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Synthesis of Reactive Vinylsulphonyl Azo Dyes for Application in Optical pH Sensing

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ABSTRACT

This work describes the application of a synthon (GM1) capable of forming a variety of reactive azo dyes useful as pH indicators. GM1 possesses two functional groups, namely (a) an amino group which allows diazo coupling to form an indicator chromophore, and (b) a 2-hydroxyethylsulphonyl group which, after activation, allows its immobilization on cellulose. GM1 has been diazo coupled with various aromatic phenols and amines to give a variety of pH probes. After the 2-hydroxyethylsulphonyl group has been converted into a reactive vinylsulphonyl group, the dye has been covalently linked to the hydroxy groups of cellulose. We use a novel type of a transparent solid film consisting of a polyester support covered with a thin layer of cellulose acetate which, during immobilization, is converted into cellulose. Cellulose membranes coloured with such pH indicating dyes can be applied for optical pH sensing. All dyes are characterized, both in dissolved and immobilized form, in terms of optical properties in the acid and conjugate base form, pK_a values and indicator properties. Semiempirical AM1 calculations have been performed in order to compute

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deprotonation energies of three representative dyes, and data were compared with experimental pK_a's

1 INTRODUCTION

The determination of the pH of aqueous solutions is an important aspect of analytical chemistry and is routinely performed in most laboratories, using glass electrodes. Nevertheless, under conditions of low ionic strength or high pH, the accuracy of glass electrodes is limited. Photometric measurements offer an attractive alternative for on-line monitoring of pH. Corresponding devices are called optrodes and can have advantages over electrodes.[1]

The principle of pH-optrodes is straightforward; a pH-sensitive dye is immobilized on the tip of an optical fibre or on a planar membrane support which may be placed in a flow-through cell. A change in the pH of the solution passing the cell leads to a change in the absorption of the coloured membrane, which is detected using a spectrophotometer, sometimes in conjunction with an optical fibre arrangement. Compared to electrochemical pH detectors, optrodes show fairly fast response time, high reproducibility and a very stable signal, but have a limited dynamic range and are cross sensitive to ionic strength.[1–3]

This paper describes the synthesis of functionalized azo dyes of the Remazol type[4,5] and their immobilization on cellulose. Dyes of that kind are used in textile chemistry because they can be covalently fixed to the substrate. However, textile chemists and consumers expect the colour of the fabric to be the same at any condition and pH. Therefore, the available Remazol dyes are not intended for, or suitable for, use in optical pH determination. The latter requires the dye to possess a functional group (such as a phenolic hydroxy group) with an appropriate pK_a value, and to give an intense change in colour with pH. Furthermore, dyes used in sensor chemistry are expected to possess absorption wavelengths in the 550 to 800 nm range in order to be compatible with available light-emitting diodes (LEDs). Last, but not least, pH indicators for optical sensing should be covalently linked to the support in order to prevent leaching. The work described here was performed accordingly in view of these aspects.

2 EXPERIMENTAL

2.1 Apparatus

Melting points were obtained on a Gallenkamp melting point apparatus and were uncorrected. ^{1}H-NMR spectra were acquired on a Varian XL

200 Gemini or on a Bruker 360 spectrometer with chemical shifts given in delta (ppm) relative to TMS. In most cases, protons of aromatic amino and hydroxy groups were hydrogen-bonded and delta values could not be determined. Mass spectra were obtained on a Finnigan 4500. Elemental microanalyses were performed on a Carlo Erba 1106 CHN microanalyser. The pH of buffer solutions (see Section 2.2) was measured with a Metrohm 632 pH-meter. The absorption spectra of coloured membranes and dissolved dyes, as well as measurements for the determination of pK_a, were performed on a Shimadzu UV-2101-PC spectrophotometer.

2.2 Reagents

All reagents were of analytic reagent grade. The buffer used in this work contained 0·04 M sodium acetate, 0·04 M boric acid, 0·04 M sodium dihydrogen phosphate, and 0·1 M sodium sulphate. The pH was adjusted using 1·0 N sodium hydroxide and 6 N sulphuric acid. Column chromatography was performed with Silica gel 60 (63–200 μm, Merck). The membranes used for immobilization were transparent overhead foils (Hewlett Packard, prod. no. 17703T). The films consisted of a layer of cellulose acetate (10 μm thickness) fixed on to a transparent polyester support (Mylar).

