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pH-dependent absorption and fluorescence spectra of hydroxyaryl-squarylium dyes

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Abstract

A range of symmetrical hydroxy-substituted 1,3-diarylsquarylium dyes has been prepared and the light absorption and emission characteristics of the dyes examined. Whereas dyes formed from 3-*N*,*N*-dialkylaminophenols show intense fluorescence, analogous compounds derived from polyhydric phenols show little or no fluorescence. Those dyes with multiple hydroxy substituents in the aryl rings are very pH sensitive and readily dissociate in solution to give progressively mono-anionic, di-anionic and tri-anionic species as the pH is increased. In view of these observations, previous literature structures for these species have been reappraised. The pronounced spectral differences between the various prototropic species in solution have potential analytical applications. For example, one derivative can be used to detect water in solvents colorimetrically, and another shows strong fluorescence from its dianionic form, and can be used as a pH fluorescence probe or indicator. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Squarylium dyes; pH indicators; Flourescence

1. Introduction

The 1,3-squarylium dyes have unique chromophoric properties and have found many specialised uses as functional dyes, for example in optical data recording materials [1] as near-infrared absorbers [2], as photoconductors in electrophotography [3], and as fluorescent dyes for biolabelling [4]. The dyes generally show a very narrow absorption band in the visible spectrum ($\Delta\lambda_{1/2}$ ca. 30 nm) with a characteristically high molar absorption coefficient ($\varepsilon_{\max} > 10^5$ 1 mol^{-1} cm^{-1}). The amino-substituted diaryl derivatives **1a** are relatively rigid molecules and consequently, often show some degree of fluorescence in solution. The fluorescence efficiency of these compounds does not become high unless additional rigidity is introduced by hydrogen bonding, as with the *ortho*, *ortho′* -bis-hydroxyaryl derivatives **1b**. For example, **1b**, $R = Et$, has $\lambda_{\max} = 640$ nm in dichloromethane, fluoresces with a quantum yield of 0.86, and has a Stokes shift of only 24 nm [5]. The long wavelength absorption/emission properties and high fluorescence efficiencies of such dyes have made them of potential value in the area of biolabelling.

In contrast, very few 1,3-diaryl derivatives are known which contain only hydroxy substituents in the rings, although such compounds show an interesting dependence of their absorption and

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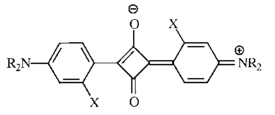
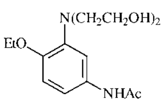


1 a; X = H
1 b; X = OH



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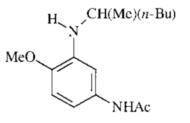

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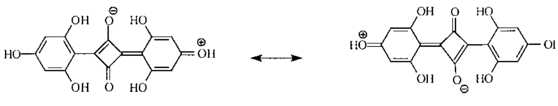
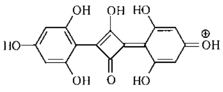


2d



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fluorescence spectra on pH. Those materials investigated to date are based on the dye derived from phloroglucinol, i.e. 1,3-bis(2,4,6-trihydroxyphenyl)squaraine, which was first described by Treibs and Jacob in 1965 [6]. Das et al. have examined some of the fluorescence characteristics of this dye [7], and Ramaiah and coworkers have considered its bromo and iodo derivatives as potential photodynamic therapy sensitizers [8]. However, certain of the structural assignments made by these authors to the species formed in solution at different pH values are at variance with the predictions of colour and constitution theory, and need reconsideration. Therefore, it was of interest to synthesise a range of 1,3-diaryl-squarylium dyes with poly-hydroxy functionality, in order to determine the true nature of the various prototropic forms in solution, and also to examine the pH-dependence of their absorption and fluorescence spectra in more detail.

