Fluorescent Dyes Bound to Hydrophilic Copolymers: Applications in Aqueous Halide Sensing

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ABSTRACT: A range of halide-sensitive fluorophores were bound to two hydrophilic copolymers. Thin films of the copolymers swelled in the aqueous media, allowing dye fluorescence to be dynamically quenched by the diffusion of halide ions. The resultant sensor films were characterized in terms of their hydrophilicity, sensitivity, and selectivity toward halide. The sensor films were reversibly capable of determining aqueous bromide and iodide at a mildly alkaline pH with typical 90% response times of 30–70 s and a shelf life in excess of 2 years. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 76: 603–615, 2000

Key words: fluorescence; hydrophilic; copolymer; halide; sensor

INTRODUCTION

Halide determination is important in many areas of everyday life, including foodstuffs,[1,2] industry,[3,4] and medicine.[5–8] Our particular interest in plastic halide sensors lies in their potential use in photographic processing equipment, where halide concentration is an important control parameter. Photographic processing is often carried out in mildly alkaline solutions, with halide concentrations ≤ 0.1 mol dm^{-3}.

Over the past 10 years there has been extensive literature on the development and characterization of luminescent-based plastic sensors, especially for the detection of oxygen,[9–12] carbon dioxide,[13,14] and ammonia.[15–16] However, relatively little attention has been paid to thin-film aqueous halide sensors.[17–19] Most notable to date is the work by Bright et al.,[18] who have used cellulose supports to immobilize harmane dyes and the work by Jiwan et al.,[19] who have used sol–gel matrices to support quinoline dyes.

The use of fluorescence quenching of immobilized dyes as a method of halide detection was first utilized by Wolfbeis et al.[17] in 1984, where quinoline dyes were immobilized onto a glass substrate, although the fluorescence quenching of fluorophores by halide ions was first described by Stokes[20] as early as 1869, when he observed that the fluorescence of quinine in dilute sulfuric acid was reduced after the addition of hydrochloric acid. The process that he observed is now commonly referred to as "dynamic fluorescence quenching," in which 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[21,22] where

$$I_0/I = 1 + k_q\tau^0[Q] \tag{1}$$

which can be used to obtain values of $k_q\tau^0$ (the Stern–Volmer constant, K_{SV}) by plotting I_0/I as a function of $[Q]$. I_0 and I are the fluorescence intensities in the absence and presence, respectively, of Q; k_q is a specific constant describing

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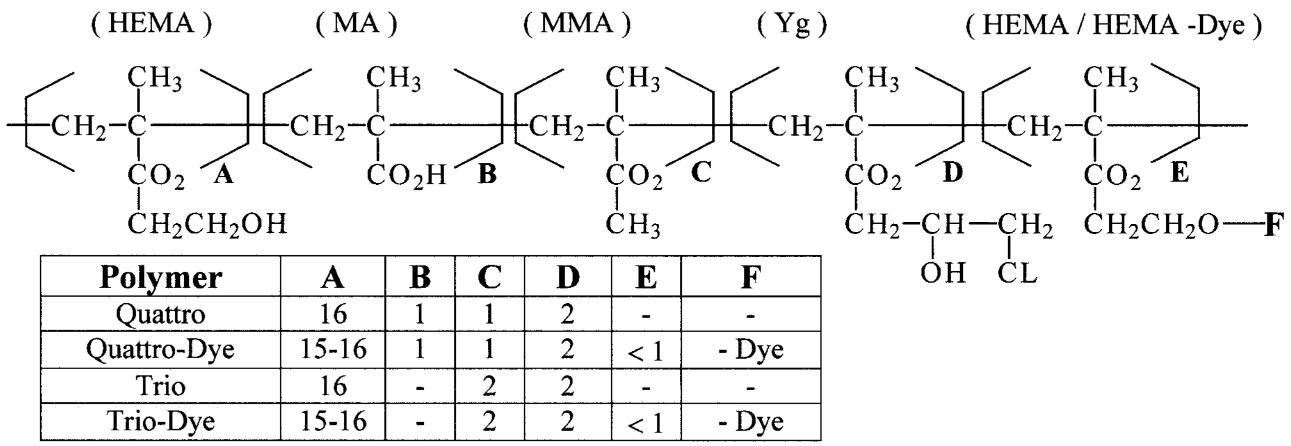


Polymer	A	B	C	D	E	F
Quattro	16	1	1	2	-	-
Quattro-Dye	15-16	1	1	2	< 1	- Dye
Trio	16	-	2	2	-	-
Trio-Dye	15-16	-	2	2	< 1	- Dye

Figure 1 Structural formulae of the Quattro copolymer poly(2-hydroexthyl methacrylate)$_{16}$-*co*-(methyl methacrylate)$_1$-*co*-(methacrylic acid)$_1$-*co*-(3-chloro-2-hydroxypropyl methacrylate)$_2$, ratios (16:1:1:2), dye bound to the Quattro copolymer, the Trio copolymer poly(2-hydroxyethyl methacrylate)$_{16}$-*co*-(methyl methacrylate)$_2$-*co*-(3-chloro-2-hydroxypropyl methacrylate)$_2$, ratios (16:2:2), and dye bound to the Trio copolymer.

bimolecular collisional deactivation of electronic energy; and τ^0 the natural fluorescence lifetime.

We have selected a hydrophilic copolymer, known as Quattro,[23] as the polymeric support for our halide-sensitive fluorophores. The copolymer ($\approx$50 microns dry) typically swells (100–200 microns in aqueous media, which is ideal for aqueous anion sensors. This copolymer can be readily coated on a variety of surfaces and can be crosslinked *in situ.* It is cheaply produced and easily disposed of, which are important considerations in throwaway-type sensors, but more important, the dye and dye counterions are readily soluble in this copolymer. A detailed description of the importance of each comonomer unit in the Quattro copolymer has been discussed previously;[23] a few of the key features are reviewed below.

