SOME EFFECTS OF PHOSPHATE BUFFERS ON THE EXCITED STATE PROTOTROPIC EQUILIBRIA OF INDAZOLE

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Summary

Fluorometric titrations in the presence of phosphate buffers have indicated that the proton transfer equilibrium in the absence of buffers in the S_1 state is not truly observed for the indazole molecule. The absorption and fluorescence spectra in various solvents are observed and discussed.

1. Introduction

Alder [1] and Longworth *et al.* [2] have observed that the fluorescence spectrum of the indazole cation (IH_2^+) is broad and the band maximum exhibits a large red shift compared with that of the neutral indazole molecule (IH). They have also calculated ΔpK_a (= $pK_a - pK_a^*$) for the cation–neutral molecule equilibrium by using the Förster cycle method and the average of the absorption and fluorescence band maxima. Similar results have been observed for the acid–base characteristics of benzimidazole [1, 2] and it was later pointed out by Kondo and Kuwano [3] that the large red shift in the fluorescence spectrum of the benzimidazole cation is due to the reversal of the L_b and L_a excited singlet states (*i.e.* the more polar L_a state is stabilized more for the benzimidazole cation in polar solvents) and is not due to the formation of the stoichiometric complex between solute and solvent [4].

The present study was carried out to resolve the points discussed above for indazole and to establish the effect of solvents on the absorption and fluorescence spectra. The work was also extended to a higher pH range to study the neutral molecule–anion equilibrium. The effect of buffers on the equilibria

$$IH_2^+ \overset{pK_a(I)}{\rightleftharpoons} IH + H^+ \quad (I)$$

$$IH \overset{pK_a(II)}{\rightleftharpoons} I^- + H^+ \quad (II)$$

has also been investigated.

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2. Experimental details

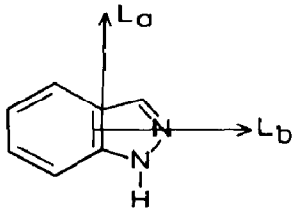
IH was prepared by the method suggested in the literature [5]. The purity of the compound was established by the presence of the same fluorescence maxima at different wavelengths of excitation. BDH Spectrograde methanol, analytical grade H_2SO_4 and NaOH and Aldrich trifluoroacetic acid (TFA) were used as received. Analytical grade acetonitrile (Merck) and *n*-heptane (BDH) were further purified as described in the literature [6]. Triply distilled water was used for the preparation of aqueous solutions. Phosphate buffers were used because acetate buffers were found to quench the fluorescence. The total analytical concentration of the buffer ions, *i.e.* $[H_2PO_4^-] + [HPO_4^{2-}]$, was maintained constant for each fluorometric titration.

The absorption spectra were recorded with a Cary 17D spectrophotometer. The fluorescence measurements were made on a scanning spectrofluorometer, made in our laboratory, and the details are available elsewhere [7]. Excitation and emission monochromators were calibrated using a calibration low pressure mercury lamp. pH values in the range 1 - 13 were measured on a Toshniwal pH meter model Cl-44A.

The bandwidth of the excitation was 8 nm and the excitation wavelength λ_{exc} used was 290 nm. In the fluorometric titrations, the isosbestic wavelength (275 nm) was used for the measurements of the relative fluorescence intensities at the analytical wavelength as a function of pH. The solutions for absorptiometric and fluorometric titrations were prepared immediately before the measurements were taken. The concentrations of the solutions were of the order of 10^{-4} - 10^{-5} M.

3. Results and discussion

The absorption spectra of IH, IH_2^+ and I^- are shown in Fig. 1, and the absorption maxima $\bar{\nu}_{max}$(abs) of the various peaks are listed in Table 1. With regard to Platt's notation for indazole,



the L_b peaks of IH are slightly red shifted with an increase in the polarity of the solvent, whereas those of the L_a band are totally unaffected. The L_b and

