Chloride sensitive probes for biological applications

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Abstract

Three fluorescent probes have been produced by the quaternisation of 6-methylquinoline with methyl bromide, methyl iodide and 3-bromo-1-propanol. The probes are water-soluble and their fluorescence is quenched by modest concentrations of chloride ions. One of the dyes, dye **1**, has a chloride Stern–Volmer constant of 255 mol^{-1} dm^3, which is one of the highest reported. Fluorescence quantum yields of the dyes have been determined using quinine sulphate as a reference and are all found to be similar lying in the range 0.5–0.64. Applications for chloride determination in biological systems using these probes are discussed. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

By considering previous work [1–3] and subsequently realising structural–reactivity relationships of the quinoline nucleus, we have successfully produced 3 dyes with improved chloride sensitivity, (Fig. 1). The dyes have been characterised both in terms of their fluorescence properties and their chloride sensitivity, which is effectively depicted by the magnitude of the Stern–Volmer constant, K_{SV}.

For a single emitting dye species in solution, "dynamic" or more commonly referred to as "collisional" fluorescence quenching, is known to follow Stern–Volmer kinetics, Eq. (1).

$$\frac{F'}{F} = \frac{\tau'}{\tau} = 1 + K_q \tau'[Q] = 1 + K_{SV}[Q] \qquad (1)$$

Here, F', τ' and F, τ are the fluorescence intensities and lifetimes in the absence and presence of quencher, Q, respectively. K_{SV} is the Stern–Volmer constant, the magnitude of which governs the halide concentration range detectable and K_q is the bimolecular quenching constant.

2. Experimental

2.1. Instrumentation and Stern–Volmer analysis

All reagents were purchased from the Aldrich Chemical Company and were used as received. The purity of 6-methylquinoline was verified using thin layer chromatography. ^{1}H NMR spectra were recorded on a Jeol GSX400 (400 MHz). IR spectra were recorded on a Nicolet 5DXC FTIR spectrometer and absorption spectra on a Perkin Elmer UV–vis spectrometer, Lambda 2. Elemental analysis and positive-ion electrospray mass spectroscopy were carried out by Crosfield Ltd.
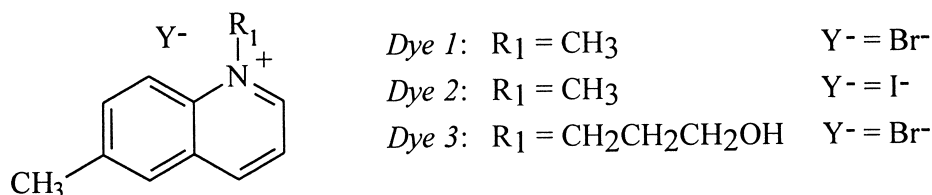
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Fig. 1. Structural formulae of dyes **1**–**3**.

Steady-state Stern–Volmer constants were determined by exciting 10^{-5} mol dm^{-3} dye solutions at 350 nm and determining the fluorescence intensity measured at 443 nm (integrated for 5 s until the standard error was better than 0.25%) as a function of halide concentration. Stern–Volmer constants were calculated using data collected in the halide concentration range 0.1–0.01 mol dm^{-3} (a region of important medical interest [1]), using the Microsoft Excel least squares linear regression analysis program, where regression coefficients were typically unity, i.e. $R^2 = 1$. All samples were measured in $4 \times 1 \times 1$ cm plastic cuvettes (Hughes and Hughes).

2.2. Quantum yield determination of dyes 1–3

The fluorescence quantum yield, ϕ, of dyes **1**–**3** was determined using quinine sulphate (99.9%) in 0.5 M, 1 N sulphuric acid as a reference standard, $\phi = 0.546$ [4]. Subsequently, ϕ for dyes **1**–**3** was calculated from Eq. (2).

$$\phi = \phi_R \frac{F}{F_R} \frac{OD_R}{OD} \frac{n^2}{n_R^2} \qquad (2)$$

where, F and F_R denote the integral of the sample and reference corrected fluorescence spectra respectively, OD is the optical density (which is typically < 0.05 to avoid inner filter effects) and n is the refractive index of the medium. A refractive index correction was deemed unnecessary as both the standard and sample were measured in sulphuric acid.

