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Spectroscopic behaviour of 8-hydroxy-1,3,6-pyrenetrisulphonate immobilized in ethyl cellulose

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Abstract

The spectroscopic behaviour of 8-hydroxy-1,3,6-pyrenetrisulphonate (HPTS) immobilized in an ethyl cellulose film and in aqueous phosphate pH buffers have been studied using fluorescence and electronic absorption spectra. The absorption, excitation and emission peak maxima of the acidic and basic forms of HPTS in ethyl cellulose and aqueous solution have been determined and compared. The deprotonation of the excited acidic form of HPTS is suppressed in ethyl cellulose but not in aqueous solution condition. HPTS in an aqueous solution is more acidic than in an ethyl cellulose film. Less number of water molecules and salt effect in ethyl cellulose are used to explain this observation. The acid dissociation constants of HPTS in an aqueous phosphate solution and an ethyl cellulose film are 7.31 and 8.43, respectively at 25 °C. © 1997 Elsevier Science S.A.

Keywords: 8-Hydroxy-1,3,6-pyrenetrisulphonate; Ethyl cellulose; Spectroscopic behaviour

1. Introduction

8-Hydroxy-1,3,6-pyrenetrisulphonate (HPTS) has been considered as one of the most potential indicators for pH and CO_2 determinations because of its excellent photostability, high quantum yield, long-wave excitation maximum and large Stokes' shift [1]. One of the key features of this molecule is the excitation of both acidic (HPTS) and basic (PTS^-) forms in aqueous medium resulting in similar fluorescence emission spectra. This is accounted for by the rapid deprotonation of the electronically excited HPTS* into excited PTS* with subsequent fluorescence emission [2].

$$\begin{array}{ccc} HPTS^* & \rightleftharpoons & PTS^{-*} + H^+ \text{ (deprotonation)} \quad (1) \\ \downarrow\uparrow & & \downarrow\uparrow \\ HPTS & \rightleftharpoons & PTS^- + H^+ \quad (2) \end{array}$$

This characteristic provides the basis for an internal reference scheme in optical pH sensing so as to counteract the adverse effect of photodegradation, leaching and instrumental fluctuations [3]. With the recent advent of fibre-optic technology, there is a growing interest in fibre-optic sensors. 8-Hydroxy-1,3,6-pyrenetrisulphonate immobilized in a solid support attached to the distal end of an optical fibre has been successfully developed to detect CO_2 [4,5]. The working principle is based on the absorbance or fluorescence change of HPTS upon exposure to CO_2. Dissolved CO_2 alters the pH of an immobilized buffer in the sensor film with subsequent change in the ratio of the acidic to basic forms of HPTS.

The spectroscopic properties of the acidic and basic forms of HPTS moving from aqueous to immobilized conditions have not been much studied. Any change of the spectroscopic properties can affect the sensitivity and working range of a pH sensor based on immobilized HPTS. On the other hand, the spectroscopic properties of HPTS in dimethylsulphoxide–water [6] and alcohol–water [7] mixtures have been reported. It has been shown that the deprotonation of the excited HPTS* in water medium is suppressed upon addition of nonaqueous organic solvents. Similarly, this observation may likely occur in a film immobilized with HPTS. In the present paper the spectroscopic behaviour of immobilized HPTS in a polymeric film of ethyl cellulose is reported and compared with HPTS in aqueous phosphate solutions.

2. Experimental

2.1. Chemicals

Trisodium 8-hydroxy-1,3,6-pyrenetrisulphonate (NaPTS) was purchased from MTM Research. Disodium hydrogen

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phosphate, ethanol, ethyl cellulose, methanol, monosodium dihydrogen phosphate, silver(I) oxide, sodium hydroxide, tetraoctylammonium bromide, toluene, tributyl phosphate and trisodium phosphate were obtained from Aldrich. Water was deionized by the Purite R0200-Stillplus HP system.

