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A halide sensor based on the quenching of fluorescence of an immobilised indolium salt

Chris D. Geddes*

Department of Chemistry, University of Wales Swansea, Singleton Park, Swansea SA2 8PP, UK

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Abstract

A halide sensitive indolium salt has been produced by the quaternisation of Harmane with 1,3-propane sultone. The product, 1-methyl-2(sulfonatopropyl)-9*H*-pyrido[3,4-b] indolium (SPI), is readily water soluble and in the presence of aqueous halide ions is fluorescence quenched, enabling halide concentrations to be determined using Stern–Volmer kinetics. The fluorophore has been immobilised within a hydrophilic *Quattro* copolymer, so-called due to its four constituent co-monomers, to produce a halide sensor. The sensor film is sensitive to modest bromide and iodide concentrations with a 90% response time to molar halide of ≈2 min. The sensor film is completely reversible, which can potentially allow for the continuous monitoring of halide in industrial and medical applications, where the 90% fluorescence recovery time to distilled water is ∼5 min. The interference of other aqueous anions on sensor performance is discussed. The sensor film was found to have a shelf life in excess of 2 years. Opportunities for multiphoton transdermal halide sensing are discussed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Fluorescence quenching; Harmane; Harman; Aribine; Halide; Bromide; Iodide; Multiphoton halide sensing

1. Introduction

Since the first report of an optical halide sensor based on the quenching of fluorescence of an immobilised fluorophore in 1984 by Urbano et al. [1], many subsequent halide sensors have been developed and reported in the research literature [2–6]. Most halide sensors have typically used quinoline analogues since some analogues are quenched by chloride, which is generally considered more difficult to sense than bromide or iodide simply because the fluorescence of most other fluorophores is unperturbed by aqueous chloride ions [7]. However, from a medical point of view we are fortunate as physiological chloride concentrations are typically greater than free bromide and iodide levels and are therefore measurable by a few probes. In industrial processes, however, halide levels may vary considerably, allowing a greater choice of fluorophores to be used. In this paper, the use of an indolium salt for halide sensing is described (Fig. 1) which is sensitive to modest bromide and iodide concentrations but is unperturbed by similar chloride concentrations. This allows for the sensing of both aqueous Br^- and I^- ions in the presence of aqueous Cl^- ions. The particular interest in the sensor film described here lies in its potential use in photographic processing equipment, where halide concentration is an important control parameter. Hence, Stern–Volmer constants are quoted at a solution pH of 10, although the sensor film is sensitive and therefore usable over a broader pH range. Opportunities for multiphoton transdermal physiological halide sensing are also discussed.

The quenching of fluorescence was first described by Stokes as early as 1869 when he observed that the fluorescence of quinine in dilute sulphuric acid was reduced after the addition of hydrochloric acid, i.e. chloride ions. The process that he observed is now commonly referred to as dynamic or collisional fluorescence quenching and is known to follow Stern–Volmer kinetics [7]:

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

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

For an aqueous halide sensor, the correct choice of polymeric support to immobilise the halide sensitive dye is crucial. Halide sensor supports should typically: allow the

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* Present address: Department of Physics and Applied Physics, The University of Strathclyde, John Anderson Building, 107 Rottenrow, Glasgow G4 ONG, UK. Fax: +44-141-552-2891.

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

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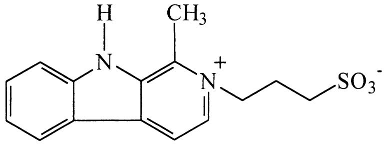

Fig. 1. The SPI molecule, 1-methyl-2(sulfonatopropyl)-9*H*-pyrido[3,4-b] indolium.

rapid diffusion of aqueous halide ions; be insoluble in the medium of interest but if possible swell to allow greater halide diffusion; readily solubilise the fluorophore producing a homogeneous class of fluorophore; be coatable on a variety of surfaces such as on the tip of a fibre optic bundle; be cross-linkable in situ; be chemically unperturbed by aqueous halide ions or pH, as well as being cheap and relatively simple to produce. One particular polymer that fulfils most of these criteria is the *Quattro* copolymer. The Quattro copolymer was first synthesised by Anderson et al. [8] and many subsequent analogues by Geddes and Douglas [2,5]. An explanation of the importance of each co-monomer unit in Quattro with regard to its role in aqueous halide sensing is described below.