2.3 Synthesis of reactive azo dyes

2.3.1 1-Hydroxy-4-[4-(2-hydroxyethylsulphonyl)-phenylazo]-naphthalene (1)
3·2 g (13·4 mmol) of GM1[6–9] were mixed with 4·5 ml (27 mmol) of 6 N hydrochloric acid and cooled to below 5°C. To this, a solution of 0·88 g (12·8 mmol) of sodium nitrite in 5 ml of water was slowly introduced. The resulting solution was added to 1·84 g (12·8 mmol) of 1-hydroxynaphthalene in 20 ml of cold acetic acid, and the mixture was stirred for 4 h. The red precipitate was filtered with suction, washed with 20 ml of 100% acetic acid to remove traces of 1-hydroxynaphthalene and then washed with 100 ml of cold water. Yield 3·6 g (79%). Recrystallisation from acetic acid-water (9 : 1) gave red crystals m.p., 239°C. ^{1}H-NMR (DMSO): δ (ppm) 8·61 (d, 1 H, =CH—), 7·62–8·28 (m, 8 H, =CH—), 6·85 (d, 1 H, =CH—), 4·94 (t, 1 H, —OH), 3·73 (q, 2 H, —CH_2—), 3·49 (t, 2 H, —CH_2—). MS: *m/z* (%): 356 (25%, $M^{\cdot+}$); 143 (100%, base peak). Calc'd for $C_{18}H_{16}N_2O_4S$: C, 60·69; H, 4·53; N, 7·86; found: C, 60·55; H, 4·65; N, 7·87.

2.3.2 1-Hydroxy-2-[4-(2-hydroxyethylsulphonyl)-phenylazo]-naphthalene (2)
An ice-cooled diazonium chloride solution, prepared from 0·32 g (1·34 mmol) of GM1, 0·45 ml (2·7 mmol) of 6 N hydrochloric acid and 0·088 g

(1·28 mmol) of sodium nitrite dissolved in 0·5 ml of water, was added to a solution of 0·19 g (1·34 mmol) of 1-hydroxynaphthalene, 0·45 g (4·2 mmol) of sodium carbonate and 0·056 g (1·4 mmol) of sodium hydroxide in 5·0 ml of water at 5°C. The resulting mixture was stirred for 1 h, after which 5·0 ml of water was added, and the mixture then acidified with 6 N hydrochloric acid. The red precipitate was filtered off, washed with 20 ml of cold water and dried at 60°C. Pure **2** was isolated by column chromatography (Silica gel 60, chloroform—acetone, 5 : 3), taking the first orange fraction, and further purified by chromatography using petroleum ether–ethyl acetate (1 : 1, v/v) as the eluent; red crystals m.p.: 216 °C.

^{1}H-NMR (DMSO): δ (ppm) 8·34 (d, 1 H, =CH—), 9·97 (t, 3 H, =CH—), 7·55–7·78 (m, 4 H, =CH—), 7·22 (s, 2 H, =CH—), 4·94 (t, 1 H, —OH), 3·74 (q, 2 H, —CH_2—), 3·52 (t, 2 H, —CH_2—). MS: *m/z* (%): 356 (15%, $M^{\cdot+}$); 143 (100%, base peak). Calc'd for $C_{18}H_{16}N_2O_4S$: C, 60·69; H, 4·53; N, 7·86; found: C, 60·68; H, 4·67; N, 7·76.

2.3.3 Potassium l-hydroxy-4-[4-(2-hydroxyethylsulphonyl) -phenylazo]-naphthalene-2-sulphonate (3)

A diazonium chloride solution was made in the usual way, from 5·0 g (21 mmol) of GM1, 7·0 ml (42 mmol) of 6 N hydrochloric acid and 1·38 g (20 mmol) of sodium nitrite dissolved in 8 ml of water. The diazonium solution was added to a cooled solution of 5·4 g (20 mol) of potassium l-hydroxynaphthalene-2-sulphonate, 1·4 g (25 mmol) of potassium hydroxide and 8·7 g (63 mmol) of potassium carbonate in 50 ml of water. The resulting mixture was stirred for 1 h. The precipitated dye was dissolved by adding small amounts of potassium hydroxide and a saturated solution of potassium chloride (50 ml) was added to the dye solution, and the mixture then acidified with 6 N hydrochloric acid. Following this procedure, the dye precipitated in a form that was easily filtered off and it was washed with 20 ml of cold water; yield 5 g (48%). Recrystallization from methanol–water (2 : 1) gave purple needles, which were dried at 60°C; m.p. *c.* 246°C (decomp).

^{1}H-NMR (DMSO): δ (ppm) 12·34 (s, 1 H, —OH, arom.), 8·97 (d, 1 H, =CH—), 8·1–8·4 (m, 6 H, =CH—), 7·7–7·9 (m, 3 H, =CH—), 4·93 (t, 1 H, —OH), 3·74 (q, 2 H, —CH_2—), 3·52 (t, 2 H, —CH_2—). Calc'd for $C_{18}H_{15}KN_2O_7S_2$ + 2·5 H_2O: C, 41·61; H, 3·88; N, 5·39; found: C, 41·87; H, 4·06; N, 5·38.