2. Experimental

The structures of the dyes investigated (**2**) are summarised in Scheme 1. Visible absorption spectra were recorded on a Perkin–Elmer Lambda 15 spectrophotometer, and fluorescence spectra on a Perkin–Elmer LS5 spectrofluorimeter. Relative fluorescence quantum yields were measured against **2a** as standard, the quantum yield for which has been determined as 0.86 in dichloromethane (DCM) [5]. The values were estimated by comparing the emission intensity of each dye at λ_{max} (fluor) with the corresponding emission intensity from the standard dye (**2a**) under conditions of equal light absorption. This assumes that the spectral response of the red light detector is constant over the relatively narrow range of wavelengths involved, and thus the quoted quantum yields should be regarded as approximate only.

2.1. *Synthesis of dyes. General procedure*

Squaric acid (0.20 g, 1.75×10^{-3} mol) was refluxed in a mixture of 1-butanol (40 ml) and toluene (20 ml) for 30 min, and water was removed azeotropically using a Dean–Stark trap. The appropriate nucleophilic aromatic compound (3.0×10^{-3} mol) was then added and refluxing continued for 1–2 h. The course of the reaction was monitored by thin layer chromatography (t.l.c.) using silica gel as adsorbent. When the reaction was judged to be complete the reaction mixture was cooled, concentrated by rotary evaporation, and the precipitated product filtered off and dried in an oven at 70°C.

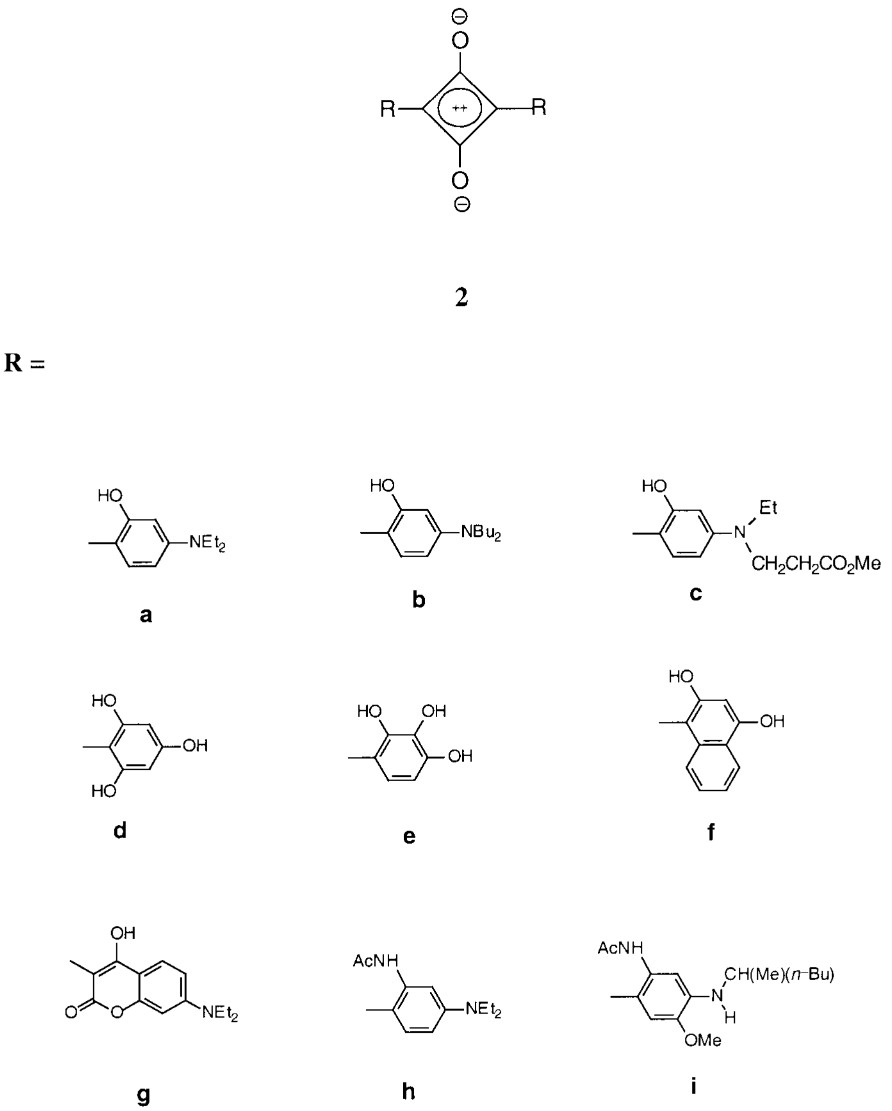
Purification was carried out by recrystallisation or column chromatography over silica gel. In the latter case DCM containing 0–10% ethanol was used as eluent. Purity was confirmed by t.l.c. and the dyes were characterised by FAB mass spectrometry (Table 1).

