

Physics

STUDY OF ASSOCIATION OF MOLECULES OF RHODAMINE 6G BY  
FLUORESCENCE POLARIZATION

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The fluorescence and polarization spectrum (per cent  $P$  vs. Wavelength of emission) of rhodamine 6G in glycerine-water mixture (50-50 per cent by volume) is reported for concentrations varying from  $10^{-6}$  g/cc to  $10^{-2}$  g/cc at room temperature (30 °C). It is observed from the fluorescence spectra that with increase in concentration, the emission band becomes broader and shifts towards the longer wavelengths. It is noticed that the value of polarization no more remains constant, over the entire emission band as is expected, if there exists only one type of emission oscillator. On the contrary, with the increase in concentration, the value of polarization on the longer wavelength side increases. This has been explained as due to association of molecules. It is further suggested that in cases where it is not possible to distinguish/identify the presence of different species in a solution by using fluorescence emission spectra, the polarization spectrum could be used.

INTRODUCTION

MOLECULAR association has been observed in solutions of many organic substances and specially in solutions of dyes. The association of dye molecules leads to basic changes in the optical properties like the excitation wavelength, emission wavelength, polarization etc. The process of association of dye molecules in solution is determined primarily by the structure of the dye molecules and the nature of the solvent. Bocharov and Levshin (1963) studied the association of rhodamine 6G in aqueous as well as alcoholic solutions and showed that though association occurs in water, intense association occurs in binary mixtures of polar and non-polar solvents. Baranova (1965), Levshin (1965), Levshin and Akbarova (1968), Levshin *et al.* (1968) and Levshin and Nizamov (1970) also observed association of the dye molecules at high concentrations and showed that the increase in concentration of the dye leads to the deformation of the absorption spectra with a consequent appearance of a new peak on the short wavelength side. Levshin and Nizamov (1970) reported the monomer absorption peak at 535 nm and a new peak due to associated molecules at 510 nm. The fluorescence spectrum has been reported to have broadening towards longer wavelengths. Raicinskyte and Vesiene (1973) showed that the association of the dye molecules is due to the presence of two active NH groups in rhodamine 6G. The information about association of molecules is usually obtained by observing the distortions in either the absorption or emission curves. This method however, does not work satisfactorily when the

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emission spectra from two species (monomers & dimers) in a solution overlap too much. For the same reason, the use of absorption spectra for the study of aggregation is not quite satisfactory when the absorption spectra due to two species have an extensive overlapping.

In the present investigation, therefore, authors have studied the effect of association of the molecules on the polarization spectrum of fluorescence. The results obtained indicate that the polarization spectrum, in some cases, provides a simpler and more sensitive method for identifying the existence of aggregated molecules in the solution.

#### MATERIALS AND METHOD

Rhodamine 6G sample used was of high grade spectral purity obtained from E. Merck, Germany. Glycerol used was of AR quality supplied by BDH which was further checked for any possible contamination by observing its absorption and fluorescence spectra. The choice of glycerine-water mixture (50-50 per cent by volume) stemmed from the consideration that in a high viscosity solvent, the polarization is higher but the association is low. In the present case, since the association of molecules is to be studied through polarization measurements, a glycerine-water mixture was preferred. The polarization of fluorescence was measured by Aminco Bowman Spectrophoto-fluorometer at room temperature (30 °C). The instrument was calibrated with 0.1N H<sub>2</sub>SO<sub>4</sub> solution of quinine

TABLE I

*Percentage polarization of rhodamine 6G in glycerine-water mixture (50-50 per cent by volume) for different concentrations within the fluorescence band 530 nm-600 nm*  
 $\lambda_{\text{excitation}} = 500\text{nm}$