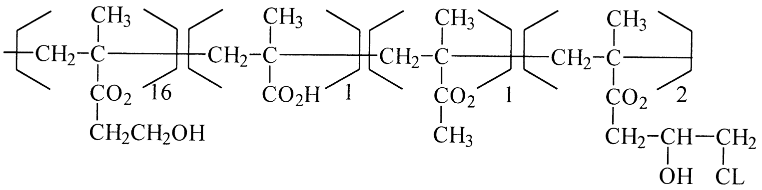

Fig. 2. The structural formulae of the Quattro copolymer poly(2-hydroxyethyl methacrylate)$_{16}$-Co-(methacrylic acid)$_1$-Co-(methyl methacrylate)$_1$-Co-(3-chloro-2-hydroxypropyl methacrylate)$_2$, ratios 16:1:1:2, i.e. HEMA$_{16}$MA$_1$MMA$_1$YG$_2$. The copolymer composition is based on complete conversion of the initial monomer feed concentrations during synthesis.

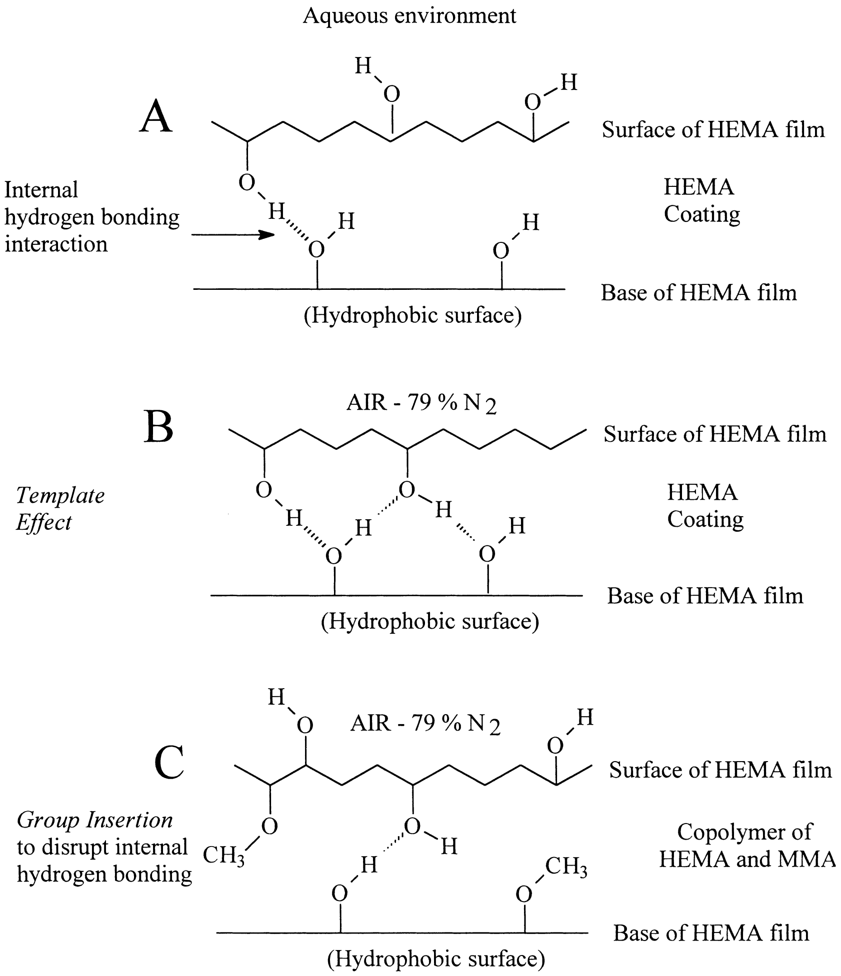

Fig. 3. The conformation assumed by HEMA hydroxyl groups for: (A) A HEMA polymer in an aqueous environment; (B) A HEMA polymer cast in air on a hydrophobic surface; (C) A copolymer of HEMA and MMA cast in air on a hydrophobic surface.