Dye **2a** was obtained in 78% yield, m.p. 257–259°C (lit. [9] 257°C), and dye **2h** in 78% yield, m.p. 254–256°C (lit. [10] 255°C).

3. Results and discussion

3.1. *Synthesis of dyes 2*

The structures of the dyes synthesised are summarised in Scheme 2. 1,3-Diaryl squarylium dyes



Scheme 1.

can be synthesised by condensing a dialkylsquarate ester (usually 1-butyl) with a suitably nucleophilic aromatic compound (usually an *N*,*N*-dialkylaniline) using the procedure of Sprenger and Ziegenbein [11]. The squarate ester is prepared in situ from squaric acid and the alcohol by heating in a solvent such as benzene or toluene with continuous removal of water by azeotropic distillation. Success of the reaction is very dependent on a strongly nucleophilic aryl species, and consequently relatively few hydroxyaryl dyes have been described, in marked contrast to the well known *N*,*N*-dialkylaminoaryl derivatives. Although the reaction of squaric acid with 3-*N*,*N*-dialkylaminophenols proceeds readily (giving for example **2a** from 3-*N*,*N*-diethylaminophenol), several hydroxy groups appear to be necessary in the case of reactions with purely phenolic compounds, and the dye **2d** derived from phloroglucinol is one of the few such dyes to be reported [6]. As hydroxyaryl

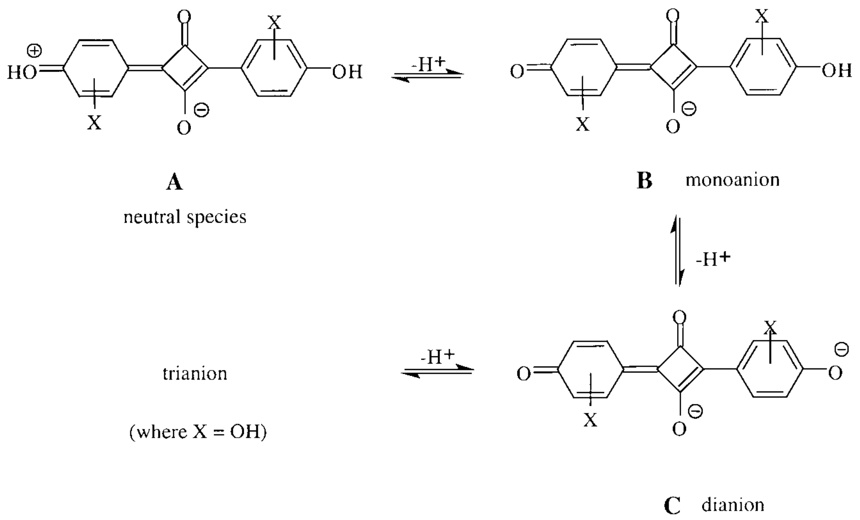
Table 1
Yieds and characterisation data for squarylium dyes **2**

Dye **2**	Yield (%)	M.p. (°C)	Mass spectrum (FAB)
b	49	192–193	$m/z = 520$; C**30**$H_{44}N_2O_4$ requires $M = 520$
c	68	205–208	$m/z = 525$; $C_{28}H_{32}N_2O_8$ requires $M + H = 525$[a]
d	59	340–345	$m/z = 331$; $C_{16}H_{10}O_8$ requires $M + H = 331$
e	–[c]	–[c]	$m/z = 330$; $C_{24}H_{10}O_{58} = M = 330$
f	68	326–328	$m/z = 399$; $C_{24}H_{14}O_6$ requires $M + H = 399$[b]
g	–[c]	–[c]	$m/z = 545$; $C_{30}H_{28}N_2O_8$
i	70	233–236	$m/z = 6097$; $C_{34}H_{46}N_4O_6$ requires $M + H = 607$

[a] Microanalysis: Found: C, 64.3; H, 6.35; N, 5.2%; $C_{28}H_{32}N_2O_8$ requires C, 64.1; H, 6.1; N, 5.2%.