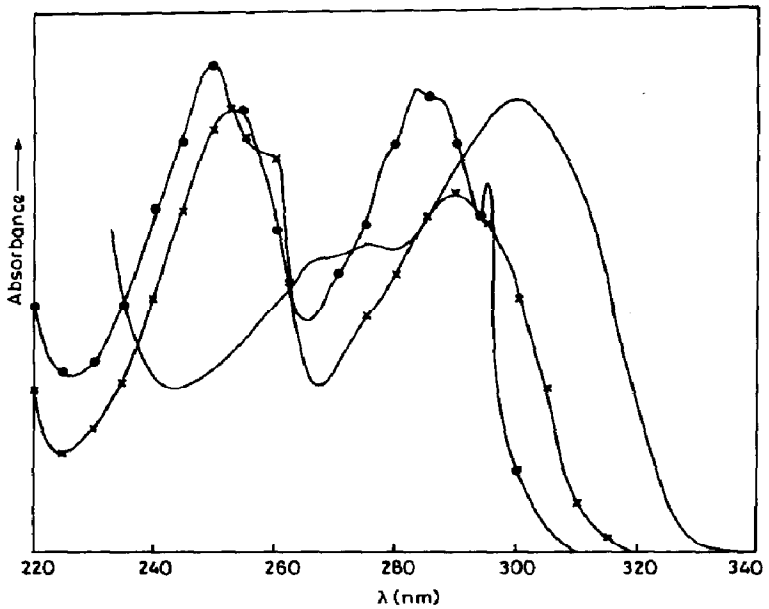


Fig. 1. Absorption spectra of IH (—●—), IH_2^+ (—x—) and I^- (——).

TABLE 1

Absorption and fluorescence maxima of indazole in various solvents

Hexane		*Acetonitrile*		*Methanol*		*Water*	
$\bar{\nu}_{max}$(abs) (cm^{-1})	$\bar{\nu}_{max}$(fluo) (cm^{-1})	$\bar{\nu}_{max}$(abs) (cm^{-1})	$\bar{\nu}_{max}$(fluo) (cm^{-1})	$\bar{\nu}_{max}$(abs) (cm^{-1})	$\bar{\nu}_{max}$(fluo) (cm^{-1})	$\bar{\nu}_{max}$(abs) (cm^{-1})	$\bar{\nu}_{max}$(fluo) (cm^{-1})
40160	33783	40160	33557	40160	33500	40160	33333
39062	32573	39062	32414	39062	32312	39062	32154
36231	31948	36101	31645	35971	31446	35971	31397
35460		35398		35273		35273	
34843		34782		34662		34662	
34071		34013		33840		33840	

L_a peaks of IH_2^+ are slightly red shifted compared with those of IH but the shape of and the energy difference between the two transitions remain the same, *i.e.* the bands in IH_2^+ are well separated. The absorption spectrum of IH is unaffected by increases in the pH up to 12.5 but on further increases the red shift in the L_a band is much more than that in the L_b band and the red shift in the L_a band appears as a shoulder of the L_b band system. The interaction of the solvent and the protonation (which is an extreme case of hydrogen bond formation) can increase the dipole moment in the horizontal direction. These results indicate that the increase in the dipole moment in the horizontal direction is not very great, thus suggesting again that the L_b state is not very polar, as was also observed for benzimidazole [3]. However, under basic conditions, *i.e.* for deprotonation, it appears from the results

