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New fluorescent indolium and quinolinium dyes for applications in aqueous halide sensing

Chris D. Geddes [a,b,*], Peter Douglas [a], Christopher P. Moore [c],
Trevor J. Wear [c], Peter L. Egerton [c]

[a] *Department of Chemistry, University of Wales Swansea, Singleton Park, Swansea, SA2 8PP, UK*
[b] *Department of Physics and Applied Physics, The University of Strathclyde, John Anderson Building, 107 Rottenrow, Glasgow, G4 0NG, UK*
[c] *Kodak Research Division, Kodak Ltd., Headstone Drive, Harrow, Middlesex, HA1 4TY, UK*

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Abstract

Bromide and teraphenylborate salts of six new highly fluorescent dyes, produced by the reaction of two heterocyclic nitrogen bases with 8-bromo-octanoic acid, 11-bromo-undecanoic acid and 15-bromo-pentadecanoic acid have been prepared. Unlike the bases themselves, the quaternary salts are water soluble and have fluorescence characteristics independent of pH in the pH range 7–11. Both the fluorescence intensity and fluorescence lifetime of these dyes are reduced in the presence of aqueous halide ions, allowing halide concentrations to be determined accurately at concentrations of importance to both medicine and industry. All the dyes have been characterised in terms of steady state fluorescence spectra and steady-state Stern-Volmer analysis. The Stern–Volmer constants, (K_{SV}), for the dyes are compared to those obtained for some commercially available dyes. The prospects for using these dyes in halide sensor devices are discussed. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Halide determination is important in many areas of everyday life, including industry [1,2] and medicine [3–6]. Most biological fluids are complicated mixtures which include inorganic electrolytes. In plasma and muscle cells, electrolyte composition is normally fairly constant, but in gastric/pancreatic juices, sweat, saliva, and urine it may vary considerably, particularly when affected by illness. A good example of this is cystic fibrosis. Few diseases can be so readily diagnosed as cystic fibrosis, which is characterised by a high chloride concentration in a patient's sweat and saliva. Accurate determination of halide concentrations in blood [3], serum [4], plasma [5], and urine [6] is, therefore, evidently important.

The fluorescence quenching of fluorophores by halide ions was first described by Stokes [7] as early as 1869, when he observed that the fluorescence of quinine in dilute sulphuric acid was reduced after the addition of hydrochloric acid. This process that he observed is now commonly

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[*] Corresponding author. Present address: Department of Physics and Applied Physics, The University of Strathclyde, Glasgow, UK. Fax: +44-(0)-141-552-2891.

E-mail address: c.d.geddes@strath.ac.uk (C.D. Geddes)

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referred to as 'dynamic fluorescence quenching' where both the lifetime and intensity of fluorescence are reduced in the presence of a quencher, Q. This process is known to follow Stern–Volmer kinetics [8,9] where:

$$I_o/I = 1 + k_q\tau^0[Q] \quad (1)$$

which can be used to obtain values of $k_q\tau^0$ (the Stern–Volmer constant, K_{SV}) by plotting I_o/I as a function of $[Q]$; I_o and I are the fluorescence intensities in the absence and presence of Q, respectively, k_q is a specific constant describing the bimolecular collisional deactivation of electronic energy and τ^0 the natural fluorescence lifetime.

The fluorescence of many dyes is known to be quenched by aqueous halide; the sensitivity to halide, and hence the halide concentration range detectable, is given by the magnitude of K_{SV}. At present there is a need for water soluble dyes with large Stern–Volmer constants, many commercially available dyes having relatively small Stern–Volmer constants.

In this paper we describe the synthesis of 12 new fluorescent dyes together, with a study of their sensitivity and selectivity to aqueous halide.

2. Experimental

2.1. Materials

All reagents were purchased from the Aldrich Chemical Company and were used as received, except for 15-bromo-pentadecanoic acid which was a gift from Dr. John Maud, University of Wales, Swansea.