2.2. Fabrication of the HTPS film

The incorporation of pH-sensitive dyes with some solid supports based on ion pairing provides a useful way of immobiiization of dyes into plastic film [8]. Tetraoctylammonium ions (TOA^+) formed ion-pairs with anionic dyes (in this case, NaPTS) at high pH and extracted into toluene has been described elsewhere [9]. Solid residues of ion-pairs (TOA^+PTS^-) were subsequently obtained by evaporating off the toluene under ambient conditions.

$$TOA^+Br^- + Na^+PTS^- \rightarrow TOA^+PTS^- + NaBr \quad (2)$$

In order to neutralize any acidic residue in ethyl cellulose and provide an immobilized buffer in the HTPS film, a basic methanoic solution of 0.5 M of tetraoctylammonium hydroxide (TOAOH) was prepared by stirring together 1.37 g of TOA^+Br^- and 1.16 g of Ag_2O in 5 ml of methanol for 5 h. The solution was then decanted from the sedimented oxide and used for membrane fabrication. A film cocktail was prepared by mixing 1.0 g of ethyl cellulose, 3.1 mg of TOA^+PTS^-, 2.0 ml of ethanol, 2.0 ml of TOAOH, 1.0 ml of tributyl phosphate and 10.0 ml of toluene. A 40–50 μm thick membrane immobilized with TOA^+PTS^- was subsequently cast on a glass slide using a dip-coating technique. For the fluorescence measurements, the concentration of TOA^+PTS^- was 100-fold fewer than that in ethyl cellulose for absorbance measurements.

2.3. Instrumentation

Various phosphate (0.05 M) pH buffers were prepared and the pH measurements were taken on a CD 640 digital pH meter (WPA Linton Cambridge). The ultraviolet–visible (UV–Vis) absorption spectra were measured on a UV 2 UV–Vis spectrophotometer (Unicam) at 25 °C equipped with a Fujitsu DX 2150 dot matrix printer (Fujitsu). All the fluorescence spectra were recorded on a Perkin-Elmer LS 5 spectrofluorophotometer at 25 °C in conjunction with a Perkin–Elmer GP 100 graphics plotter.

3. Results and discussion

3.1. Absorption spectra

The absorption spectra of HPTS in various pH phosphate buffers are shown in Fig. 1. Two absorption bands are found with an isosbestic point at 416 nm. At high pH values, an absorption band appears with peak maxima (λ_{max}) at 454 and 368 nm, revealing the presence of the basic form of

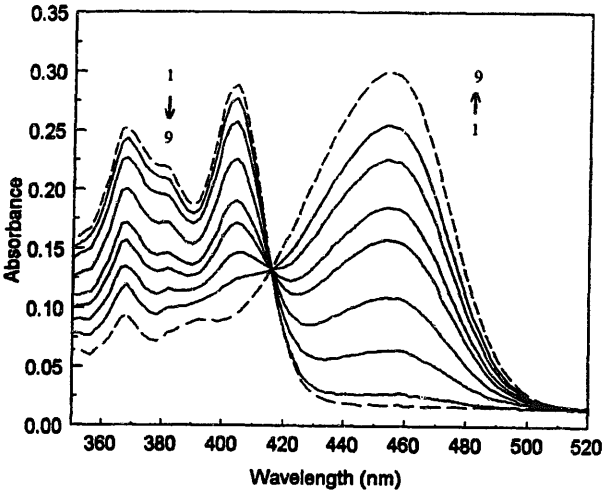

Fig. 1. Absorption spectra of 15.5 μM HPTS in various pH phosphate buffers. (1) 5.00; (2) 6.00; (3) 6.70; (4) 7.00; (5) 7.30; (6) 7.48; (7) 7.75; (8) 8.00; (9) 10.0.

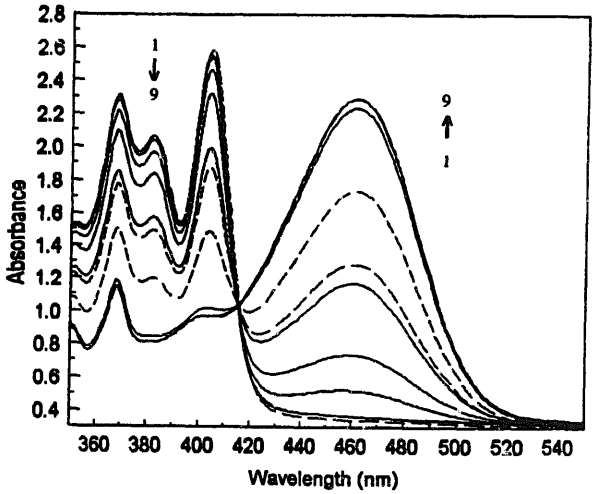

Fig. 2. Absorption spectra of HPTS ethyl cellulose film in various pH phosphate buffers. (1) 5.00; (2) 6.00; (3) 7.00; (4) 7.50; (5) 8.00; (6) 8.38; (7) 8.62; (8) 9.00; (9) 11.0.