The HEMA (2-hydroxyethyl methacrylate) comonomer unit is the principle source of hydroxyl groups that fulfil a dual function in the final coated copolymer film. First, they render the polymer susceptible to well-known activation chemistries and to subsequent dye attachment. And, second, their presence confers hydrophilicity to the coated copolymer, which is an important consideration in aqueous-sensing applications. In an aqueous environment it is the specific conformation assumed by the HEMA monomer unit rather than the general molecular structure that renders the copolymer hydrophilic. In this conformation the pendent —OH groups tend to be drawn to the surface, where they are stabilized by H-bonding interactions with the surrounding water molecules. However, when films are cast in air (air = ~79% N_2) on a hydrophobic surface, the resultant polymer films are very different. The —OH groups are now buried within the polymer, a conformation thought energetically favorable. This *template effect* is not uncommon and has been reported elsewhere.[24,25] One way to disrupt this internal hydrogen bonding, known as *group insertion*, is to insert a further comonomer, methyl methacrylate (MMA), which lacks the —OH substituent, thus increasing copolymer hydrophilicity when films are cast in air. Methacrylic acid (MA) was included to increase hydrophilicity and provide anionic sites on the copolymer, while 3-chloro-2-hydroxypropyl methacrylate (Yg) was essential to confer the base-induced crosslinkability of the copolymer. The structure of this random quattropolymer, $(\mathrm{HEMA})_{16}(\mathrm{MMA})_1(\mathrm{MA})_1(\mathrm{Yg})_2$, is shown in Figure 1, where the molar ratios of the component monomers are 16:1:1:2, based on the complete conversion of the monomer feed concentrations.

Early unbound-dye (referring to dye mixed in the copolymer solution before casting and crosslinking unlike bound dye, which is covalently attached to copolymer) sensor film studies, where the dye is simply mixed with a copolymer and crosslinking agent, showed significant amounts of dye leaching from the films[26] during fluorescence quenching measurements. To address this problem, the dyes have been covalently attached to the copolymer to give bound-dye sensor films (Fig. 1). Dye counterions have also been changed to improve dye–polymer compatibility[12–14] and to remove the possibility of fluorescence quenching by the counterions themselves. We have also examined the suitability of another new copolymer, Trio $[(\mathrm{HEMA})_{16}(\mathrm{MMA})_2(\mathrm{Yg})_2]$, which is based on the Quattro copolymer (Fig. 1), comprising three comonomer units.

In this article several sensor-film formulations are discussed. The sensor films are reversibly capable of determining aqueous bromide and iodide with ≈4% and 2% accuracy, respectively, at concentrations of around 10^{-3} mol dm^{-3} and are more sensitive than previous plastic sensor fabrications.[18]

EXPERIMENTAL

Materials

The synthesis of dyes 1–12 has been described previously.[26] Dye synthesis gives bromide salts (dyes 1–6), and counterion exchange was carried out to remove the bromide counterions that otherwise might themselves act as quenchers. The counterion BPh_4^- was chosen as an organophilic counterion (dyes 7–12) in the hope that counterion exchange would also improve polymer–dye compatibility.[12–14] All chemicals were purchased from the Aldrich Chemical Company and used as received except for the monomers 3-chloro-2-hydroxypropyl methacrylate (Yg), 2-hydroxy-ethyl methacrylate (HEMA), and methyl methacrylate (MMA), which were purchased from Poly Sciences. All monomers were nitrogen purged to remove oxygen prior to polymerization.

Number average molecular weights of copolymers were determined by size-exclusion chromatography, using Polymer Laboratories poly(methyl methacrylate) standards, covering the range 1.21 k → 1.4*M*.

Preparation of Quattro

The synthesis of the Quattro copolymer poly(2-hydroxyethyl methacrylate)$_{16}$-*co*-(methyl methacrylate)$_1$-*co*-(methacrylic acid)$_1$-*co*-(3-chloro-2-hydorxypropyl methacrylate)$_2$, ratios (16:1:1:2), has been described previously.[23] The glass transition temperature (T_g) was 122°C, (M_n = 91.8 k), and (M_w/M_n = 1.66).

Preparation of Trio

Poly (2-hydroxyethyl methacrylate)$_{16}$-*co*-(methyl methacrylate)$_2$-*co*-(3-chloro-2-hydroxypropyl methacrylate)$_2$, ratios (16:2:2), based on complete conversion of the feed concentrations, was synthesized as follows.

A three-necked flask fitted with a condenser, nitrogen inlet, and mechanical stirrer was charged with the following: 62.44 g (0.48 mol) 2-hydroxyethyl methacrylate; 6.35 g (0.06 mol) methyl methacrylate; 10.85 g (0.06 mol) 3-chloro-2-hydroxypropyl methacrylate; 4.21 g p-toluene sulfonic acid monohydrate; 0.79 g *bis*(4-*tert*-butyl cyclohexyl) peroxydicarbonate; and 250 mL (9:1 v/v) ethanol–methyl cellosolve.

The solution was continuously stirred at 50°C for 12 h. The copolymer was recovered by precipitation into an excess of diethyl ether and dried under vacuum at 40°C. T_g was 100°C, M_n = 111 k, and M_w/M_n = 1.31. ^{1}H-NMR and IR were consistent with the proposed product.

Dye Attachment to the Quattro and Trio Copolymers (Bound Sensor Films)

Dyes 1–12 and rhodamine B (Table I) were covalently bound to Quattro and Trio through an ester linkage formed between the hydroxyl groups of HEMA and the carboxylic acid functional groups of the dyes (Fig. 1). The chemistry of this simple attachment process using both DCC (dicyclohexylcarbodiimide) and DMP (4-dimethylaminopyridine) has been reviewed many times.[27–30] It was observed that solely activating the dye with DCC–DMP followed by slow addition to the respective copolymer solution typically resulted in a larger percentage of dye attachment. Although the percentage of dye attachment could simply be increased by increasing the concentrations of DCC–DMP, the percentage of dye attachments was kept low in order to both minimize the potential change in copolymer characteristics and to eliminate the possibility of dye–dye energy transfer.[31] Table I shows the quantities of dye, DCC, and DMP in 50 mL DMF (dimethylformamide), which were added to 5 g of Quattro or Trio in 50 mL of DMF. The mixtures were heated under reflux and argon at 70°C for 2 days. The mixtures were then precipitated into H_2O, dissolved in warm ethanol, and precipitated into diethyl ether. This procedure was repeated several times to remove any unbound dye. The dyed copolymers were then dried under vacuum at 40°C for 12 h and powdered using a blender. Elemental analysis, X-ray fluorescence spectroscopy, and ^{1}H-NMR indicate that the bound-dye polymer ratio for Quattro was <1% (w/w). DMF did not crosslink the copolymers. The crosslinking, casting, and curing of bound-dye copolymers for use as sensors is described below.