$\lambda_{\text{em}}$ (nm)	Concentration (g/cc)					
	$1.7 \times 10^{-6}$	$1.7 \times 10^{-5}$	$5.6 \times 10^{-5}$	$1.7 \times 10^{-4}$	$1.7 \times 10^{-3}$	$1.7 \times 10^{-2}$
530	6.9	6.5	6.5	7.6	—	—
534	7.0	7.2	7.2	8.0	—	—
538	7.2	7.1	7.6	8.0	—	—
542	7.3	7.6	7.8	8.2	—	—
546	7.3	7.3	7.9	7.8	—	—
550	7.1	7.2	7.7	8.0	—	—
554	7.2	7.3	8.2	8.0	—	—
558	7.0	7.0	7.9	8.7	—	—
562	6.7	6.3	7.9	9.7	15.0	18.4
566	6.4	6.0	8.0	11.0	16.5	22.2
570	5.7	5.7	8.3	12.0	17.9	23.2
574	—	—	8.5	12.0	18.4	24.0
578	—	—	—	12.2	18.3	24.6
582	—	—	—	11.8	18.6	27.3
586	—	—	—	12.0	18.5	29.4
590	—	—	—	12.5	18.9	29.7
594	—	—	—	13.5	20.5	30.3
600	—	—	—	15.0	21.0	30.3

sulphate. The corrections for Xenon Lamp (Melhuish, 1962) and photomultiplier tube (White *et al.*, 1960) were applied. In order to reduce absorption due to inner-filter effect at high concentrations, fluorescence was observed by the front surface excitation technique instead of the usual right-angle geometry. Also to prevent scattering and reflection of the excitation beam into the analyser monochromator, the samples were placed at a slight angle to the excitation beam. Though the maximum intensity of fluorescence, at low concentrations, was obtained by excitation with 524 nm, in the present case, the polarization measurements were made by exciting the sample with a broad band centred around 500 nm having a band spread of  $\pm 25$  nm. This was done in order to avoid the contributions of scattered and reflected light to fluorescence and to excite simultaneously monomers and the aggregated molecules which have a lower excitation wavelength. Keeping the excitation monochromator at 500 nm, the emission monochromator was varied from 530 nm to 590 nm. The polarization was calculated using the following relation adopted by Azumi and McGlynn (1962) :

$$P = \frac{I_{EE} - I_{EB} (I_{BE}/I_{BB})}{I_{EE} + I_{EB} (I_{BE}/I_{BB})} \quad \dots(1)$$

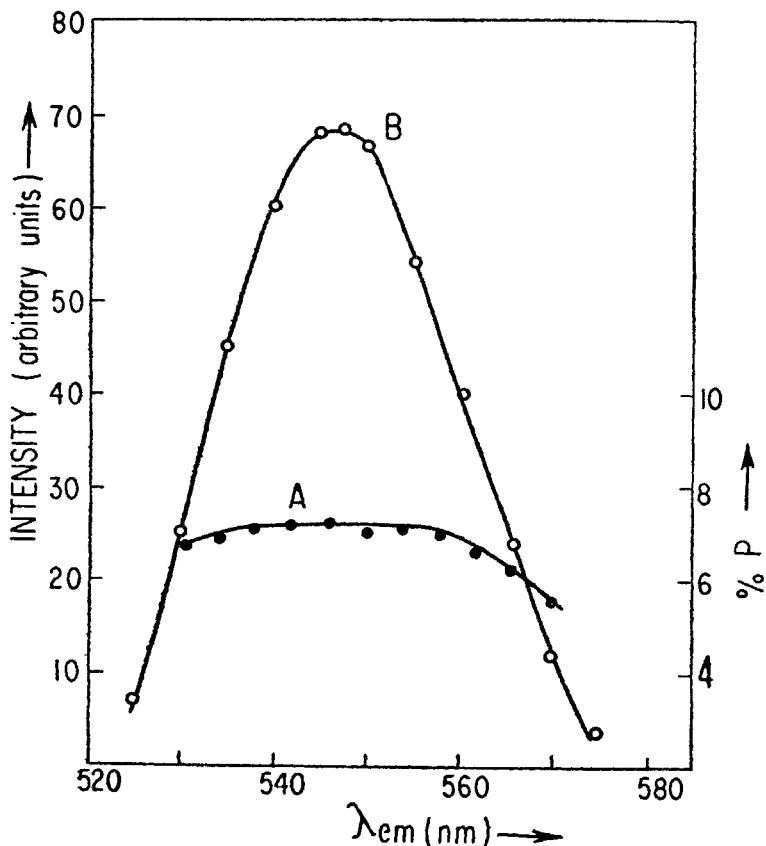


FIG. 1. (A) Percentage polarization vs. emission wavelength, (B) Intensity vs. emission wavelength for concentration  $1.7 \times 10^{-6}$  g/cc.