2.3. Synthetic procedure

The nitrogen heteroatom of 6-methylquinoline was quaternised with methyl bromide, methyl iodide (iodomethane) and 3-bromo-1-propanol to produce dyes **1**–**3** respectively (Fig. 2), which was based on the procedures previously described by Wolfbeis and Urbano [5] and Geddes et al. [3,6].
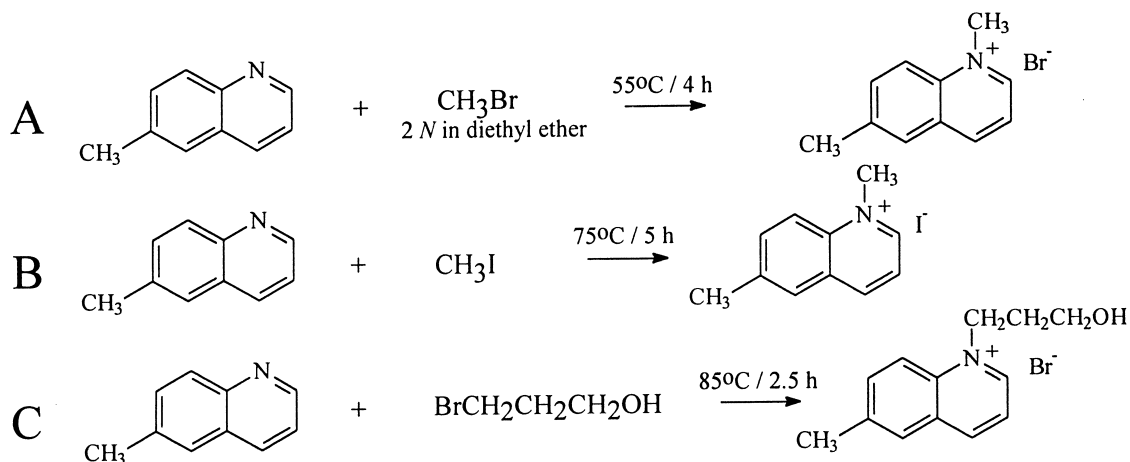

Fig. 2. The quaternisation of the nitrogen heteroatom of 6-methylquinoline with A, methyl bromide; B, methyl iodide; and C, 3-bromo-1-propanol to produce dyes **1**–**3** respectively.

This procedure has several advantages in that both the products and heterocyclic starting material can easily be separated as they are polar and hence water soluble and relatively nonpolar respectively. This also allows for simple and quick bench top product purity checks using thin-layer chromatography.

2.3.1. Synthesis of dye 1

6-Methylquinoline (9.98 g, 69.7 mmol) and (60 ml, 120 mmol) methyl bromide (2 N in diethyl ether) were heated under reflux at 55°C, under the exclusion of moisture for 4 h. After cooling and the addition of 75 ml diethyl ether the mixture was stirred for 1 h and then the ether decanted. Continual washings of the product with diethyl ether gave 4.54 g (27.35%) white product. The structure was characterised by: ^{1}H NMR (D$_2$O): δ 2.5 (s, 3H), 4.5 (s, 3H), 7.8–9.0 (m, 6H); IR (KBr): 1531, 1589, 2341, 2360 and 3005 cm^{-1}; UV–vis (pH 7, H$_2$O): λ_{max} 320 nm and ms: m/z 158 (M$^+$). Anal. calcd for C$_{11}$H$_{12}$NBr: C, 55.49; H, 5.04; N, 5.88. Found: C, 54.54; H, 4.94; N, 5.57.

2.3.2. Synthesis of dye 2

6-Methylquinoline (9.98 g, 69.7 mmol) and (9.95 g, 70.1 mmol) methyl iodide were heated under reflux at 75°C for 5 h. After cooling and the addition of 50 ml diethyl ether the mixture was stirred for 1 h and then the ether decanted. Continual washings of the product with diethyl ether gave 17.52 g (88.17%) yellow product. The structure was characterised by: ^{1}H NMR (D$_2$O): δ 2.5 (s, 3H), 4.5 (s, 3H), 7.8–9.0 (m, 6H); IR (KBr): 1531, 1584, 2336, 2370 and 2981 cm^{-1}; UV–vis (pH 7, H$_2$O): λ_{max} 320 nm and ms: m/z 158 (M$^+$). Anal. calcd for C$_{11}$H$_{12}$NI: C, 46.35; H, 4.21; N, 4.91. Found: C, 46.16; H, 4.19; N, 4.74.