Unbound Sensor Films

Dissolved into 2.5 mL of ethanol by stirring for ≈ 2 h at room temperature was 0.5 g uncrosslinked copolymer (Quattro or Trio), followed by the addition of 0.01 g of dye and an additional 2 h stirring. In order to minimize dye–dye energy transfer[31] within sensor films, optical densities were kept very low, ≤ 0.01. The crosslinking of unbound sensor films is described below.

Polymer Crosslinking, Casting, and Curing

For both bound- and unbound-dye sensor films, crosslinking was carried out by the addition of 0.125 g of a polymer crosslinking agent, which was added dropwise with rapid stirring to 0.5 g of copolymer (uncrosslinked polymer for unbound- and dyed polymer for bound-dye films) and then dissolved in 2.5 mL of ethanol. Several crosslinking agents were examined. The effect of the crosslinking agent on sensor performance is discussed later. Films were then rapidly cast by sliding a drop of copolymer mixture between two glass slides, with the slides then separated and the polymer films cured in a Gallenkamp BS2 fan-assisted oven at 140°C. After curing, films were washed in distilled water to remove excess crosslinking agent. The crosslinking agents did not quench the fluorescence of dyes 1–12 or the rhodamine dyes.

The thickness of dry films, ≈50 microns, was determined using a Digi-Cal dead-weight micrometer, averaging more than 10 points on the surface of the film.

Dye Leaching

Sensor films cast on glass microscope slides were immersed in 1 L of buffered water (borax buffer, pH 10) at 20°C. Buffers of pH 10 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 deionized water.[32] 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. For sensor films where comparisons were made between the percentage or type of crosslinking agent used, films with the same initial dry thickness were used.

Polymer Film Swelling Measurements

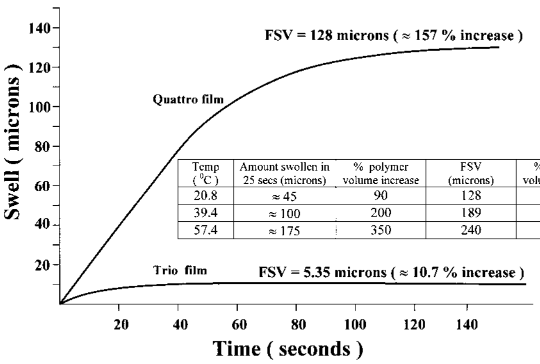
Swelling measurements were performed with a *swellometer* on copolymer films to determine their hydrophilicity and the optimum degree of crosslinking. Dry films were mounted in the sample holder of the swellometer and the dry film thickness measured, effectively zeroing the instrument. Lowering the sample into the swelling media activated a small tappet that continually moved up and down, probing the surface of the copolymer film and providing swelling data as a function of time. The temperature was 19 ± 0.5°C.

Steady-State Stern–Volmer Analysis

All steady-state emission and excitation spectra for dye solutions and sensor films were recorded on a Jobin–Yvon JY3D spectrofluorimeter. All excitation spectra were corrected with respect to the Xenon arc lamp profile. Steady-state Stern–Volmer analysis of dye solutions 1–12 was carried out at 21°C, pH 10 (borax buffer), using halide concentrations in the range 1–10^{-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. While Stern–Volmer analysis for dye solutions was carried out with a spectrofluorimeter, described above, analysis of sensor films was carried out using an optical flow cell, described previously by the authors.[26,33] The flow cell allowed aqueous halide solutions to be pumped over the surface of the copolymer films; the extent of fluorescence quenching was monitored orthogonal to the plane of excitation. A stepwise increase in halide concentration allowed the Stern–Volmer quenching constant, K_{SV}, to be determined for the respective dye–polymer combination.

Sensor Film Response Times

Sensor films were cast from solutions made up in the following ratios: 2.5 mL ethanol and 0.5 g copolymer with 2 parts per hundred copolymer (pph) dye (for unbound films) and 25 pph tripropylamine (TPA). They were cured in a hot and dry environment for 12 h. Sensor film response times were determined using an optical flow cell.[33] At the point of halide injection into the flow cell, the



Temp (°C)	Amount swollen in 25 secs (microns)	% polymer volume increase	FSV (microns)	% polymer volume increase
20.8	≈ 45	90	128	157
39.4	≈ 100	200	189	378
57.4	≈ 175	350	240	480

Figure 2 Swelling profiles for both Quattro and Trio films. Films were cast from solutions made up from the following: 2.5 mL ethanol; 0.5 g copolymer; 25 pph TPA; cured at 140°C for 12 h. All films had the same initial thickness, ≈50 microns. [Insert: the swelling of Quattro films as a function of temperature. FSV—final swollen volume of copolymer (microns)].

digital storage oscilloscope was triggered, and a measurement was taken of the variation in fluorescence intensity with time.

RESULTS AND DISCUSSION

Quattro and Trio Swelling

Swelling measurements were undertaken on Quattro and Trio films to assess film hydrophilicity. Figure 2 shows the typical swelling profile for both Quattro and Trio films cast from solutions containing 2.5 mL ethanol, 0.5 g copolymer, and 25 pph TPA and that were cured at 140°C for 12 h (film formulations are discussed later). It was found that Quattro films with a dry thickness of approximately 50 microns typically swell ≈100–200 microns compared to similar Trio films, which swell significantly less, ≈5–10 microns. The differences in the extents of swelling between these two copolymers is attributed to the lack of MA rather than the extra ≈5% w/w MMA in the Trio copolymer, as the addition of MMA is thought to increase film hydrophilicity.[23] It would appear that the presence of MA units has a significant effect on copolymer hydrophilicity.

Temperature-dependent swelling measurements on the same films (Fig. 2) show a greater swelling rate at higher temperatures. It is interesting to note that the final swollen volume (FSV) of the films is also greater at higher temperatures.

