BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 44, 967—970 (1971)

The Effect of Pressure on the Association of Dye Molecules in an Aqueous Solution[1]

Keizo SUZUKI and Masao TSUCHIYA[2]

Department of Chemistry, Faculty of Science and Engineering, Ritsumeikan University, Kita-ku, Kyoto

(Received October 12, 1970)

The effect of pressure on the association of rhodamine B (RB) and methylene blue (MB) in water has been investigated on the basis of their absorption spectra under hydrostatic pressures up to 4500 atm at room temperatures. Pressure increases the association of these dyes. The volume changes accompanying the 2 monomer$\rightleftarrows$dimer process are calculated. The values are -10.4 cc/mol and -10.6 cc/mol in RB and MB respectively. These negative values are inconsistent with the suggestions by Rohatgi and Singhal and by Mukerjee and Ghosh that hydrophobic bonds are responsible for the associations of RB and MB. It is more plausible to assume that the hydrogen bond is the main binding force in the dye associations. London's dispersion force and the increase in the dielectric constant of water with the increase in the pressure may also assist the dye associations.

Dye association in an aqueous solution has, in general, been deduced from nonconformity with Beer's law. Various mechanisms have been advanced for the association of dyes. However, no definite mechanism has been put forward to explain the forces holding the dye molecules together. Mukerjee and Ghosh[3] have recently suggested the contribution of the hydrophobic bond in the formation of the dimer from the fact that urea decreases the association of methylene blue. Rohatgi and Singhal[4] also considered the hydrophobic bond as responsible for the association of rhodamine B because of the positive entropy change (+5.3 eu). If the hydrophobic bond is responsible for dye association, as they suggested, it could be expected that the equilibrium would shift to the monomer side under a high pressure, because the formation of the hydrophobic bond accompanies a positive volume

---

[1] Presented at the 20th Annual Meeting of the Chemical Society of Japan, Tokyo, 1967, and the 21st Annual Meeting of the same society, Osaka, 1968.

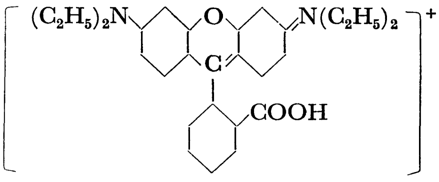
[2] Present address: Laboratory of Biology and Chemistry, College of Naval Architecture of Nagasaki, Nagasaki.

[3] P. Mukerjee and A. K. Ghosh, *J. Phys. Chem.*, **67**. 193 (1963).

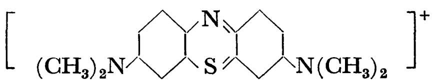
[4] K. K. Rohatgi and G. S. Singhal, *ibid.*, **70**, 1695 (1966).

change.[5,6]

In this communication we will attempt to interpret the nature of bonding in the association of two dyes, rhodamine B and methylene blue, by measuring the absorption spectra of these aqueous dye solutions under high pressure. No study of the effects of applied pressure on the dye association has yet been reported. The molecular formulas of these dyes may be represented as follows:



Rhodamine B (RB)



Methylene blue (MB)

Experimental

Methods. High-pressure optical equipment of the Drickamer type[7] was used, along with a Hitachi-Perkin Elmer 139 type spectrophotometer. The absorption cell used is shown in Fig. 1, and the dye solution was sealed with mercury. Silicone oil was used as a pressure medium. The concentration of the dye solution under pressure was corrected by using the compressibility data of water.[8] The concentration of the solutions was limited to below *ca.* 10^{-3} mol/*l* in such a manner so as to form an isosbestic point, indicating that primarily there are present only two absorbing species (monomer and dimer) at 1 atm.

Materials. The rhodamine B (RB, Sumitomo Chemical Co.) and the methylene blue (MB, Nakarai Chemical Co.) were recrystallized twice, from acetone and ethanol respectively, and then dried at 110°C. The purity of the samples was judged from the values of their peak wavelengths at the monomer band. These values are tabulated in Table 1, along with the values for reference.

