

New fluorescent quinolinium dyes — applications in nanometre particle sizing

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Received 13 July 1999; accepted 16 August 1999

Abstract

Bromide, iodide and tetraphenylborate quaternary salts of four new highly fluorescent dyes have been produced by the reaction of the 6-methoxyquinoline heterocyclic nitrogen base with 3-bromo-1-propanol, methyl iodide and methyl bromide with one of the dyes having its counter-ion exchanged for the tetraphenylborate ion. Three of the dyes, unlike the base, are readily water soluble, the tetraphenylborate salt only slightly water soluble and all are fluorescent over a broad pH range. The dyes have been characterised in terms of their water solubility, their steady-state fluorescence spectra and their bi-exponential fluorescence lifetimes, which are all found to be in the range ≈ 10 –30 ns. We have found that the cationic dyes readily bind to negatively charged colloidal silica particles and because of the suitably long fluorescence lifetimes we have been able to determine the particle size of Du Pont's SM30 colloidal silica using time-resolved fluorescence anisotropy. The average particle diameter, determined using three of the dyes, was 6.8 ± 0.6 nm, which compares well with Du Pont's mean value of 7 nm. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Fluorescent dyes; Fluorescence anisotropy; Nanometre particle sizing; Ludox colloidal silica

1. Introduction

Over the past 15 years quinolinium type dyes, particularly those based on the 6-methoxyquinoline nucleus, have assumed an ever more important role, their uses being multifarious [1–5]. They have been used by Geddes et al. [1,2] supported in polymeric thin films, by Jiwan and Soumillion [3] bound in sol–gel films and by Urbano et al. [4] immobilised on the surface of glass slides, as sensor transduction elements for the determination of aqueous halide ions, since their fluorescence is

known to be dynamically quenched by halide ions. Wolbeis et al. have reported their use as a titration indicator in argentometry [5]. 6-Methoxyquinoline also plays an important role in pharmaceutical applications, the cinchona alkaloid quinine, which is used as an antimalarial and the quinidine derivatives which are used as antipyretics and depressants of cardiac fibrillation, are all derived from the 6-methoxyquinoline nucleus [6].

In this paper we present the synthesis of four new fluorescent dyes, based on the 6-methoxyquinoline nucleus and a further application for these type of dyes, namely, particle sizing. By introducing these dyes into a colloidal dispersion of silica particles, Ludox SM30, and measuring the time-resolved anisotropy, we were able to measure a mean silica particle size. This was possible as the

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anisotropy decay is interpreted in terms of one rotational correlation time corresponding to cationic dye bound to negatively charged silica particles. The dyes shown in Fig.1 have been named, in accordance with their emission wavelength maxima in H₂O, pH 7.

2. Theory

The theory and application of fluorescence depolarisation has been reviewed [7,8], the relevant parts are summarised below.

Basically, by recording vertically and horizontally polarised fluorescence decay curves, $F_V(t)$ and $F_H(t)$, orthogonal to vertically polarised excitation, an anisotropy function $R(t)$ can be generated, as shown in Eq. (1).

$$R(t) = \frac{F_V(t) - F_H(t)}{F_V(t) + 2F_H(t)} \quad (1)$$

For an unbound spherical rigid rotor in an isotropic solvent, or in our case, for a dye bound to rotating silica particles, the decay of $R(t)$ which describes depolarisation of the fluorescence due to Brownian rotation can be expressed by Eq. (2).

$$R(t) = R_0 \exp(-t/\tau_r) \quad (2)$$

where R_0 is the initial anisotropy with a maximum value of 0.4 and, in the simplest case, τ_r is described by the Stokes–Einstein equation [Eq. (3)],

$$\tau_r = \eta V/kT \quad (3)$$

in which η is the viscosity, $V = 4/3\pi r^3$, the hydrodynamic molecular volume, T the temperature and k the Boltzmann constant.