Dye Attachment

Table I shows the quantities of dye, copolymer, DCC, DMP, and the respective polymer yields for the dye-attachment procedure. Approximately 10–18% w/w of the HEMA comonomer units were targeted for attachment using these quantities, but less than 1% w/w of the dye was actually bound to Quattro, and ≈2–4% w/w was bound to Trio. The percentage of dye attachments were estimated from ^{1}H-NMR, FTIR, X-ray fluorescence spectroscopy, mass spectroscopy, and nitrogen elemental analysis in which the dye was the only source of nitrogen in the recovered polymer. It was found that using smaller concentrations of either dye or DCC–DMP resulted in no detectable attachment, while using larger quantities resulted in copolymers with larger molecular-weight distributions that were difficult to cast, resulting in sensor films with very small Stern–Volmer quenching constants. Table II shows typical M_n and T_g values for both the Quattro and Trio copolymers before and after dye attachment. Both the T_g and M_n are larger for copolymers after dye attachment.

Dye attachment yields are higher for the Trio copolymer than for the Quattro copolymer. This is as expected, since MA can compete for HEMA hydroxyl groups in the dye-attachment process. This competition reaction is probably the cause of self-crosslinking in the Quattro copolymer during dye attachment.

Polymer-swelling measurements on Quattro- and Trio-bound films (Table III) show that the bound-dye films swell significantly less than the virgin copolymers, probably because of an increase in the crosslinking density of the copolymers during dye attachment. It is also interesting to note that bound-dye films with BPh_4^- counterions typically swell less than the corresponding dye with a Br^- counterion, probably a result of the presence of large hydrophobic BPh_4^- groups.

Fluorophore leaching is a typical problem associated with polymeric-based dye sensors when a sensor film is immersed in solution. Unbound-dye sensor films soaked in buffered water (pH 10, 20°C) for 5 h typically showed a few percent dye loss. However, bound-dye sensor films show no dye loss, even after very long immersion times (Fig. 3).

Sensor Film Formulations

A study of film formulations using both Quattro and Trio revealed that films cast from solutions

Table I Quantities of Dye, DCC and DMp in 50 mL DMf, which were added to 5g Quattro or Trio in 50 mL DMF. The reaction mixtures were Continuously Stirred under argon for 2 Days.

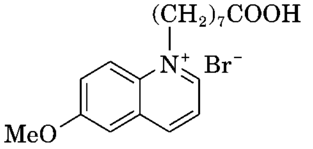
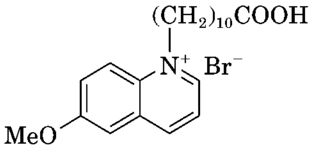
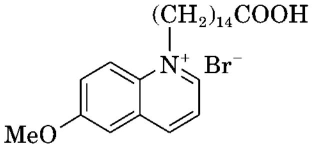
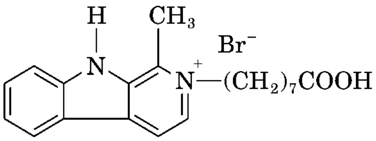
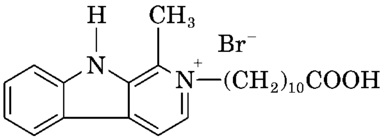
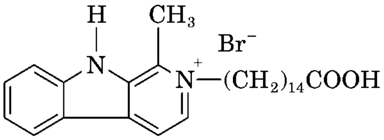
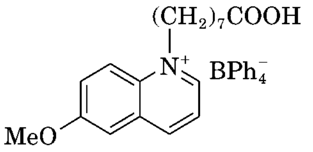
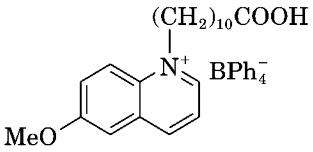
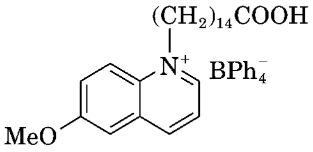
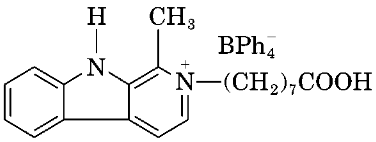
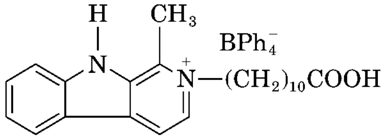
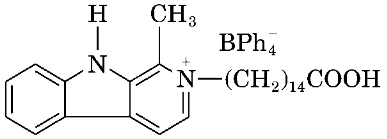
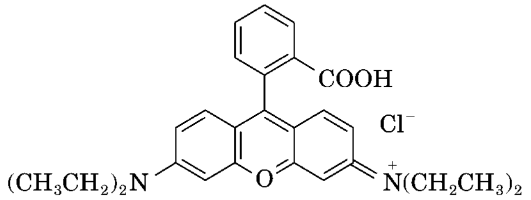
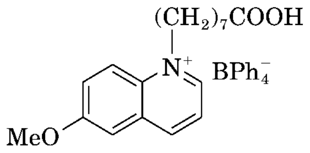
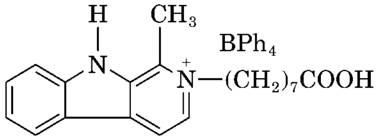
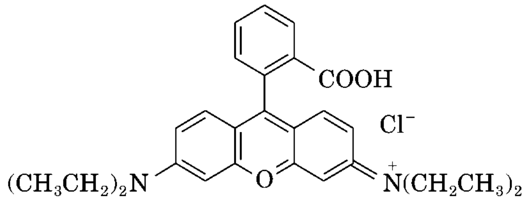
Polymer	Dye Structure	Dye	DCC	DMP	Yield
Quattro		Dye 1 0.363 g (0.95 mmol)	0.196 g	0.012g	3.21 g (59.9%)
Quattro		Dye 2 0.403 g (0.95 mmol)	0.196 g	0.012 g	3.67 g (68.0%)
Quattro		Dye 3 0.456 g (0.95 mmol)	0.196 g	0.012 g	3.60 g (65.9%)
Quattro		Dye 4 0.384 g (0.95 mmol)	0.196 g	0.012 g	3.34 g (62.0%)
Quattro		Dye 5 0.424 g (0.95 mmol)	0.196 g	0.012 g	3.20 g (58.9%)
Quattro		Dye 6 0.477 g (0.95 mmol)	0.196 g	0.012 g	3.35 g (61.2%)
Quattro		Dye 7 0.592 g (0.95 mmol)	0.197 g	0.012 g	3.28 g (58.7%)
Quattro		Dye 8 0.632 g (0.95 mmol)	0.197 g	0.012 g	3.50 g (62.1%)
Quattro		Dye 9 0.685 g (0.95 mmol)	0.197 g	0.012 g	3.23 g (56.8%)
Quattro		Dye 10 0.613 g (0.95 mmol)	0.196 g	0.116 g	2.23 g (39.7%)