TABLE 1. PEAK WAVELENGTH AT EACH M-BAND OF RB AND MB

Dye	Wavelength (mμ)	
	This work	Ref.
RB	554 (30°C)	554 (30°C)[a] 555 (—)[b]
MB	665 (18°C)	656 (27°C)[c] 664 (25°C)[d] 660 (20°C)[e] 665 (30°C)[f]

a) Rohatgi and Singhal (1966)[4]
b) Glowacki (1964)[9]
c) Rabinowitch and Epstein (1941)[16]
d) Bergmann and O'Konski (1963)[22]
e) Mukerjee and Ghosh (1963)[3]
f) Braswell (1968)[21]

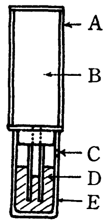


Fig. 1. Optical cell.
A: Glass cell, B: Sample solution, C: Entrance and exit of pressure medium, D: Mercury, E: Holder of mercury made of poly vinylchloride tubing.

Results and Discussion

Figure 2 shows the spectra of aqueous RB solutions at concentrations from 10^{-3} to 10^{-5} mol/*l* at 1 atm. The absorption bands at 554 and 524 mμ correspond to those of the monomer (M) and the dimer (D) respectively, and an isosbestic point appears at 533 *mμ*. These spectra coincide well with those in the literature.[9]

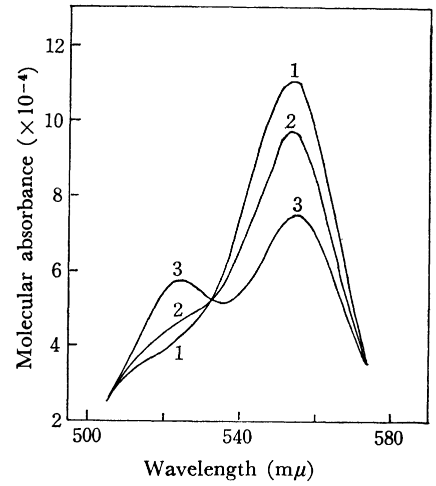


Fig. 2. Effect of concentration on absorption spectra of RB in aqueous solution at 1 atm and 30°C.

Concn. of RB (mol/*l*)
1 1×10^{-5}
2 1×10^{-4}
3 1×10^{-3}

Figure 3 shows the absorption spectra of RB (5.00×10^{-5} mol/*l*) under pressures of up to 4500 atm. It can be found from this figure that both the absorption bands shift to the red and that the absorption of the M-band decreases, while the absorption of the D-band increases, with an increase in the pressure. That is, the influence of the pressure is similar to the increase in the concentration, as is shown in Fig. 2, with the exception of the band shift. Such a band shift is a general phenomena observed in the absorption spec-

---

5) W. Kauzmann, *Advan. Protein Chem.*, **14**, 1 (1959).

6) G. Némethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962); *J. Chem. Phys.*, **36**, 3401 (1962).

7) K. Suzuki, Y. Miyosawa, and C. Suzuki, *Arch. Biochem. Biophys.*, **101**, 225 (1963).

8) P. W. Bridgman, *Proc. Amer. Acad. Arts and Sci.*, **48**, 309 (1912).

9) J. Glowacki, *Acta Phys. Polonica*, **26**, 905 (1964).

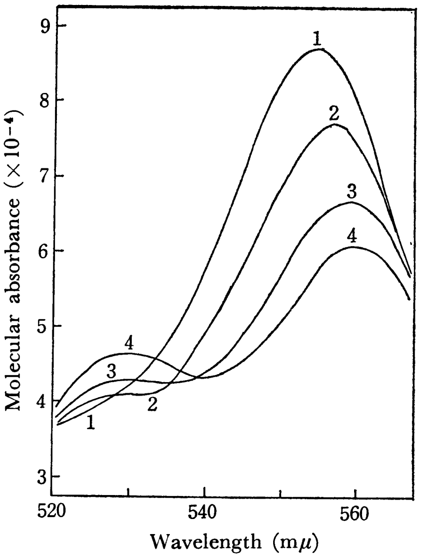


Fig. 3. Effect of pressure on the absorption spectra of RB in aqueous solution (5×10^{-5} mol/l) at 30°C.