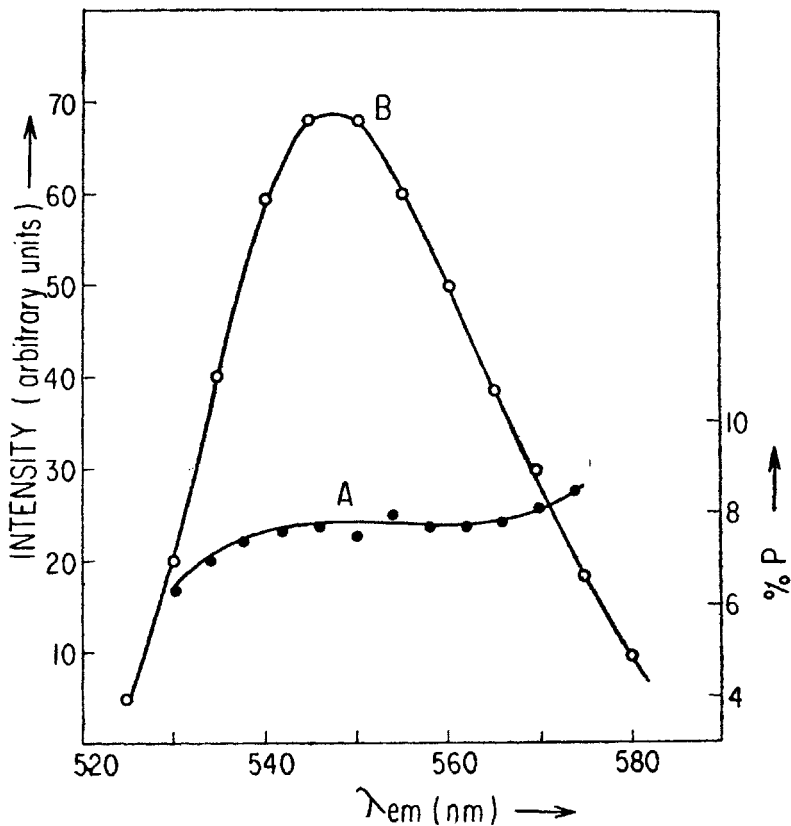


FIG. 2. (A) Percentage polarization vs. emission wavelength. (B) Intensity vs. emission wavelength for conc.  $5.6 \times 10^{-5}$  g/cc.

where  $I$  is the observed intensity, the first and the second subscripts refer to the orientation of the polarizer and the analyzer. The results obtained for polarization are given in Table I and shown in Fig. 1(A), 2(A), 3(A) and 4(A).

#### DISCUSSION

Perrin (1929) has defined the polarization of fluorescence as a function of viscosity, molecular volume, temperature and life time of the excited state as :

$$\left( \frac{1}{P} - \frac{1}{3} \right) = \left( \frac{1}{P_0} - \frac{1}{3} \right) \left( 1 + \frac{RT\tau}{\eta V} \right) \quad \dots(2)$$

Assuming the molecule to be of quasispherical shape, because many organic (aromatic) molecules are compact, fused ring structures, the above relation can be approximated (Hercules, 1967) as :

$$P = \left( \frac{1}{P_0} - \frac{1}{3} \right)^{-1} \frac{V}{10^4 + V} \quad \dots(3)$$

