LETTERS TO THE EDITOR

ENVIRONMENTAL EFFECT ON THE FLUORESCENCE OF MERCURIDIBROMO-SODIUM FLUORESCEIN

THE universal interaction between solute-solvent molecules is due to collective influence of the solvent as a dielectric medium. It depends on the dielectric constant D and refractive index n of the solvent and dipole moment μ of the solute molecule. Because of the variation of electron density in different electronic states, an interaction with the environment affects differently the various electronic states of a molecule. A shift in the spectrum may result due to such differences in the two combining states. Pertinent theoretical treatments within the zeroth order approximations for calculating the Stoke's shift (difference between the absorption and fluorescence wavenumbers) have been given by several authors[1–6]. In the present investigation, the absorption and fluorescence frequency shifts are studied for Mercuridibromosodium-fluorescein in different solvents for a constant concentration $\sim 10^{-7}$ g/cc at room temperature 25° C using Aminco Bowman Spectrophotofluorometer. The results obtained are explained in terms of Onsager theory[1] of reaction field. Expressing in wave numbers, the Stoke's shift is given by

$$\bar{\nu}_a - \bar{\nu}_f = \triangle\bar{\nu} = \frac{2(\mu_e - \mu_g)^2}{hca^3}\left\{\frac{D-1}{2D+1} - \frac{n^2-1}{2n^2+1}\right\}$$

$$= \frac{2(\mu_e - \mu_g)^2}{hca^3} \cdot \varphi(D, n) \qquad (1)$$

where

$$\varphi(D, n) = \frac{D-1}{2D+1} - \frac{n^2-1}{2n^2+1};$$

μ_g and μ_e are dipole moments of the ground and excited molecules respectively, D is the dielectric constant, n refractive index and a is the radius of the cavity in which the solute molecule resides.

In the present study the dye used was of spectroscopic grade purity and the solvents used were of AR quality obtained from BDH/EM and were further checked by absorption studies for any possible contamination. The concentration used ($\sim 10^{-7}$ g/cc) was low enough to avoid reabsorption of the emitted light and possible changes in the wavelength due to inner filter effect. The entrance and exit slits of the spectrophotofluorometer were adjusted to give a reasonable deflection and a resolution for absorption and emission wavelengths of the order of ± 1 nm. The effect of scattering and background for each solvent was checked by using the blank solution in the cuvette and was found to be less than 0·5%. The corrections for Xenon Lamp and photomultiplier tube were also applied[7–8]. The peak absorption and emission wavelengths were located and the results are given in Table I and shown in Figs. 1 and 2.