	Pressure (atm)
1	1
2	1800
3	3600
4	4500

trum under high pressure; it may be attributed to a solvent effect deduced from the increase in the dielectric constant of the solvent.[10,11] If the shift at each pressure is restored to 1 atm, an isosbestic point can be demonstrated at 533 mμ, as in Fig. 2. We can, then, assume that only the dimer is formed in the experimental ranges of pressure and concentration.

The equilibrium constant, K, of 2 monomer $\rightleftarrows$ dimer was calculated according to the method of Rohatgi and Singhal.[4] The values of K from 1 to 4500 atm are tabulated in Table 2. The volume change accompanying the dimerization, ΔV, is calculated from the following relation;

$$\Delta V = -\boldsymbol{R}T\left(\frac{\partial \ln K}{\partial P}\right)_T$$

Table 2. Effect of pressure on the equilibrium constant K of 2 monomer $\rightleftarrows$ dimer of RB (30°C) and MB (20°)

Pressure (atm)	Equilibrium constant K (l/mol)[a]	
	RB ($\times10^3$)	MB($\times10^4$)
1	6.01	5.56
900	7.48	8.52
1800	10.1	10.7
2700	14.8	15.1
3600	23.2	27.7
4500	39.8	42.2

a) The volume change accompanying association calculated from the above data is −10.4 cc/mol in RB and −10.6 cc/mol in MB.

The value of ΔV thus calculated is −10.4 cc/mol.

If the dimer is formed by a hydrophobic bond, as has been suggested by Rohatgi and Singhal,[4] the volume change accompanying the dimerization should be positive, because the formation of the hydrophobic bond is accompanying by a positive volume change, as has been reported by Kauzmann[5] and Scheraga *et al.*[6] Even if the participation of hydrophobic interaction in the dimerization process can not be neglected, another interaction, such as a hydrogen bond, should be predominant. The structure of the RB molecule fully admits the possibility of hydrogen-bond formation by the carboxyl group. It is plausible to assume that the hydrogen bond is concerned in the dimerization process of RB, because the formation of the hydrogen bond is accompanied by a negative volume change.[12–15]

Figure 4 represents the pressure effect on the absorption spectrum of MB in an aqueous solution. Absorption peaks appear at about 665 and 610 mμ; they correspond to the M-band and the *D*-band respectively, as has been reported by Rabinowitch and Epstein[16] and by others.[3,17–23] Both the absorption

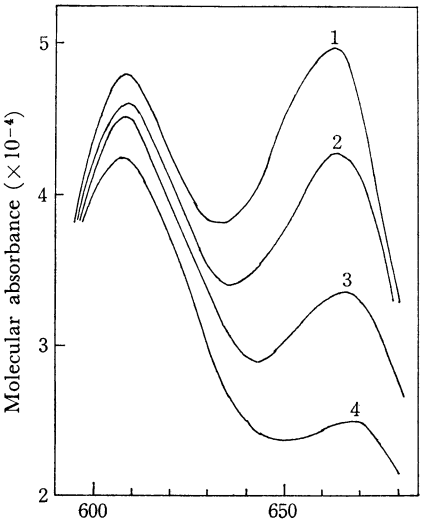


Fig. 4. Effect of pressure on the absorption spectra of MB in aqueous solution (1×10^{-4} mol/l) at 18°C.

	Pressure (atm)
1	1
2	900
3	2700
4	4500

---

[10] F. G. Wick, *Proc. Amer. Acad. Arts and Sci.*, **58**, 555 (1923).

[11] W. W. Robertson, S. E. Babb, and F. A. Matson, *J. Chem. Phys.*, **26**, 367 (1957).

