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Halide sensing using the SPQ molecule

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Abstract

The fluorescent and halide sensitive 6-methoxy-*N*-(3-sulfopropyl)quinolinium (SPQ) molecule has been synthesised and incorporated into a hydrophilic copolymer to produce a halide sensor film. The sensor film typically swells in aqueous media allowing dye fluorescence to be quenched by the diffusion of aqueous halide ions at high pH. The steady-state Stern–Volmer constants, K_{SV}, for the sensor film towards chloride, bromide and iodide (pH 10) are 24, 65 and 123 mol^{-1} dm^3, less than half the pH 10 solution values of 79, 144 and 236 mol^{-1} dm^3, respectively. The reduction in K_{SV} values of the immobilised dye is discussed and are compared to K_{SV} values for SPQ immobilised in a hydrophobic copolymer. Fluorescence lifetime data, obtained using the single photon counting technique, of SPQ sensor films is also presented. The sensor films are reversibly capable of determining aqueous iodide, bromide and chloride with ≈1.5, 2.5 and 5% accuracy, respectively, at concentrations around 0.001 mol dm^3, where chloride concentrations in blood, sweat and some photographic processes are typically in the range 0.1–0.001 mol dm^3. The hydrophilic sensor films 90% response time to molar halide solution was typically 30 s. The sensor films were found to have a shelf life in excess of 2 years. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Fluorescence quenching; Halide; Chloride; Bromide; Iodide; Thin film; Sensors; SPQ molecule

1. Introduction

The importance of halide sensing to both medicine and industry over the past 10 years has become evident with many new sensors reported in the research literature [1–4].

One sensing mechanism that has attracted a lot of attention is the collisional quenching of fluorescence [5]. Halide collisional quenching, or often referred to as dynamic quenching, requires that a fluorescent dye is sensitive to quenching by a halide ion, which results in a decrease in fluorescence intensity and lifetime, τ, which can be described by the Stern–Volmer equation [5], Eq. (1), although the quenching of fluorescence was first described by Stokes in 1869, when he observed that the fluorescence of quinine in dilute sulphuric acid was reduced after the addition of hydrochloric acid.

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

Here, F', τ' and F, τ are the intensities 2nd lifetimes in the absence and presence of quencher, Q, respectively, K_{SV} is the Stern–Volmer constant, the magnitude of which determines the halide concentration range detectable and k_q is the bimolecular quenching constant.

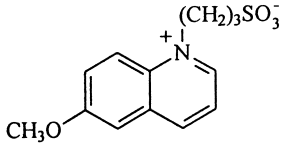
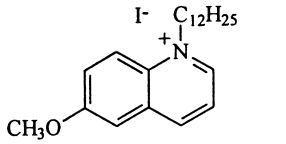
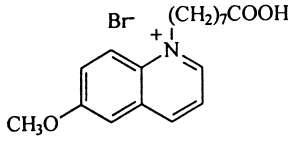
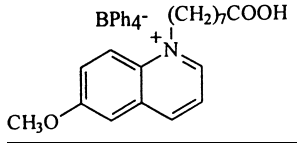
It is almost a case of historical coincidence when we realise that the first solution quenching studies performed by Stokes used the quinine molecule and that the first halide sensor, which involved the immobilisation of a fluorophore on a glass substrate [3] reported over 100 years later, also used a quinine derivative, namely, 6-methoxy-*N*-(3-sulfopropyl)quinolinium, better known today as SPQ in the research literature. It was perhaps using the hint that quinine, which contains a quinolinium ring, was sensitive to halide, that both SPQ and many other new compounds containing this ring were subsequently made. Table 1, which is meant to be informative rather than exhaustive, summarises a few quinolinium type dyes that have been synthesised along with their corresponding Stern–Volmer constants. Krapf et al. [6] have studied the structure-activity relationships for 6-methoxyquinoline and similar nuclei and have shown that the SPQ molecule offers both good water solubility and a good all round halide sensitivity.