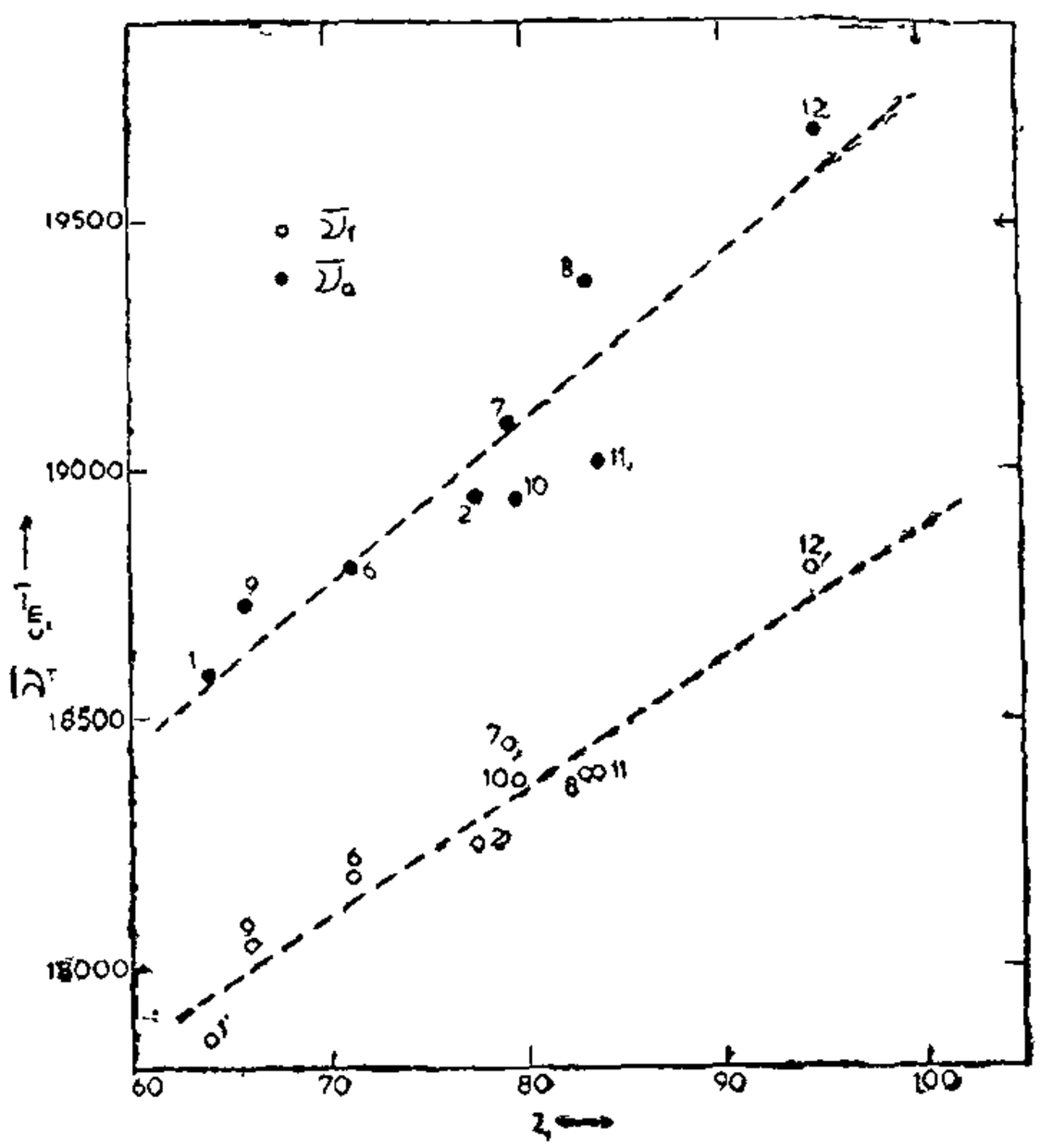

FIG. 1. $\bar{\nu}_a$ and $\bar{\nu}_f$ versus Z.