2.3.3. Synthesis of dye 3

6-Methylquinoline (5.06 g, 35.3 mmol) and (4.82 g, 34.7 mmol) 3-bromo-1-propanol were heated under reflux at 85°C for 2½ h. After cooling and the addition of 30 ml acetone, the mixture was stirred for 1 h and then the acetone decanted. Continual washings of the product with acetone gave 5.91 g (60.40%) white product. The structure was characterised by:
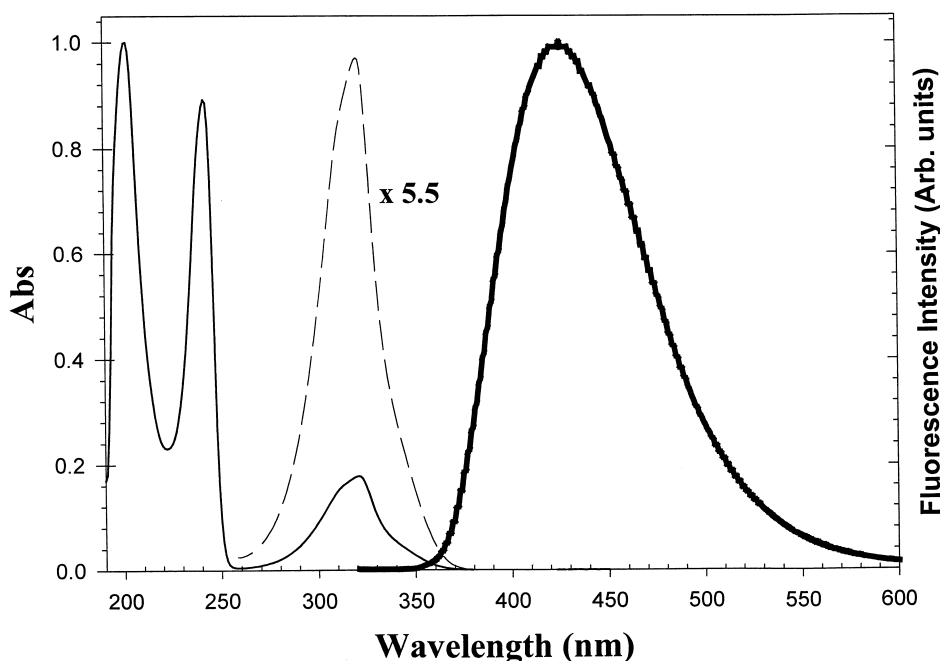

Fig. 3. Absorption (——) and emission (——) spectra of dye **1** in 0.5 M, 1.0 N H$_2$SO$_4$. The absorption band (– – –) at 320 nm has been multiplied by a scaling factor of 5.5 for clarity.

Table 1
Absorption λ_{abs} and emission λ_{em} wavelength maxima for dyes **1–3** in various solvents[a]

Solvent	Dye **1**		Dye **2**		Dye **3**	
	λ_{abs} max (nm)	λ_{em} max (nm)	λ_{abs} max (nm)	λ_{em} max (nm)	λ_{abs} max (nm)	λ_{em} max (nm)
H_2O, pH 7	320	420	320	420	320	422
0.5 M, 1 N H_2SO_4	320	422	320	422	321	425
Methanol	320	433	320	431	320	434
Propanol	320	431	321	432	321	433
Butanol	320	430	321	431	321	432

[a] The excitation wavelength λ_{ex}, was 320 nm for all dyes. The full width half maximum (FWHM) values for dyes **1–3** in 0.5 M, 1 N H_2SO_4 are 87.6, 87.5 and 87.6 nm respectively.

^{1}H NMR (D_2O): δ 2.3 (m, 2H), 2.5 (s, 3H), 3.6 (t, 2H), 5.0 (t, 2H), 7.8–9.0 (m, 6H); IR (KBr): 1516, 1594, 2336, 2360, 2947 and 3272 cm^{-1}; UV–vis (pH 7, H_2O): λ_{max} 320 nm and ms: m/z 202 (M^+). Anal. calcd for $C_{13}H_{16}NOBr$: C, 55.34; H, 5.68; N, 4.97; O, 5.68. Found: C, 55.06; H, 5.65; N, 4.74.