It is generally observed that for a hindered molecular rotation, such as for a fluorophore bound to a macromolecule, which rotates on a

much longer time scale than the fluorescence, or in an ordered media such as a lipid bilayer membrane in the gel phase, the fluorescence is only partially depolarised, leading to a residual anisotropy function R_∞ , given by Eq. (4).

$$R(t) = (R_0 - R_\infty) \exp(-t/\tau_r) + R_\infty \quad (4)$$

In this case, τ_r reflects the vicinal viscosity close to the binding site which may be markedly different from that in bulk solution described by Eq. (2).

We have found that Eq. (2) provides the best description of dyes in Ludox SM30, with Eq. (4) providing only an adequate description, often showing evidence for over parameterisation, such as negative residual anisotropy values.

3. Experimental

3.1. Materials

All reagents were purchased from the Aldrich Chemical Company and used as received.

3.2. Sample preparation

All aqueous dye solutions were made from doubly distilled water to give concentrations of 10^{-5} mol dm⁻³. All other solvents were spectroscopic grade, where available. For silica particle size determinations, dye salts were stirred with Ludox SM30, without dilution, to give concentrations of $\approx 10^{-5}$ mol dm⁻³, cast into $1 \times 1 \times 4$ cm polystyrene cuvettes (Hughes and Hughes Ltd) and immediately sealed to prevent autogelation, a consequence of water loss from colloidal silicas.

3.3. Instrumentation

¹H NMR spectra were recorded on a Jeol GSX400 (400 MHz) spectrometer and chemical shifts are reported in δ ppm relative to tetramethylsilane in deuteriochloroform. IR spectra were recorded on a Nicolet 5DXC FTIR spectrometer and absorption spectra on a Perkin–Elmer UV–vis spectrometer, Lambda 2. Steady-state fluorescence emission and excitation spectra for dye solutions were recorded

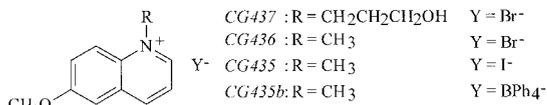


Fig. 1. New fluorescent dyes.

on a Perkin–Elmer luminescence spectrometer LS50B, and all emission spectra are uncorrected with respect to the instrumental profile. Steady-state fluorescence emission spectra for dye salts in Ludox SM30 colloidal silica were recorded on a Jobin Yvon SPEX Fluoromax 2, where the spectra are corrected for both the instrumental profile and the low background intrinsic fluorescence from Ludox SM30. The intrinsic fluorescence had no noticeable effect on the time-resolved fluorescence decay kinetics. Elemental analysis and positive-ion electrospray mass spectroscopy were carried out by the Science Support Group, ICI, Wilton.

Time-resolved fluorescence measurements were made using the single photon counting technique [9]. The excitation source was a hydrogen filled coaxial nanosecond flashlamp [10] with wavelength selection obtained using a monochromator with 20 nm bandpass. Unless otherwise stated, excitation was centred at 345 nm. Fluorescence emission was selected by a 390 nm cut-off filter and Glan–Thompson polarisers, which were automatically toggled between the two polarisations for anisotropy measurements and detected using a Philips XP2020Q Photomultiplier. The instrumental response was 1.67 ns fwhm. Non-linear least squares impulse deconvolution analysis of the fluorescence anisotropy data was performed using the IBH software library with a χ^2 goodness of fit criterion.