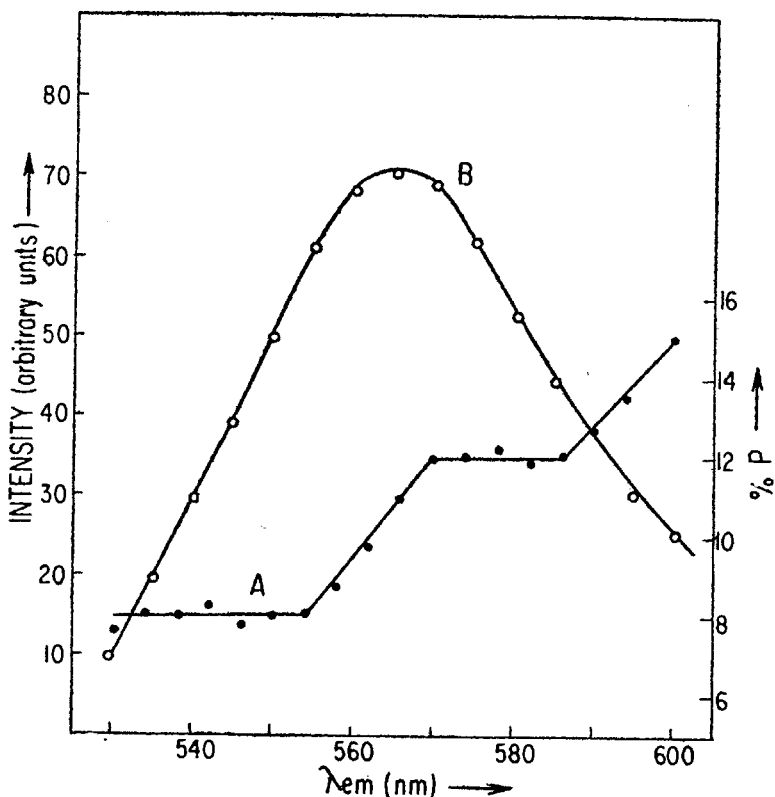


FIG. 3. (A) Percentage polarization vs. emission wavelength. (B) Intensity vs. emission wavelength for conc.  $1.7 \times 10^{-4}$  g/cc.

$$= \left( \frac{1}{P_0} - \frac{1}{3} \right)^{-1} 10^{-4} V \quad \dots(4)$$

Eqn. (4) shows that polarization  $P$  is directly proportional to molecular volume  $V$  which for a given specimen remains constant. But if a substance in solution exists in more than one form of molecular aggregation,  $V$  will be different and therefore, polarization may show a change in the overlapping emission band due to these different molecular forms. The polarization of the aggregated molecule can also be different due to a change in the value of  $\tau$ . The fluorescent properties viz., emission and excitation wavelengths, quantum yield and polarization change with increase in the concentration of the fluor. These changes may be due to (1) re-absorption of the emitted light and (2) formation of aggregates. The effect due to the first has been reduced in the present case by using front face geometry and hence the observed changes in percentage polarization must be essentially due to the second. Further, Klochkov and Neporent (1962) have suggested that the changes in the degree of polarization with emission wavelength can be explained by the existence of at least two centres, the value of  $P$  being different for each one

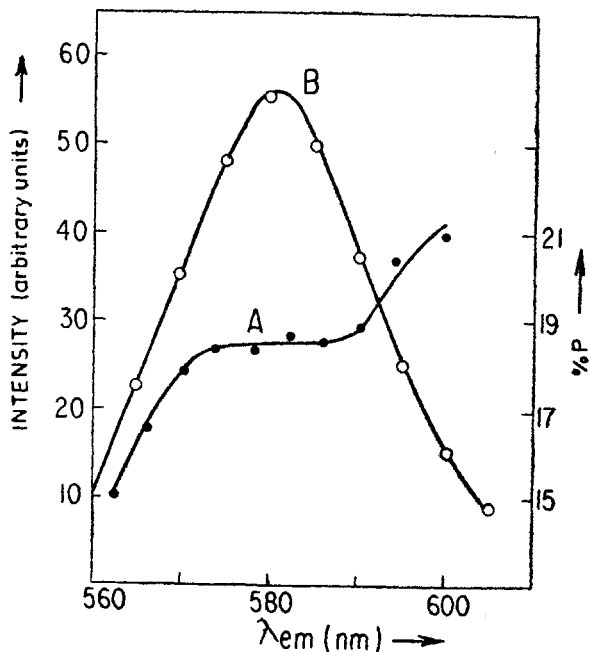


FIG. 4. (A) Percentage polarization vs. emission wavelength. (B) Intensity vs. emission wavelength for conc.  $1.7 \times 10^{-3}$  g/cc.

