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Journal of Photochemistry and Photobiology A: Chemistry 114 (1998) 235–239

Journal of Photochemistry and Photobiology A:Chemistry

Spectroscopic behaviour and protolytic equilibrium of fluorescein immobilized in ethyl cellulose

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Received 14 May 1997; received in revised form 7 January 1998; accepted 8 January 1998

Abstract

The spectroscopic behaviour and protolytic equilibria of fluorescein (FL) dianion immobilized in polymeric films of ethyl cellulose have been studied using fluorescence and electronic absorption spectroscopies. The absorption, excitation and emission peak maxima of the dianionic form of FL in a polymeric film of ethyl cellulose are red-shifted when compared with in aqueous medium. The bathochromic shift can be explained by hydrogen bonding effects between the dianionic form of FL and surrounding water molecules as the concentration of water in a hydrophobic polymeric film of ethyl cellulose is less. Only one single protolytic step is observed for neutral FL in polymeric membrane phase. The acid dissociation constant of FL in ethyl cellulose film is found to be 5.00–5.13 at 25°C.

Keywords: Fluorescein dianion; Ethyl cellulose; Spectroscopic behaviour; Protolytic equilibrium

1. Introduction

Fluorescein (FL) is a xanthene dye which is widely used in industry and commerce. It is also employed as a tracer in environmental monitoring work due to its favourable photophysical characteristics, i.e., strong fluorescence when it is radiated by ultraviolet/visible (UV/Vis) light. The absorption and fluorescence spectra of FL molecules are very much affected by their chemical environment and surrounding pH conditions. Owing to its pH-sensitive spectroscopic properties and the recent advent of fibre-optic technology, there has been a growing interest in the development of fibre-optic pH [1–3], CO_2 [4–6] chemosensors and biosensors [7]. As a result, fluorescein or its derivatives have been chosen as the pH-sensitive dyes for fabrication of optical sensors. The working principle is based on the absorbance or fluorescence change of FL upon pH change or exposure to different levels of CO_2. Dissolved CO_2 can alter the pH of an immobilized buffer in the sensor film with subsequent change in the ratio of the acidic to basic forms of FL.

The spectroscopic properties and protolytic equilibria of the acidic and basic forms of FL switching from aqueous to immobilized polymeric support conditions have not been much studied. Any change of these spectroscopic and protolytic properties can seriously affect the sensitivity and working range of a pH-sensitive optical sensor based on a polymeric support immobilized with FL. On the other hand, the spectroscopic properties and protolytic equilibria of FL in ethyl acetate, acetone [8], alcohol/water [9], acetone/water [10] and dimethylsulphoxide/water [11] mixtures have been reported. It has been shown that the ionization of FL in aqueous medium is heavily influenced by the addition of nonaqueous organic solvents. Fluorescein immobilized in a polymeric support is also likely to experience a chemical microenvironment that is similar to organic solvents. Consequently, the changes of the spectroscopic properties and protolytic equilibria of FL probably happen when it is switched from an aqueous solution to a polymeric film conditions. In the present paper the protolytic equilibria and spectroscopic behaviour of a polymeric membrane of ethyl cellulose immobilized with FL is reported and compared with FL in aqueous solution conditions. The findings can possibly be used to explain the spectroscopic and protolytic equilibria of FL in other solid polymeric support material.

2. Experimental

2.1. Chemicals

Disodium hydrogen phosphate, ethanol, fluorescein, ethyl cellulose, methanol, monosodium dihydrogen phosphate, sil-

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1010-6030/98/$19.00
PII S1010-6030(98)00220-2

ver (I) oxide (Ag_2O), sodium hydroxide, tetraoctylammonium bromide (TOA^+Br^-), toluene, tributyl phosphate and trisodium phosphate were obtained from Aldrich. Water was deionized by the Purite R0200–Stillplus HP system.