The synthesis of SPQ was first described by Wolfbeis and Urbano in 1982 for use as a fluorescent standard in the near-neutral pH range [7], and its immobilisation onto a glass support produced the first halide sensor of this type [3]. The sensor devices were able to detect chloride, bromide and iodide in aqueous solution. The errors in determination of halide in the concentration range 0.01–0.1 mol dm^{-3} were

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Table 1
Recent quinolinium type molecules that have been used for halide sensing and their corresponding Stern–Volmer constants, K_{SV}'s

Fluorophore	Fluorophore	Halide	Solvent	Solution K_{SV}	Supported K_{SV}		Matrix	Reference
					K_{SV1}	K_{SV2}		
	6-Methoxy-*N*-(sulfopropyl)quinolinium	Cl^-	H_2O, pH 7.4	118	–[a]	–	–	[6]
		Br^-		175	–	–	–	
		I^-		276	–	–	–	
	*N*-dodecyl-6-methoxyquinolinium iodide	Cl^-	MeOH	536	0.15[b]	25.6	Sol–gel	[2]
		Br^-		583	0.46[b]	64.3		
		I^-		850	2.96[b]	281		
	6-Methoxy-*N*-(8-octanoic acid)quinolinium bromide	Cl^-	H_2O, pH 10	52	16[c]	Nq	Organic polymer	[4]
		Br^-		225	64[c]	11[d]		
		I^-		634	192[c]	66[d]		
	6-Methoxy-*N*-(8-octanoic acid)quinolinium tetraphenyl borate	Cl^-	H_2O/EtOH, pH 10	20	Nq	Nq	Organic polymer	[4]
		Br^-		70	10[c]	5[d]		
		I^-		218	47[c]	29[d]		

[a] "–" Indicates SPQ was not supported in the matrix, Nq: indicates no detectable quenching.

[b] To obtain the Stern–Volmer constants, the data has been fitted to a Stern–Volmer function which describes the heterogeneous quenching of dye in the sol–gel matrix: $F/F' = f_1/(1 + K_{SV1}[Q]) + f_2/(1 + K_{SV2}[Q])$, where f_1 and f_2 are the relative weighting factors.

[c] Unbound fluorophore (fluorophore mixed with copolymer and crosslinking agent and then cast).

[d] Fluorophore covalently bound to copolymer.

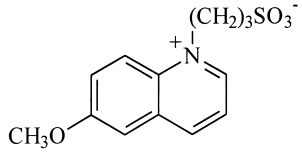


Fig. 1. 6-Methoxy-*N*-(3-sulfopropyl)quinolinium (SPQ molecule).

1% for iodide and 1.5% for bromide. The error for the determination of chloride at concentrations around 100 mM which is the level in normal blood is reported as being ≈5%. The 95% response time (the average response time for a fluorescence signal to change by 95%) was ≈40 s. They also observed that there was a 10–20% decrease in the K_{SV}'s of the immobilised SPQ as compared to SPQ free in solution. This effect has also been observed by the author using similar quinolinium type dyes in solution, covalently bound and ''loosely'' immobilised in hydrophilic hydrogels, Table 1, [4]. Whilst, inorganic SiO_2 polymers (glass) have been used as the support for previous SPQ sensors [2,3], it is somewhat surprising that organic polymers, which offer several advantages such as low cost, castability and a diverse range to choose from, have not until now been considered.

In this paper, the SPQ molecule, Fig. 1, has been synthesised and incorporated into a hydrophilic copolymer to produce a halide sensor. The hydrophilic copolymer *Quattro* [1] has been chosen as the support. This copolymer (≈50 μm dry) typically swells ≈100–200 μm in aqueous media which is ideal for aqueous anion sensors. It is readily coatable on a variety of surfaces including glass, and therefore, can be potentially used with fibre optic devices as well as having the added attraction of being crosslinkable in situ. It is cheap to produce and easily disposed of, which is an important consideration in ''throw away'' type sensors, but more importantly, SPQ is readily soluble in the Quattro copolymer. The suitability of another copolymer, *Trio* [2], is also examined and the diffusion kinetics within this relatively more hydrophobic copolymer, as compared to Quattro, are discussed. A detailed description of the importance of each comonomer unit in both Quattro and Trio and their relative hydrophilicity has been discussed previously [4,8].

The Stern–Volmer kinetics for the sensor film are reported at pH 10, since the particular interest in these sensors lies in their use in photographic processing solutions, where halide concentration is an important control parameter. The sensors reported here offer improved stability as compared to previous work [3], in that no dye degradation is observed after long term usage, and after initial film washing, no SPQ leaches from the sensor films. The response times to halide are also found to be slightly better although the overall sensitivity of the sensor films is comparable to previous work [3].

