Zimm theory cannot be expected to be applicable. The acid-modified albumin is certainly not a random coil, though it may have internal viscoelastic responses in view of the flexibility revealed by fluorescence depolarization.[29] In principle, the viscoelastic properties of the molecule itself could be derived from those of the solution, though such a calculation has been formulated only for spherical molecules at infinite dilution.[32]

The steady-state compliance of the albumin solutions is also given in Table III. It is much smaller than for the paramyosin solutions but increases rapidly with protein concentration.

Conclusions

In dilute paramyosin solutions, the viscoelastic behavior can be attributed to rigid-body orientation of the

(32) R. Cerf, *J. Chem. Phys.*, **20**, 395 (1952).

elongated molecules and can be described fairly well by the Cerf–Scheraga theory. Exposure to concentrated glycerol without glycine appears to increase the molecular flexibility slightly. In concentrated bovine serum albumin solutions, viscoelastic behavior appears only after molecular modification by aging in concentrated glycerol or by slight acidification. Here the viscoelasticity appears to arise primarily from intramolecular flexibility rather than rigid-body orientation. It cannot be described by any molecular theory at present. Such measurements may have promise for obtaining additional information about intramolecular flexibility in biological macromolecules.

Excimer Fluorescence in Liquid Phenol, p-Ethylphenol, and Anisole[1,2]

S. S. Lehrer and Gerald D. Fasman[3]

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The fluorescence emission spectra of phenol, p-ethylphenol, and anisole have been investigated. An additional fluorescence band in the liquid state has been observed which was shifted 5200–5600 cm.$^{-1}$ to lower frequency from that found for the dilute solution band. This new band is due to the emission of excited dimers or excimers in distinction to the monomer band, the latter being due to the emission from excited monomers. From the temperature dependence of the emission of each band, the heat and entropy of activation for the photoassociation, ΔH_a and ΔS_a, of phenol and anisole have been determined. For phenol $-\Delta H_a = 4.5 \pm 0.5$ kcal./mole and $\Delta S_a = -25 \pm 3$ cal./mole deg. For anisole $-\Delta H_a = 5.1 \pm 0.6$ kcal./mole and $\Delta S_a = -22 \pm 3$ cal./mole deg. The variation of the wave length of maximum fluorescence for these compounds in the liquid and solid states and in different solvents has been interpreted as being due to hydrogen bonding. This bonding appears to be stronger in the excited state than in the ground state.
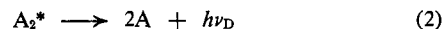
Introduction

The appearance of a new emission band in concentrated solutions of certain aromatic hydrocarbons is due to photoassociation and subsequent emission at

higher wave lengths by an excited dimer or excimer[4,5]

$$A^* + A \longrightarrow A_2^* \tag{1}$$

$$A_2^* \longrightarrow 2A + h\nu_D \tag{2}$$

This emission competes with the monomer fluorescence

$$A^* \longrightarrow A + h\nu_M \tag{3}$$

and monomer quenching processes

$$A^* \longrightarrow A \tag{4}$$

The excimer can also dissociate more readily at higher temperatures

$$A_2^* \longrightarrow A + A^* \tag{5}$$

and relax nonradiatively

$$A_2^* \longrightarrow 2A \tag{6}$$

to re-form the dissociated molecules in the ground state.

The probability of excimer formation is generally higher the longer the lifetime of the excited monomer, the more concentrated the solution, and the lower the temperature. In these systems, the transition from the ^1L_b excited singlet state to the ^1A ground state is associated with a weaker transition moment than the transition from the ^1L_a state. Since the emission occurs from the lowest excited state, a longer lifetime is apparent when the ^1L_b state is lower than the ^1L_a state.[5] Most of the compounds that have produced excimer emission have a lower ^1L_b state.