HPTS. At low pH values, another absorption band with λ_{max} at 404 and 368 nm and a shoulder peak at 382 nm shows the existence of the acidic form of HPTS. The absorption spectra of HPTS immobilized in ethyl cellulose in various pH phosphate buffers are given in Fig. 2. Similarly, the basic form of HPTS with λ_{max} of 460 and 368 nm appears in high pH values. At low pH values, the acidic form of HPTS with λ_{max} of 404, 382 and 368 nm exists. An isosbestic point is at 415 nm. The absorption spectra of the acidic form of HPTS is better resolved in ethyl cellulose than in aqueous medium because the movement of the acidic form molecules are more restricted in a rigid polymeric film than in water. λ_{max} peak of 382 nm is clearly located in ethyl cellulose whereas only a shoulder peak is observed in aqueous medium. The absorption spectra of the basic form of HPTS in ethyl cellulose is similar to in aqueous solution, with the exception that the λ_{max} appears slightly red-shifted by 6 nm. The bathochromic shift can be explained by hydrogen bonding effects between

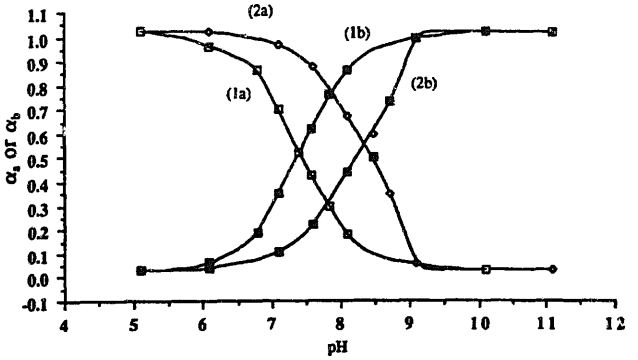

Fig. 3. Plot of α_a and α_b of HPTS in phosphate buffers and ethyl cellulose film against pH. Phosphate buffer: (1a) α_a vs. pH; (1b) α_b vs. pH; Ethyl cellulose film: (2a) α_a vs. pH; (2b) α_b vs. pH.

the basic form of HPTS and surrounding H_2O molecules as the concentration of water in a hydrophobic polymeric film of ethyl cellulose is less. The ground state of the basic form of HPTS in aqueous medium is stabilized more by hydrogen bond formation than its first excited state [10].

The response mechanism of an immobilized HPTS dye polymeric film in different pH buffers can be visualized as a cation-exchange system and has been described by Morf et al. [11]:

$$TOA^+PTS^-_{(org)} + H^+_{(aq)} \rightleftharpoons HPTS_{(org)} + TOA^+_{(aq)} \quad (3)$$

where $TOA^+PTS^-_{(org)}$ and $HPTS_{(org)}$ are the basic and acidic forms of HPTS in the polymeric membrane phase, respectively, and $H^+_{(aq)}$ and $TOA^+_{(aq)}$ are hydrogen and tetraoctylammonium ions in aqueous solution, respectively.

If the absorbances of the acidic and basic forms of HPTS in aqueous or membrane phase follow Beer–Lambert's law:

$$\alpha_a = (A - A_b)/(A_a - A_b) \quad (4)$$

$$\alpha_b = (A - A_a)/(A_b - A_a) \quad (5)$$

where A is the measured absorbance, and, A_a and A_b are the limiting absorbance values for the acidic and basic forms of HPTS, respectively. α_a and α_b are the ratios of the concentration of HPTS in the acidic and basic forms, respectively, to the total concentration of HPTS present. Applying Eqs. (4) and (5), the α_a and α_b values of HPTS in aqueous and membrane phases at 404 and 454 nm (aqueous)/460 nm (membrane), respectively, can be plotted as a function of pH (Fig. 3). At low pH values, α_a is equal to 1 and α_b is equal to 0 when all the HTPS present in acidic form. Likewise at high pH values, α_a is equal to 0 and α_b is equal to 1 when all the HPTS molecules exist in basic form. The acid dissociation constants (pK_a) of HTPS in aqueous and membrane phases can be easily obtained at pH values when α_a and α_b are equal to 0.50. From Fig. 3, the pK_a of HPTS in a 0.05 M phosphate solution and in an ethyl cellulose film are found to be 7.31 and 8.43, respectively. The diminished acidity of the immobilized HPTS in its electronic ground state is probably a result of hydrogen bonding effect. As mentioned above, the number of water molecules surrounding the HPTS molecules is less in an ethyl cellulose film than in an aqueous medium. Enhanced hydrogen bonding in aqueous solutions would stabilize the conjugate base (phenolate form) with respect to the conjugate acid (phenol form) and make prototrophic dissociation easier.