[b] Microanalysis: Found: C, 72,2; H, 3.75%; $C_{24}H_{14}O_6$ requires C, 72.4; H, 3.5%.

[c] Could only be isolated in pure form in solution.



Scheme 2.

squarylium dyes should also show pH-dependent light absorption and emission properties, it was of interest to examine the synthesis and properties of a range of derivatives of this type. Further information was also sought concerning the synthetic limitations of the reaction between squaric acid and phenolic compounds employing the classical reaction conditions of Sprenger and Ziegenbein, but in which the benzene co-solvent was replaced by toluene for obvious safety reasons.

Aryl derivatives containing a single hydroxy group would only react if an amino group were also present in the ring, preferably *meta* to the hydroxy group, as in the case of 3-*N*,*N*-diethylamino-, 3-*N*,*N*-dibutylamino-, and 3-*N*-ethyl-*N*-(2-methoxycarbonylethyl)-phenols, which gave dyes **2a–c** respectively. In contrast, 4-*N*,*N*-dialkylaminophenols are unreactive and, for example, 4-*N*,*N*-dimethylaminophenol gave only a trace of a coloured product, indicating the reluctance of the reaction to take place in the absence of a strong electron releasing group *para* to the position of attack. Two hydroxy groups in a phenyl ring appear to be insufficient to facilitate an efficient reaction, and although resorcinol did give traces of a red dye ($\lambda_{max} = 565$ nm in ethanol) on prolonged reaction, it was not possible to isolate pure material. Treibs and Jacob attempted to react resorcinol with squaric acid by melting the two together and heating at 250°C, but they obtained only a tri-addition product [12].

Resorcinol derivatives with electron withdrawing groups in the 6-position (e.g. acetyl, benzoyl, formyl) not surprisingly also failed to give any trace of coloured products, although 6-carboxy-1,3-dihydroxybenzene did react very slowly to give trace amounts of a red dye with typical squarylium characteristics. In contrast, trihydroxybenzenes have sufficient reactivity to take part in reactions with squaric acid, and Treibs and Jacob conducted a reaction between 1,3,5-trihydroxybenzene with squaric acid in refluxing 60% acetic acid and obtained **2d** in 67% yield. Under our reaction conditions this dye was isolated in 59% yield, and we were also able to convert 1,2,3-trihydroxybenzene and squaric acid to dye **2e** in 22% yield. Electron withdrawing groups in the 1,2,3-trihydroxybenzene system had a pronounced inhibiting effect, and both 4-benzoyl- and 5-carboxy-1,2,3-trihydroxybenzene failed to react, even after prolonged reaction times (12–24 h).

Reactivity was somewhat increased with the naphthol system, and 1,3-dihydroxynaphthalene gave the blue dye **2f** in 68% yield, in contrast to the low reactivity of resorcinol. However, 1-naphthol, 2-naphthol and 2,3-dihydroxynaphthalene were unreactive.

Interestingly, 7-*N*,*N*-diethylamino-4-hydroxycoumarin showed sufficient nucleophilicity at the 3-position of the coumarin ring to undergo condensation, albeit very inefficiently, in spite of the remoteness of the amino group from the reaction centre and the deactivating effect of the ester carbonyl group. Thus dye **2g** was isolated in very low yield.

