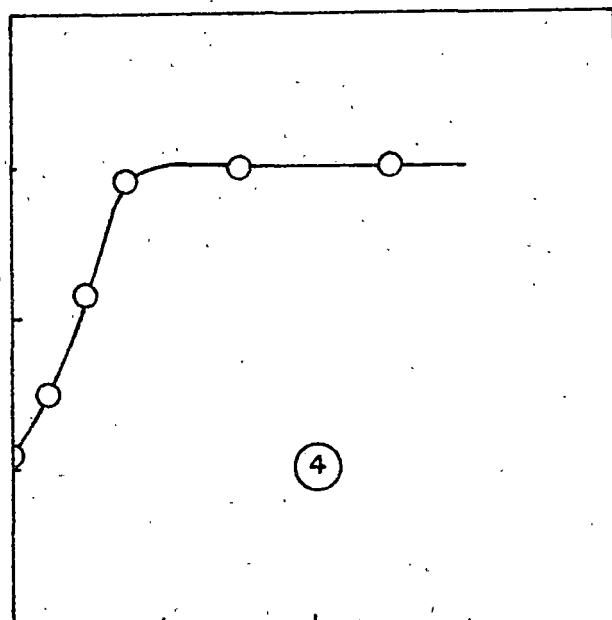
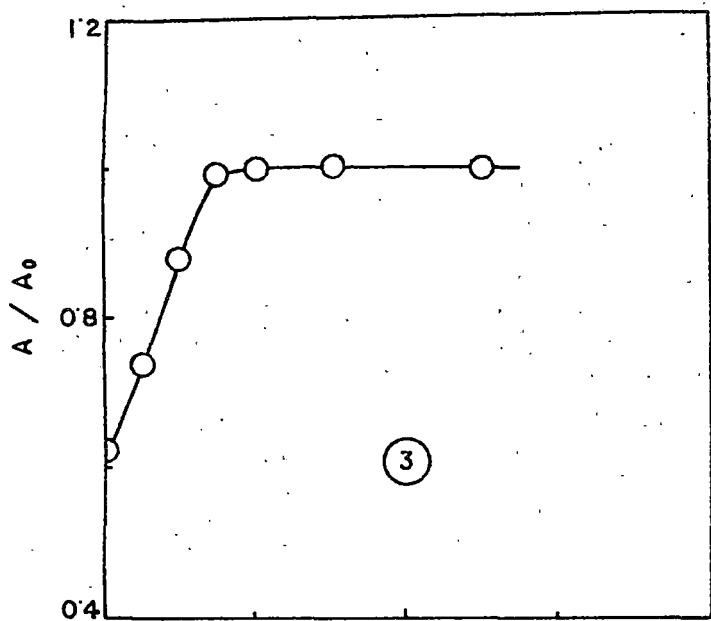


FIG.37b. INFLUENCE OF VARIOUS ORGANIC SOLVENTS ON DYE -DNA COMPLEX. 5.006×10^{-4} M DMT. 8×10^{-6} M DNA - PHOSPHATE. 1: ME - THANOL 2: ETHANOL 3: n - PROPANOL 4: ISO - PROPANOL. A_0 IS THE ABSORBANCE OF DYE IN THE ABSENCE OF DNA.



V % OF ORGANIC SOLVENT

FIG.37c. INFLUENCE OF VARIOUS ORGANIC SOLVENTS ON DYE - DNA COMPLEX. 5×10^{-4} M MB. 8×10^{-6} M DNA - PHOSPHATE. 1: METHANOL 2: ETHANOL 3: n-PROPANOL 4: ISO-PROPANOL. A_0 IS THE ABSORBANCE OF DYE IN THE ABSENCE OF DNA.