2.2. Fabrication of immobilized FL film

The incorporation of a pH-sensitive anionic dye with a polymeric support based on ion pairing and solvent extraction provides a simple way of immobilization of an anionic dye into a polymeric support [12,13]. Briefly, tetraoctylammonium ions, which (TOA^+) formed ion-pairs with fluorescein dianion (FL^{2-}) at high pH values, were extracted into toluene. Solid residues of ion-pairs $(TOA^+)_2FL^{2-}$ were subsequently obtained by evaporating off toluene under ambient conditions. Infrared data was run to confirm the structure of $(TOA^+)_2FL^{2-}$ as shown in Fig. 1.

$$2TOA^+Br^- + 2Na^+ + FL^{2-} \rightarrow (TOA^+)_2FL^{2-} + 2NaBr \quad (1)$$

In order to neutralize any acidic residue in ethyl cellulose and provide an immobilized buffer in the FL film, a basic methanolic solution of 0.5 M of tetraoctylammonium hydroxide (TOA^+OH^-) 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, 3.0 mg of $(TOA^+)_2FL^{2-}$, 2.0 ml of ethanol, 2.0 ml of TOA^+OH^-, 1.0 ml of tributyl phosphate and 10.0 ml of toluene. A 40–50 μm thick membrane immobilized with $(TOA^+)_2FL^{2-}$ was subsequently cast on a glass slide using a dip-coating technique. For the fluorescence measurements, the concentration of $(TOA^+)_2FL^{2-}$ 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 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 UV-Vis absorption spectra of $(TOA^+)_2FL^{2-}$ immobilized in ethyl cellulose film immersed into various pH phosphate buffers are given in Fig. 2. Since TOA^+ ions and the other materials are transparent in the visible light region, the absorption band observed should correspond to the absorption band of FL^{2-} ions with an absorption peak maximum (λ_{max}) of 510 nm and a shoulder peak (λ_S) of 482 nm. The absorption spectra of FL^{2-} ions immobilized in ethyl cellulose is similar to that in aqueous solution, with the exception that the λ_{max} appears red-shifted by 20 nm [14]. The bathochromic shift can be explained by hydrogen bonding effects between the dianionic form of FL and surrounding water molecules as the concentration of water in a hydrophobic polymeric film of ethyl cellulose is less. The electronic ground state (S_0) of the dianionic form of FL in aqueous medium is stabilized more by hydrogen bond formation than its electronic first excited state (S_1) [15].

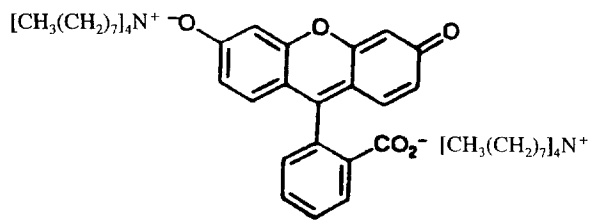
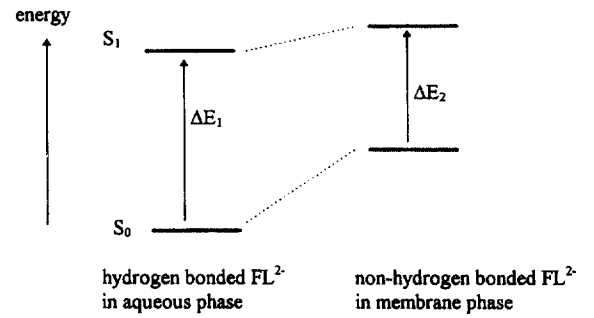


Fig. 1. The chemical structure of the tetraoctylammonium ion and fluorescein dianion ion pair.



The electronic transition energies of FL^{2-} ions from S_0 to S_1 states in aqueous phase (ΔE_1) is larger than that in membrane phase (ΔE_2).