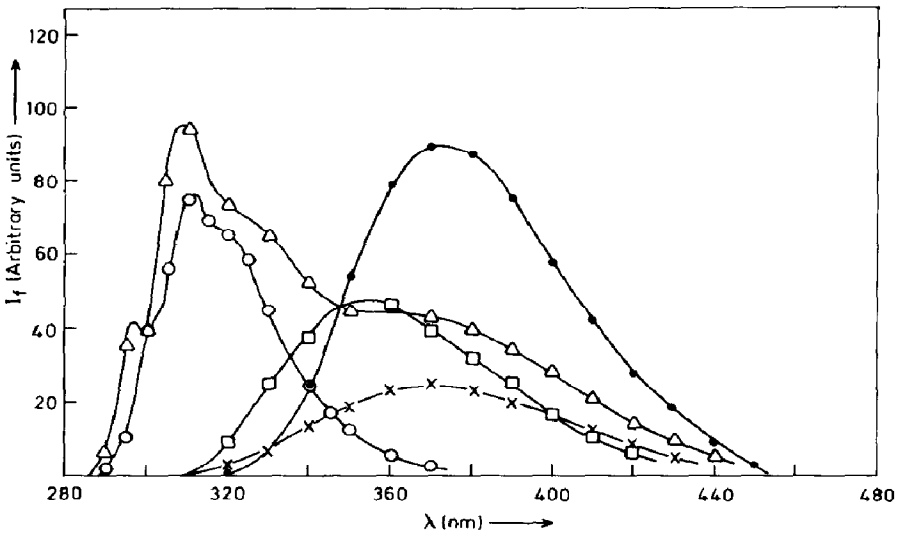


Fig. 2. Fluorescence spectra of IH, IH_2^+ and I^- in various media: ○, water (pH 7.0); △, acetonitrile with 0.01 M TFA; ×, water (pH 0.0); □, *n*-heptane with 0.01 M TFA; ●, water (pH 14.3).

that the dipole moment increases in the transverse direction and thus stabilizes the more polar L_a state in polar media.

The fluorescence spectra of IH, IH_2^+ (in H_2SO_4) and I^- (pH 14.3) (in NaOH) are shown in Fig. 2. Figure 2 also represents the fluorescence spectra of IH_2^+ in *n*-heptane and acetonitrile containing 0.01 M TFA. The fluorescence band maxima in various solvents are listed in Table 1 and those of IH_2^+ in various media are listed in Table 2. The $\bar{\nu}_{max}$(fluo) values of IH at pH 7, IH_2^+ and I^- are in good agreement with the reported values [1, 2]. The red shift observed in $\bar{\nu}_{max}$(fluo) and the Stokes shift ($\bar{\nu}_{max}$(fluo) − $\bar{\nu}_{max}$(abs)), calculated from the data of Table 1 with increasing polarity or increasing hydrogen bonding capacity of the solvents, are small. This indicates that the dipole moment of IH in the S_1 state is not very different from that in the S_0 state. The large red shift observed in IH_2^+ and I^- could be due to (i) an actual change in the pK_a values, (ii) formation of a stoichio-

TABLE 2

Absorption and fluorescence maxima of the indazole molecule and the indazole cation in various media

System	IH_2^+		IH^a	
	$\bar{\nu}_{max}$(abs)	$\bar{\nu}_{max}$(fluo)	$\bar{\nu}_{max}$(abs)	$\bar{\nu}_{max}$(fluo)
Water (pH 0.0)	34482	27027	35273	32154
n-heptane (0.01 M TFA)	34296	28571	35460	32573
Acetonitrile (0.01 M TFA)		26666	35398	32414

[a]These data are in pure water (pH 7.0) and solvents without TFA.

metric complex between the solvent and solute in the S_1 state or (iii) the reversal of the L_b and L_a electronic states, as observed for the benzimidazole cation. The conclusion for the benzimidazole cation was based on the facts that the L_a and L_b states are nearly degenerate in the polar medium and no differences were observed in the spectra with changes in the concentration of benzimidazole or changes in the environment. The differences between the L_b and L_a absorption band maxima of IH_2^+ and IH are nearly identical, *i.e.* they are well separated in both cases. The observed fluorescence spectra of IH_2^+ with different concentrations of IH, H_2SO_4 and $HClO_4$ in water were the same. IH in *n*-heptane containing 0.01 M TFA gave a broad fluorescence band with a maximum at 355 nm, indicating the formation of IH_2^+, whereas the acetonitrile solution under similar conditions gave the fluorescence spectra of IH (λ_{max} = 309 nm) and IH_2^+ (λ_{max} = 355 nm). These results clearly indicate that the large red shift in the fluorescence spectrum of IH_2^+ is due to the change in pK_a. This is convincing since in general, when $\pi^* \leftarrow \pi$ is the lowest energy transition, charge migration takes place from the carbocyclic ring to the heterocyclic ring, concentrating the charge more on the pyridinic nitrogen atom, thereby making it more basic in the S_1 state. The results in acetonitrile are self-explanatory as complete formation of IH_2^+ is not possible because TFA is a weak acid in this solvent. The small red shift of IH_2^+ in *n*-heptane and water is due to solvent relaxation but it indicates that IH_2^+ is more polar than IH in the S_1 state.