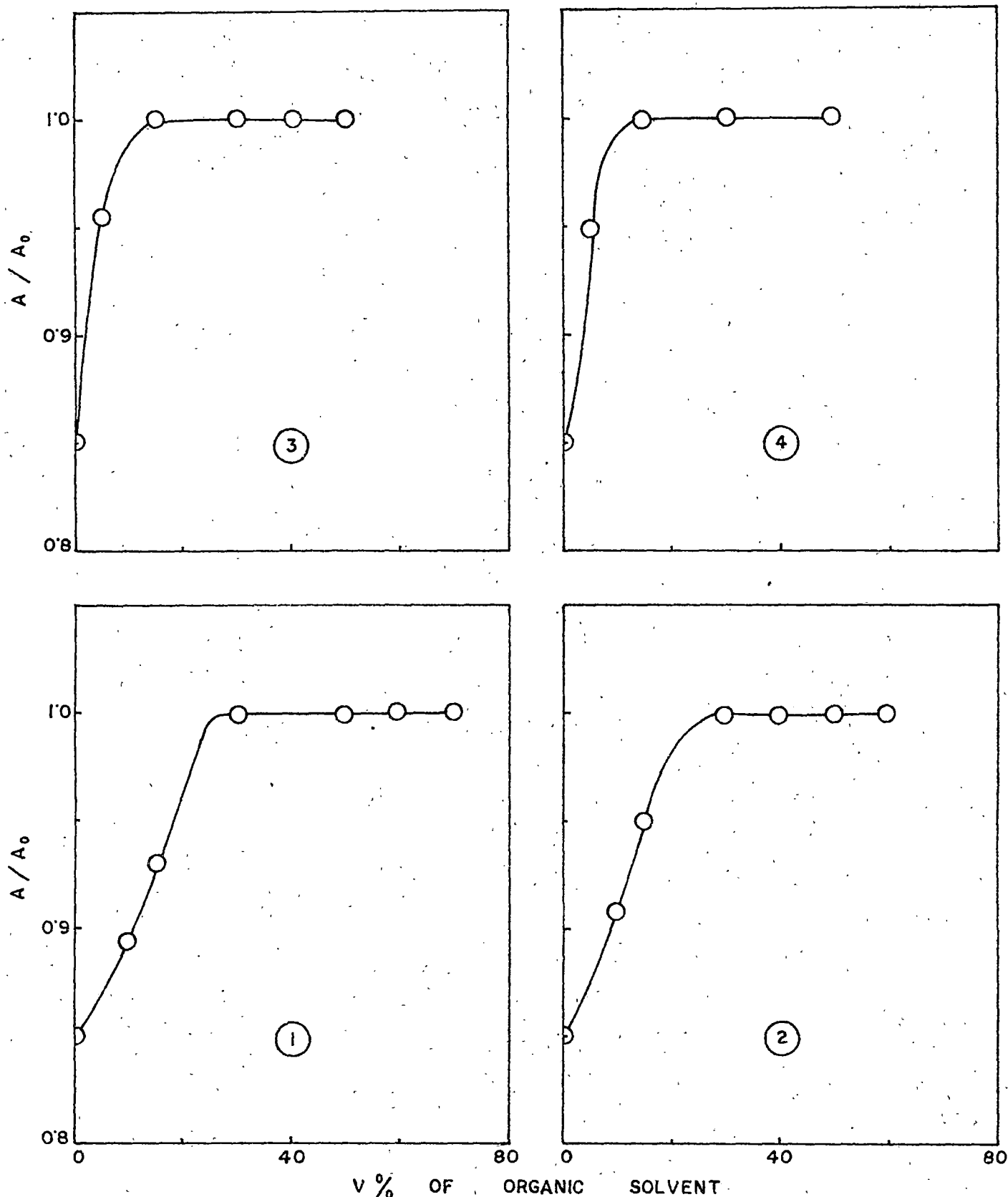


FIG.37d. INFLUENCE OF VARIOUS ORGANIC SOLVENTS ON DYE - DNA COMPLEX. 4.99×10^{-4} M TET. 8×10^{-6} M DNA - PHOSPHATE. 1: METHANOL 2: ETHANOL 3: n-PROPANOL 4: ISO-PROPANOL. A_0 IS THE ABSORBANCE OF DYE IN THE ABSENCE OF DNA.