Excimer emission has been observed in the molten and crystalline states where, of course, the concen-
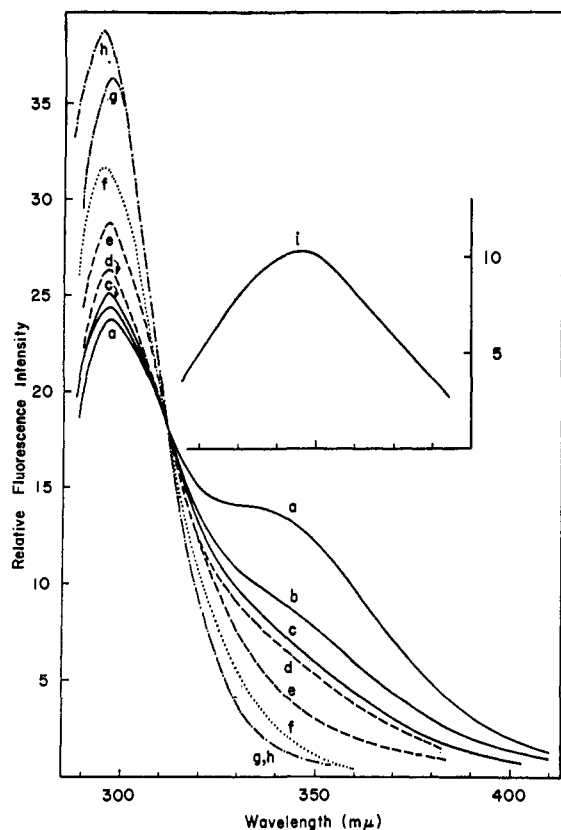
Figure 1. Fluorescence emission spectra of phenol: liquid hemi-hydrate, ———— (a) 8.7°, (b) 28.9°, (c) 39.3°, (i) excimer; liquid, --------- (d) 44.1°, (e) 87.1°; dilute in water, ..., (f) 25°; crystalline, —·—·—· (g) phenol, 25°, (h) hemihydrate, 3.8°.

tration is maximum.[6-9] In crystals the presence or absence of the emission (if it occurs in fluid media) has been used as a criterion for the presence or absence of the parallel stacking of rings.[9]

The observation of excimer emission in liquid benzene and toluene was the first example of this phenomenon occurring in a single ring system,[8] and one may expect that such observations will be extended to other systems of substituted benzene molecules when the above spectroscopic and concentration conditions are met. In this work we report the observation of excimer emission in phenol, p-ethylphenol, and anisole in the liquid state, and the absence of excimer emission in crystalline phenol, phenol hemihydrate, and p-ethylphenol.[10]

Experimental Section

The emission spectra were measured with a Zeiss ZFM 4C spectrofluorometer using a Zeiss motorized, wave length drive and a Brown recorder. The spectra were corrected for instrumental variations of sensitivity with wave length by the use of a concentrated (≃5 g./l.) rhodamine B solution in ethylene glycol as a quantum counter to measure the spectral distribution

(6) J. B. Birks and J. B. Aladekomo, *Spectrochim. Acta*, 20, 15, (1964).
(7) B. Stevens and T. Dickinson, *J. Chem. Soc.*, 5492 (1963).
(8) T. V. Ivanova, G. A. Mokeeva, and B. Ya. Sveshnikov, *Opt. Spectry.*, 12, 325 (1962).
(9) B. Stevens, *Spectrochim. Acta*, 18, 439 (1962).
(10) A preliminary observation of excimer emission in liquid phenol was reported earlier; see S. S. Lehrer and G. D. Fasman, *Biopolymers*, 2, 199 (1964).

of the xenon-excitation lamp. The calibrated xenon lamp was then used as a source of radiation to determine the sensitivity of the system. Melhuish has described a similar method.[11]

The temperature was varied in the 0–100° range by circulating heated or cooled water through a copper cuvette holder and in the −40–0° range by circulating gaseous nitrogen produced by boiling liquid nitrogen. Thermal contact was made to two 2-mm. quartz stoppered cuvettes similarly positioned. Fluorescence measurements were made with one cuvette while the temperature was read to ±0.5° using a Veco thermistor in contact with the same substance in the second cuvette. The thermistor resistance was monitored with a modified Sargent bridge. For these concentrated solutions, it was necessary to use the tilted position of the Zeiss holder in order to observe the emission from the same face of the cuvette which is exposed to the exciting radiation. The band widths of the emission monochrometer were in the 3–6-mμ range at 300 mμ and excitation was at 270 or 275 mμ.