of them. The present polarization measurements indicate such a situation and hence the polarization spectrum at higher concentration could be accounted for as due to the association of the molecules. Fig. 1-A corresponds to conc.  $1.7 \times 10^{-6}$  g/cc for which  $\lambda_{ex} = 500$  nm. In this case, the percentage polarization remains almost constant between 530 nm and 554 nm which indicates that the emission is due to only one variety, possibly monomers. This observation is also supported by the emission spectrum which has a single peak at 547 nm. Fig. 2-A corresponds to conc.  $5.6 \times 10^{-5}$  g/cc in which case the percentage polarization remains constant upto 562 nm and beyond this, percentage of  $P$  increases slightly. This now indicates that in addition to monomers, there is some other fluorescing centre, with a higher  $\lambda_{em}$  and  $P$ , in the solution. This fluorescing centre could be a dimer and the upward bend in the polarization spectrum in Fig. 2A corresponds to the onset of aggregation of molecules. The emission spectrum Fig 2-B for this concentration also lends support to this view. Comparing the emission spectrum of 2-B with that of 1-B, the emission spectra of 2-B is found broader indicating that the emission is not entirely due to one variety but on the longer wavelength side there is a contribution from the associated molecules as well. Also, the similarity between the two emission curves 2-B and 1-B except distortion on the longer wavelength side in 2-B indicates that the emission curve is essentially due to monomers i.e., the number of monomers as compared to dimers in solution is still large at this concentration ( $\sim 5 \times 10^{-5}$  g/cc). Fig. 3-A corresponds to conc.  $1.7 \times 10^{-4}$  g/cc. In this case there are two clear steps in the polarization spectrum. The first step corresponding to polarization value of 8 extending from 534 nm to

554 nm which possibly represents the contribution from monomers, while the second step with polarization value of 12 extending from 570 nm to 586 nm may be due to the associated molecules. The variation in percentage polarization between 554 nm to 570 nm could be due to the simultaneous contribution from both the varieties i.e., the monomers and the aggregated molecules. The emission curve (Fig. 3-B) for this concentration also lends support to this view. Comparing the emission curves of 1-B, 2-B, and 3-B, it is seen that the curve 3-B is comparatively more broad. It is due to the overlapping of the emission spectra of monomers and dimers. An isolated observation of this broad emission band cannot give any conclusive evidence of the formation of aggregates in the solution. On the other hand the polarization spectrum leaves no doubt about the formation of dimers and their simultaneous existence along with the monomers. Fig. 4 corresponds to