An alternative ring substituent to hydroxy that can hydrogen bond to the squarylium carbonyl groups is the acetylamino group, and 3-acetylamino-*N*,*N*-dialkylarylamines condensed readily with squaric acid. The products of this reaction proved to be almost as fluorescent as their hydroxy counterparts, and, for example, **2h** had a fluorescence quantum yield of 0.51. To attempt to push the λ_{max} of such a dye to longer wavelengths, attempts were made to introduce an additional alkoxy group into the aryl ring, and thus the reaction was attempted with 5-acetylamino-2-ethoxy-*N*,*N*-bis(hydroxyethyl)aniline **(3)**. The reaction proved extremely slow however, and although traces of dye were detected, no product could be isolated. The low reactivity can be attributed to steric interaction between the dialkylamino group and the *ortho* ethoxy group in **3**, which is relieved by partial rotation of the amino group out of conjugation with the benzene ring. The resultant loss of ring activation would be sufficient to inhibit the condensation reaction with squaric acid. In agreement with this suggestion, it was found that the arylamine **4** which has a secondary amino group, and is thus less sterically hindered, reacted readily with squaric acid to give the strongly bathochromic dye **2i** ($\lambda_{max} = 702$ nm in dichloromethane).

3.2. Light absorption and emission properties of neutral dyes

It was apparent that the colour and fluorescence properties of those compounds with a hydroxy group *para* to the squarylium residue, i.e. dyes **2e–f**, were very dependent on solvent pH, and even solvent polarity, which could be attributed to the facile ionisation of the *para* hydroxy group in the dye molecule. Thus, initially the light absorption and emission characteristics of all the dyes **2** were measured under conditions in which ionisation did not take place. This could be achieved by using pure DCM as solvent, or in ethanol containing a trace of hydrochloric acid when solubility in DCM was low. The light absorption and fluorescence properties of the undissociated neutral dyes are summarised in Table 2.

It can be seen that the dyes range from magenta to cyan in colour, and the dependence of λ_{max} on substituents follows the expected trend for donor-acceptor chromophores of this type. Thus dyes containing only hydroxy donor groups generally absorb at shorter wavelengths than the amino-substituted dyes. Dye **2f**, which is derived from 1,3-dihydroxynaphthalene, is unusual in this respect and is cyan in colour ($\lambda_{max} = 663$ nm), reflecting the higher polarisability of the naphthalene residue relative to the benzene ring. The extended conjugation in the coumarin dye **2g**, which has $\lambda_{max} = 680$ nm, provides a significant

Table 2
Visible absorption and fluorescence spectroscopic properties of undissociated dyes **2**

Dye **2**	λ_{max} (abs) (nm)	ε_{max}/l mol^{-1} cm^{-1})	Solvent	λ_{max} (fluor) (nm)	Stokes shift (nm)	Quantum yield
a	640	387,000	DCM	664	24	0.86
b	649	390,000	DCM	661	12	0.41
c	636	373,000	DCM	664	18	0.83
d	562	160,000	EtOH[a]	–[b]	–	–
e	568	–[c]	EtOH[a]	–[b]	–	–
f	663	120,000	EtOH[a]	696	33	0.09
g	680	–[c]	DCM	722	42	0.27
h	672	359,000	DCM	698	26	0.51
i	702	321,000	DCM	732	30	0.50

[a] Containing a trace of hydrochloric acid.

[b] No detectable fluorescence.

[c] Could only be isolated in pure form in solution.

bathochromic effect, and can be compared with the simple *N*,*N*-diethylamino dye **2a**, which has $\lambda_{max} = 640$ nm. Interestingly the dye **2h**, with an acetylamino group in place of the hydroxy group in **2a**, exerts almost as large a bathochromic effect, and absorbs at 672 nm. A significant factor in this must be the strong intramolecular hydrogen bond that can exist between the NH proton of the acetylamino group and the adjacent squarylium carbonyl oxygen, which is also responsible for the strong fluorescence observed from **2h**. By combining the effect of an *ortho* acetylamino group with the additional electron donating strength of a methoxy group in the aryl ring, it was possible to shift the absorption band into the near-infrared region, and dye **2i** had $\lambda_{max} = 702$ nm in dichloromethane. Extinction coefficients of dyes with a *para* hydroxy donor group are significantly lower than those with a *para* amino group.