Phenol (Baker) and p-ethylphenol (Eastman) were purified by vacuum sublimation. Anisole (Eastman) was distilled at reduced pressure (b.p. 63–64°). Solvents were of spectroscopic grade or were distilled and were free of fluorescent contaminants. Phenol hemihydrate was prepared following the procedure of Smits and Maarse.[12] In this work, seeds of the hemihydrate were produced by quenching a solution of phenol containing 9% H$_2$O (slight excess) to Dry Ice temperature. Some seeds were then added to the phenol solution at 10° and the resulting crystals were air dried on filter paper (m.p. 15.6–15.8°) and transferred to a cuvette. The crystallization procedure was then repeated in the cuvette. p-Ethylphenol did not produce a hemihydrate when treated in a similar manner.

Results and Discussion

The fluorescence emission spectra of phenol under various conditions are seen in Figure 1. The spectra of crystalline phenol (g), crystalline phenol hemihydrate (h), and dilute phenol in water (f), are very similar to each other and consist of a single narrow peak. In the liquid phase (a–e), a pronounced shoulder is observed. As the temperature is raised, the intensity of the shoulder near 340 mμ decreases, the intensity of the major band at 297 mμ increases, and the spectra of the liquid at various temperatures have an isosbestic point at 312 mμ. All other curves were normalized at 312 mμ to show the change in shape. In pure liquids of polycyclic aromatics, essentially excimer fluorescence is observed near the melting point.[6,7] In the liquid phenol near the melting point (d), only an increased "tail" fluorescence is seen. These general observations of the relative intensities of monomer and excimer bands are similar to the case of liquid benzene.[8] A lower observation temperature was possible by supercooling the molten hemihydrate, thereby increasing the ratio of excimer to monomer fluorescence (F_D/F_M). The shape of the excimer emission, curve i in Figure 1, was obtained by sub-

(11) W. H. Melhuish, *J. Opt. Soc. Am.*, 52, 1256 (1962).
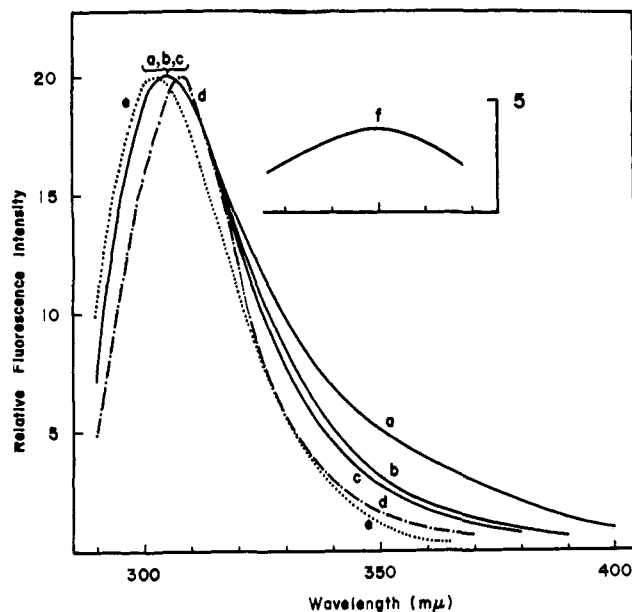(12) A. Smits and J. Maarse, *Proc. Koninkl. Ned. Akad. Wetenschap. Amsterdam*, 20, 100 (1911).

Figure 2. Fluorescence emission spectra of p-ethylphenol: liquid, ——— (a) 14% H_2O, 19.7°, (b) 44.2°, (c) 86.5°, (f) excimer; crystalline, —·—·—· (d) 25°; dilute in water, ... (e) 25°.