Table I *Continued*

Polymer	Dye Structure	Dye	DCC	DMP	Yield
Quattro		Dye 11 0.642 g (0.95 mmol)	0.196 g	0.116 g	3.71 g (65.8%)
Quattro		Dye 12 0.695 g (0.95 mmol)	0.196 g	0.116 g	3.10 g (54.4%)
Quattro		Rhodamine B 0.456 g (0.95 mmol)	0.196 g	0.012 g	3.48 g (63.8%)
Trio		Dye 7 0.589 g (0.95 mmol)	0.196 g	0.012 g	2.56 g (45.8%)
Trio		Dye 10 0.611 g (0.95 mmol)	0.196 g	0.012 g	2.02 g (36.0%)
Trio		Rhodamine B 0.456 g (0.95 mmol)	0.196 g	0.012 g	3.63 g (66.5%)

made up in the following ratios, proved the best for practical casting: 2.5 mL ethanol and 0.5 g copolymer with 2 pph dye (unbound films) and 25 pph of a crosslinking agent. Films containing a smaller quantity of copolymer were found to be too dilute for practical casting, while films containing >1 g copolymer in 2.5 mL ethanol were typically too viscous for casting. The introduction of 2 pph dye into the mixture made no notable change to the viscosity of the copolymer solution nor did it hinder film casting. The addition of a >50 pph crosslinking agent caused the mixture to crosslink too quickly.

Both Quattro and Trio copolymers can be crosslinked *in situ* by the addition of a suitable base[23] followed by curing at a temperature of ≈140°C. TEA (triethylamine; Bpt. 89°C) and TPA (Bpt. 157°C), both suitable bases, were found to be soluble in the copolymer solution and sufficiently water soluble, which allowed the films to be washed after curing in order to remove the excess crosslinking agent.

Figure 4 shows the static leaching of unbound rhodamine 6G from films crosslinked with TEA and TPA when immersed in 1 L of buffered water (pH 10, 20°C). Films crosslinked with TEA show more significant dye leaching, which is thought to be a result of incomplete crosslinking, as the TEA quickly evaporates at 140°C. TPA was used to crosslink films in subsequent sensor studies.

Table II Molecular Weights and Glass Transition Temperatures for Some Quattro and Trio Copolymers Before and After Dye Attachment. T_gs are Quoted as the Mean of Five Measurements

Polymer	$\bar{M}_n$	T_g [Stdv]
Quattro	91.8 k	121.6 [5.6]
Quattro–Dye 7	126 k	125.2 [3.4]
Quattro–Dye 8	125 k	123.8 [8.1]
Quattro–Dye 10	106 k	132.1 [1.7]
Quattro–Rhodamine B	95.0 k	140.6 [4.9]
Trio	111 k	99.9
Trio–Dye 7	168 k	135.3 [2.5]
Trio–Dye 10	103 k	142.4 [1.1]
Trio–Rhodamine B	141 k	146.3 [3.1]

The effect of crosslinking on film swelling (Fig. 5) was studied using films that were cast from solutions containing 2.5 mL ethanol and 0.5 g Quattro with 2 pph dye and 5 → 100 pph TPA and that were cured at 140°C. The results show a minimum swelling volume occurs when ≥25 pph TPA is used, suggesting that films have reached a maximum crosslinking density with this concentration of base. Dye-leaching studies on unbound-dye sensor films containing different amounts of a TPA crosslinking agent show significant dye loss from films containing <25 pph TPA; hence a compromise between polymer swelling and dye leaching was reached, further films being cast from solutions containing 25 pph TPA. Similar results were also observed for Trio films made from solutions with the same composition. Sensor films used throughout the rest of this work were therefore cast from solutions containing 2.5 mL ethanol and 0.5 g copolymer with 2 pph dye (unbound films) and 25 pph TPA.

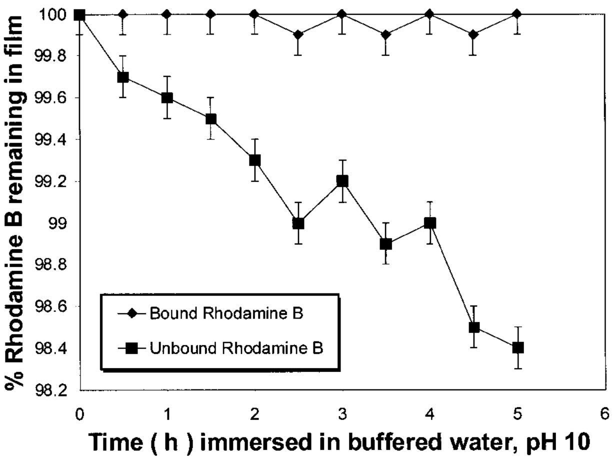


Figure 3 Static leaching of rhodamine B unbound and bound films in buffered water (pH 10). Films were cast from solutions made up from the following: 2.5 mL ethanol; 0.5 g copolymer; 25 pph TPA; cured at 140°C for 12 h. All films had the same initial thickness, ≈50 microns, and the same initial optical density.