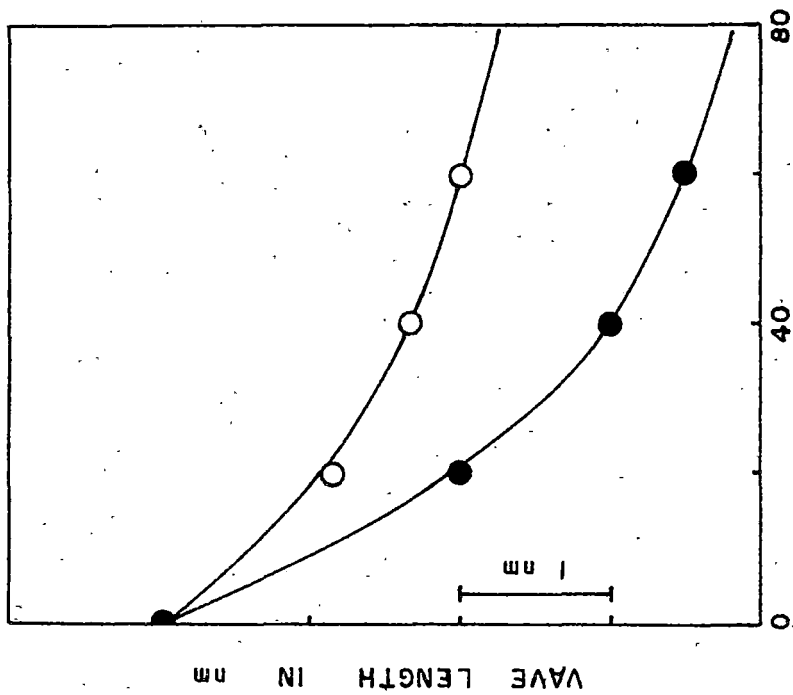


FIG.38. SHIFTS OF THIONINE ● AND MB ○ MAXIMA TOWARDS THE RED INDUCED BY ETHANOL.

of various organic solvents [Figs. 37(a), 37(b), 37(c) & 37(d)]. The relative effect of various organic solvents was characterized by the volume percentage, which caused reduction of the relative absorbance intensity change.

The effectiveness of the organic solvent increases with increasing hydrocarbon content (Table-19).

Table-19

Compound	Methanol	Ethanol	n-Propanol	iso-Propanol
Rhionine	39%	38%	16%	22%
RII	25%	26%	11%	15%
RII	17%	17%	8%	10%
RII	16%	14%	3%	5%

The results demonstrate the importance of hydrophobic forces for the formation of the complexes of the dyes with BHA in the aqueous solution. The decrease in the dye binding can be attributed to competition of organic solvent molecules with the dyes for hydrophobic interaction with the BHA bases. Since denaturation of BHA by organic solvents takes place at a higher solvent concentration, the BHA is assumed to be in the double helical state in the concentrations of the organic solvents, which suppress efficiently

the dye binding at room temperature, the decreased binding ability can not be due to the denaturation of DNA. It is reasonable to assume that the hydrophobic forces stabilizing the dye-DNA complex is much weaker in organic solvent than those stabilizing the double helical conformation of DNA.

The hydrophobic character of the dyes should increase with increase in hydrocarbon content. The dye with longer chain length will, therefore, compete with the solvent molecule more effectively for binding with DNA. As a consequence, the solvent concentration for suppressing dye binding with the DNA bases should be higher for RII. But experimentally the reverse order has been attained. This perhaps suggests that although the increase in hydrophobic character of the dyes should result in effective dye-DNA binding, the steric factor is more important in such interactions since the dye with bulky substituent is easily removed from DNA molecule. This may be due to the fact that the distance between the reacting sites of the dye and DNA widens as bulkier group is substituted in the dye resulting in weak binding.

Denaturation of the dye-DNA complexes in the presence of organic solvents:

The question whether solvent induced alterations in the DNA secondary structure may be responsible for the reduced binding ability needs consideration. It seems rather improbable for the following two reasons:

1. All experiments performed with thermally denatured DNA coincide with the finding that its binding ability for acridine dyes is not significantly altered in comparison with native DNA (137).

2. Denaturation of DNA by organic solvents occur generally at higher solvent concentrations than those which are effective in the restriction of complex formation (Table-20).

Table- 20

Solvent	Dielectric Constant	Vol % solvent for denaturation mid points taken from Ref.
Methanol	32.0	80
Ethanol	25.8	80
n-Propanol	22.0	80
iso-propanol	19.0	80

On the otherhand, the CD measurement of Brems and Monnaerts (138) yielded deviations from the B-form towards the A-form of DNA under the influence of low concentrations of organic solvents, far below the denaturation region. But again it seems unlikely that this might be the reason for the observed expressive changes in binding properties since acridine binds effectively both natural and synthetic poly-

nucleotides, the latter ones often have an A-type helical conformation.

The Effect of Organic Solvent on the Eye Spectra:

It is shown in Fig. 38 that by increasing organic solvent content, the long wave length band of the dye is red shifted. Whether alteration of the properties of the dyes induced by organic solvents is responsible for reduced binding of dyes should be discussed.

1. The red shift of the longest wave length band with increasing solvent polarity excludes the possibility that this band corresponds to an $n \rightarrow \pi^*$ transition or that, the dye aggregates in solution, because in both the cases blue shift is observed.

2. Specific interactions leading to the formation of organic solvent-dye complexes can be excluded, since the shift is continuous depending on the composition of the solvent.

3. Studies (139) have shown that in the asymmetric molecules the dipole moment is not changed substantially after excitation and thus it is probable that the shift could be due to changed solute-solvent interactions attributable solely to the change of dipole moment after excitation.