Figure 3. Fluorescence emission spectra of liquid anisole: (a) $-33.8°$, (b) $-26.8°$, (c) $-16.8°$, (d) $+2.0°$, (e) $+23.5°$, (f) $+51.7°$.

tracting curve e, normalized at 297 mμ, from curve a. The peak of the excimer emission is at 345 mμ.

The fluorescence spectra of p-ethylphenol in the liquid state as a function of temperature, in the crystalline state, and in dilute aqueous media are shown in Figure 2, where all spectra have been normalized at the peak because no isosbestic point was obtained. In this system photoassociation occurs only to a small extent, probably because the steric hindrance of the ethyl groups makes close interaction between rings difficult.[5] The same type of behavior was reported for toluene compared to benzene.[8] As in the case of phenol, a supercooled solution effected by adding a small amount of water to liquid p-ethylphenol ($\simeq$14% H_2O) produced a greater F_D/F_M ratio because it allowed a lower observation temperature in the liquid state. The shape of the excimer emission (curve f in Figure 2) was approximated by subtracting (e) from (a) after shifting (e) 3 mμ to higher wave length. The excimer peak appears at 350 mμ.

The fluorescence results for liquid anisole as a function of temperature are seen in Figure 3. Since the melting point for anisole ($-37.3°$) is lower than that of phenol or the hemihydrate, a greater range of temperature was available for the observation of the excimer emission. The curves intersected at an isosbestic point at 332 mμ, and the excimer peak is at 345 mμ.

No special precautions were taken to exclude oxygen during the above measurements. However, the effect of saturating liquid anisole with oxygen, air, and nitrogen was measured at 2 and 17°. It was found that $F_{O_2}/F_{N_2} = 0.5$ and $F_{air}/F_{N_2} = 0.7$ for both excimer and monomer fluorescence; that is, the quenching of monomer and excimer emission by oxygen was about the same.

It has been pointed out by Stevens and Ban[13] that the enthalpy, ΔH_a, and entropy, ΔS_a, of association can be determined from the temperature dependence if the

(13) B. Stevens and M. I. Ban, *Trans. Faraday Soc.*, **60**, 1515 (1964).
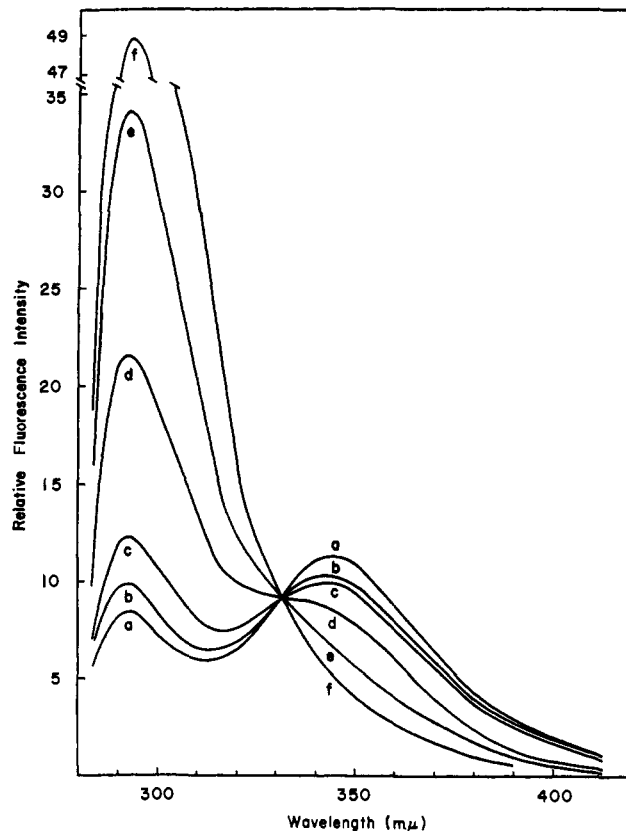
condition $k_5 \gg k_6 + k_2$ is satisfied (these k values are the rate constants associated with reactions 1–6 above). That is, if an equilibrium can be set up between excited monomers and dimers, the above values can be calculated by the usual formula adapted for these systems[13] (subject to the above condition)