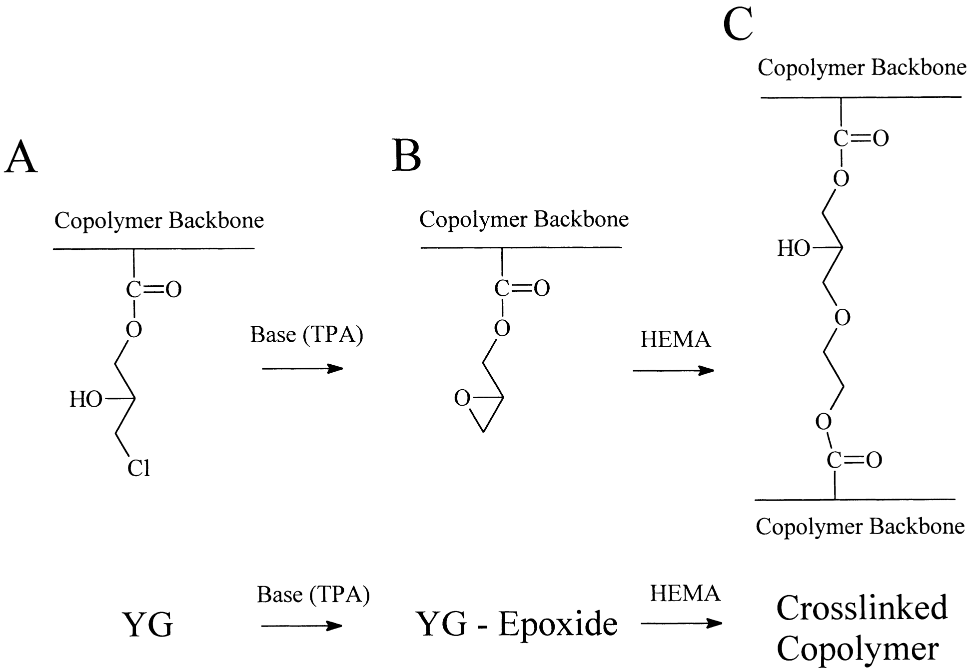

Fig. 4. Cross-linking of the Quattro copolymer: (A) YG co-monomer unit; (B) epoxide formed from YG on the addition of base; (C) cross-linked Quattro copolymer formed from the reaction of epoxide and deprotonated HEMA. TPA — tripropylamine.

1.1. The Quattro copolymer

The hydrophilic copolymer, Quattro, consists of four co-monomer units (Fig. 2). The HEMA (2-hydroxyethyl methacrylate) co-monomer unit is the principal source of hydroxyl groups in the coated polymer, fulfilling a dual function. Firstly, the presence of hydroxyl groups renders the copolymer hydrophilic an important consideration for an aqueous sensor matrix, and secondly, they render the copolymer susceptible to well-known activation chemistries and to subsequent dye attachment. In previous sensor work, Geddes and Douglas [2] described dye attachment to the Quattro copolymer through these HEMA hydroxyl groups to produce bound dye[1] sensor films.

In an aqueous environment it is the specific conformation assumed by the HEMA co-monomer units rather than the general molecular structure that renders the copolymer hydrophilic and therefore useful as a sensor matrix. In an aqueous environment the pendent –OH groups tend to be drawn to the surface where they are in turn stabilised by hydrogen bonding interactions with the surrounding water molecules (Fig. 3A). However, when the films are cast in air which is predominantly nitrogen based, on a hydrophobic surface, the resultant copolymer films are very different. The hydroxyl groups now adopt a more energetically favourable position and bury themselves within the copolymer (Fig. 3B). This drastically reduces the hydrophilicity of the coated copolymer and its usefulness as a sensor matrix. One way to disrupt this *template effect*, which is not uncommon and has been reported elsewhere [9], is to introduce another co-monomer unit into the copolymer, which lacks an –OH substituent and therefore disrupts the internal film hydrogen bonding, namely methylmethacrylate (MMA) (Fig. 3C). This enables films to be cast on hydrophobic surfaces and in air. Information pertaining hydroxyl group orientation was obtained by coupling cyano-type dyes to the surface of films through their available hydroxyl groups and after washing away unbound dye, bound dye concentrations and hence hydroxyl group orientation could be estimated from absorption measurements [10]. Hamilton contact angle measurements [11] enabled the link between hydroxyl group orientation and film hydrophilicity to be realised. Methacrylic acid (MA) was introduced into the copolymer to both increase hydrophilicity and to provide anionic sites on the copolymer backbone (which would allow for the non-specific adsorption of proteins when used in medical applications [8]), whilst 3-chloro-2-hydroxypropyl methacrylate (Yg) was introduced to confer the base-induced cross-linkability of the copolymer. The cross-linking of the Quattro copolymer, which is a result of the reaction of the resulting epoxide formed from Yg on the addition of base with deprotonated HEMA hydroxyl groups (Fig. 4) enables the copolymer to be coated on a variety of surfaces as well as preventing unbound dye leaching from sensor films. The effect of the cross-linking density of Quattro towards halide diffusion has previously been addressed by Geddes and Douglas [2] as has, a detailed discussion about the ideal Quattro film formulation.

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[1] Unbound dye refers to dye mixed in the Quattro copolymer before cross-linking, casting and curing as compared to bound dye, where the dye is covalently bound to the Quattro copolymer.

2. Experimental

2.1. Dye solutions

All chemicals were purchased from Aldrich and used as received. All measurements were performed in pH 10 borax buffer, which was made by mixing 50 ml of 0.025 mol dm^{-3} borax (sodium tetraborate), 18.3 ml of 0.1 mol dm^{-3} NaOH and 31.7 ml of doubly distilled deionised water [12]. The dye concentration in buffer was 5×10^{-6} mol dm^{-3}. A brief study of SPI in solution revealed that the degassing of samples was unnecessary as oxygen was not found to quench SPI fluorescence to any detectable extent. The purity of the heterocyclic nitrogen base, Harmane, was verified using thin-layer chromatography. Harmane, 1-methyl-9*H*-pyrido(3,4-b) indole, is sometimes also referred to as Aribine or Harman in the research literature.