3.2. Fluorescence spectra

Fig. 4 shows the fluorescence excitation (EX) and emission (EM) spectra of HTPS in various pH phosphate buffers. It is clear that the excitation spectra look similar to the absorption spectra (Fig. 1). An isoemissive point is observed at EX 417 nm and EM 510 nm when both the acidic and basic forms of HPTS have the same fluorescence intensities. At low pH values, an excitation band appears with peak maximum (λ_{EX}) at 404 nm indicating the formation of the acidic form of HPTS. At high pH values, another excitation band presents with λ_{EX} at 457 nm revealing the formation of the basic form of HPTS. Both the acidic and basic forms of HTPS in aqueous solution have similar emission spectra with peak maxima (λ_{EM}) at 510 nm. This observation matches with Eq. (1) in that there is the rapid deprotonation of the electronically excited acidic form into the excited basic form of HPTS with subsequent fluorescence emission. However, a small amount of the acidic form of HPTS can still fluoresces without deprotonation. A small shoulder emission peak at about 440 nm is observed (Fig. 4(c)) which is attributed to the emission band of the acidic form of HPTS [12,13]. This band diminishes as the pH is raised accompanying with the conversion of the acidic into basic forms of HPTS.

The excitation and emission spectra of immobilized HPTS in various pH phosphate buffers are depicted in Fig. 5 and Fig. 6. An emission band with λ_{EM} at 515 nm and excitation band with λ_{EM} at 468, 402 and 370 nm are clearly seen in Fig. 5. The excitation and emission bands of the basic form of HPTS increase as the pH increases. The excitation spectra also match with the absorption spectra in Fig. 2. In contrast to the observation of the acidic form of HPTS in aqueous solution, the deprotonation of the acidic form of HPTS in ethyl cellulose is suppressed. An excitation band with λ_{EX} at 402 and 369 nm and an emission band with λ_{EM} at 429 nm of the acidic form of HPTS are shown in Fig. 6. As the pH increases, an additional emission band at 515 nm, characteristic of the green fluorescence of the excited basic form of HPTS, appears. The excitation spectra of the acidic form of HPTS match with the absorption spectra shown in Fig. 2. The depressing of deprotonation of the excited acidic form of HPTS can be explained by the decrease in local water molecules surrounded the excited acidic HPTS in ethyl cellulose [14]. There is insufficient water molecules available to hydrate and stabilize the dissociated proton from the excited acidic HPTS leading to the strong recombination of H^+ and excited PTS^- [15]. Secondly, the local salt concentration in ethyl cellulose film is less than in aqueous phosphate buffers. Although the ethyl cellulose film was immersed in the phosphate buffers, the local electrolyte concentration is still lower than in aqueous solution due to the phosphate salt being