In their non-dissociated forms, the *para*-hydroxy substituted dyes **2d**–**f** showed a much reduced tendency to fluoresce than the *para*-amino derivatives, and in fact only the naphthalene dye **2f** showed any detectable fluorescence (quantum yield ca. 0.09). In contrast, the 4-amino derivatives showed fluorescence quantum yields ranging from 0.41 to 0.86, the parent dye **2a** having the highest value. The acetylamino group proved as effective as the hydroxy group in providing the requisite intramolecular hydrogen bonding for fluorescence, and dyes **2h** and **2i** both showed high quantum yields. Stokes shifts were slightly larger for the *ortho*-acetylamino dyes than for the *ortho*-hydroxy derivatives, but were still small. However, the coumarin dye **2g** did show a large Stokes shift (42 nm) whilst retaining a reasonable quantum yield (0.27). Consequently, this cyan dye satisfies one of the important criteria for a biolabelling fluorophore, but unfortunately it could be synthesised only in low yields.

*3.3. pH-Dependent spectroscopic properties of dyes **2d**–**f***

The three polyhydroxy dyes **2d**–**f** showed similar acid/base equilibria in solution due to the presence of readily dissociating hydroxy substituents. It can be safely assumed that the structure of these dyes in the non-dissociated state, i.e. in neutral or weakly acidified aprotic solvents, is as represented by structure **A** in Scheme 2, and as shown for **2d** below. Thus the chromophore is neutral and electronically symmetrical, with a positive charge shared equally by the terminal hydroxy groups, and with a negative charge in the central ring delocalised over both carbonyl groups.

The partial positive charge on the hydroxy groups accounts for their high acidity, and the high degree of electronic symmetry accounts for the very narrow bandwidth and the high extinction coefficient of the visible absorption band. This conclusion is at variance with the mono-protonated structure **5** for the dye **2d** in its narrow band/high extinction coefficient prototropic form assumed by earlier workers [7,8]. Structure **5** cannot be correct for the following reasons:

a. The narrow band/high extinction species exists in aqueous solution under very weakly acid conditions ($\text{pH} < 1$ up to ca. 5). However, protonation of the 4-aminoaryl squarylium dyes (e.g. **2a**) requires very strongly acidic conditions ($< \text{pH } 1$), and protonation of hydroxy analogues to give a structure such as **5** would be even more difficult due to the lower basicity of the oxygen. Thus, under weak acid conditions the species cannot be protonated and must have the neutral structure **2d**.
b. Unlike **2d**, structure **5** is cationic and thus can only exist as a salt with an appropriate counter-anion. When pure solid **2d** is prepared under neutral, non-ionic conditions so that it cannot possibly have a salt structure, and is dissolved in rigorously dried acetone, it shows a single sharp peak at 558 nm with the characteristic narrow half-bandwidth of ca. 30 nm.

The intensely absorbing neutral species from dyes **2d–f** have very high p*K*a values, and loss of a proton can occur spontaneously in non-acidic protic solvents, or aprotic solvents containing trace amounts of water, to give the monoanionic species **B** (Scheme 2). Thus, for example, a solution of **2d** in dry acetone shows a dramatic change in colour from intense purple to orange-red on addition of 0.5% water. The formation of the monoanions from **2d–f** is in each case characterised by a large hypsochromic shift and an increase in the absorption band width.

Careful addition of a base (e.g. a dilute solution of KOH in ethanol) to a solution of the monoanionic species can remove a second proton to give a dianion, **C**, accompanied by a further dramatic change in the spectrum. Thus the absorption band now shifts to an even longer wavelength than that of the original neutral chromophore, and the narrow band width is regained. With higher concentrations of base a third proton can be removed, and the absorption band of the resultant trianion moves hypsochromically, to wavelengths intermediate between the values for the neutral and mono-anionic species. The trianions are unstable and their solutions fade irreversibly over ca. 5–10 minutes at room temperature. The spectral changes accompanying these processes are illustrated for dye **2d** in Fig. 1. The various prototropic equilibria taking place with these dyes are summarised in Scheme 2. It should be noted that these structural assignments differ significantly from those of Das et al., who presumed structure **5** for **2d** [7]. Table 2 gives the λ_{max} and half-bandwidth values of the various species formed in solution.