2.2. Steady-state analysis

Steady-state emission spectra were recorded on a Jobin-Yvon JY3D spectrofluorometer. Absorption measurements were performed using a Philips Unicam PU8620 UV/VIS/NIR spectrophotometer. Steady-state Stern–Volmer analysis of SPI solutions was carried out using a Jobin-Yvon JY3D spectrofluorometer at 20°C, pH 10 (borax buffer) using halide concentrations in the range 10^{-1}–10^{-2} mol dm^{-3}. The Stern–Volmer constants were calculated using Eq. (1) and the Axum Graphics linear regression program. Steady-state Stern–Volmer analysis of SPI sensor films was carried out using an optical flow cell.

2.3. The optical flow cell

A 1 cm × 1 cm “cup” with a quartz window and two rubber rings was used to trap the sensor film and give a sealed flow cell, through which aqueous halide solutions were pumped over the SPI sensor film (Fig. 5). Light from a 100 W stabilised tungsten halogen lamp was passed through a band pass filter and made incident on the film at 90° to the plane of the slide support. Because of entrapment of fluorescence emission by total internal reflection within the glass support, the emitted light was conveniently collected from one edge of the slide, passed through a suitable cut off filter and made incident on a Hammamatsu R928 photomultiplier. The photomultiplier signal was stored using a Gould OS4020 storage oscilloscope. The optical set-up which houses the flow cell is shown in Fig. 6. To maintain an overall constant refractive index upon halide injection, i.e. a constant solution–solution, a constant solution–film interfacial refraction [13,14] and a constant ionic strength, all quenching measurements were carried out using solutions containing 1 mol dm^{-3} sodium nitrate. (The SPI sensor film was not quenched by 1 mol dm^{-3} sodium nitrate solution.) The film was firstly blanked with the high ionic strength background and the system calibrated (i.e. the position of zero fluorescence quenching determined) before the halide solution was injected into the cell. To determine SPI sensor film Stern–Volmer constants, K_{SV}'s, from plots of F'/F as a function of $[Q]$ (where F' and F are the fluorescence intensities in the absence and presence of the quencher Q, respectively), known concentrations of halide were injected into the flow cell, stepwise (starting from the most dilute halide solution), until sufficient data points had been collected to produce a plot. Least-squares linear regression analysis of the data was performed using the Axum Graphics software package. Further details of the optical flow cell and advantages of this optical geometry have been discussed by Geddes et al. [14] recently.

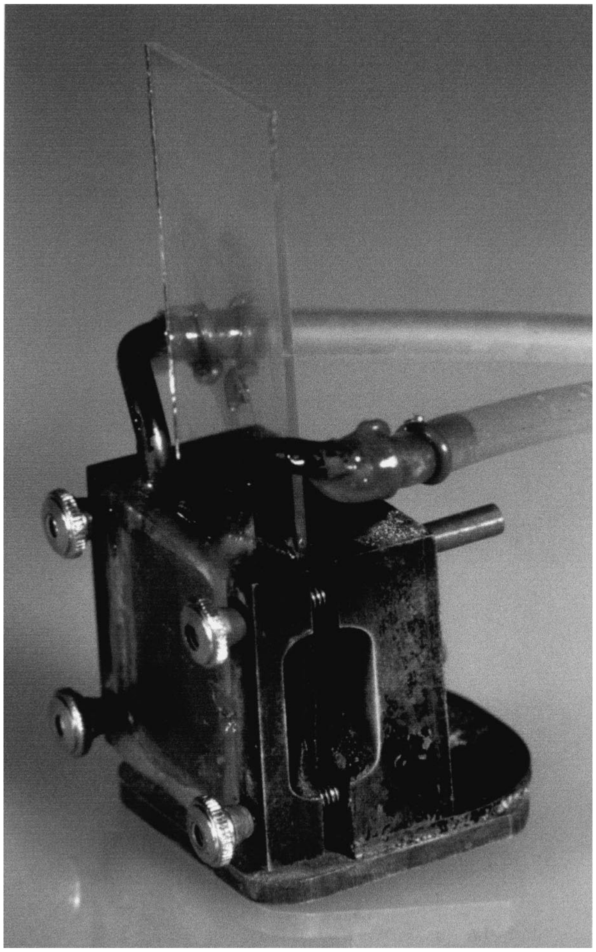

Fig. 5. The optical flow cell.