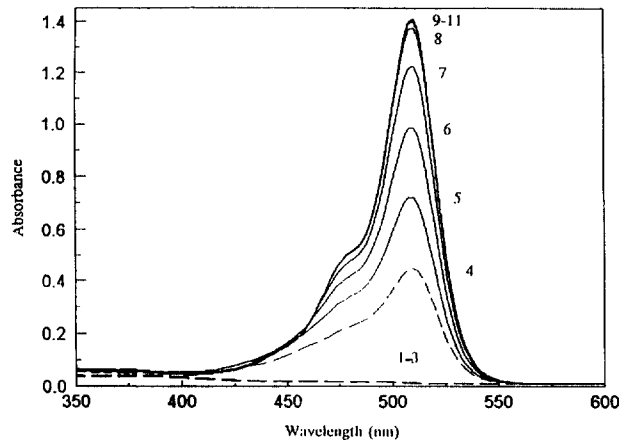


Fig. 2. Absorption spectra of immobilized $(TOA^+)_2FL^{2-}$ ethyl cellulose film immersed into various pH phosphate buffers. 1: 4.00; 2: 4.52; 3: 4.70; 4: 4.90; 5: 5.00; 6: 5.50; 7: 6.00; 8: 7.00; 9: 8.00; 10: 10.0; 11: 13.0.

The absorbance of immobilized FL^{2-} ions decreases with lowering pH value. The protolytic equilibria of FL with two ionization steps in aqueous solution are depicted in Fig. 3 [11]:

$$H_2FL+H_2O\rightarrow HFL^-+H_3O^+ \qquad pK_{a1}=4.24, \qquad (2)$$

$$HFL^-+H_2O\rightarrow FL^{2-}+H_3O^+ \qquad pK_{a2}=6.33. \qquad (3)$$

where K_{a1} and K_{a2} are the first and second acid dissociation constants of FL in an aqueous solution [16]. However, the two ionization steps are combined into one single step especially when FL presents in an organic solvent medium [17]. It has been reported that the neutral form of FL can exist as three different tautomers, i.e., zwitterion, quinonoid and lactone. Unlike the zwitterion and quinonoid tautomers with absorption bands in the visible light region, the lactonic form is colorless [18]. The colorless lactonic form is usually the dominant tautomer present in non-hydroxylic organic solvents such as ethyl acetate and acetone [8,10]. Neutral lactonic form, which is the preferable species, exist in a hydrophobic microenvironment at low pH values as it is more lipophilic than monocation, zwitterion and quinonoid species of FL. Fluorescein dianions immobilized in a polymeric film of ethyl cellulose also experience a hydrophobic chemical microenvironment that is similar to that in an organic solvent conditions. Thus, the change in absorbance of FL^{2-} ions immobilized in an ethyl cellulose polymer film from high to low pH values can be explained by the conversion of the dianionic form of FL to the colorless neutral lactonic form in one single protolytic step.

The response mechanism of an immobilized FL^{2-} dye polymeric film in different pH buffers can be visualized as a cation-exchange system [19]:

$$(TOA^+)_2FL^{2-}_{(poly.)}+2H^+_{(aq.)}\leftrightarrow H_2FL_{(poly.)}+2TOA^+_{(aq.)} \qquad (4)$$

where $(TOA^+)_2FL^{2-}_{(poly.)}$ and $H_2FL_{(poly.)}$ are the dianionic and neutral lactonic forms of FL in the polymeric membrane phase, respectively. $H^+_{(aq.)}$ and $TOA^+_{(aq.)}$ are hydrogen and tetraoctylammonium ions in aqueous solution, respectively.

If the absorbances of the dianionic form of FL in membrane phase follow the Beer–Lambert law, then the ratio (α) of the concentration of FL in the dianionic form to the total concentration of FL can be expressed as the following equation:

$$\alpha=(A-A_b)/(A_1-A_b) \qquad (5)$$

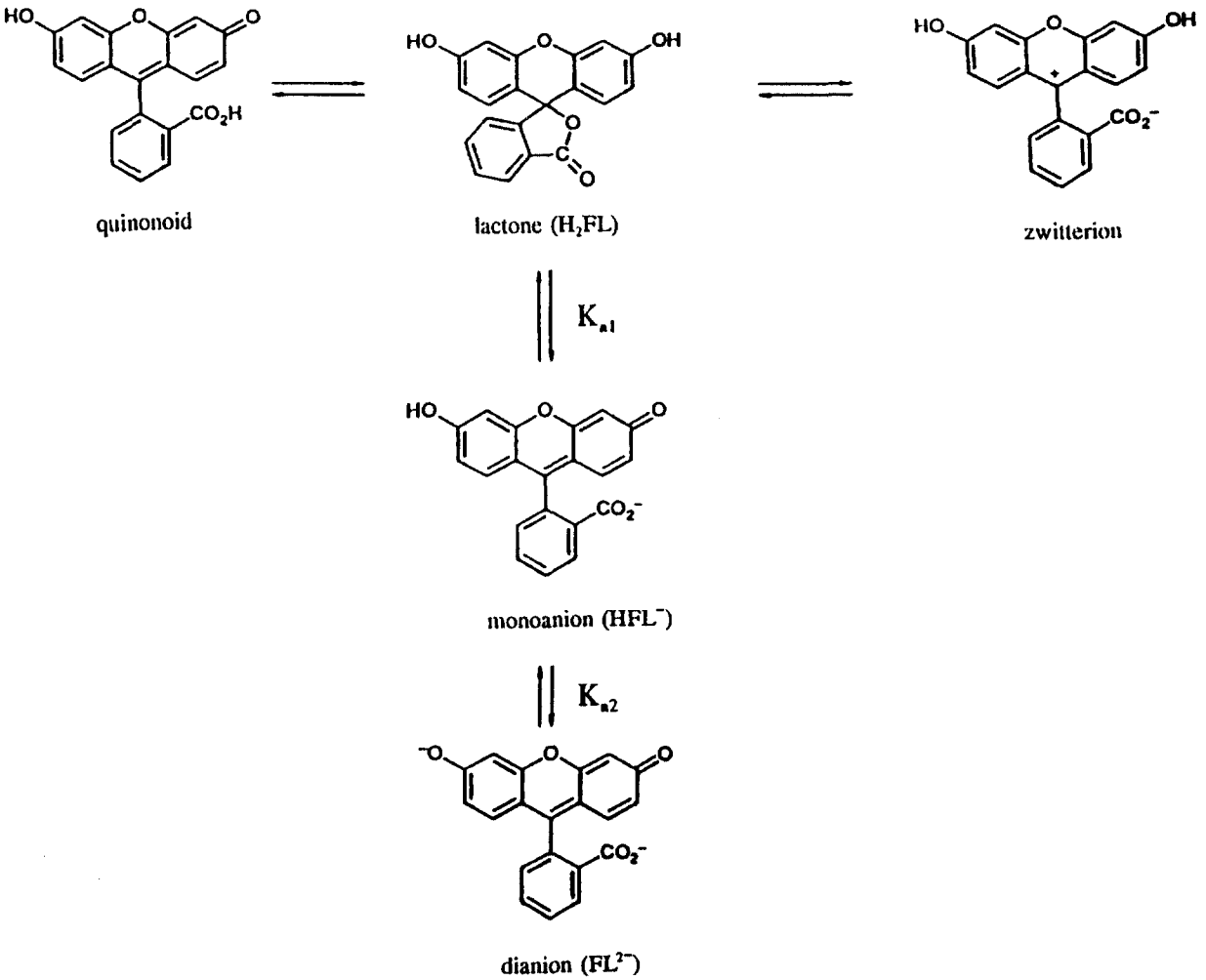


Fig. 3. The protolytic equilibria of FL in aqueous solution [11]. K_{a1}, K_{a2} are the first and second acid dissociation constants of FL.

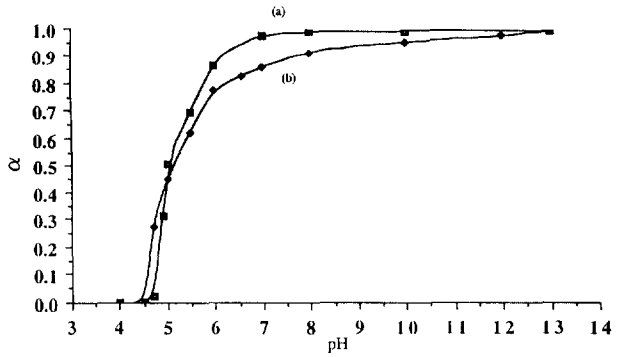


Fig. 4. Plot of α against pH. (a) Absorption measurements. (b) Fluorescence measurements.