[1] *Quattro* — a hydrophilic copolymer comprising of four comonomer units [4].

[2] *Trio* — a hydrophobic copolymer comprising of three comonomer units [4].

2. Experimental

2.1. Materials

All chemicals were purchased from the Aldrich Chemical Company and used as received. 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 [9].

2.2. Instrumentation and data analysis

Steady-state fluorescence excitation and emission spectra for both SPQ solutions and sensor films were recorded on a Spex Fluorolog-2 FL340 spectrofluorometer where excitation wavelength maxima are corrected with respect to the xenon arc lamp profile. Absorption measurements for both SPQ solutions and sensor films were recorded on a Philips Unicam PU8620 UV–VIS spectrometer.

Steady-state Stern–Volmer analysis of SPQ solutions was carried out at 21°C, pH 10, using halide concentrations in the range 1–0.04 mol dm^{-3}. Subsequently, the Stern–Volmer quenching constants for aqueous halide ions were calculated using the Axum Graphics linear regression program. Whilst, Stern–Volmer analysis for aqueous SPQ solutions was carried out on the above spectrofluorometer, analysis of sensor films was carried out using an optical flow cell described previously by Geddes and co-workers [10]. The flow cell allows aqueous halide solutions, 1–10^{-3} mol dm^{-3}, to be pumped over the surface of the copolymer films, the extent of fluorescence quenching is monitored orthogonal to the plane of excitation. A step-wise increase in halide concentration allows the Stern–Volmer quenching constant, K_{SV}, Eq. (1), to be determined.

Time-resolved fluorescence measurements, made using the single photon counting technique [5], were performed using an Edinburgh Instruments, FL900 fluorescence lifetime spectrometer. The excitation source was hydrogen filled coaxial nanosecond flashlamp [11] with wavelength selection obtained using a monochromator. Fluorescence emission was selected using a monochromator and/or a cut-off filter with a Glan–Thompson polariser positioned at the magic angle. The emission was detected using a photomultiplier. The instrumental response was ≈1.5 ns fwhm. Deconvolution analysis of the fluorescence lifetime data was performed using Edinburgh Instruments deconvolution library with a χ^2 goodness of fit criterion.

2.3. Sensor film preparation

The synthesis of the Quattro copolymer, poly(2-hydroxyethyl methacrylate)$_{16}$-Co-(methacrylic acid)$_1$-Co-(methyl methacrylate)$_1$-Co-(3-chloro-2-hydorxypropyl methacrylate)$_2$, ratios (**16**:**1**:**1**:**2**), and the Trio copolymer, poly(2-hydroxyethyl methacrylate)$_{16}$-Co-(methylmethacrylate)$_2$-Co-(3-chloro-2-hydroxypropyl methacrylate)$_2$ ratios

Copolymer	A	B	C	D
Quattro	16	1	1	2
Trio	16	-	2	2

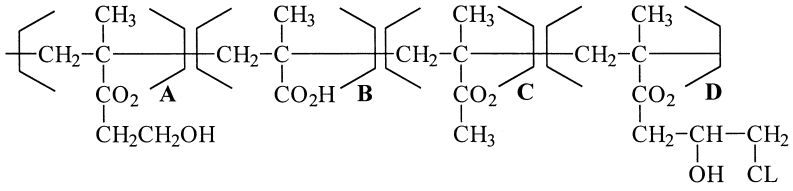


Fig. 2. The structural formulae of the Quattro copolymer poly(2-hydroxyethyl methacrylate)$_{16}$(**A**)-Co-(methacrylic acid)$_1$(**B**)-Co-(methyl methacrylate)$_1$(**C**)-Co-(3-chloro-2-hydorxypropyl methacrylate)$_2$(**D**), ratios (**16**:**1**:**1**:**2**) and the Trio copolymer poly(2-hydroxyethyl methacrylate)$_{16}$(**A**)-Co-(methyl methacrylate)$_2$(**C**)-Co-(3-chloro -2-hydroxypropyl methacrylate)$_2$(**D**), ratios (**16**:**2**:**2**). Copolymer compositions are originally based on complete conversion of the initial monomer feed concentrations [4].