Table III Polymer Swelling Data for Some Quattro and Trio Bound-Dye Films and the Virgin Copolymers. Films were Cast from Solutions Made Up in the Following Ratios: 2.5 mL Ethanol; 0.5 g Copolymer; 25 pph TPA; Cured at 140°C for 12 h. All Films had the Same Initial Thickness, ≈50 microns

Polymer	% Increase in Polymer Volume
Quattro	157
Quattro–Dye 1	135
Quattro–Dye 7	128
Quattro–Dye 8	120
Quattro–Dye 9	118
Quattro–Dye 10	69
Quattro–Rhodamine B	135
Trio	10.7
Trio–Dye 7	2.3
Trio–Dye 10	1.8
Trio–Rhodamine B	2

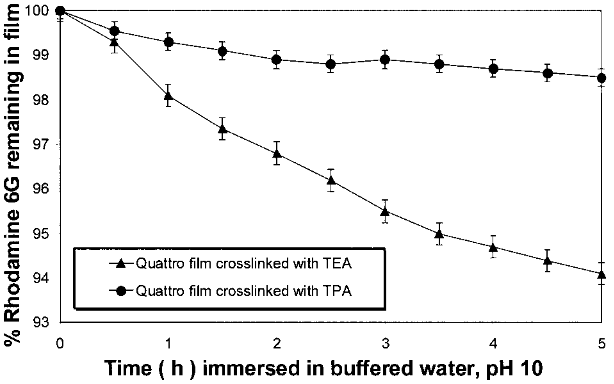


Figure 4 Static leaching of rhodamine 6G unbound films crosslinked with TEA and TPA. The percentage dye remaining in films has been calculated from the decrease in optical density of films, as a function time, at 10 points on the surface of the films. Values are quoted as the mean of 10 points, and error bars as three times the standard deviation. The films were cured at 140°C. All films had the same initial thickness, ≈50 microns.

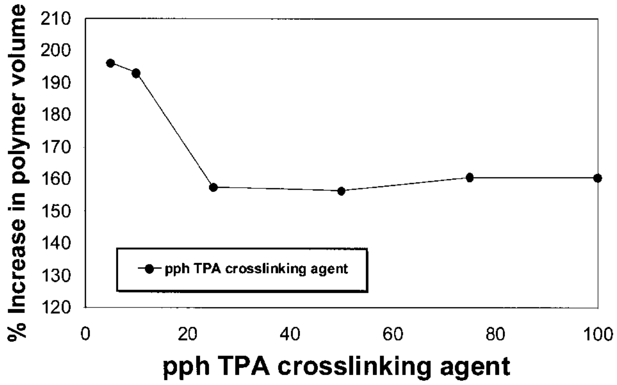


Figure 5 Percentage increase in Quattro swelling volume with increasing quantities of TPA crosslinking agent. The films had the same initial thickness, ≈ 50 microns.

Stern–Volmer Analysis of Sensor Films

The excitation and emission wavelength maxima for rhodamine dyes and dyes 1–12, in solution, unbound and bound to copolymer are shown in Table IV. The excitation and emission spectra for the unbound and bound dyes are the same as in solution, indicating little or no electronic interaction between copolymer supports and the fluorescent dye molecules. Dyes 1–12 typically show ≈100 nm Stokes-shifted fluorescence maxima, and the film optical densities were ≤0.01. These two facts eliminate the possibility of dye–dye energy transfer,[31] which otherwise might have complicated the fluorescence quenching kinetics.

All solution Stern–Volmer plots for dyes 1–12 and the rhodamine dyes are linear, with good linear regression correlation coefficients. However, Stern–Volmer plots for both unbound- and bound-dye films show negative deviations under conditions of very efficient quenching—for example, at halide concentrations approximately >0.1 mol dm,$^{-3}$ (Fig. 6). Even so, for halide concentrations in the range of our technological interest, that is, <0.1 mol dm^{-3}, both bound- and unbound-dye sensor films gave Stern–Volmer plots with good linearity (Fig. 6). It is generally observed that quenching-based luminescent sensors that are supported in organic or inorganic polymers exhibit nonlinear Stern–Volmer quenching behavior. Two common explanations of the non-

Table IV **Excitation (λ_{ex}) and Emission (λ_{em}) Wavelength Maxima for Rhodamine Dyes and Dyes 1–12 in Solution (pH 10, Borax Buffer), Unbound and Bound to Quattro and Trio Copolymers (Wet Films–H_2O, pH 10). Rhodamine 6G was not Bound to Copolymer. Dye Structures can be Seen in Table I.**

	Solution			Unbound Dye Sensor Films			Bound Dye Sensor Films	
Dye/Quattro	λ_{ex} (nm)	λ_{em} (nm)	Dye Solubility in Water	λ_{ex} (nm)	λ_{em} (nm)	Dye Solubility in Unbound Polymer	λ_{ex} (nm)	λ_{em} (nm)
1	360	460	Very Good	360	460	Very Good	360	460
2	360	460	Very Good	360	460	Very Good	362	460
3	360	460	Very Good	360	460	Very Good	360	460
4	390	460	Good	390	470	Very Good	390	470
5	390	460	Good	390	470	Very Good	390	470
6	390	460	Good	390	470	Very Good	390	470
7	360	460	Good	360	460	Very Good	360	460
8	360	460	Good	360	460	Very Good	360	460
9	360	460	Good	360	460	Very Good	360	460
10	390	460	Fair	390	470	Good	390	470
11	390	460	Fair	390	470	Good	390	470
12	390	460	Fair	390	470	Good	390	470
Rhodamine B	550	580	Very Good	550	590	Very Good	550	590
Rhodamine 6G	530	550	Very Good	530	560	Very Good	not bound	
Dye/Trio								
7	360	460	Good	360	460	Good	360	460
10	390	460	Fair	390	470	Good	390	470
Rhodamine B	550	580	Very Good	550	590	Good	550	590

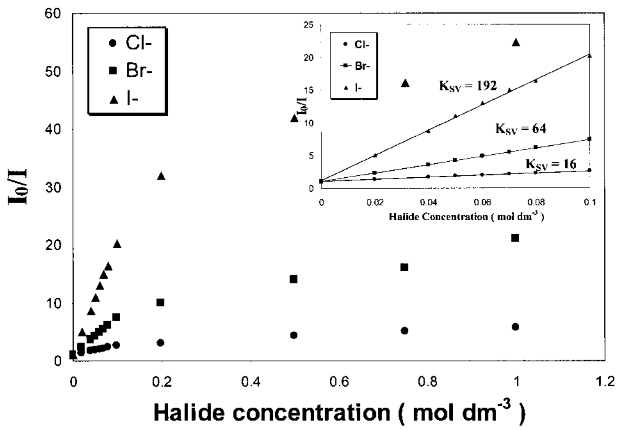


Figure 6 Stern–Volmer plots for unbound dye 1 sensor films, quenched by aqueous halide ions at 21°C, pH 10. (Insert: The plots are linear at halide concentrations < 0.1 mol dm^{-3}.)

linearity[9–12] are either *multisite dye binding* or the *nonlinear solubility properties* of the dye in the support. The halide concentration at which Stern–Volmer plots deviate from linearity varied from dye to dye, and since no information on dye binding or dye–polymer solubility could be obtained, this is not discussed further.