3.4. Synthesis of CG437: (6-methoxy-1-(3-propanol) quinolinium bromide)

1.90 g (12 mmol) of 6-methoxyquinoline and 1.68 g (12.1 mmol) of 3-bromo-1-propanol were heated under reflux for 2.5 h at 70°C. After cooling, 30 ml of acetone was added and the mixture was stirred continuously for 16 h. After continual washing with acetone, the resulting white precipitate was recovered to give 2.27 g (64% yield) product. The structure was characterised by: $^1\text{H NMR}$ (D_2O): δ 2.1 (**m**, 2H), 3.4 (**t**, 2H), 3.8 (**s**, 3H), 4.7 (**t**, 2H), 7.4–8.5 (**m**, 6H); IR (KBr): 1591, 1622, 1696 and 3270 cm^{-1} ; UV-VIS: (pH 7, H_2O): λ_{max} 345 nm (ϵ 3195) and ms: m/z 218 (M^+). Anal. calcd for $\text{C}_{13}\text{H}_{16}\text{NO}_2\text{Br}$: C, 52.34; H, 5.37; N, 4.69; O, 10.74. Found: C, 52.0; H, 5.50; N, 4.70; O, 10.0.

3.5. Synthesis of CG436: (6-methoxy-1-methyl-quinolinium bromide)

4.99 g (31.3 mmol) of 6-methoxyquinoline and 15 ml (30 mmol) of methylbromide (2 M in diethyl ether, anhydrous) were heated under reflux, under an argon atmosphere, at 80°C for 4 h. Whilst cooling, 100 ml of diethyl ether was added and the mixture stirred continuously for a further 18 h. After continual washing with diethyl ether, the resulting cream precipitate was recovered to give 0.16 g (2.12% yield) product. The structure was characterised by: $^1\text{H NMR}$ (D_2O): δ 4.1 (**s**, 3H), 4.7 (**s**, 3H), 7.7–9.1 (**m**, 6H); IR (KBr): 1526, 1595, 1621, 1692, 3230, 3406 and 3456 cm^{-1} ; UV-vis: (pH 7, H_2O): λ_{max} 342 nm (ϵ 3836) and ms: m/z 174 (M^+).

3.6. Synthesis of CG435: (6-methoxy-1-methyl-quinolinium iodide)

1.82 g (11.4 mmol) of 6-methoxyquinoline and 1.30 g (9.1 mmol) of methyl iodide were heated under reflux, at 75°C for 1 h. Whilst cooling, 50 ml of diethyl ether was added and the mixture stirred continuously for 16 h. After continual washing with diethyl ether, the resulting yellow precipitate was recovered to give 2.28 g (83% yield) product. The structure was characterised by: $^1\text{H NMR}$ (D_2O): δ 3.8 (**s**, 3H), 4.4 (**s**, 3H), 7.5–8.8 (**m**, 6H); IR (KBr): 1597, 1625, 1643, 1694 and 3053 cm^{-1} ; UV-vis: (pH 7, H_2O): λ_{max} 342 nm (ϵ 4006) and ms: m/z 174 (M^+). Anal. calcd for $\text{C}_{11}\text{H}_{12}\text{NOI}$: C, 43.85; H, 3.99; N, 4.65; O, 5.32. Found: C, 43.00; H, 3.70; N, 4.60; O, 5.1.

3.7. Synthesis of CG435b: (6-methoxy-1-methyl-quinolinium tetraphenylborate) (counter-ion exchange of CG435)

5 g (14.6 mmol) sodium tetraphenylboron in 50 ml doubly distilled water was pipetted slowly into 100 ml of doubly distilled water containing 1 g (3.3 mmol) of CG435. The resulting white precipitate was recovered and continual washing with doubly distilled water gave 1.36 g (83% yield) white product. The structure was characterised by: IR (KBr): 1592, 1620, 1700 and 3001 cm^{-1} ; UV-vis: (MSO): λ_{max} 345 nm and ms: m/z 174 (M^+).