where A is the measured absorbance, and, A_b and A_l are the background absorbance value of the polymer film and the limiting absorbance value for the dianionic form of FL, respectively. Applying Eq. (5), the α value of FL in membrane phase monitored at 510 nm can be plotted as a function of pH (Fig. 4a). At low pH values, α is equal to 0 when all the FL molecules present in the neutral lactonic form. The acid dissociation constant (pK_a) of FL in membrane phase containing a very small amount of water can be easily obtained at a pH value when α is equal to 0.50. The pK_a value is defined as the acid dissociation constant of the neutral lactonic form of FL from a polymeric film to an aqueous medium under the experimental conditions and is depicted in Eq. (4). From Fig. 4a, the pK_a of FL in an ethyl cellulose film is found to be 5.00.

3.2. Fluorescence spectra

The fluorescence excitation and emission spectra of immobilized $(TOA^+)_2FL^{2-}$ ethyl cellulose film in various pH phosphate buffers are shown in Fig. 5. An excitation band with peak maximum (λ_{EX}) of 512 nm and a shoulder peak (λ_S) of 484 nm, and, an emission band with peak maximum (λ_{EM}) of 536 nm corresponding to the dianionic form of FL are clearly observed at high pH values. The fluorescence

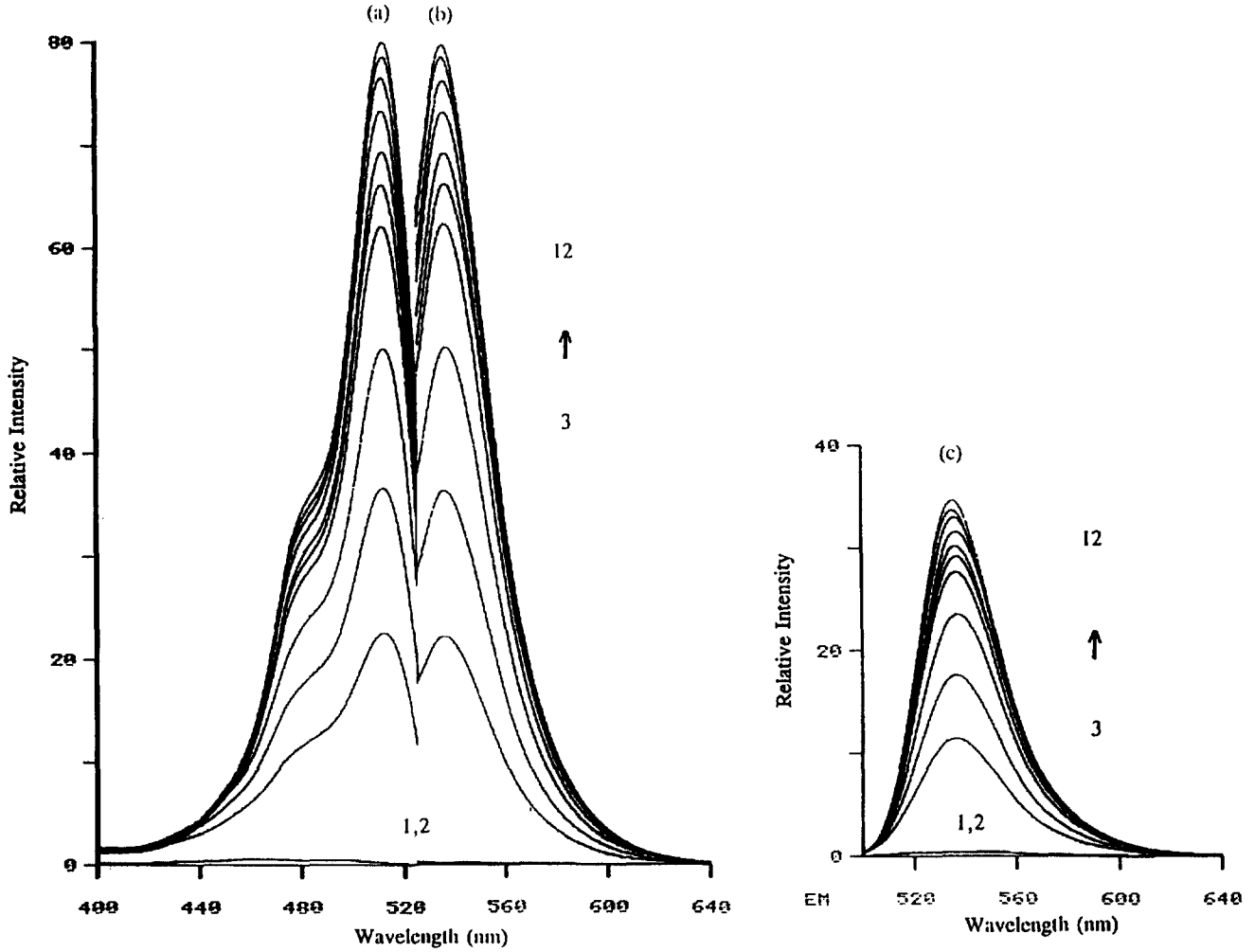


Fig. 5. Fluorescence excitation and emission spectra of immobilized $(TOA^+)_2FL^{2-}$ ethyl cellulose film immersed into various pH phosphate buffers. 1: 4.00; 2: 4.50; 3: 4.70; 4: 5.00; 5: 5.50; 6: 6.00; 7: 6.57; 8: 7.00; 9: 8.00; 10: 10.0; 11: 12.0; 12: 13.0. (a) Excitation spectra with emission wavelength of 536 nm. (b) Emission spectra with excitation wavelength of 512 nm. (c) Emission spectra with excitation wavelength of 484 nm.

Table 1
Spectroscopic properties and acid dissociation constants of FL in aqueous solution and ethyl cellulose film

Spectroscopic properties	FL^{2-} in	
	Aqueous solution [14]	Ethyl cellulose film
Absorption, λ_{max} (nm)	490	510
Excitation, λ_{EX} (nm)	491	512
Emission, λ_{EM} (nm)	513	536
pK_a	4.24[a], 6.33[b]	5.00[c], 5.13[d]

[ab]First and second acid dissociation constants of FL, respectively.
[cd]Determined by absorption and fluorescence data, respectively.