2.2. Instrumentation

^{1}H NMR spectra were recorded on a Varian Unity plus (400 MHz) spectrometer. Chemical shifts are reported in δ ppm relative to tetramethylsilane in deuteriochloroform. All IR spectra were recorded on a Perkin–Elmer PE2000R NIR FT-Raman spectrometer. All absorption measurements were performed using a Philips Unicam PU8620 UV/VIS/NIR spectrophotometer.

2.3. Stern–Volmer analysis

Steady-state emission and excitation maxima were recorded on a Jobin-Yvon JY3D spectrofluorimeter. Steady-state Stern–Volmer analysis of dyes **1**–**12** was carried out at 21°C, pH 10, using halide concentrations in the range $1 \rightarrow 10^{-4}$ mol dm^{-3}. Subsequently, the Stern–Volmer quenching constants for dyes **1**–**12** with aqueous halide ions were calculated using the Axum Graphics linear regression program. pH 10 buffers were made by mixing 50 ml of 0.025 mol dm^{-3} borax, 18.3 ml of 0.1 mol dm^{-3} NaOH and 31.7 ml of doubly distilled deionised water [10]. The Stern–Volmer constants reported here are for aqueous solutions at pH 10, although the dyes are sensitive to halide in the pH range 7–11, where both the K_{SV} and fluorescence lifetime are fairly constant.

2.4. Synthesis of dyes 1–3

Dyes **1**–**3** were produced by the reaction of the 6-methoxy quinoline heterocyclic nitrogen base with 8-bromo-octanoic acid (dye **1**), 11-bromo-undecanoic acid (dye **2**) and 15-bromo-pentadecanoic acid (dye **3**). In all cases 7.2 mmol of each of the starting materials was heated under reflux for 4 h at 110°C, after which the mixtures were allowed to cool, and 50 ml of acetone was added. This was followed by stirring for 1 h, after which the acetone was decanted off. The dyes were washed with diethyl ether and dried under vacuum. Characterisation data for the dyes is shown below.

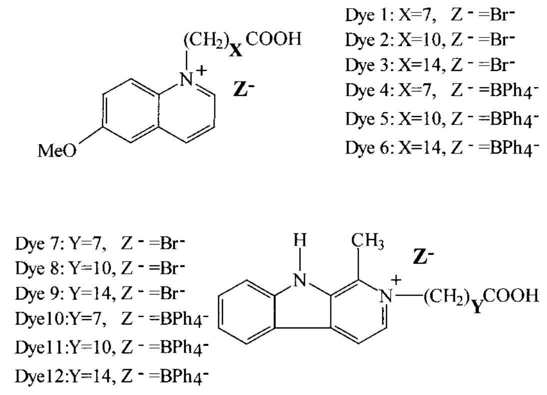

Fig. 1. Dyes **1**–**12**.

Dye 1 ^{1}H NMR (deuterium oxide): δ 1.2–2.4 (m, 12H), 4.1 (s, 3H), 5.1 (t, 2H), 7.6–9.2 (m, 6H); ir (potassium bromide): 1696, 2856 and 2932 cm^{-1}. Anal calcd for $C_{18}H_{24}NO_3Br$: C, 56.55; H, 6.33; N, 3.66; found: C, 56.61; H, 6.30; N, 3.65%.

Dye 2 ^{1}H NMR (methyl-d$_3$ alcohol-d): δ 1–2.4 (m, 18H), 4.0 (s, 3H), 4.8 (s, 1H), 5.0 (t, 2H), 7.6–9.2 (m, 6H); ir (potassium bromide): 1697, 2853, 2929 and 3293 cm^{-1}. Anal calcd for $C_{21}H_{30}NO_3$-Br: C, 59.44; H, 7.13; N, 3.30; found: C, 59.43; H, 7.14; N, 3.28%.

Dye 3 ^{1}H NMR (dimethyl-d$_6$ formamide-d): δ 1.5 (m, 26H), 2.3 (t, 2H), 4.1 (2, 3H), 5.3 (t, 2H), 7.8–9.8 (m, 6H); ir (potassium bromide): 1696, 2849 and 2919 cm^{-1}. Anal calcd for $C_{25}H_{38}NO_3Br$: C, 62.49; H, 7.97; N, 2.92; found: C, 62.13; H, 7.98; N, 3.01%.

