Electrochemical immobilization of a pH sensitive fluorescein derivative: synthesis and characterization of a fluorescein-derivatised polythiophene

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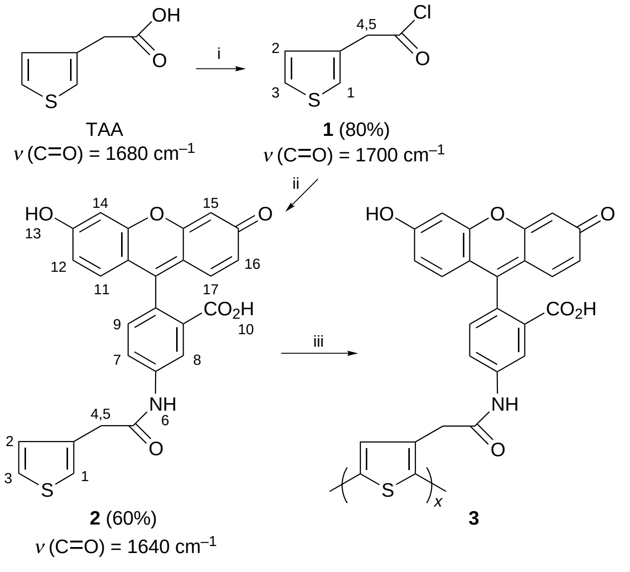
The electrochemical immobilization of a pH sensitive fluorescein derivative was achieved by preparing a fluorescein-substituted thiophene species, followed by its electropolymerization on a Pt electrode.

Immobilization of fluorescein[1] for the preparation of fibre-optic pH sensors has been achieved using several techniques.[2] Simple entrapment in sol-gel matrices has been reported by several workers.[3] Fluorescein derivatives have also been used to create a covalent link between the indicator and the optical fibre surface.[2] The photochemical covalent immobilization of fluoresceinamine has been reported for the preparation of fibre-optic chemical sensors (FOCSs) for pH measurement.[4] Here we report the first example of the electrochemical immobilization of a pH sensitive fluorescein derivative.

Electropolymerization is a simple and attractive approach for the immobilization of fluorophores in the form of a polymer on a substrate surface. The process of electropolymerization can be controlled by electrode potential and allows accurate control of the amount of fluorophore immobilized. In this work the electrochemical immobilization of fluoresceinamine is being undertaken in order to prepare a FOCS for pH and to study the effect of electrode potential on the fluorescence properties of the new polymer.

Scheme 1 shows the reaction for the preparation of fluorescein-derivatised polythiophene. The new monomer for electropolymerization was prepared using thiophene-3-acetic acid (TAA), a well characterized compound for preparing conducting polymers.[5] From TAA, thiophene-3-acetyl chloride (TAC) **1** was prepared *via* reaction with thionyl chloride.[6]‡ TAC was then reacted with fluoresceinamine, to yield **2**.§



Scheme 1 *Reagents and conditions:* i, excess $SOCl_2$, 60 °C, 2 h (ref. 6); ii, fluoresceinamine, acetone, reflux, then $CHCl_3$–H_2O extraction; iii, electrochemical polymerisation (ref. 7)

Polymer **3** was prepared *via* the electrochemical polymerization on Pt coated glass in MeCN using the method reported for the preparation of poly-TAA.[7]¶ Polymer **3** was produced as a golden film. Under the experimental conditions used, the polymer film produced is probably over-oxidised and consequently non-conducting. However, the electrical properties of the polymer are not of interest here and will not be discussed further.

Fluorescein itself in aqueous solution occurs in cationic, neutral and anionic forms, making its absorption and fluorescence properties strongly pH dependent.[1] It has been shown to be electrochemically active in non-aqueous media.[8] At neutral and high pHs in aqueous media the equilibrium shown in Fig. 1 dominates.

The fluorescence intensity ratio *vs.* pH curves for sodium fluorescein (NaFlu) and for **2** in aqueous solution are given in Fig. 2. The emission wavelength for **2** was identical to that of NaFlu and had a value of 515 nm. The two titration curves have very similar characteristics and indicate that the modification of fluorescein with thiophene has little effect on the pH sensitivity of the fluorescein moeity. The solid lines are theoretical plots generated by fitting Eqn. (1) to the data. This model yields approximations for the pK_a of the indicators. For NaFlu the pK_a is 6.3 and that for **2** it is 6.2. The reported value for fluorescein is in the range 6.2–6.9.[1]

Fig. 2 also shows the pH titration curve for **3**. Polymer **3** shows a well-defined titration with a lower pH sensitivity compared to NaFlu and **2** in solution. The decrease in sensitivity is attributed to small changes in the fluorescence properties of the fluorescein moiety in its new environment. This phenomenon is also observed for photochemically immobilized fluorescein in hydrogel films.[4]

This work has for the first time reported an electrochemical immobilization method for a pH sensitive fluorescein derivative. We have shown that the pH sensitive polymer has similar response characteristics to that of fluorescein in solution. Current investigations are concerned with the effect of electrode potential on the fluorescence properties of the polymer and the preparation of copolymers with TAA. Indole and pyrrole derivatives as alternative monomer units are also being investigated.