2.4. Synthesis of 1-methyl-2(sulfonatopropyl)-9H-pyrido-[3,4-b] indolium (SPI)

The synthesis of SPI was first described by Wolfbeis and Urbano [15] in 1982 for use as a fluorescent standard in the near neutral pH range and was achieved by fusing Harmane with 1,3-propane sultone at 130°C for 1 h. On the addition of DMF a precipitate is formed which after collection and

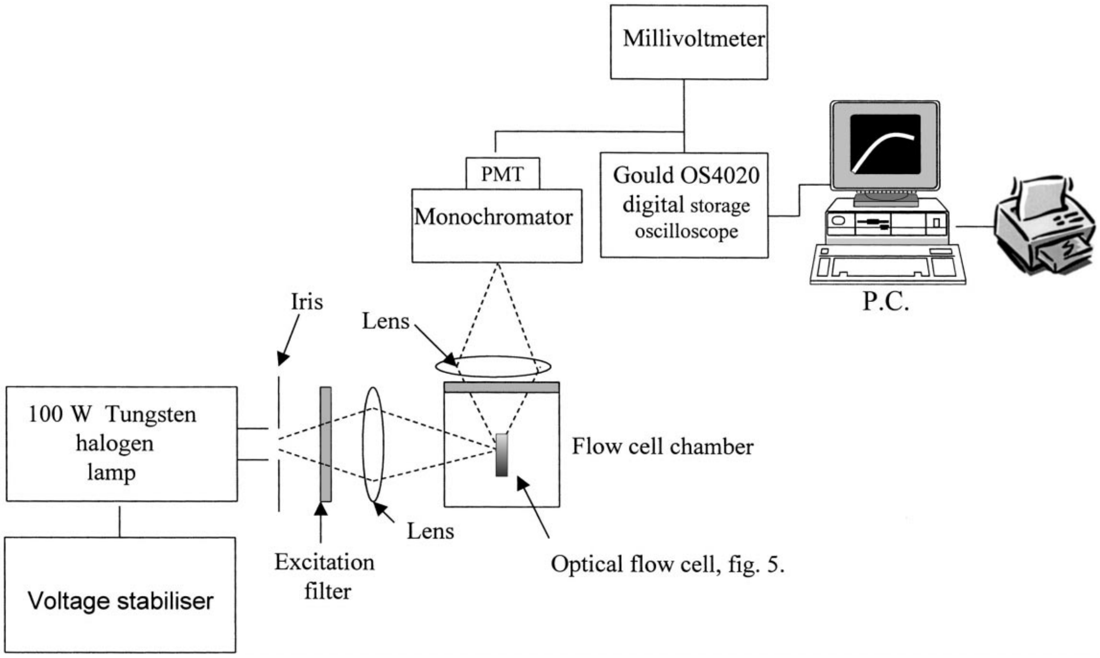

Fig. 6. The optical set-up, which incorporates the optical flow cell. PMT — Hammamatsu R928 photomultiplier tube.

washing can be recrystallised from a 9:1 (v/v) ethanol:water mixture. The quaternisation of the nitrogen heteroatom of Harmane with 1,3-propane sultone to form the zwitterionic inner salt is shown in Fig. 7. SPI was successfully synthesised giving a similar reaction yield and spectroscopic characterisation.

2.5. Preparation of SPI sensor films

Sensor films were cast from solutions comprising of 2.5 ml ethanol, 0.5 g Quattro copolymer, 2 pph (parts per hundred resin) SPI and 25 pph TPA (tripropylamine), and a suitable cross-linking agent [2]. Sensor films were cast by sliding a drop of copolymer solution between two glass microscope slides, the slides then separated and the copolymer sensor films then cured at 140°C in a Gallenkamp BS2 oven. Film dimensions on glass slides were typically 1 cm × 1 cm for quenching experiments. After curing, the films were washed in distilled water to remove excess cross-linking agent. TPA did not quench the fluorescence of SPI. Film composition, casting and curing has been discussed in detail by Geddes and Douglas [2] previously.

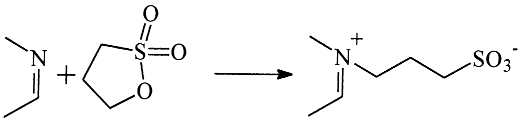

Fig. 7. Quaternisation of the nitrogen heteroatom of Harmane with 1,3-propane sultone to form the zwitterionic inner salt.

2.6. SPI leaching from sensor films

Sensor films, which were cast on glass microscope slides, were immersed in 1 l of buffered water at 20°C. The volume of buffer was such that any dye leached could be considered infinitely diluted thus avoiding any potential equilibrium between unbound dye and leached dye in solution. The percentage of dye remaining in films was calculated from the decrease in optical density of films as a function of time. Film optical densities were ∼0.01. The thickness of dry films, ≈50 μm, was determined using a Digi-Cal dead-weight micrometer at different positions on the surface of the sensor film. The film thickness was reassuringly constant enabling dye leaching to be studied in this regard.