2.5. *Synthesis of dyes **4–6***

The counter-ion exchange of dyes **1–3** to produce dyes **4–6** was achieved by dissolving the dyes in a minimum amount of hot water and pipetting the resultant solution into a saturated solution of sodium tetraphenylborate. The white precipitate was filtered, washed with distilled water to remove sodium bromide, and dried under vacuum at 40°C. Relevant characterisation data for the dyes is given below:

Dye 4 Anal. calcd for $C_{42}H_{48}NO_3B$: C, 81.15; H, 7.08; N, 2.25; found: C, 80.15; H, 6.93; N, 2.15%.

Dye 5 Anal. calcd for $C_{45}H_{54}NO_3B$: C, 81.44; H, 7.54; N, 2.11; found: C, 81.12; H, 7.51; N, 2.06%.

Dye 6 Anal. calcd for $C_{49}H_{62}NO_3B$: C, 81.78; H, 8.07; N, 1.95; found: C, 81.52; H, 7.99; N, 1.63%.

2.6. *Synthesis of dyes **7–9***

Dyes **7–9** were produced by the reaction of the harmane heterocyclic nitrogen base with 8-bromo-octanoic acid (dye **7**), 11-bromo-undecanoic acid (dye **8**) and 15-bromo-pentadecanoic acid (dye **9**). 1.65 mmol of each of the required starting materials was heated under reflux for 1 h at 120°C. After cooling for 1 h the resulting yellow solids were filtered and treated with 50 ml diethyl ether and vigorously stirred for a further 1 h. The yellow precipitates were recovered and recrystallised from methanol. Characterisation data for the dyes is shown below.

Dye 7 ^{1}H NMR (methyl-d$_3$ alcohol-d): δ 1.2–1.8 (m, 12H), 3.1 (s, 3H), 4.7 (t, 3H), 4.7–8.4 (m, 6H), 8.5 (s, 1H); ir (potassium bromide): 1627, 1726, 2851 and 2928 cm^{-1}. Anal calcd for $C_{20}H_{25}N_2O_2$-Br: C, 59.26; H, 6.22; N, 6.91; found: C, 59.3; H, 6.24; N, 6.95%.

Dye 8 ^{1}H NMR (dimethyl-d$_6$ sulphoxide): δ 1.2–1.5 (m, 18H), 3.3 (s, 3H), 4.7 (t, 2H), 7.0–8.7 (m, 6H); ir (potassium bromide): 1339, 1734, 2852, 2919 and 3074 cm^{-1}. Anal calcd for $C_{23}H_{31}$-N_2O_2Br: C, 61.74; H, 6.98; N, 6.26; found: C, 61.67; H, 7.00; N, 6.25%.

Dye 9 1H NMR (dimethyl-d$_6$ formamide-d): δ(1.4 (m, 26H), 3.4 (s, 3H), 4.9 (t, 2H), 7.4–8.8 (m, 6H); ir (potassium bromide); 1335,1732,1900, 2849, 2917 and 3075 cm^{-1}. Anal calcd for $C_{27}H_{39}N_2O_2Br$: C, 64.41; H, 7.81; N, 5.56; found: C, 64.42; H, 7.85; N, 5.49%.

2.7. *Synthesis of dyes **10–12***

The counter-ion exchange of dyes **7–9** to produce dyes **10–12** was carried out in the same manner as for dyes **4–6**. Relevant characterisation data for the dyes is given below.

Dye 10 Anal. calcd for $C_{44}H_{49}N_2O_2B$: C, 82.0; H, 6.99; N, 4.35; found: C, 80.07; H, 6.89; N, 4.36%.

Dye 11 Anal. calcd for $C_{47}H_{55}N_2O_2B$: C, 82.21; H, 7.43; N, 4.08; found: C, 79.82; H, 7.12; N, 4.31%.