$$\ln K_a = \frac{\Delta S_a}{R} - \frac{\Delta H_a}{RT} = \ln \frac{F_D{}^i}{F_M{}^i[A]}$$

where [A] is the fluorophore concentration and $F_D{}^i$ and $F_M{}^i$ are the excimer and monomer fluorescence at the isosbestic point, respectively. The temperature range over which this equilibrium holds is defined by the presence of an isosbestic point and the linear dependence of $\ln F_D{}^i/F_M{}^i[A]$ vs. $1/T$. Using this method the values in Table I were obtained and compared with corresponding values for pyrene obtained by Stevens and Ban.[13]

As shown by Stevens and Ban,[13] the ratio of the radiative decay constants, k_2 and k_3, of dimer and monomer, respectively, can be calculated with the above data. That is

$$\frac{k_2}{k_3} = \frac{F_M{}^i}{\int F_M d\lambda} \frac{\int F_D d\lambda}{F_D{}^i}$$

The values calculated were $k_2/k_3 = 6.2$ for phenol and $k_2/k_3 = 0.34$ for anisole. The higher ratio obtained for phenol can possibly be explained by a lower monomer fluorescence in phenol owing to self-quenching facilitated by hydrogen bonding which is not possible in anisole. The quenching of phenol fluorescence by various bases has previously been attributed to

Table I. Enthalpies, Entropies, and Frequency Shifts of Photoassociation

Material	Concn.,[a] M	Temp., °C.	$-\Delta H_a$, kcal./mole	ΔS_a, cal./mole deg.	ν_M^{max} (liquid) − ν_D^{max}, cm.$^{-1}$	ν_M^{max} (hexane) − ν_D^{max}, cm.$^{-1}$
Pyrene (in ethanol)	0.01	60–85	9.4 ± 1.0	−18.5		6100[b]
Phenol heimihydrate (liquid)	9.7	8–40	4.5 ± 0.5	−25 ± 3	4700	5600
Anisole (liquid)	8.6	−34–+24	5.1 ± 0.6	−22 ± 3	5100	5500
p-Ethylphenol (liquid)					4200	5200

[a] Calculated by assuming the density equal to 1 for the liquids over the temperature range studied. [b] Data from ref. 9; note that ethanol is the solvent.

hydrogen bond formation.[14] This explanation is supported by the observation that at 52° where excimer fluorescence was small, the relative fluorescence intensity of phenol was about one-tenth that for anisole.

The difference in frequency between the monomer and excimer peaks is listed in the last two columns in Table I. In the pure liquid the monomer emission band is shifted to lower frequency as compared to a dilute solution of the same substance in a nonpolar solvent such as n-hexane. The frequency difference falls in the range of many polycyclic systems (5000–6000 cm.$^{-1}$) when the monomer frequency peak in n-hexane is used for the comparison (last column, Table I).

In this work it was generally observed that the wave length of maximum fluorescence, λ_{max}, of the monomer band depended upon the solvent and the state of the system. The results are summarized in Table II.

Table II. Wave Length of Maximum Fluoresence of the Monomer Band[a]

Material	State or solvent[b]	λ_{max}, mμ
Phenol	n-Hexane (dilute)	289
	Methanol (dilute)	294
	Water (dilute)	295
	Liquid	297
	Crystalline	297
Phenol hemihydrate	Liquid	297
	Crystalline	295
p-Ethylphenol	n-Hexane (dilute)	296
	Water (dilute)	303
	Liquid	305
	Crystalline	308
Anisole	n-Hexane (dilute)	290
	Methanol (dilute)	290
	Liquid	292

[a] Excited at 270 or 275 mμ. [b] The absorbance was <1 for dilute solutions in a 1-cm. cuvette at the excitation wave length.