2.7. SPI sensor film response times

Response times for the sensor film to respond to aqueous halide were determined using the optical flow cell and set-up [14], Figs. 5 and 6, respectively. At the point of halide injection into the flow cell, the digital storage oscilloscope was triggered and the response curve observed. Ninety per cent response times, the time for the fluorescence signal to change by 90%, are quoted. This is in accordance with the previous work [2,14]. The reversibility of the sensor films was studied by washing the sensor films with nitrate solution after quenching. As the halide was removed, fluorescence recovery was observed. Ninety per cent fluorescence recovery times are quoted.

Table 1
Absorption (λ_{abs}), emission (λ_{em}) wavelength maxima and the solubility of SPI in various solvents and unbound[a] in the Quattro copolymer (wet films — H_2O, pH 10)

Solvent	λ_{abs} (nm)	λ_{em} (nm)	SPI solubility in solvent	Unbound SPI sensor film		SPI solubility in copolymer film
				λ_{abs} (nm)	λ_{em} (nm)	
Borax buffer (pH 10)	390	410, 460	Very good	390	500[b]	Very good
Distilled H_2O (pH 7)	372	414, 484	Very good	–[c]	–	NA[d]
Methanol	374	412, 482	Very good	–	–	NA
Ethanol	374	407, 475	Very good	–	–	NA

[a] Unbound dye refers to SPI mixed in the copolymer solution before casting and curing. SPI was therefore not covalently bound to the copolymer backbone.

[b] $\lambda_{ex} = 390$ nm.

[c] Not measured.

[d] Not applicable.

3. Results and discussion

The absorption and emission wavelength maxima for SPI in various solvents and in the sensor film can be seen in Table 1. The emission wavelength maxima is red-shifted in the film ≈40 nm with respect to pH 10 solution. Also only one broader emission band is observed in films as compared to solution where two emission bands are observed, although only one discrete ground state species is present.

SPI solution and sensor film Stern–Volmer plots were linear in the halide concentration range studied, i.e. 0.1–0.01 mol dm^{-3}. The solution and film K_{SV}'s can be seen in Table 2. One noticeable feature is the decrease in K_{SV}'s for the SPI sensor film as compared to solution. This is attributed to the smaller halide diffusion rate in the sensor film, i.e. the K_q term in K_{SV} (Eq. (1)).

Whilst not shown in Table 2, increasing the solution temperature had the effect of increasing K_{SV}'s, which can be explained twofold. Firstly, increasing the temperature will increase the halide diffusion rate, i.e. for dynamic quenching $K_q \propto T/\eta$ [7] and secondly, the Quattro film thickness has been shown previously by Geddes and Douglas [2] to increase (swell) with increasing solution temperature, e.g. for a 50 μm film the final swollen thickness at 20.8 and 57.4°C was 128 and 240 μm, respectively, which would also result in a greater diffusion rate within the film. Hence, the temperature dependence of K_{SV}'s is likely to be complex.

Individual SPI sensor films were able to determine 0.01 mol dm^{-3} iodide and bromide concentrations with ≈2 and ≈3% error, respectively. Film fluorescence was unperturbed by 0.1 → 0.01 mol dm^{-3} chloride. The sensitivity of the film was in the order $I^- > Br^-$.

Stern–Volmer constants of multiple sensor films, of the same composition, were determined to investigate SPI homogeneity. All films tested gave very similar K_{SV}'s, indicating a homogeneous distribution of SPI within the sensor film. In one paper, Zhu et al. [3] immobilised quinine in cellulose to produce a halide sensor. They report that plots of $F'/F - 1$ vs $[Q]$ for multiple films do not extrapolate to the same position on the $F'/F - 1$ axis, which is indicative of an inhomogeneous distribution of quinine within the cellulose support. Therefore, each individual sensor film would need to be separately calibrated before use. With SPI–Quattro sensor films this is not the case, indicating very good SPI solubility in films as well as demonstrating the necessity for a careful choice of polymeric support.

The 90% response time for an SPI sensor film to molar halide solutions was ≈120 s (Table 3). The response times to smaller halide concentrations were longer. The film was found to be reversible by washing with distilled water, the dye fluorescence recovering as the halide was removed. To return film fluorescence to its original unquenched intensity required washing with 1 mol dm^{-3} nitrate solution for approximately 290–330 s (Table 3) where the volume of nitrate used was typically 10 times that of halide. It is interesting to see from Table 3 that the film response times for both bromide and iodide are similar. At first one might expect that the response to bromide would be quicker, given the greater mobility of bromide in solution because of its relatively small

Table 2
Stern–Volmer constants for SPI in solution and in unbound sensor films[a]

Conditions for solution studies	Steady-state Stern–Volmer constants (mol dm^{-3})			
	Solution		Unbound sensor films	
	Br^-	I^-	Br^-	I^-
H_2O (pH 10)	3	204	2	112

[a] For sensor film studies, films were exposed to aqueous halide at pH 10. SPI was not quenched by aqueous chloride ions at the concentrations employed here.