(**16**:**2**:**2**), Fig. 2, have been described previously by Geddes and Douglas [4]. For the Quattro copolymer, the glass transition temperature (T_g) was 122°C, $\overline{M_n} = 91.8k$, and $\overline{M_w}/\overline{M_n}$ (the polydispersity) = 1.66.

For copolymer crosslinking, 0.5 g uncrosslinked Quattro or Trio copolymer was dissolved in 2.5 ml of ethanol by stirring for ≈2 h at room temperature, followed by the addition of 0.01 g dye (two parts per 100 copolymer, pph) and a further 2 h stirring. In order to minimise dye–dye energy transfer [5] within sensor films optical densities were kept very low, ≤0.01. The sensor film formulation, where the percentage copolymer, dye and crosslinking agent used has been optimised, has been discussed by the author previously [4]. The copolymer crosslinking mechanism has also been discussed previously [4]. After the addition of 25 pph tripropylamine (TPA) films were rapidly cast by sliding a drop of copolymer mixture between two glass microscope slides, the slides then separated and the polymer films cured in a Gallenkamp BS2 fan assisted oven at 140°C. After curing, films were washed extensively with distilled water to remove the excess TPA crosslinking agent as SPQ is known to be quenched by some amines [5]. The thickness of dry films was determined to be ≈50 μm using a Digi–Cal dead weight micrometer.

2.4. Dye leaching

Sensor films cast on glass microscope slides were immersed in one litre of buffered water (borax buffer, pH 10) at 20°C. The percentage dye remaining in films was calculated from the decrease in optical density of films as a function of time. Measurements were made using a Philips Unicam PU8620 UV–VIS spectrometer. Initial optical densities were typically ≤0.01.

2.5. Sensor response

Sensor films were cast from solutions made up in the following ratios: 2.5 ml ethanol; 0.5 g copolymer; 2 pph SPQ; 25 pph TPA and cured in hot/dry environment for 12 h. Sensor film response times were determined using the optical flow cell [10]. At the point of halide injection into the flow cell, the digital storage oscilloscope was triggered and the variation in fluorescence intensity measured with time. From the trace, the 90% response time was determined, i.e. the time for a ≈90% change in fluorescence signal, which is in accordance with previous reports [4].

3. Results and discussion

The excitation and emission wavelength maxima for SPQ in various solvents and the Quattro copolymer (i.e. sensor films) is shown in Table 2. The emission spectra are typically broad and structureless with $\lambda_{em(max)} \approx 440$ nm. They are similar to the emission spectra of protonated 6-methoxyquinoline, where $\lambda_{em(max)} \approx 445$ nm [12]. Dyes also based on the 6-methoxyquinoline nucleus have recently been reported with similar absorption and emission wavelength maxima as well as similar fluorescence lifetimes [13]. An interesting feature of the SPQ molecule and in fact an important factor in this work is the large Stokes shift in the fluorescence emission spectrum with respect to the excitation, ≈100 nm. This allows the use of cut-off filters rather than interference filters with only very little loss in excitation and emission energy and also reduces the like-

Table 2
Excitation, $\lambda_{ex(max)}$ and emission, $\lambda_{em(max)}$, wavelength maxima for SPQ in various solvents and the Quattro copolymer

Medium	$\lambda_{ex(max)}$ (nm)	$\lambda_{em(max)}$ (nm)
pH 10	320, 348	442[b]
Methanol	317, 345	444[c]
Ethanol	317, 347	442[c]
Propan-1-ol	317, 347	440[c]
Quattro film[a]	318, 352	421[d,e], 457[d]

[a] Spectra were recorded on wet films. Films were immersed in distilled water for 1 min prior to spectra being taken.
[b] $\lambda_{ex} = 348$ nm.
[c] $\lambda_{ex} = 345$ nm.
[d] $\lambda_{ex} = 318$ nm.
[e] $\lambda_{ex} = 352$ nm.