[12] S. D. Hamann, "Physico-chemical Effects of Pressure," Butterworths Sci. Publications, London (1957), p. 147.

[13] E. Fishman and H. D. Drickamer, *J. Chem. Phys.*, **24**, 548 (1956).

[14] J. Osugi and Y. Kitamura, *Rev. Phys. Chem. Jap.*, **35**, 25 (1965).

[15] K. Suzuki, Y. Miyosawa, M. Tsuchiya, and Y. Taniguchi, *ibid.*, **38**, 63 (1968).

[16] E. Rabinowitch and L. F. Epstein, *J. Amer. Chem. Soc.*, **63**, 69 (1941).

[17] G. Holst, *Z. Physik. Chem.*, **A182**, 321 (1938).

[18] L. Michaelis and S. Granick, *J. Amer. Chem. Soc.*, **67**, 1212 (1945).

bands decrease with an increase in the pressure, and no isosbestic point appears. It has been known that the absorption decreases under high pressure in other cases.[11] It should be mentioned, however, that the ratio of the optical density at the D-band (D_d) to that of the M-band (D_m), D_d/D_m, increases with the pressure. Moreover, no other absorption band besides the M-band and the D-band can be found at the shorter wavelengths 600 mμ to 400 mμ. The equilibrium constant, K, can be calculated according to the method of Rohatgi and Singhal,[4] as in RB. The values are tabulated in Table 2. The volume change accompanying the dimerization, $\varDelta V$, is calculated to be -10.6 cc/mol from Eq. (1).

The MB results also show that the dye association is promoted by compression, as in the case of RB. It has been found that the presence of urea and alcohol decreases the self-association of MB.[3] Therefore, the effect of the pressure on the association of MB is the opposite of the effects of urea and alcohol. From the viewpoint of the value of $\varDelta V$ (negative sign), the association of MB can not be interpreted in terms of a hydrophobic bond, as has been suggested by Mukerjee and Ghosh.[3]

If the value of $\varDelta V$ is taken into account, hydrogen bonding may be concerned in the self-association of MB. The structure of MB, however, does not admit the possibility of direct hydrogen bonding. A hydrogen bond through sandwiched water molecules may be involved in this phenomenon, as has been suggested by previous investigators.[23,24]

London's dispersion force could also cause a stacking of dye molecules so as to decrease the volume when the monomer units are in a sandwich with the principal molecular axes parallel, as has been suggested by Förster[25] and by Bergmann and O'Konski.[22] The increase in the dielectric constant with an increase in the pressure[26] may assist the association of dye ions, for an increase in the dielectric constant reduces the coulombic repulsive forces between the ionic parts of the dye ion.[27]

The authors wish to express their thanks to Professor W. Kauzmann of Princeton University for his valuable suggestions. The financial support given by the Ministry of Education is also gratefully acknowledged.

---

[19] T. Vickerstaff and D. R. Lemin, *Nature*, **157**, 373 (1946); D. R. Lemin and T. Vickerstaff, *Trans. Faraday Soc.*, **43**, 491 (1947).

[20] M. Schubert and A. Levine, *J. Amer. Chem. Soc.*, **77**, 4197 (1955).

[21] E. Braswell, *J. Phys. Chem.*, **72**, 2477 (1968).

[22] K. Bergmann and C. T. O'Konski, *ibid.*, **67**, 2169 (1963).

[23] J. A. Bergeron and M. Singer, *J. Biophys. Biochem. Cytol.*, **4**, 433 (1958).

[24] S. E. Sheppard and A. L. Geddes, *J. Amer. Chem. Soc.*, **66**, 2003 (1944).

[25] T. Förster, *Naturwissenschaften*, **33**, 166 (1946).

[26] B. K. P. Scaife, *Proc. Phys. Soc.* (London), **B68**, 790 (1955).

[27] R. F. Tuddenham and A. E. Alexander, *J. Phys. Chem.*, **66**, 1839 (1962).