The Stern–Volmer constants, K_{SV}, increase for all dyes in solution when going from chloride to bromide to iodide (Table V). For dyes 1–3 and dyes 7–9, there is a general decrease in halide sensitivity as the alkyl chain length increases from $C_7 \rightarrow C_{14}$. Stern–Volmer constants for BPh_4^- salts are significantly smaller than those for Br^- salts, showing that the counterion has an effect on sensitivity. This may simply be a size effect, with the larger counterion sterically hindering halide diffusion to the dye. Or it may be related to the lower degree of film swelling, which is found for films containing the BPh_4^- counterion, or the organosoluble BPh_4^- ion may make the dye soluble in more hydrophobic regions of the polymer, inaccessible to aqueous halide. The effect of the counterion is also observed for the harmane dyes, dyes 4–6 and 10–12. However, the harmane dyes

Table V Stern–Volmer Constants for Dyes in Solution (Either H_2O/pH 10 or a 50 : 50 v/v Mixture of Ethanol and Buffered Water) and in Unbound- and Bound-Dye Sensor Films. (For all Film Studies, Films were Exposed to Aqueous Halide at pH 10). '—' Indicates no Detectable Quenching. Rhodamine 6G was not Bound to Copolymer

Dye in Quattro Sensor Films	Conditions for Solution Studies	Steady-State Stern–Volmer Constants (mol^{-1} dm^3) Solution			Steady-State Stern–Volmer Constants (mol^{-1} dm^3) Unbound Films			Steady-State Stern–Volmer Constants (mol^{-1} dm^3) Bound Films		
		Cl^-	Br^-	I^-	Cl^-	Br^-	I^-	Cl^-	Br^-	I^-
1	H_2O/pH 10	52	225	634	16	64	192	—	11	66
2	H_2O/pH 10	34	150	480	3	14	53	—	—	—
3	H_2O/pH 10	34	127	398	4	14	28	—	—	—
4	H_2O/pH 10	—	3	191	—	2	15	—	2	7
5	H_2O/pH 10	—	5	198	—	2	15	—	—	—
6	H_2O/pH 10	—	3	192	—	2	14	—	—	—
7	Ethanol/H_2O pH 10	20	70	218	—	10	47	—	5	29
8	Ethanol/H_2O pH 10	14	55	165	—	11	22	—	4	19
9	Ethanol/H_2O pH 10	11	27	139	—	6	3	—	1	8
10	Ethanol/H_2O pH 10	—	1	139	—	—	8	—	—	1
11	Ethanol/H_2O pH 10	—	1	143	—	—	7	—	—	1
12	Ethanol/H_2O pH 10	—	1	141	—	—	7	—	—	1
Rhodamine 6G	H_2O/pH 10	—	—	24	—	—	4	not bound		
Rhodamine B	H_2O/pH 10	—	—	5	—	—	1	—	—	1
Dye in Trio sensor films										
7	Ethanol/H_2O pH 10	20	70	218	—	—	11	—	—	—
10	Ethanol/H_2O pH 10	—	1	139	—	—	4	—	—	1
Rhodamine B	H_2O/pH 10	—	—	5	—	—	1	—	—	—