Table 3
Fluorescence kinetic data[a] for the SPQ molecule in various solvents and immobilised in the Quattro copolymer film

Medium	τ_1 (ns), %$_1$	τ_2 (ns), %$_2$	τ_3 (ns), %$_3$	χ^2 [b]
pH 10[c]	20.7, 66.18	32.1, 33.82	–	0.99
Methanol[c]	17.0, 19.78	24.0, 80.22	–	1.07
Ethanol[c]	15.7, 26.00	20.5, 74.00	–	1.10
Propanol[c]	14.3, 46.50	21.5, 53.50	–	1.11
Quattro[d,e]	2.05, 4.43	8.33, 37.25	22.1, 58.32	0.93
Quattro[d,f]	2.40, 6.85	9.35, 38.16	22.9, 54.99	0.93

[a] The intensity of fluorescence, I, at time, t, for SPQ in various solvents has been fitted to: $I(t) = B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2)$, where B_1 and B_2 are the relative amplitudes of each fluorescent component. For SPQ sensor films, the intensity of fluorescence has been fitted to: $I(t) = B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2) + B_3 \exp(-t/\tau_3)$.
[b] χ^2 — Goodness of fit criterion.
[c] $\lambda_{ex} = 337$ nm, $\lambda_{em} = 460$ nm.
[d] $\lambda_{ex} = 316$ nm, $\lambda_{em} = 430$ nm.
[e] Quattro film 1 (dry).
[f] Quattro film 2 (dry).

lihood of energy transfer [5] within sensor films, a potential complication which could occur due to the type of optical geometry used in the flow cell [10].

The emission spectrum for a SPQ sensor film shows an ≈20 nm hypsochromic shift as compared to SPQ solutions and there is evidence for an additional peak at ≈457 nm, when excited at 318 nm. This suggests either two ground state species, or more likely one ground state species located in two different environments within the sensor film. The excitation spectrum is similar to that in solution with bands at 318 and 352 nm, as compared with 320 and 348 nm respectively, Table 2.

Although the emission spectra for protonated 6-methoxyquinoline and SPQ are similar their excited state fluorescence decay kinetics are different. At pH ≈1, protonated 6-methoxyquinoline, shows mono-exponential fluorescence decay kinetics with $\tau \approx 20$ ns [12], whilst, SPQ is bi-exponential with lifetimes, τ_1 and τ_2 solvent dependent, in the range ≈14–32 ns, Table 3. The bi-exponential nature of the 6-methoxyquinoline nucleus and some of its quaternary salts has been reported by several authors previously [12,13].

Fluorescence decay kinetics for SPQ-Quattro sensor films are complicated, where a 3-exponential function was the simplest found to give an acceptable fit to the fluorescence decay data. Table 3 shows the fluorescence lifetime data for two dry SPQ films, both showing similar complicated kinetics. Distribution functions of lifetimes were also tested but did not provide a simpler analysis since they gave either very broad distributions, with mean $\tau \approx 10$ ns, or distributions centred around two mean lifetimes, $\tau_1 \approx 3$–4 ns and $\tau_2 \approx 14$–17 ns, respectively. Such complicated behaviour prevented the determination of the dynamic K_{SV}'s from the time-resolved data although collisional, ''dynamic'', quenching was thought to be the quenching mechanism as no changes in the shape of the SPQ sensor films absorption spectrum was observed in the presence of different concentrations of aqueous halide and as a function of temperature. (Collisional quenching only affects the excited state of the SPQ molecule and hence for collisional quenching no changes in the absorption spectrum are observed [5].) Instead, steady-state Stern–Volmer analysis was used.

Solution steady-state Stern–Volmer plots for SPQ quenched by aqueous halide ions at pH 10 are linear with good linear regression coefficients, i.e. $R^2 \approx 1$, Fig. 3. The solution K_{SV}'s for chloride, bromide and iodide are 79, 144 and 236 mol^{-1} dm^3, Table 4, which are similar to values reported previously at a lower pH, 118, 175 and 276 M^{-1}, respectively [6]. The slight differences are thought to be due to the pH of the quenching medium. Stern–Volmer plots for sensor films, Fig. 4A, show negative deviations under conditions of very efficient quenching, i.e. at halide concentrations >0.1 mol dm^{-3}, which has been observed previously