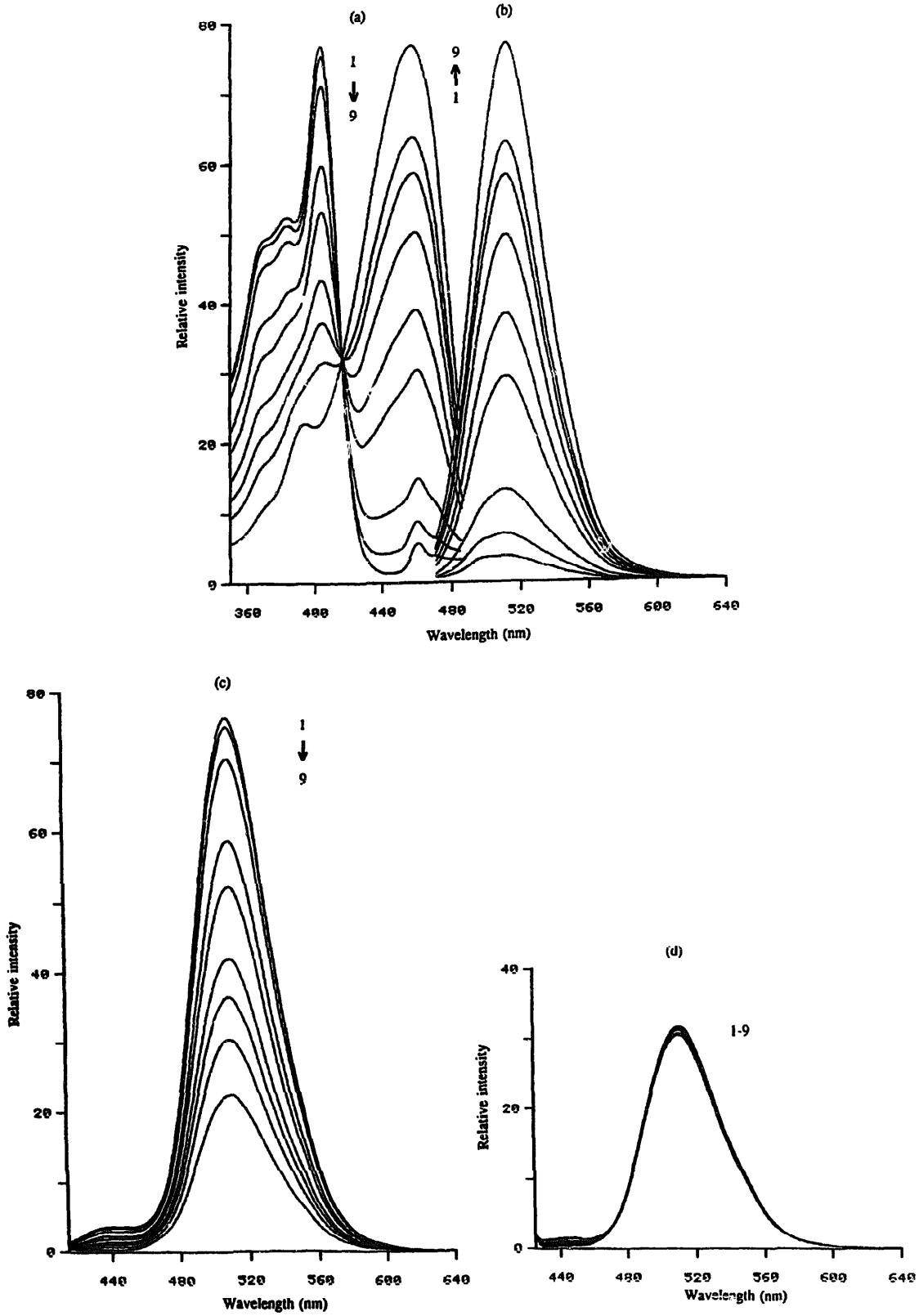

Fig. 4. Fluorescence spectra of 0.18 μM HPTS in various pH phosphate buffers. (1) 5.02; (2) 6.02; (3) 6.54; (4) 7.06; (5) 7.32; (6) 7.59; (7) 7.80; (8) 8.07; (9) 10.1. (a) Excitation spectra: EX 350–485 nm; EM 510 nm; (b) Emission spectra: EX 457 nm; EM 470–640 nm; (c) Emission spectra: EX 404 nm; EM 415–640 nm; (d) Emission spectra: EX 417 nm; EM 425–640 nm.

insoluble in a more hydrophobic ethyl cellulose film. The higher salt concentration has the effect of screening the proton's Coulombic attraction to the excited PTS^- thus diminishing the geminate recombination and increasing the yield of deprotonation of excited HPTS in aqueous solution than in ethyl cellulose [16]. Using the data from Figs. 4–6 and