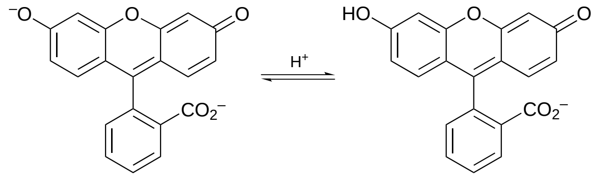


Fig. 1 Dominant equilibrium of fluorescein at neutral and high pH. Fluorescein has a high molar absorbitivity at 488 nm, a large fluorescence quantum yield and high photostability. An attractive feature of this indicator is that it has an excitation maximum at 488 nm, and a pH insensitive shoulder at 430 nm making it suitable for use in the 'ratiometric' mode which overcomes problems in measurement associated with photobleaching, light intensity fluctuation in the excitation source, ionic strength and indicator concentration (ref. 9).

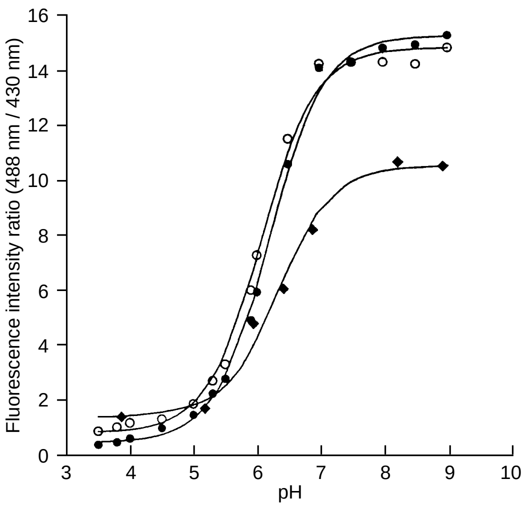


Fig 2 pH Titration curve for (○) **2** (pK_a = 6.1), (◆) **3** (pK_a = 6.3) and (●) NaFlu (pK_a = 6.2). The overall equilibrium can be written. HIn $\rightleftharpoons$ H^+ + In^-, where HIn (not fluorescent) and In^- (fluorescent) are the protonated and deprotonated forms of the indicator respectively. The pH is related to the fluorescent intensity by pH = pK_a + log[$I/(I_o - I)$] (ref. 2) where the fluorescence intensity (or intensity ratio) I = [In^-] and I_o = [In^-]$_o$ where I_o is the maximum fluorescence intensity. This equation can be re-written in terms of I to give eqn. (1):

$$I = \frac{I_o}{1 + (10^{\mathrm{pH} - \mathrm{p}K_a})^{-1}} + B$$

An additional term B is introduced where B is the fluorescence intensity when [In^-] = 0. The solid lines are theoretical fits using eqn. (1).

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Notes and References

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‡ *Experimental procedure* for **1**:[6] The reaction of TAA (1.00 g, Acros, 98%) with $SOCl_2$ (0.8 cm^3) at 60 °C for 2 h in dry conditions gave an orange–brown liquid **1** (the excess $SOCl_2$ was evaporated *in vacuo* at 35 °C) with a yield of *ca.* 80%. δ_H (360 MHz, [$^2H_6$]acetone) 7.24 (d, H1), 7.21 (d, H3), 7.05 (s, H2), 4.25 (s, H4, H5).

§ *Experimental procedure* for **2**: Compound **1** (0.74 g) was reacted with fluoresceinamine (4.00 g) in boiling acetone (125 cm^3) and cooled. Chloroform (100 cm^3) and de-ionised water (100 cm^3) were added to the reaction mixture, which was transferred to a separation funnel with 25 cm^3 of 5% NaOH. The aqueous layer containing the product was removed and 100 cm^3 of 5% HCl was added. Vacuum filtration and recrystalisation from EtOH–H_2O (1 : 1) gave orange crystals of **2** with a yield of *ca.* 60%. δ_H(360 MHz, [$^2H_6$]acetone 9.82 (s, H10), 8.94 (s, H13), 8.42 (s, H15), 7.96 (s, H14), 7.48 (d, H9), 7.33 (d, H7), 7.18–7.16 (2d, H2, H3), 6.82–6.76 (2d, H11, H12), 6.7–6.68 (2d, H8, H1), 6.62–6.59 (2d, H16, H17), 3.79 (s, H4, H5), 2.83 (s, H6).

¶ *Experimental procedure* for polymer **3**: Electrochemical cell consisted of a Pt coated glass working electrode, Ag/Ag^+ reference electrode and Pt gauze counter electrode. The electrolyte solution was 0.10 mol dm^{-3} tetrabutylammonium perchlorate (TBAP) and 0.021 mol dm^{-3} **2** in MeCN. Compound **2** was deposited using cyclic voltammetry. The potential was applied *via* an Oxford Electrodes potentiostat at 100 mV s^{-1} between −0.2 and 2.6 V *vs.* Ag/Ag^+ reference electrode for 20 cycles. A golden film was produced on the electrode surface.

Fluorescence emission intensities were measured on a Perkin-Elmer LS50B luminescence spectrophotometer. The fluorescence intensity ratio was measured using excitation at 430 and 488 nm The emission wavelength was 515 nm.

Platinum deposition on glass was achieved using thermal vapour deposition.

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