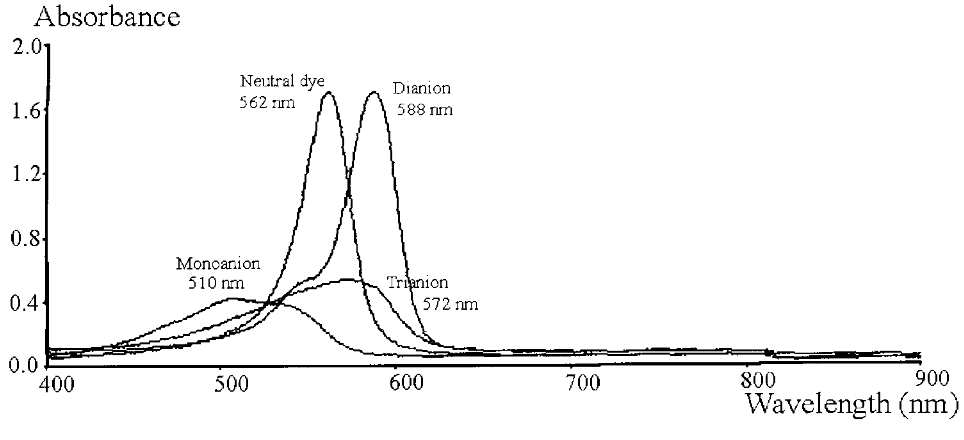


Fig. 1. Visible absorption spectra of **2d** and its derived anions in ethanol formed by addition of increasing amounts of KOH.

The spectral changes observed can be explained readily in terms of simple resonance theory. As noted previously, the parent neutral dyes **A** (see Scheme 2) are electronically symmetrical, which means that they will show minimal geometry change between the ground and first excited state, and thus they have a characteristically narrow first absorption band. Dissociation gives the mono-anion **B**, which is electronically unsymmetrical (in certain respects resembling an undissociated oxonol dye), and this not only induces a hypsochromic shift, but also causes band broadening. Loss of the second proton to give dianion **C** restores electronic symmetry and thus the chromophore again affords a narrow bandwidth. At the same time, the stronger electron donating strength of -O$^-$ compared to -OH means that dianion **C** will absorb at even longer wavelengths than the parent compound **A**.

Dye **2f** showed the most pronounced pH-induced colour change, with the brilliant cyan neutral species changing to red on dissociation. This corresponds to a hypsochromic shift of ca. 117 nm in ethanol, and provides as a very sensitive indicator system for detecting the presence of small proportions of water in organic solvents. For example, when **2f** was dissolved in dry acetone and increasing amounts of water added, the spectrum changed progressively, with a clear isosbestic point, and the dye was completely dissociated when 0.5% (by volume) of water was present. This permits accurate analysis of water content over the range 0–0.5%. The spectral changes in acetone are shown in Fig. 2.

As noted previously, the neutral dyes **2d** and **2e** were not fluorescent, and **2f** showed only weak fluorescence with a quantum yield of 0.09. None of the mono-anions derived from **2d**–**f** were fluorescent, which is not surprising in view of the unsymmetrical nature of these species and their very broad visible absorption bands. However, the dianion from **2d** did show reasonably intense red fluorescence [$\lambda_{\max}$ (fluor) = 611 nm in ethanol; quantum yield ca. 0.2]. Fig. 3 shows the variation in the absorbance of a solution of **2d** in ethanol, measured at the $\lambda_{\max}$ (abs.) of its dianion (588 nm),

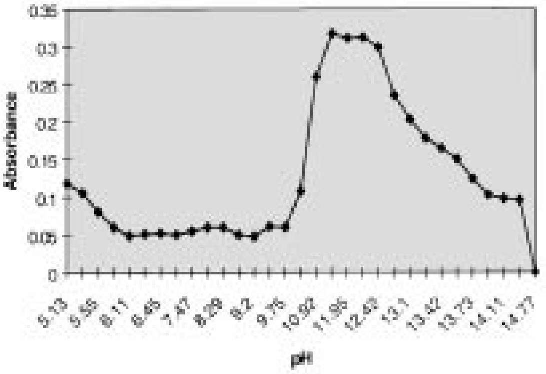


Fig. 3. Changes in absorbance (at 588 nm) with pH for a solution of **2d** in ethanol.