4. Results and discussion

Table 1 shows the absorption and emission wavelength maxima of the dyes in different solvents. One interesting feature is the large Stokes shifted emission wavelength maxima with respect to the absorption wavelength maxima, ≈ 100 nm. This has also been observed with similar 6-methoxyquinoline quaternary salts [2] where the authors have utilised this Stokes-shifted fluorescence in sensor applications to reduce the possibility of dye–dye energy transfer [1,11]. However, these previously synthesised dyes contained long hydrophobic carboxylic acid chains which renders them less suitable for particle sizing than the new dyes described here. The dyes were all found to be highly fluorescent over the measured range, pH 1–11, and were all water soluble except for CG435b which was only sparingly soluble in water. A degree of organophilic character may enable CG435b to be used for particle size measurements in organic solvents where aqueous probes are not suitable. In Ludox SM30, the dyes typically show ≈ 10 nm hypsochromic shift compared to the dyes in other solvents (Table 1), which has been attributed to a possible dye–silica particle interaction, as no change in the emission maxima is observed in pH 10 NaOH solution. (Ludox SM30 is a dispersion of colloidal silica particles in NaOH, pH 10.2).

Whilst the steady-state emission spectra indicate one ground state species, analysis of the fluorescence decays of the “CG” dyes indicated that

they are bi-exponential with both lifetimes, τ_1 and τ_2 solvent dependent, in the range 10–30 ns (Table 2). The bi-exponential nature of 6-methoxyquinoline and its related molecules has been reported by many authors [6,12–14] and has been attributed to solvent relaxation processes on a nanosecond time scale, and since dye impurities have been eliminated in the analytical analysis, the bi-exponential behaviour of these dyes is not discussed further. The bi-exponential lifetimes were accounted for in the anisotropy analysis.

The anisotropy analysis for dyes in Ludox SM30 are shown in Table 3. We found that Eq. (2) provided the best description of the data. The particle sizes in Table 3 have been calculated using Eq. (3), where the fluid viscosity, η , was taken as 1 cp, i.e. the viscosity of water at 20°C, rather than the bulk viscosity value of 5 cp [15]. The microviscosity, i.e. the viscosity surrounding dye–silica particles, rather than the bulk viscosity to calculate silica particle sizes, has been used previously to determine the diameter of primary particles in acidic hydrogels using a *NIR*-dye with ≈ 2 ns fluorescence lifetime [16,17]. In the particle size determination we have made no allowance for the additional size of the dye molecule/s, although simple consideration of the dye structures (Fig. 1) shows that they are relatively small in comparison (dye diameter ≈ 4 – 5 Å, assuming a carbon–carbon bond length of 1.2 Å). Table 3 shows that these probes are capable of determining Ludox silica particle sizes with a degree of precision $\leq \pm 0.6$ nm, giving an average

Table 1
Absorption and emission wavelength maxima for 6-methoxyquinoline type dyes in various solvents and Ludox SM30 at 20°C

Solvent	CG437		CG436		CG435		CG435b	
	λ_{ABS} (nm)	λ_{EM} (nm)	λ_{ABS} (nm)	λ_{EM} (nm)	λ_{ABS} (nm)	$\lambda_{\text{EM}}^{\text{a}}$ (nm)	λ_{ABS} (nm)	$\lambda_{\text{EM}}^{\text{a}}$ (nm)
H ₂ O, pH 7	317,345	437	315,342	436	315,342	435	Slightly soluble	
Methanol	316,347	444	315,346	436	315,346	440	315,346	441
Ethanol	316,345	442	316,347	437	316,346	439	315,347	439
Propanol	317,347	440	316,346	437	317,346	437	316,347	439
Acetonitrile	316,347	440	315,345	437	315,345	437	315,346	436
Acetic acid	317,346	437	316,346	432	315,345	435	317,345	436
Ludox SM30 ^c	319,352	428 ^b	319,349	427 ^b	320,350	427 ^b	Slightly soluble	

^a Excitation wavelength = 345 nm. λ_{ABS} , absorption wavelength maxima (nm); λ_{EM} , emission wavelength maxima (nm).

^b Excitation wavelength = 347 nm.

^c Ludox SM30 is a dispersion of colloidal silica particles, average diameter 7 nm, in NaOH, pH 10.2 [15].