Table 3
SPI sensor film 90% response times to molar bromide and iodide solutions and the 90% reversible/recovery response times to molar nitrate

Response time (s) to 1 mol dm^{-3}		Reversible response times (s)[a] using 1 mol dm^{-3} nitrate solution	
Br^-	I^-	Br^-	I^-
120	120	≈290	≈330

[a] The sensor films already quenched by halide in Column 1 were used to obtain the reversible response times in Column 2.

ionic volume and low mass. This therefore must report the microscopic structure of the Quattro copolymer and suggests a compact cross-linked polymer network, which significantly reduces the mobility of both halides to a similar extent. Of course, this has the added attraction of reducing dye leaching from unbound dye sensor films, which is an important consideration given that bound dye films, which do not leach dye, typically have significantly smaller K_{SV}'s [2].

One problem typically associated with immobilised dye sensors is that of fluorophore leaching when immersed in solution. The unbound SPI sensor film described here, when soaked in buffered water at pH 10 for a few hours, showed only a small percentage dye loss, ≈1–2%. However, after being immersed about 5 h, the sensor film showed no change in optical density indicating no further dye loss. For a practical working sensor, the initial dye loss is not necessarily a problem as one can either pre-soak the sensor prior to use or by frequently measuring the fluorescence intensity of the solution containing the analyte, i.e. obtaining F' in Eq. (1), one can compensate for the dye loss. This is possible because the calculated halide concentration is dependent on the fluorescence ratio, F'/F, rather than on the absolute fluorescence intensity of the sensor film.

It was shown previously by Geddes and Douglas [2] that increasing the cross-linking density of the Quattro copolymer had the detrimental effect of reducing sensor film K_{SV}'s. Hence, 25 pph TPA was used to cross-link the sensor film which was a compromise between the extent of dye leaching and film K_{SV}'s. Reducing the pH from 10 to 7 had no noticeable effect on the leaching rate of SPI from the sensor film.

The stability of the sensor film was studied using the optical flow cell, where the fluorescence intensity of the SPI film was monitored during constant 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 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.

As for most sensors it is important to characterise and quantify any potential interferences before a sensor is applied in practice. A good example of this is molecular oxygen, which is a well-known quencher of many fluorophores. As a result, solutions of most long-lived fluorescent species have to be degassed prior to being studied. However, for both Harmane and SPI, no fluorescence quenching is observed with molecular oxygen. The effects of other potentially interfering species on the SPI sensor film, which can be present in industrial processes, were studied. No interferences were observed with 0.5 mol dm^{-3} sulphate, phosphate and nitrate ions. However, 0.5 mol dm^{-3} sulphite and chloride ions were found to quench SPI sensor film fluorescence. (For interference studies the sensor film was firstly blanked with pH 10 buffer only, before the halide was injected into the flow cell.) 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 [16] in 1983. The K_{SV}, Eq. (1), is dependent upon the specific interaction of quencher and fluorophore. However, if more than one kind of quencher is present, e.g. halide and an interference, then the contribution of all quenchers to the fluorescence ratio F'/F is

$$\frac{F'}{F} = 1 + K_{SV1}[Q_1] + K_{SV2}[Q_2] + \cdots + K_{SVn}[Q_n] \quad (2)$$

where K_{SV1} and K_{SV2} are the Stern–Volmer constants for halide and interference ions, respectively. To determine the unknown halide concentration, then the K_{SV}'s have to be determined before hand from independent plots of F'/F vs