Dye 12 Anal. calcd for $C_{51}H_{63}N_2O_2B$: C, 82.48; H, 7.95; N, 3.77; found: C, 79.13; H, 7.69; N, 3.69%.

3. Results and discussion

Quaternisation of the heterocyclic nitrogen bases produced water soluble dyes with large Stern–Volmer constants, reflecting the sensitivity of dyes **1–12** to aqueous halide, Table 1. The Stern–Volmer constants determined for some commercial dyes is also shown in Table 1 for comparison.

Stern–Volmer constants for dyes **4–6** and **10–12** are smaller than those of dyes **1–3** and **7–9**, respectively, showing that the counter ion has an effect on sensitivity. This may simply be a size effect, the larger BPh_4^- counter ion sterically hindering halide diffusion to the dye. There is a decrease in halide

Table 1
Stern–volmer constants for dyes **1–12** and some commercial dyes, pH 10, 21°C

	Steady-state Stern–Volmer constants (mol^{-1} dm^3)[a]		
Dye	Cl^-	Br^-	I^-
1	52	225	634
2	34	150	480
3	34	127	398
4	20	70	218
5	14	55	165
6	11	27	139
7	–[b]	3	191
8	–	5	198
9	–	3	192
10	–	1	139
11	–	1	143
12	–	1	141
Some commercial dyes			
Rhodamine B	–	–	5
Acridine Orange	–	1	51
Eosin B	–	–	5
Acridine Yellow	–	4	50
Rose Bengal	–	0.5	13

[a] Stern-volmer constants are precise to < 5%.

[b] – indicates no sensitivity.

sensitivity for all dyes as the alkyl chain lengths ‘X’ and ‘Y’ increase.

To ascertain dye selectivity, interferences were studied by using 0.5 mol dm^{-3} standard solutions of various anions. Sulphite and the *pseudo*halide, isothiocyanate, were shown to be effective fluorescence quenchers with large Stern–Volmer constants. Whilst one would not normally expect to find these ions in serum and urine, other *pseudo*halides, such as the anaesthetic ‘Halothane’ may be present and therefore detectable by these dyes. No interferences were observed with nitrate, sulphate or phosphate ions.

Table 2 shows the excitation and emission wavelength maxima of dyes **1**–**12** and their solubility in mildly alkaline solution, (pH 10). It was generally observed that the BPh_4^- dye salts were slightly less water soluble than the corresponding bromide salts. Whilst at first this may seem a hindrance in aqueous halide sensing, it is generally observed that sensor devices, where dyes are immobilised in polymeric supports, require a degree of organophilic character to achieve compatibility within the support [11,12,13], and hence the BPh_4^- salts may give better polymer compatibility than the Br^- salts.

Table 2
Excitation, λ_{ex}, and emission wavelength, λ_{em}, maxima for dyes 1–12 and their solubility in mildly alkaline solution, pH 10

Dye	Wavelength maxima		Water solubility
	λ_{ex} (nm)	λ_{em} (nm)	
1	360	460	Very good
2	360	460	Very good
3	360	460	Very good
4	360	460	Good
5	360	460	Good
6	360	460	Good
7	390	460	Good
8	390	460	Good
9	390	460	Good
10	390	460	Fair
11	390	460	Fair
12	390	460	Fair

Dyes **1**–**12** are highly fluorescent with emission wavelength maxima of ≈ 460 nm, some 70–100 nm Stokes shifted from their absorption maxima. The ease of discrimination between both the excitation and emission wavelengths (which would minimise dye–dye energy transfer kinetics if the dyes were immobilised within a support [14]), their good water solubility and their sensitivity/selectivity towards halide ions, offers good prospects for use in halide sensor devices.

4. Conclusions

We have described the synthesis and characterisation of 12 new highly fluorescent water soluble dyes, which are sensitive to aqueous halide at concentrations of both commercial and physiological interest, i.e. $\leqslant 0.1$ mol dm^{-3}.

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