It has been shown that hydrogen bonding of phenol to polar solvents affect electronic transitions, causing a shift to higher wave lengths. This has been observed in the absorption spectrum of phenol and naphthol,[15,16] and in the absorption and fluorescence of anthrol.[17] The difference in λ_{max} of fluorescence for phenol and p-ethylphenol in n-hexane and water is a reflection of

this type of interaction. The red shift in λ_{max} in water compared to n-hexane was 700 cm.$^{-1}$ for phenol and 800 cm.$^{-1}$ for p-ethylphenol. As seen in Table II, anisole does not show this effect.

The absorption spectra of liquid phenol and anisole were determined in order to compare the effects of hydrogen bonding on the absorption spectra with the effects on fluorescence spectra (Table II). The spectra were run using a thin layer ($\simeq 1$ μ) of liquid formed by placing a drop of liquid between two optical quartz plates. The phenol absorbed some water from the atmosphere which prevented crystallization at room temperature. Little or no difference in the peaks of the vibrational structure was noted between dilute phenol in n-hexane and liquid phenol. The wave length of maximum absorption was at 271 mμ in both cases. For the liquid some broadening of the vibrational structure was apparent. The spectrum of liquid anisole was very similar to liquid phenol and the wave length of maximum absorption was 272 mμ. These results are similar to those reported by Ito.[18] He also reported that the small blue shift in the phenol absorption spectrum which occurred as the concentration was increased to about 6 M in CCl$_4$ was due to a phenol–phenol interaction. However, an alternate interpretation of this blue shift is possible by an examination of Ito's spectra. The λ_{max} of the vibrational peaks of the concentrated phenol in CCl$_4$ were the same as the λ_{max} of the peaks of dilute phenol in n-hexane.[18] The work reported here for liquid phenol also illustrated this fact. A red shift relative to phenol in n-hexane can also be noticed in Ito's spectra of the dilute phenol solution in CCl$_4$ which was not discussed.[18] This is probably due to a phenol–CCl$_4$ interaction. Upon increasing the phenol concentration in CCl$_4$ the λ_{max} of the peaks revert to values found for phenol in n-hexane, or for liquid phenol. This is the result of a change in the environment from that of CCl$_4$ to that of liquid phenol as the concentration is increased. The previous solvent effect is thereby eliminated, seemingly causing a "blue shift."

These observations can be interpreted in terms of the Franck–Condon principle as applied by Pimentel[19] to hydrogen-bonded systems. Even though there is little shift in the absorption spectrum for phenol as the concentration is increased to the pure liquid, the fluorescence monomer emission maximum is shifted 700–900 cm.$^{-1}$ (Table II). Pimentel's equations[19]

(14) R. W. Cowgill, Biochem. Biophys. Res. Commun., 16, 332 (1964).
(15) S. Nagakura and M. Gouterman, J. Chem. Phys., 26, 881 (1957).
(16) H. Baba and S. Suzuki, J. Chem. Phys., 35, 1118 (1961).
(17) S. Suzuki and H. Baba, ibid., 38, 348 (1963).

(18) M. Ito, J. Mol. Spectry., 4, 125 (1960).
(19) G. C. Pimentel, J. Am. Chem. Soc., 79, 3323 (1957).

(eq. 7 and 8) can be applied to these data. These

$$\Delta \nu_a = \nu_a - \nu_a^0 = W_0 - W_1 + w_1 \simeq 0 \qquad (7)$$

$$\Delta \nu_e = \nu_e - \nu_e^0 = W_0 - W_1 - w_0 \simeq -800 \, cm.^{-1} \qquad (8)$$

equations relate the absorption frequency shift, $\Delta \nu_a$, and the fluorescence frequency shift, $\Delta \nu_e$, to the hydrogen-bond energy in the ground and excited states, W_0 and W_1, respectively, and to the Franck–Condon energies in the ground and excited states, w_0 and w_1, respectively. These relations indicate that the hydrogen-bond energy is greater in the excited state than in the ground state. Since all the energies are positive, the maximum difference in energy, $W_1 - W_0$, is about 800 cm.$^{-1}$ or $\simeq 2$ kcal./mole.