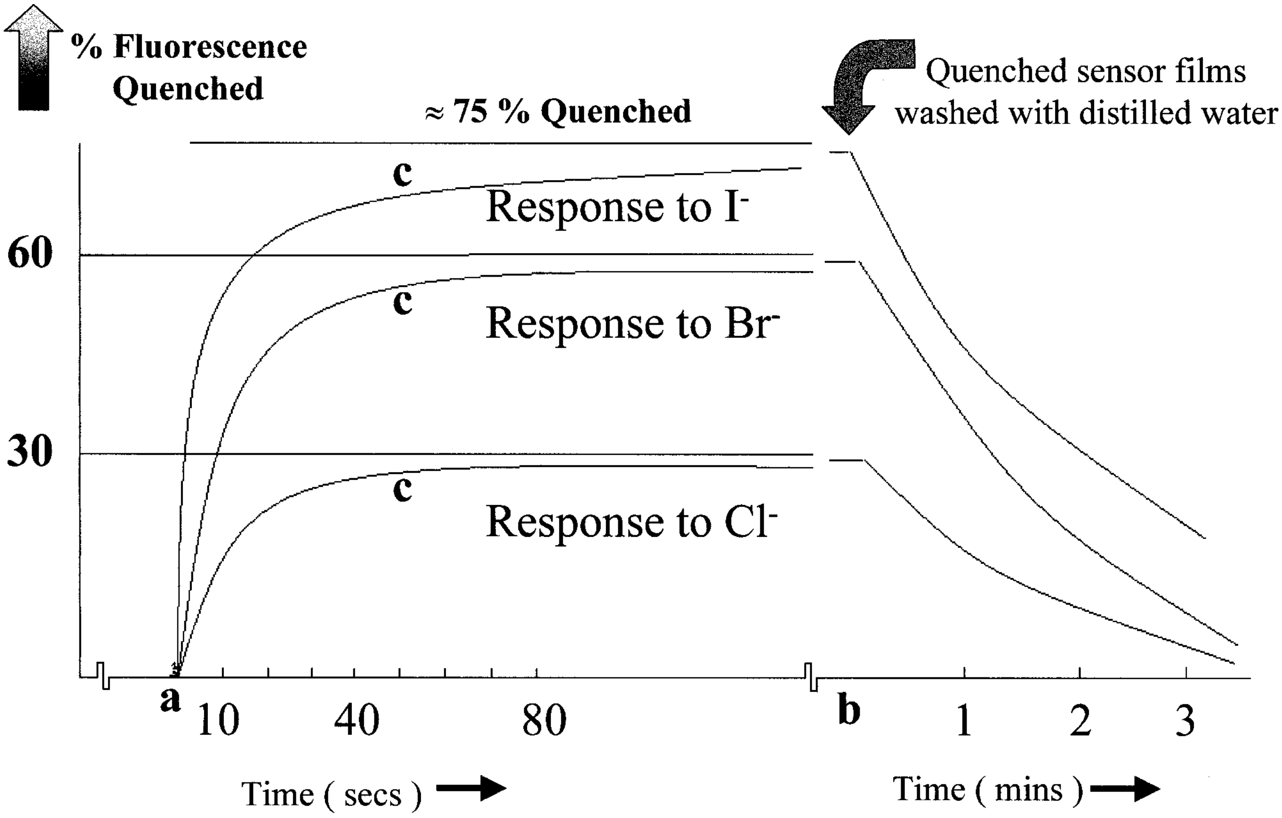


Figure 7 Unbound dye 1 sensor film response to molar halide solutions. The same film is used for all 3 response curves. Washing quenched films with distilled water returns the films to their original fluorescence intensity: (a) indicates the point of halide injection at time, $t = 0$, (b) indicates the point of distilled water injection into the flow cell, and (c) indicates the ≈90% sensor response point.

do not show any effect of chain length on Stern–Volmer constants.

The Stern–Volmer constants for sensor films are much lower than those for the corresponding dyes in solution. This is probably a result of lower halide diffusion rate in the copolymer films. Similarly, the Stern–Volmer constants are significantly smaller in bound compared to unbound films, thought to be the result of the increased crosslinking of the copolymer during the dye-attachment process, which is expected to lead to a lower rate of halide diffusion.

A comparison between Quattro and Trio sensor films shows smaller Stern–Volmer constants for Trio films. It is thought that the increased hydrophobicity of Trio as compared to Quattro results in reduced film sensitivity toward halide.

Although all studies reported here are for aqueous solutions at pH 10, the sensors are also effective in the pH range of 7–11. However, there are some small changes to Stern–Volmer constants over this pH range.

Sensor Film Response Times

The 90% response times (the time for a 90% change in the respective fluorescence signal) of unbound-dye films to molar halide are found to lie in the range of 30–70 s. Figure 7 shows a typical response to molar halide solutions for a dye 1 unbound sensor film. The reversibility of the sensor film is demonstrated by washing the film with distilled water; the dye fluorescence returns as the halide is removed.

The results show slightly longer response times for bound as compared to unbound films (Table VI). Again this is thought to be the result of a smaller rate of halide diffusion from the increased crosslinking of the copolymer after dye attachment. A comparison between Quattro and Trio sensor films shows a typically longer response time for Trio films, which is thought to be because of the increased hydrophobicity of Trio as compared to Quattro.

Table VI Sensor Film 90% Response Times to Molar Halide Solutions. '—' Indicates No Response Obtained. N/M—Not Measured. Rhodamine 6G was Not Bound to Copolymer

	Response Time to 1 mol dm^{-3}			Response Time to 1 mol dm^{-3}		
	Cl^-	Br^-	I^-	Cl^-	Br^-	I^-
Dye in Quattro Sensor Films	(Seconds) Unbound 90%			(Seconds) Bound 90%		
1	50	50	50	—	55	50
2	50	50	50	—	—	—
3	50	50	50	—	—	—
4	—	65	65	—	70	70
5	—	70	—	—	—	—
6	—	60	—	—	—	—
7	—	50	50	—	50	40
8	—	50	50	—	60	50
9	—	50	50	—	50	45
10	—	—	60	—	—	60
11	—	—	60	—	—	—
12	—	—	60	—	—	—
Rhodamine B	—	—	35	—	—	40
Rhodamine 6G	—	—	30	not bound		
Dye in Trio sensor films						
7	N/M	N/M	N/M	—	—	80
10	N/M	N/M	N/M	—	—	90
Rhodamine B	N/M	N/M	N/M	—	—	70

It is interesting to note that no sensor film showed its fluorescence to be 100% quenched, even when very thin sensor films and high halide concentrations were used. This "background fluorescence" could be due to dye buried in the polymer, which is not accessible to halide ions, and this may also account for the negative deviation of Stern–Volmer plots at a high halide concentration, that is, >0.1 mol dm^{-3}.

Sensor Film Selectivity

To ascertain sensor film selectivity, interferences were studied by using 0.5 mol dm^{-3} standard solutions of various anions. Sulfite and the *pseudo*halide isothiocyanate were shown to be effective fluorescence quenchers with large Stern–Volmer constants. No interferences were observed with nitrate, sulfate, or phosphate ions.

CONCLUSIONS

We have described several sensor film formulations based on the attachment of 13 dyes to two hydrophilic copolymers. The sensor film formulations have been optimized in terms of their hydrophilicity and sensitivity toward aqueous halide ions.

A comparison of the Quattro and Trio copolymers shows that Trio gives an increased dye binding efficiency, from $<1\%$ w/w with Quattro to 2–4% w/w with Trio. However, MA had a more substantial role in determining film hydrophilicity than first thought, as the Trio films were only swollen by $\approx 10\%$ in aqueous media compared to ≈ 100–500% for Quattro films with the same formulation. This reduction in swelling appears to result in a reduction in the sensitivity of bound-dye Trio films to aqueous halide.

Thin film sensors, based on the Quattro copolymer, are sensitive to aqueous halide ions at a mildly alkaline pH. The increased sensitivity over that reported by others is attributed to the large Stern–Volmer constants of some of the dyes when bound to the hydrophilic Quattro copolymer. The 90% response times have been found to be dependent on film formulation, with typical response times of 30–70 s. It has been observed that the Quattro copolymer typically has a greater swelling rate and a greater final swollen volume when immersed in a higher temperature medium. Operating these sensor films at higher temperatures may therefore decrease sensor response times and increase sensitivity towards halide ions, and it may also extend the region of linear Stern–Volmer response.

Given that the bound-dye films described here do not leach dye when immersed, they could be used for the on-line industrial monitoring of halide or the measurement of halide concentrations in blood, serum, or plasma, where solution temperatures can be greater than 20°C.

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