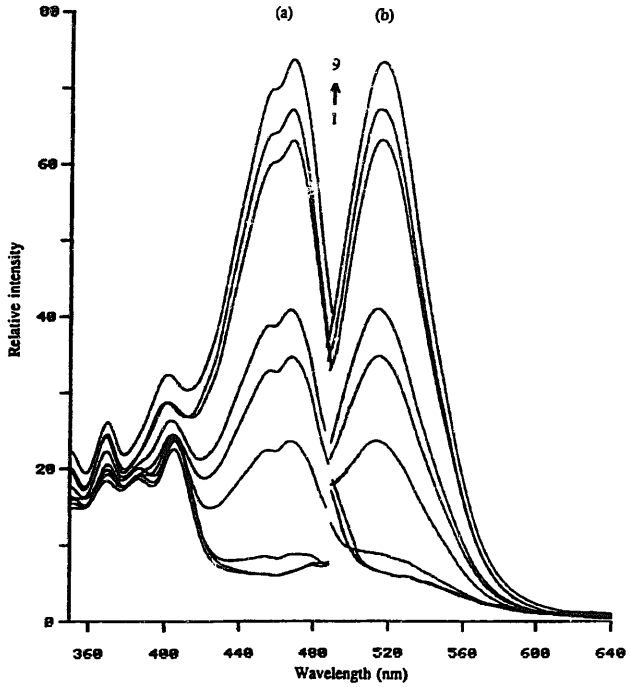

Fig. 5. Fluorescence spectra of the basic form of HPTS ethyl cellulose film in various pH phosphate buffers. (1) 5.00; (2) 6.00; (3) 7.00; (4) 8.00; (5) 8.38; (6) 8.62; (7) 9.00; (8) 11.0; (9) 0.10 M NaOH. (a) Excitation spectra: EX 350–488 nm; EM 515 nm; (b) Emission spectra: EX 468 nm; EM 490–640 nm.

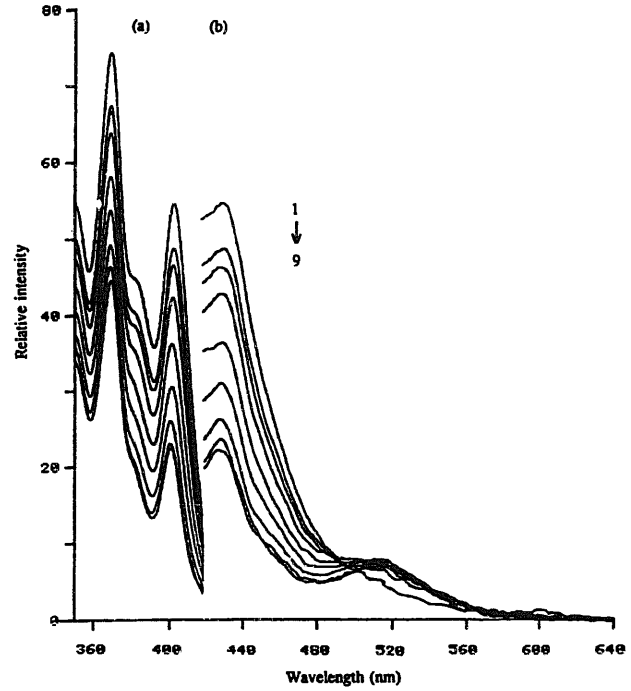

Fig. 6. Fluorescence spectra of the acidic form of HPTS ethyl cellulose film in various pH phosphate buffers. (1) 5.00; (2) 6.00; (3) 7.00; (4) 7.50; (5) 8.00; (6) 8.38; (7) 9.00; (8) 11.0; (9) 0.10 M NaOH. (a) Excitation spectra: EX 350–416 nm; EM 429 nm; (b) Emission spectra: EX 402 nm; EM 418–640 nm.

the analogous equations to Eqs. (4) and (5), graphs similar to Fig. 3 were plotted to determine pK_a of HPTS in an aqueous solution and an ethyl cellulose film. The results are not shown here but a summary of the spectroscopic properties and pK_a of HPTS in aqueous phosphate solution and ethyl cellulose film is presented in Table 1.

Table 1
Spectroscopic properties and pK_a of HTPS in phosphate solution and ethyl cellulose film at 25 °C

Spectroscopic properties		HTPS in Phosphate solution	Ethyl cellulose film
Absorption, λ_{max} (nm)	Acidic form	404, 382(s), 368	404, 382, 368
	Basic form	454, 392(s), 368	460, 404(s), 368
Isosbestic point (nm)		416	415
Excitation, λ_{EX} (nm)	Acidic form	404, 382, 368(s)	402, 382(s), 369
	Basic form	457, 395, 369(s)	468, 458(s), 402, 370
Emission, λ_{EM} (nm)	Acidic form	510	429
	Basic form	510	515
Isoemissive point (nm)		EX 417	–
		EM 510	–
pK_a		7.31	8.43

(s): shoulder peak.

4. Conclusions

The spectroscopic behaviour of HPTS immobilized in an ethyl cellulose film and in aqueous phosphate pH buffers does not show much difference with the exception that the deprotonation of the excited acidic form of HPTS is suppressed in ethyl cellulose. The emission spectra of the acidic form of HPTS immobilized in ethyl cellulose is observed and this will hamper the internal reference scheme in optical pH sensing. In addition, the pK_a values of HPTS in ethyl cellulose film is higher than in an aqueous solution condition which will certainly change the pH sensitive range of this indicator when immobilized in a polymeric film of ethyl cellulose.

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