Table 2
Bi-exponential fluorescence lifetime analysis for dyes in various solvents^a

Dye/solvent	τ_1 (ns)	(Std. Dev.) (ns)	% Amplitude B_1	τ_2 (ns)	(Std. Dev.) (ns)	% Amplitude B_2	^b χ^2
CG437/H ₂ O	18.50	0.66	38.6	28.24	0.13	61.4	1.08
CG437/methanol	10.22	1.23	5.1	21.80	5.15	94.9	1.07
CG437/ethanol	12.30	0.75	21.4	19.42	0.08	78.6	0.97
CG437/propanol	13.28	0.71	41.6	19.67	0.31	58.4	1.02
CG437/acetonitrile	13.85	0.88	16.2	23.23	0.08	83.8	1.06
CG436/H ₂ O	16.19	0.24	43.4	26.08	0.15	56.6	1.11
CG436/methanol	15.22	2.67	15.5	21.10	0.19	84.5	1.14
CG436/ethanol	14.13	0.69	49.5	19.95	0.27	50.5	1.15
CG436/propanol	11.96	0.29	43.8	19.31	0.23	56.2	1.05
CG436/acetonitrile	18.46	1.72	60.7	26.34	0.56	39.3	1.06
CG435/H ₂ O	18.12	0.30	61.9	29.84	0.26	38.1	1.20
CG435/methanol	12.84	0.87	17.7	20.53	0.08	82.3	1.11
CG435/ethanol	12.86	0.28	45.4	19.44	0.13	54.6	1.02
CG435/propanol	11.15	0.23	54.5	17.94	0.14	45.5	1.11
CG435/acetonitrile	14.45	0.60	30.8	22.14	0.11	69.2	1.06

^a The intensity of fluorescence, I , at time, t , has been fitted to: $I(t) = B_1 \exp(-\frac{t}{\tau_1}) + B_2 \exp(-\frac{t}{\tau_2})$, where B_1 and B_2 are the relative amplitudes of each fluorescent component.

^b χ^2 , goodness of fit criterion.

Table 3
Fitting of anisotropy data to Eq. (2) and the subsequent particle size determination from Eq. (3)

Dye	Fitting to Eq. (2)				Particle diameter (nm), calculated from τ_{r1} [Eqs. (2) and (3)]
	τ_{r1} (ns)	(Std. Dev.)	R_0	χ^2	
CG437	$3.39e^{-7}$	$(2.97e^{-8})$	0.2482	1.20	6.44 ± 0.56^a
CG436	$3.87e^{-7}$	$(2.54e^{-8})$	0.2526	1.15	6.72 ± 0.44^a
CG435	$4.63e^{-7}$	$(2.99e^{-8})$	0.2409	1.26	7.14 ± 0.46^a

^a Errors in particle diameter are calculated from 3 times the standard deviation of the rotational time. Average of the three measurements is 6.8 ± 0.6 nm.

diameter of 6.8 nm from the three measurements, which compares quite well with the Du Pont figure of 7 nm [15]. Ludox colloidal silicas are often used as light scattering media in fluorescence lifetime studies when determining the instrumental impulse response [10]. However, our analysis showed no evidence of scattering of the excitation light from silica particles. Current investigations are underway to determine the particle size limitations of this

technique, both from the viewpoint of scattering and the lifetime of the probes.

5. Conclusions

We have described the synthesis and characterisation of four new highly fluorescent dyes with the objective of determining the mean size of colloidal silica particles. We have demonstrated their use by measuring the mean size of Du Pont's colloidal silica, Ludox SM30, $\approx 6.8 \pm 0.6$ nm, which compares well with the manufacturers quoted value of 7 nm.

Acknowledgements

We wish to acknowledge the EPSRC for research grants, including a Post Doctoral Research Fellowship held by C.D.G. and a Ph.D. CASE Studentship with Crosfield Ltd held by K.A. The comments and support of I. McKeown and G. Earl of Crosfield Ltd are also gratefully acknowledged.

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