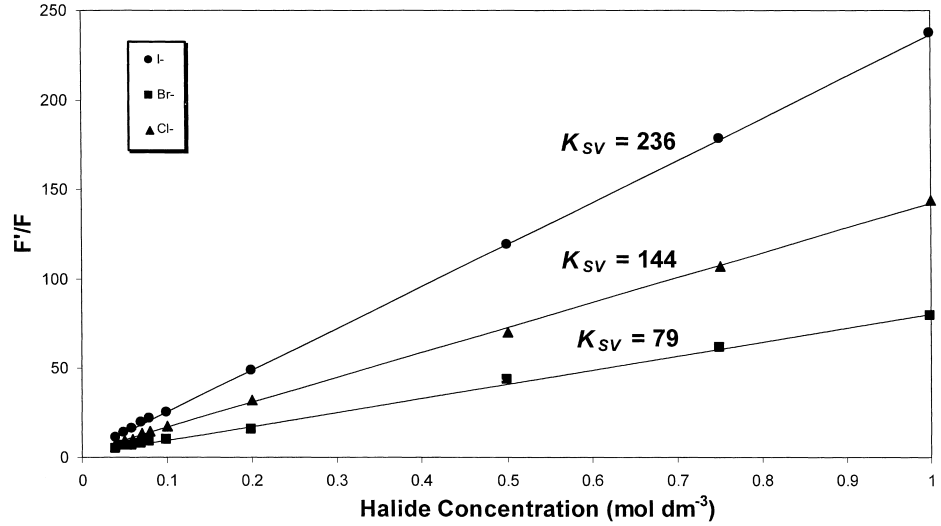


Fig. 3. Stern–Volmer plots for 10^{-5} mol dm^{-3} SPQ solutions quenched by aqueous halide ions at 21°C, pH 10. K_{SV} units — mol^{-1} dm^3.

Table 4
Stern–Volmer constants for the SPQ molecule in pH 10 solution, and the Quattro and Trio copolymer sensor films[a]

Medium	Solution K_{SV} (mol^{-1} dm^3)[a]		
	Cl^-	Br^-	I^-
H_2O/pH 10	79	144	236
Quattro copolymer	24	65	123
Trio copolymer	–[b]	10	25

[a] K_{SV}'s are accurate to within ±2%.
[b] "–" Indicates no response detected.

[4] and was attributed to the heterogeneous distribution of dye within the Quattro films. This is further supported here by the fact that fluorescence decay kinetics for SPQ in Quattro are somewhat complex as well as the appearance of an extra steady-state emission band at 457 nm when excited at 318 nm. A linear Stern–Volmer plot is generally indicative of a single class of fluorophores, all equally accessible to the quencher. However, if two fluorophore populations are present, and one class is not accessible to quencher, then negative deviations from Stern–Volmer behaviour can be observed, i.e. downward curving plots. This effect is frequently found for the quenching of tryptophan fluorescence in proteins by polar or charged quenchers where these molecules do not penetrate the hydrophobic interior of proteins, and only those tryptophan residues on the surface of the protein are quenched [5]. In contrast, a combination of both dynamic and static quenching lead to a modified form of the Stern–Volmer equation, Eq. (2), which is second-order in [Q], and is typically upward curving [5].

$$\frac{F'}{F} = (1 + K_{SV}[Q])(1 + K_{static}[Q]) \quad (2)$$

where K_{static} is the static Stern–Volmer constant.

The response of a fluorophore towards halide is known to depend slightly upon the ionic strength of the medium [5]. However, a constant nitrate background was typically used in the optical flow cell, in accordance with previous work [4,10], and so the ionic strength is though to have little effect on the Stern–Volmer kinetics reported here. Even so, for halide concentrations in the range of most biological and technological interest, i.e. 0.1–0.001 mol dm^{-3}, the sensor films gave reproducible and linear steady-state Stern–Volmer plots, Fig. 4B, which enables them to be used for halide determination in this regard. The K_{SV}'s for sensor films are typically half the pH 10 solution values and this is probably