3. Results and discussion

The absorption, λ_{abs} and emission, λ_{em} wavelength maxima for dyes **1–3** in various solvents are shown in Table 1. Typically λ_{abs} maxima for dyes **1–3** is ≈320 nm whilst λ_{em} maxima ≈420–434 nm. Fig. 3 shows the absorption and emission spectra of dye 1 in 0.5 M, 1.0 N H_2SO_4, which is characteristic for all the dyes. An interesting feature of Fig. 3 is the large Stokes shift in emission maxima with respect to the absorption maxima, ≈100 nm, indicating a significant difference in the energy gap between the S_0 and S_1 states of the dyes in absorption and emission. For sensor applications, the ease of discrimination between these two states allows for potential chloride sensors to operate at low signal to noise ratios because the loss of excitation and emission energy can be minimised using either cut-off or band pass filters as opposed to interference filters and/or monochromators which are inherently dark. Also, being able to operate potential sensors, derived from the immobilisation of dyes **1–3**, at low signal to noise ratios, is an important consideration at high quencher concentrations where deviations from Stern–Volmer behaviour typically limit the halide detection

Table 2
Quantum yield, ϕ, for dyes **1–3**

Dye	ϕ^a
1	0.50±0.05
2	0.55±0.05
3	0.64±0.05

[a] ϕ for dyes **1** and **2** is the mean of two separate determinations and for dye **3**, the mean of 3 separate determinations.

range as the fluorescence quenching kinetics are difficult to model [1,7].

Table 2 shows the quantum yields for dyes **1–3** obtained using quinine sulphate as a reference standard, i.e. Eq. (2). All ϕ values are similar, lying in the range 0.50–0.64 and are also similar to: β-carboline in 1 N H_2SO_4, $\phi = 0.60$; 2-aminopyridine in 0.1 N H_2SO_4, $\phi = 0.60$ and cresyl violet in methanol, $\phi = 0.54$ [4].

Solution steady-state Stern–Volmer constants of dyes **1–3** quenched by aqueous halide ions at pH 7, 20°C are shown in Table 3. As expected the halide sensitivity for all dyes is in the order $I^- > Br^- > Cl^-$. The Cl^- K_{SV} for dye **1** is larger than the Cl^- K_{SV} for all the other dyes and is in fact one of the highest reported in the research literature [1,4], more than double that of the SPQ[1] molecule, Cl^- $K_{SV} = 118$ M^{-1}, $\phi = 0.69$ [4], which has been used to measure intra-cellular chloride levels and ion transport across cell membranes [8] as well as being the transduction element in a recent plastic halide sensor [7]. Other notable

[1] 6-Methoxy-*N*-(3-sulfopropyl) quinolinium — SPQ [2,4,7,8].

Table 3
Steady-state Stern–Volmer constants[a], K_{SV} (mol^{-1} dm^3), for dyes
1–3 in doubly distilled deionised H_2O, pH 7, measured at 20°C

Dye	K_{SV} I^-	K_{SV} Br^-	K_{SV} Cl^-
1	645	421	255
2	370	266	162
3	526	346	219

[a] Stern–Volmer constants have been corrected to three significant figures.

chloride sensitive probes include MACA[2], Cl^- $K_{SV} = 225$ M^{-1}, MAMC[3], Cl^- $K_{SV} = 160$ M^{-1} and Lucigenin[4], Cl^- $K_{SV} = 390$ M^{-1} [4].

For immobilised dye type sensors then large K_{SV} values are also favoured since the immobilisation of the halide sensitive probe nearly always results in a smaller K_{SV} value, due to a reduction in the bimolecular quenching constant, K_q, because the accessibility of the quencher to the dye is hindered by the sensor support [7].

One other interesting feature of Table 3 can be seen from a comparison of the K_{SV} values for dyes **1** and **2** with respect to their molecular structures, where the dye counter-ion has a significant influence on the magnitude of K_{SV}. A further detailed discussion of the photophysics of these new probes will be published shortly.

4. Conclusions

The authors have described the synthesis of three fluorescence probes and characterised them in terms of their aqueous halide ion sensitivity and fluorescence quantum yield. One of the probes, dye **1**, has a Cl^- K_{SV} and ϕ of 255 mol^{-1} dm^3 and 0.50 respectively. The probes offer new opportunities to probe low aqueous physiological halide concentrations, such as those required for Cystic Fibrosis diagnosis, where Cystic Fibrosis patients typically have elevated chloride levels of > 60 mmol l^{-1} as compared to non-cystic fibrosis individuals < 40 mmol l^{-1} [1].

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[3] *N*-methylacridinium-9-methylcarboxylate — MAMC [4].
[4] *N,N′*-dimethyl-9,9′-bisacridinium nitrate — Lucigenin [4].