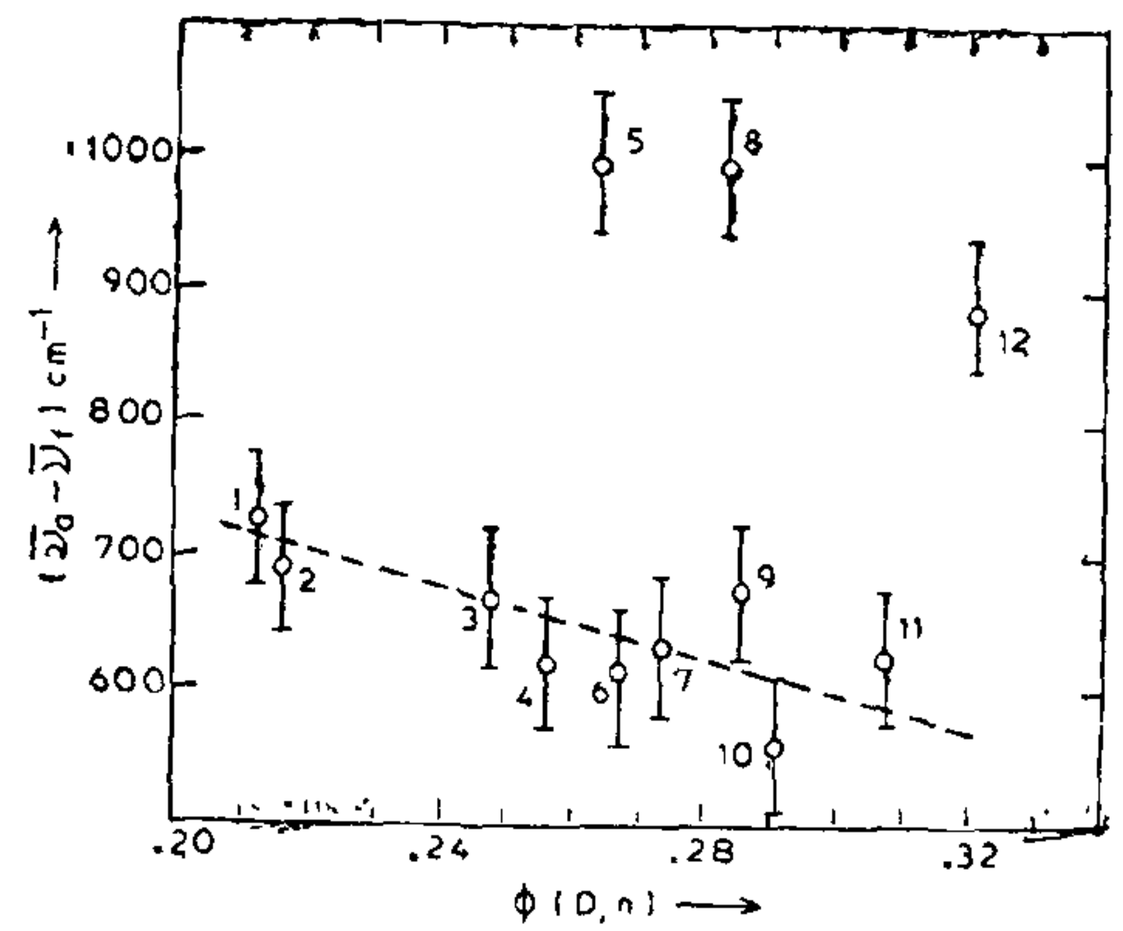

FIG. 2. $\bar{\nu}_a - \bar{\nu}_f$ versus $\varphi(D, n)$.

Table I indicates that the change in solvent produces alterations in both the λ_a and λ_f indicating that the solvent interacts with the solute in the ground state

TABLE I

Solvent	λ_a (nm)	λ_f (nm)	$\bar{\nu}_a$ (cm^{-1})	$\bar{\nu}_f$ (cm^{-1})	$\bar{\nu}_a - \bar{\nu}_f$ (cm^{-1})	φ (D, n)	Z
Pyridine	538	560	18587	17857	730	0·212	64·0
n-Butanol	528	548	18939	18248	691	0·216	77·7
Cyclohexanone	538	558	18587	17921	666	0·248	..
Amylalcohol	530	548	18867	18248	619	0·256	..
Glycerol	516	544	19379	18382	997	0·264	..
Iso-butanol	532	550	18796	18181	615	0·267	71·3
Acetic-anhydride	524	542	19083	18450	633	0·273	79·2
Formamide	516	544	19379	18382	997	0·283	83·3
Acetone	534	554	18726	18050	676	0·285	65·7
Ethanol	528	544	18939	18382	557	0·290	79·6
Methanol	526	546	19011	18387	624	0·308	83·6
Water	508	532	19685	18796	889	0·320	94·6

as well as in the excited state. The shifts in the wavelength depend upon the polarity of the solvent. It is obvious that a graph between D and $\bar{\nu}_a$ or $\bar{\nu}_f$ will not be quite linear, However, a plot of Z versus $\bar{\nu}_a$ or $\bar{\nu}_f$ as shown in Fig. 1, shows a good linear relationship. This indicates that the universal solvent polarity scale as suggested by Kosower[9] and given in terms of Z values is a better measure of the microscopic solvent polarity than D which gives the macroscopic solvent behaviour. It may be noted that the slope of lines $\bar{\nu}_a$ versus Z and $\bar{\nu}_f$ versus Z are almost equal indicating that the change in the dipole moment in the excited state from the ground state is not very large.

According to equation (1), when the Stoke's shift $\triangle\bar{\nu}$ is plotted against ϕ (D, n) of the solvent, it should be a straight line, the slope of which would give

$$2(\mu_e - \mu_g)^2/hca^3.$$

Fig. 2 shows such a plot between φ (D, n) and $\bar{\nu}_a - \bar{\nu}_f$. This straight line graph confirms the theoretical relationship stated in equation 1. Again the slope of the line here indicates that the change in the dipole moment is not very large.

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CHROMIUM (III) MYOGLOBIN

RECENT interest in the reaction of oxygen with chromium (II) porphyrin[1] and capability of inositol hexaphosphate in switching the quaternary structures of high-spin ferric hemoglobin from oxy- to deoxy- states[2,3] have prompted us to investigate the properties of chromium replaced hemoproteins. In this report we describe the preparation of chromium (III) myoglobin and its visible and electron paramagnetic resonance spectra. The chloro derivative of chromium(III) mesoporphyrin IX was prepared by known method[4]. The chromium(III) myoglobin was prepared by mixing the chloro derivative of chromium (III) and apomyoglobin in Tris-HCl buffer of pH 8. The reaction mixture was transferred immediately to a Sephadex G–25 gel permeable column equilibrated and eluted with 0·01 M potassium phosphate buffer of pH[5]. The first eluted fraction was chromium(III) myoglobin. The second fraction was excess metal porphyrin. The chromium (III) myoglobin was further purified by absorbing on to a CM–52 cellulose ion-exchange column and