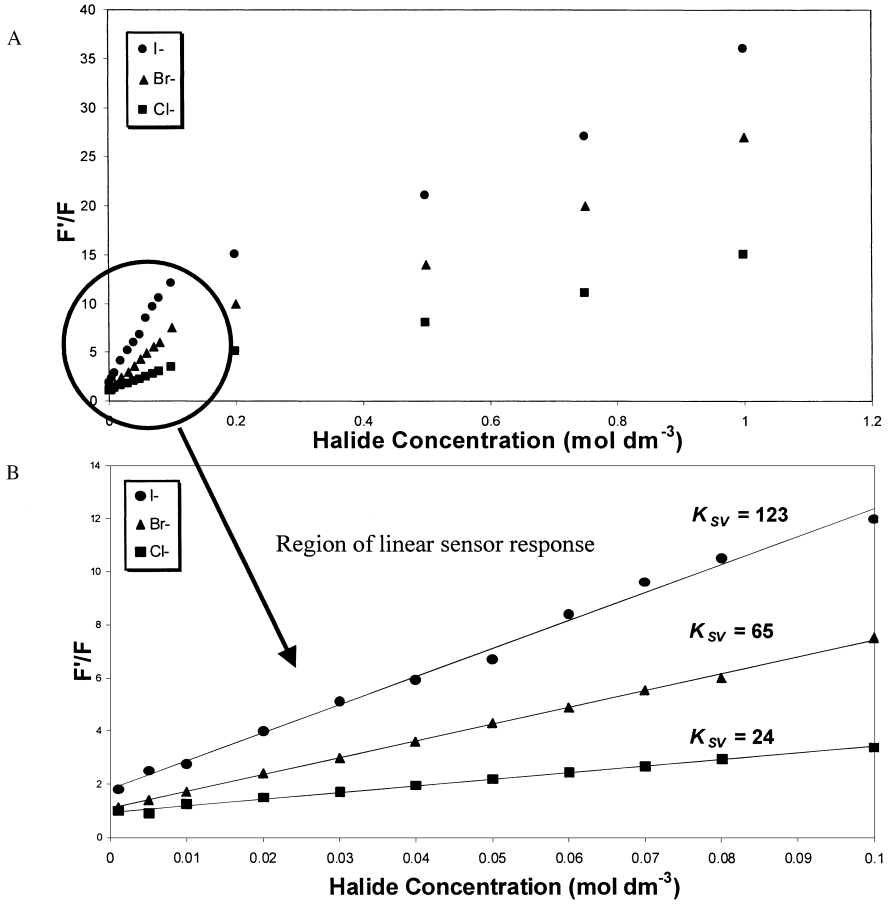


Fig. 4. (A) Stern–Volmer plots for SPQ sensor films, quenched by aqueous halide ions at 21°C, pH 10. (B) Region of linear sensor response to halide (i.e. <0.1 mol dm^{-3}). K_{SV} units — mol^{-1} dm^3.

due to both the lower halide diffusion rate in the Quattro copolymer films, i.e. the k_q term in K_{SV}, as well as a contribution from the reduced fluorescence lifetimes of SPQ in Quattro, Table 3. The K_{SV}'s for SPQ-Trio sensor films are smaller than those for Quattro. This is attributed to the fact that Trio is relatively more hydrophobic than Quattro and therefore swells less in aqueous media [4], reducing the diffusion of aqueous halide ions and subsequently reducing the K_{SV}'s. Unfortunately, fluorescence lifetime data for SPQ-Trio films is unavailable. (The hydrophilicity and swelling properties of these copolymers has been reported recently [4].) It is interesting to see from Fig. 2 that by replacing 5% methacryclic acid (w/w) with 5% (w/w) methyl methacrylate, i.e. the Trio as compared to the Quattro copolymer, then the hydrophilicity [4], swelling properties [4] and K_{SV}'s for both copolymers are quite different, which subsequently influences the magnitude of the bimolecular quenching constant, k_q. Whilst, k_q usually reflects the efficiency of quenching, here, it gives a good indication of the accessibility of quencher to the SPQ molecules, effectively reporting the local structure, enabling comparisons to be made between both copolymers. However, the lack of discrete SPQ lifetimes in sensor films restricts any further detailed characterisation.

The errors for individual halide determination using the SPQ-Quattro sensor films at concentrations of 0.001 mol dm^{-3} are typically ±1.5% for iodide, ±2.5% for bromide and ±5% for chloride. In comparison, at halide concentrations around 0.01 mol dm^{-3}, the errors are typically ±1, ±1.5 and ±4.5% for iodide, bromide and chloride, respectively. As one would expect, the sensitivity of the films is in the order: $I^- > Br^- > Cl^-$.