The cause of the large red shift in the fluorescence of I^- is still inconclusive as we could not obtain this species in any non-polar or less polar medium. The absorption data only indicate that it could be due to a reversal of the L_a and L_b states because the difference between the L_a and L_b absorption band maxima of I^- is decreased markedly. Moreover, the pyrrolic hydrogen atom becomes more acidic (see later) in the S_1 state and it is possible that the anion might be more polar in this state.

The ground state pK_a values were calculated spectrophotometrically and are listed in Table 3. The values (1.22 and 13.79), which are lower than the respective values for pyrazole (2.48 and 14.21), are consistent with the theory that electron-withdrawing groups such as phenyl decrease the basicity and increase the acidity of the molecule.

TABLE 3

Excited singlet state acidity constants for the indazole molecule

Equilibrium	pK_a				pK_a*(FT)[a] *for various concentrations*			
	pK_a	pK_a*(abs)	pK_a*(fluo)	pK_a*(av)	*0.0 M*	*0.1 M*	*0.5 M*	*1.0 M*
I	1.22	2.99	11.83	7.41 - 6.22	1.8	1.8	2.2	2.8
II	13.79	10.11	2.57	6.34	12.2	12.0	11.2	—

[a] pK_a*(FT), pK_a* calculated from fluorometric titrations.

The pK_a^*(I) and pK_a^*(II) values are calculated using absorption maxima, fluorescence maxima, the average of fluorescence and absorption maxima and the Förster cycle method [8]. These values, together with the pK_a values, are listed in Table 3. The pK_a^*(I) and pK_a^*(II) values were also obtained with the help of fluorometric titrations without a buffer and in the presence of phosphate buffers up to concentrations of 1 M and 0.5 M respectively. Higher concentration of buffers could not be used because of the saturation limits in the respective media. The values obtained are listed in Table 3. Within the limits of accuracy, our values for pK_a^*(I) calculated with the help of the Förster cycle method and by averaging the absorption and fluorescence data agree with the values given in the literature [2]. However, the difference between the value of pK_a(I) calculated using absorption maxima and that calculated using fluorescence maxima is very large and of course the value obtained by averaging the two maxima is in between the two values. The basic assumptions involved in the Förster cycle method are as follows: (a) ΔS is the same for the S_0 and S_1 states; (b) the vibrational and solvent relaxations are the same in both states; (c) the same electronic states are involved in the acid–base conjugate pair [9]. Assumption (a) is generally valid in all cases and, when it is not, the difference between the true value and the experimental value is not more than 1 pK_a unit [10]. Assumption (c) is not completely valid in our case as is clear from the data of Tables 1 and 2, but there is not much difference in the solvent relaxation of the respective species in the S_0 and S_1 states. The other possible reason could be the use of the band maxima instead of the 0–0 transition values. However, all these could not result in such a large difference between the pK_a(I) value calculated using absorption data and that calculated using fluorescence data. As stated earlier this is due to the migration of charge from the carbocyclic ring to the heterocyclic ring in the S_1 state and thereby to an increase in the charge density at the pyridine nitrogen atom, making it more basic in the S_1 state. Further support for this comes from the fluorescence spectrum of 5-aminoindazole [11] and its protonated species at the pyridine nitrogen atom. The cause of the large difference between the pK_a(II) value obtained by using absorption data and that obtained by using fluorescence data is still inconclusive but it could be due to the violation of assumption (c).