Fluorescence differences were also noted in the condensed phases. The emission maxima for the crystalline phenol hemihydrate and the dilute aqueous solution of phenol were at 295 mμ, whereas the crystalline phenol and liquid phenol maxima (hemihydrate and pure) were at 297 mμ. The crystal structures indicate that every phenol is hydrogen bonded to another phenol in the pure crystal and to a water molecule in the case of the hemihydrate.[20,21] The difference in the wave length of the fluorescence maxima presumably reflects this difference in environment in the two different crystals and the similarity of the environment of the dilute aqueous solution and the crystalline hemihydrate. The observation that the fluorescence maxima of the liquid hemihydrate and the solid phenol occur at the same wave length implies that the local environ-ments are similar; i.e., in the liquid hemihydrate the phenol molecules preferentially hydrogen bond to each other rather than to water as in the crystal. The reason that phenol usually crystallizes out of the hemihydrate liquid if cooled slowly below 10° has been explained by this preferential binding.[21]

Excimer emission was not observed for crystalline phenol, phenol hemihydrate, and p-ethylphenol. The crystal structures of phenol[20] and phenol hemihydrate[21] indicate that neighboring rings do not overlap. Therefore, the fluorescence results on p-ethylphenol may also indicate nonstacking of rings in the crystal.

In a preliminary report of the fluorescence of poly-L-tyrosine[10] the presence of a new fluorescence band near 420 mμ was interpreted as being due to excimer emission caused by the interaction of neighboring phenolic side chains. Current work has indicated that this band at 420 mμ is due to a new chemical species formed by photoirradiation of the polymer during measurement and also by the chemical treatment during the preparation. This new species has a fluorescence excitation spectrum similar to that of the phenolic chromophores. The present observation, of excimer emission at 345–350 mμ in liquid phenol and p-ethylphenol, which are appropriate models for the polymer chromophore, substantiate the current interpretation. Continuing work is in progress and will be reported elsewhere. It is to be noted that the possibility of chromophore interaction in proteins, i.e., excimer formation and phenol–phenol self-quenching, when the side chains are favorably oriented, allows for another mechanism of energy transfer and may offer an explanation for reduced quantum yields commonly observed in proteins.

(20) C. Scheringer, Z. Krist., 119, 273 (1963).
(21) B. Meuthen and M. V. Stackelberg, Z. Elektrochem., 64, 387 (1960).

Dissociation Studies in High Dielectric Solvents. VI. Conductance of Magnesium Sulfate Heptahydrate in Acetone–Formamide Mixtures at 25°[1]

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Contribution from the Department of Chemistry, University of Gorakhpur, Gorakhpur, U. P., India. Received May 26, 1965

The conductance of $MgSO_4 \cdot 7H_2O$ has been measured at 25° in 0–50% acetone-formamide mixtures. The dielectric constant, viscosity, and density of each mixed solvent from 0–100% acetone have also been measured at 25°. The conductance data have been analyzed by the Fuoss–Onsager equation for associated electrolytes and reasonable Λ^0, K_A, and a_J parameters have been obtained. A plot of log K_A vs. D^{-1} is linear. However, the Walden product decreases and a_J increases with the decrease in the dielectric constant of the mixed solvent.

Introduction

In a previous communication of this series, we have shown that magnesium sulfate is essentially unassociated ($K_A \simeq 9$) in formamide,[3] and the Fuoss–Onsager equation[4] describes the conductance data fairly satisfactorily. The behavior of this salt was further examined in dioxane–formamide mixtures, where higher pairwise association was forced in.[5] The results apart from showing the adequacy of the theory in the entire range of dielectric constants (110–21), exhibited an interesting specificity in the hydrodynamic parameter,

(3) G. P. Johari and P. H. Tewari, J. Phys. Chem., 69, 696 (1965).
(4) R. M. Fuoss and F. Accascina, "Electrolytic Conductance," Interscience Publishers, Inc., New York, N. Y., 1959.
(5) P. H. Tewari and G. P. Johari, J. Phys. Chem., 69, 2857 (1965).