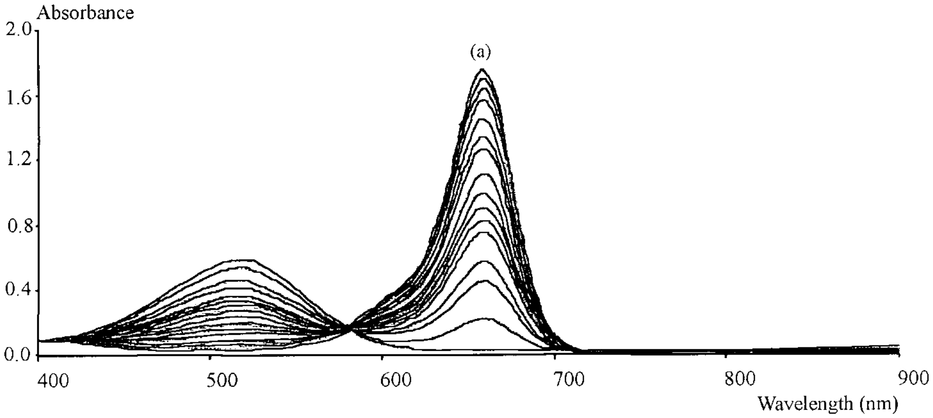


Fig. 2. Absorption spectrum of **2f** (1.4×10^{-5} M) in dry acetone [curve (a)] and the effects of progressive addition of water (up to 0.5% by volume) to the solution.

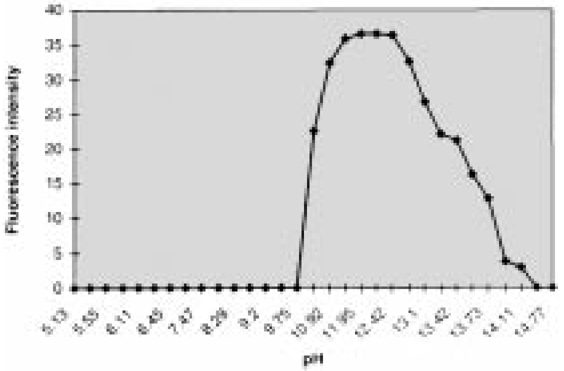


Fig. 4. Changes in fluorescence intensity [measured at λ_{max} (fluor) = 611 nm; excitation wavelength 588 nm] with pH for a solution of **2d** in ethanol.

and Fig. 4 shows the variation of the fluorescence intensity of the same solution, measured at the fluorescence maximum of the dianion (611 nm), as the pH of the solution is increased. The pH values, as measured by a pH-meter, should of course be regarded as arbitrary in view of the non-aqueous nature of the solvent, but it can be seen that with increasing pH over a nominal range of ca. 9.7 to 11.5 both the absorbance at 588 nm and the emission intensity at 611 nm show a steep rise, and these values remain constant up to ca. pH 12.5. At higher pH, the absorbance and emission values decrease, corresponding to formation of the trianion. Thus dye **2d** has potential value as a fluorescent pH probe or indicator.

4. Conclusions

1,3-Diarylsquarylium dyes show intense long wavelength fluorescence when there are amino groups in the *para*, *para'* positions and hydrogen bonding groups (-OH or -NHAc) in the *ortho*,*ortho'* positions of the aryl rings. In contrast, related dyes in which the *para* amino groups have been replaced by hydroxy groups are largely non-fluorescent, but show a strong dependence of their absorption spectra on pH. The *para*-hydroxy groups dissociate progressively in solution with increasing pH, so forming mono- and di-anionic species, with characteristic changes in the visible absorption spectra. The dye derived from squaric acid and 1,3-dihydroxynaphthalene shows a pronounced colour change from cyan to red in dry acetone on addition of up to 0.5% water, due to formation of the mono-anion, and can be used as an analytical indicator for detecting low levels of water in solvents. Whereas both the neutral and mono-anionic forms of dye **2d** (derived from squaric acid and phloroglucinol) are non-fluorescent, the dianion is strongly fluorescent and thus this dye can be used as a fluorescent pH indicator.

Acknowledgements

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