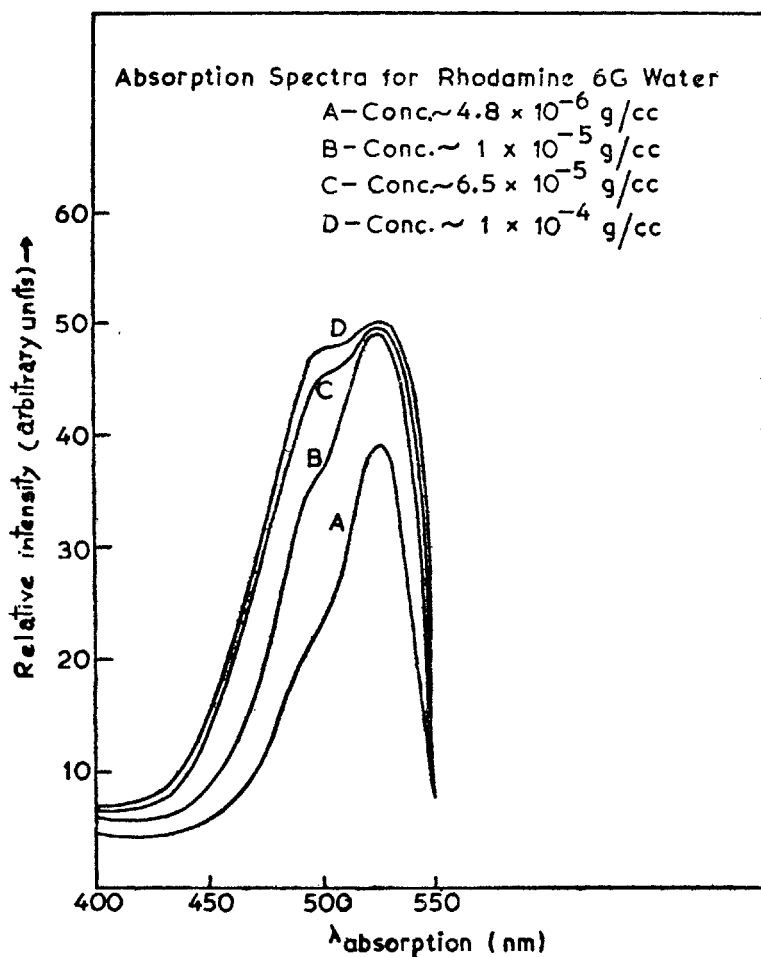


FIG. 5. Absorption spectra of Rhodamine 6G in water. (A) Conc.  $4.8 \times 10^{-6}$  g/cc. (B) Conc.  $1 \times 10^{-5}$  g/cc. (C) Conc.  $6.5 \times 10^{-5}$  g/cc. (D) Conc.  $1 \times 10^{-4}$  g/cc.

conc.  $1.7 \times 10^{-3}$  g/cc. The emission curve (Fig. 4-B) has a peak at 582 nm and the polarization curve (Fig. 4-A) shows a constant value in the region 572 nm. to 586 nm, the region of emission of the aggregated variety having peak at 582 nm. Comparing it with the emission curve of Fig. 1-B it is seen that the aggregation has caused bathochromic shift of 35 nm (from 547 nm for monomers to 582 nm for the associated molecules) in the emission wavelength. The higher value of percentage  $P$  for concentrations  $1.7 \times 10^{-3}$  g/cc and  $1.7 \times 10^{-2}$  g/cc shows that the higher aggregates are possibly formed which have their emission peaks extending beyond our range of measurement (in the present case 600 nm) but their tail extending in the region 570 nm-600 nm. It can also be noted that as the concentration increases, the contribution to the polarization spectrum of fluorescence from monomers decreases whereas that of the associated molecules increases. The absorption curves for four different concentrations viz.,  $4.8 \times 10^{-6}$  g/cc,  $1 \times 10^{-5}$  g/cc,  $6.5 \times 10^{-5}$  g/cc and  $1 \times 10^{-4}$  g/cc for the dye in aqueous solution at room temperature are shown in Fig. 5. These curves are recorded with a Beckman DB-G.T. spectrophotometer. As Fig. 5 is drawn as a composite figure from separate absorption curves only their shapes are relevant for discussion. It is seen from these curves that with increase in concentration, a deformation in the electronic absorption spectrum takes place and there appears a peak on the shorter wavelength side. For conc.  $1 \times 10^{-4}$  g/cc (Fig. 5-D), this peak at 500 nm is quite prominent. The absorption data, therefore, also lend support to the observations from the polarization spectrum.

### CONCLUSIONS

The present study shows that with increase in the concentration, the shape of the polarization spectrum of fluorescence changes. The higher value of percentage polarization on the longer wavelength side in the emission band is due to the association of molecules. In general, it suggests that this method, of using polarization spectrum will be better suited to identify two fluorescing species in a binary mixture which normally cannot be distinguished by observing the emission spectra alone which results due to an extensive overlapping of the individual emission spectra. Some work on binary mixtures of this type has been reported by us elsewhere (Pandya & Machwe, 1978). No doubt this method is much simpler, when can be put to use, as compared to the more sophisticated methods suggested by Green (1974) and O'Haver and Parks (1974), for the same purpose.

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