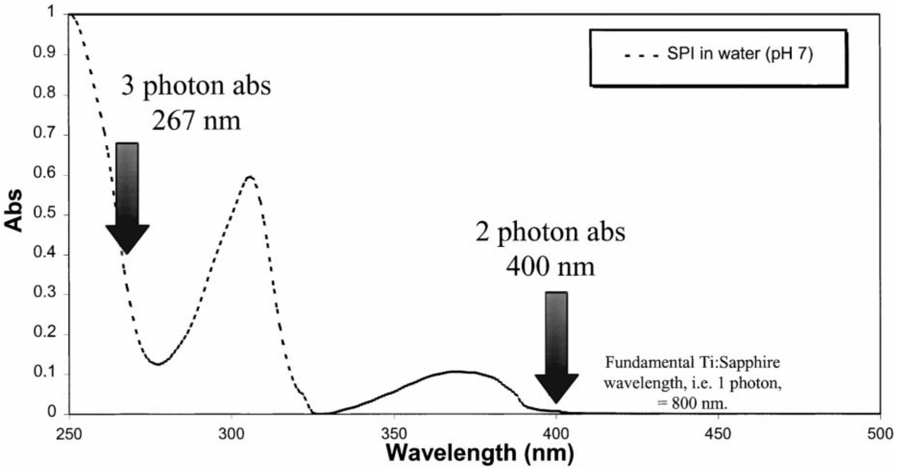

Fig. 8. One photon absorption spectrum of SPI in doubly distilled deionised water, pH 7. In energy terms, two photons at 800 nm = one photon at 400 nm and three photons at 800 nm = one photon at 267 nm.

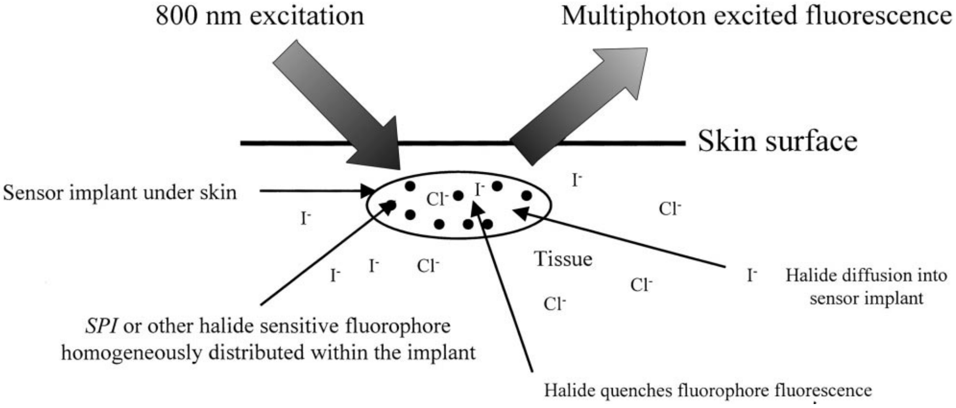

Fig. 9. The principle of multiphoton transdermal halide sensing.

[Q], where a second sensor film is also used (to solve both resultant simultaneous equations), containing a different fluorophore, which has different K_{SV}'s than SPI for both halide and interference. Therefore in practice, n unknown analyte species can be determined with n sensor films containing n immobilised fluorophores.

Response times and Stern–Volmer constants for the sensor film were recorded during a 2-year period to ascertain the shelf life. Both remained unchanged indicating sensor film stability during the 2-year period, although it is likely that the sensor film can be both stored and operated for even longer periods of time.

4. Conclusions

A halide sensitive fluorophore, SPI, has been synthesised and immobilised in a hydrophilic copolymer to produce a halide sensor. The sensor film is able to reversibly determine 0.01 mol dm^{-3} bromide and iodide with ≈97 and ≈98% accuracy, respectively. The 90% response and recovery times to molar halide solutions are ≈2 and ≈5 min, respectively, where a similar response and recovery time is observed for both halides. Increasing the analyte solution temperature reduces the response time of the sensor, but encourages more dye leaching. This is thought due to both the increased rate of dye diffusion and the subsequently greater final swollen thickness of the sensor film.

One particular advantage of the sensor film lies in the fact that it can be cast and cross-linked in situ on many surfaces such as glass or even other polymers. This allows for the potential mass production of this film for multiple disposable applications, such as in medicine or photography. Also, the apparent homogeneity of SPI in the Quattro copolymer requires for only one initial sensor calibration, which would significantly reduce production costs. However, it is envisaged that casting an SPI–Quattro film on a different surface, other than what has been tested here, i.e. glass microscope slides, may require the sensor to be studied further. This is because the nature of the surface may change the orientation of film hydroxyl groups, which in turn could effect the solubility and hence homogeneity of SPI within the film.

Although all studies reported here are for a solution of pH 10, the sensor film is sensitive over a broader pH range and therefore could be used in the near neutral pH range for medical applications. One typical clinical 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 [7]. 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 [17] of SPI to overcome this problem, e.g. the two or three photon excitation of SPI (Fig. 8) using an 800 nm regeneratively amplified Ti:sapphire laser. Near-infrared multiphoton excitation has the additional attractions of reduced dye and sample degradation and reduced scattering of the excitation light, λ^{-4} dependence. Further, it may be possible to sense physiological halide, using a sensor film implant, such as the one described here, incorporated just under human skin. This would allow for the continuous transdermal monitoring of halide levels (Fig. 9). At this time no reports of multiphoton Stern–Volmer halide sensing kinetics have been reported. Further investigations by the author are underway.

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