Fluorometric titrations give the correct values if the prototropic equilibrium is attained in the S_1 state. The fluorometric titrations also indicate that the pyridinic nitrogen atom is more basic and that the pyrrole-type nitrogen atom is more acidic in the S_1 state than in the S_0 state. This is confirmed from the appearance of the fluorescence spectrum of I^- at pH values at which I^- does not exist in the ground state; the spectrum is shown in Fig. 3. These results are consistent with the results obtained for other molecules containing nitrogen as the heteroatom and with $\pi \rightarrow \pi^*$ as the S_1 electronic state [12, 13]. However, ΔpK_a calculated from fluorescence data is very small and, to ascertain whether the true equilibrium is established in the S_1 state, fluorometric titrations were carried out in the presence of large

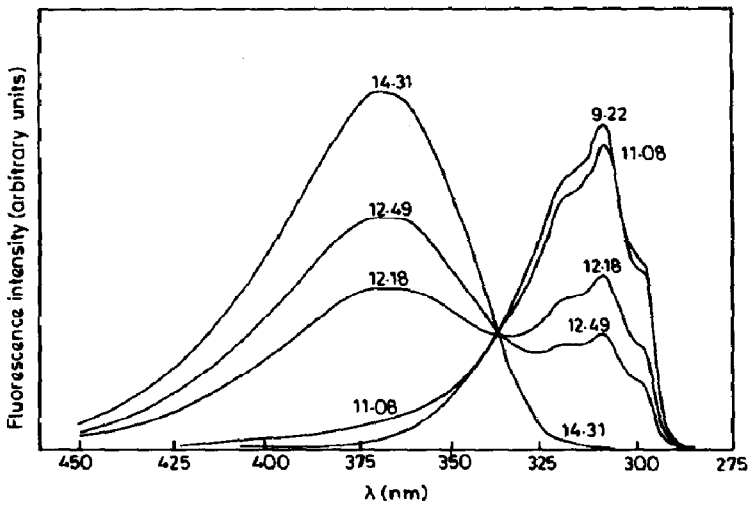


Fig. 3. Fluorescence spectrum of indazole at various pH values.

concentrations of phosphate buffers as shown in Fig. 4. Although no saturation limit is observed, the pK_a^* values calculated from the fluorometric titrations in the presence of large concentrations of buffers do change for both equilibria but true equilibrium is still not attained.

From the above results the following conclusions can be made.

(i) There is an increase in the charge density at the pyridine nitrogen atom in the S_1 state, thereby making it more basic in this state than in the S_0 state. Thus the values of pK_a^* calculated by using the Förster cycle method and fluorescence data will be closer to the true value.

(ii) Since the fluorometric titration curves, obtained in the absence of buffers, are displaced towards higher and lower pH values with increases in the concentration of buffer ions and furthermore since it is established that the buffer ions do not quench the fluorescence intensities of the excited

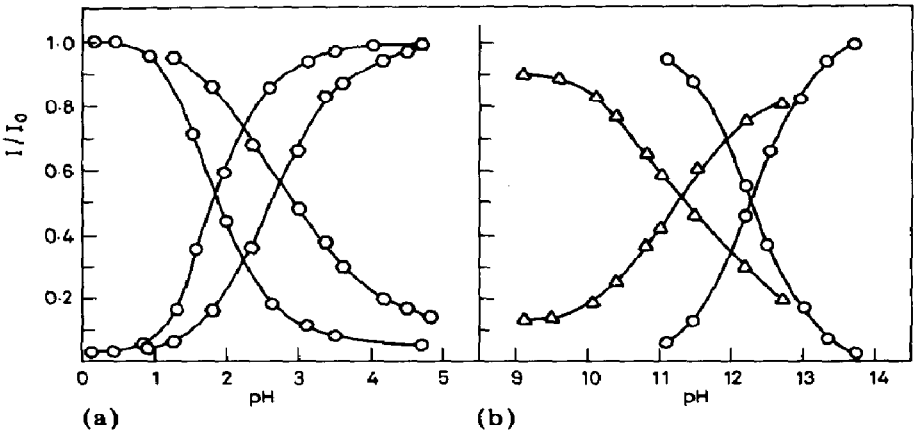


Fig. 4. Plot of relative intensities of (a) indazole and its cation and (b) indazole and its anion at various buffer concentrations: ○, without buffer; ○, 1 M buffer; △, 0.5 M buffer.

species, it is possible that the original fluorometric titration curve does not correspond to the true equilibrium condition in the S_1 state [14].

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