The 90% response times for both Quattro and Trio films are found to lie in the range 30–100 s, Table 5. The response times for Trio are typically longer than for Quattro with no response observed for the SPQ-Trio sensor film quenched by aqueous chloride. Again, this is attributed to the hydrophobicity of the Trio sensor film and has been observed previously, also using fluorophores based on the 6-methoxyquinoline heterocyclic nucleus [4]. The sensor films were found to be reversible by washing with distilled water, the dye fluorescence returning as the halide was removed. To return film fluorescence to its original unquenched intensity required washing with molar nitrate solution for approximately 3 min, where the volume of nitrate typically used was 10 times that of halide. It is interesting to note that no sensor films showed its fluorescence to be 100% quenched even when very thin films and high halide concentrations were used. This ''background'' fluorescence could be due to dye buried within the sensor films, which is not accessible by halide ions, and this may account for the negative deviations from Stern–Volmer behaviour at halide concentrations >0.1 mol dm^{-3}. Further, both the steady-state and time-resolved fluorescence data suggest multiple ground and excited state species, which is indicative of a fluorophore distributed heterogeneously throughout the sensor films. Increasing, the temperature of the quenching medium had the effect of reducing response times, which has been shown previously to be due to a greater swelling rate of the copolymer as well as resulting in a greater final swollen film volume [4]. Operating this sensor in a higher temperature medium may, therefore, reduce sensor film response times, increase sensitivity towards halide and may also extend the region of linear Stern–Volmer response.

Table 5
SPQ sensor films 90% response times to molar halide solutions

SPQ copolymer sensor films	Response time to 1 mol dm^{-3} (s)		
	Cl^-	Br^-	I^-
Quattro	30	30	30
Trio	–[a]	100	85

[a] ''–'' Indicates no response obtained.

SPQ-Quattro sensor films soaked in buffered water (pH 10, 20°C), for 5 h, typically showed only a few percent dye loss, after which time the absorbance of the sensor films remained approximately constant indicating no further dye loss. Reducing the pH to 7 had no noticeable effect on the leaching rate of SPQ from the films.

Sensor film selectivity was studied using 0.5 mol dm^{-3} solutions of various anions. No interferences were observed with sulfate, phosphate, nitrate or perchlorate ions. However, sulfite was found to be an efficient fluorescence quencher. Anion interferences can be taken into account and hence are not necessarily a problem, by making use of the *modified* Stern–Volmer equation, first proposed by Wolfbeis and Urbano in 1983 [14].

The stability of sensor films was studied using the optical flow cell [10], where the fluorescence intensity of films was monitored during irradiation with a 100 W stabilised tungsten halogen lamp. The excitation light was passed through a 320–510 nm band-pass filter and the emission monitored >520 nm using a cut-off filter. Only very slight decreases in fluorescence intensity were observed after several hours of constant irradiation, indicating good film stability, although in practice a reference sensor film could be used to correct for the decrease in fluorescence intensity due to dye degradation/bleaching. The difference in the initial fluorescence intensity of a series of SPQ sensor films was studied and found to be ±30%, which is thought to reflect the slight variation in individual film thickness.

Response times and Stern–Volmer constants for different sensor films were recorded during a 2-year-period to ascertain the ''shelf-life'' of the sensors. Both remained unchanged, indicating film stability during the 2-year-period.

4. Conclusions

A halide sensor for aqueous chloride, bromide and iodide has been produced by incorporating the SPQ molecule into a hydrophilic copolymer. The sensor film is reversibly capable

of detecting halide, with a good degree of accuracy, at concentrations of both industrial and physiological interest.

The stability and lack of SPQ leaching from the films offers good prospects for ''on-line'' industrial halide monitoring, where they could be potentially used in either quality control, e.g. to monitor halide concentrations in photographic emulsions, or even to monitor halide concentrations in industrial waste waters, an area of growing importance.

In clinical applications, one potential problem in recent years has been to design/find dyes which are both sensitive to halide ions at physiological concentrations and which also accommodate the optical properties of water and tissues. In general, the auto fluorescence from tissues or any biological sample is lower for longer excitation wavelengths [5]. At longer wavelengths, one also avoids the absorption of haemoglobin and melanin but unfortunately there are very few halide sensitive dyes that both absorb and emit within this region. The region of low absorption from 600 to 1000 nm is often called the therapeutic range and it may be possible to use multiphoton excitation of current dyes to overcome this problem, for example, 3-photon excitation of SPQ using a 800 nm Ti:Sapphire femtosecond laser. At the time of preparation of this manuscript, no reports of multiphoton Stern–Volmer kinetics with respect to halide sensing have been reported.

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