intensity decreases as the pH decreases. The decrease of fluorescence can be explained as earlier by the conversion of the strongly fluorescent dianionic form to the neutral lactonic form that has shorter emission wavelengths than the dianion. The excitation spectra also match with the absorption spectra shown in Fig. 2. The emission spectra of the FL^{2-} ions in ethyl cellulose is similar to that in aqueous solution, with the exception that the λ_{EM} appears red-shifted by 23 nm [14]. The emission spectra are bathochromic shifted which are in good agreement with the hydrogen bonding stabilization effects explained earlier. Emission spectra obtained at λ_{EX} and λ_S are similar, but different in intensity due to the different optical densities at these two excitation wavelengths. The emission spectra feature looks very similar indicating that internal conversion from the second excited state to the first excited state is efficient after excitation from the ground state to the second excited state with subsequent fluorescence occurring when electrons fall from the first excited state to the ground state [14].

Using the data from Fig. 5 and the analogical equation to Eq. (5), λ is plotted against pH (Fig. 4b). The pK_a value is found to be 5.13. A summary of the spectroscopic properties and acid dissociation constants of FL in aqueous solution and ethyl cellulose film is presented in Table 1.

4. Conclusion

The spectroscopic properties and protolytic equilibria of FL are altered when moved from an aqueous to a polymeric film media. Two individual ionization steps occur in aqueous solution whereas only one is observed in a polymeric film under the experimental conditions. The absorption and fluorescence spectra are red-shifted in polymeric film condition owing to the hydrogen bonding stabilization effects. Furthermore, the spectroscopic behaviour and protolytic equilibria of FL in organic solvents are similar to that in polymeric supports since both materials are more hydrophobic. This similarity can provide an insight to the spectroscopic behaviour of a dye when it is immobilized in a polymeric film if the spectroscopic properties and protolytic equilibria of the dye in organic solvents are available beforehand. Last but not least, the FL immobilized films discussed here can be used as pH-sensitive membranes in pH range 4.5 to 7.0 based on either absorbance or fluorescence measurements.

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