2.3.4 Sodium l-hydroxy-2-[4-(2-hydroxyethylsulphonyl)-phenylazo]-naphthalene-4-sulphonate (4)

An ice-cooled diazonium chloride solution, made from 3·2 g (13·4 mmol) of GM1, 4·5 ml (27 mmol) of 6 N hydrochloric acid and 0·88 g

(12·8 mmol) of sodium nitrite dissolved in 5 ml of water, was added to a solution consisting of 4·7 g (13·4 mmol) of sodium 1-hydroxynaphthalene-4-sulphonate (70%), 4·5 g (42 mmol) of sodium carbonate, and 0·56 g (14 mmol) of sodium hydroxide in 20 ml of water. The resulting mixture was stirred for 1 h, 20 ml of water added, and the mixture then acidified with 6 N hydrochloric acid. The red precipitate was filtered off and washed with 20 ml of cold water; yield 4·6 g (70%). Recrystallization from methanol–water (1 : 1) gave red crystals which were dried at 60°C; m.p. >300°C.

^{1}H-NMR (DMSO): δ (ppm) 8·62 (d, 1 H, =CH—), 8·34 (d, 1 H, =CH—), 7·98 (s, 4 H, =CH—), 7·7–7·9 (m, 3 H, =CH—), 4·96 (t, 1 H, —OH), 3·74 (q, 2 H, —CH_2—), 3·52 (t, 2 H, —CH_2—). MS: *m/z* (%): 356 (5%, $M^{\cdot+}$ minus —SO_3Na); 64 (100%, base peak). Calc'd for $C_{18}H_{15}NaN_2O_7S_2 \times 3H_2O$; H_2O: C, 42·19; H, 4·09; N, 5·46; found: C, 42·37; H, 3·81; N, 5·45.

2.3.5 8-Hydroxy-5-[4-(2-hydroxyethylsulphonyl)-phenylazo]-quinoline (**5**)[10]
An ice-cooled diazonium chloride solution, prepared from 3·2 g (13·4 mmol) of GM1, 4·5 ml (27 mmol) of 6 N hydrochloric acid and 0·88 g (12·8 mmol) of sodium nitrite dissolved in 5 ml of water, was slowly added to a solution of 1·85 g (12·8 mmol) of 8-hydroxyquinoline in 20 ml of acetic acid. The mixture was stirred for 5 h, and 20 ml of a saturated solution of sodium acetate then added to precipitate the dye. The crude dye was filtered off and washed with 20 ml of cold water, then with 20 ml of acetic acid, and finally with 100 ml of cold water to remove acetic acid; yield 4·3 g (90%). Recrystallization from water gave orange crystals, which were dried at 40°C; m.p. 221°C.

^{1}H-NMR (DMSO): δ (ppm) 9·3 (d, 1 H, =CH—), 9·1 (m, 1 H, =CH—), 8·12 (m, 5 H, =CH—), 7·82 (q, 1 H, =CH—), 7·25 (d, 1 H, =CH—), 4·93 (t, 1 H, —OH), 3·76 (q, 2 H, —CH_2—), 3·57 (t, 2 H,—CH_2—). MS: *m/z* (%): 357 (24%, $M^{\cdot+}$); 144 (100%, base peak). Calc'd for $C_{17}H_{15}N_3O_4S+H_2O$: C, 54·39; H, 4·56; N, 11·19; found: C, 54·64; H, 4·71; N, 11·29.

2.3.6 2-Hydroxy-1-[4-(2-hydroxyethylsulphonyl)-phenylazo]-naphthalene (**6**)[6]
This was obtained following the procedure for **4** above; m.p. 241°C methanol–water (1 : 1).

^{1}H-NMR (DMSO): δ (ppm) 8·44 (d, 1 H), 7·4–8·0 (m, 8 H), 6·76 (d, 1 H), 4·93 (t, 1 H), 3·74 (q, 2 H, —CH_2—), 3·52 (t, 2 H, —CH_2—). MS: *m/z* (%): 356 (42%, $M^{\cdot+}$); 143 (100%, base peak). Calc'd for $C_{18}H_{16}N_2O_4S$: C, 60·69; H, 4·53; N, 7·86; found: C, 60·55; H, 4·61; N, 7·78.

2.3.7 Sodium 2-hydroxy-1-[4-(2-hydroxyethylsulphonyl)-phenylazo]-naphthalene-6-sulphonate (**7**)

An ice-cooled diazonium chloride solution, made from 2·0 g (8·4 mmol) of GM1, 2·8 ml (16·8 mmol) of 6 N hydrochloric acid and 0·52 g (7·6 mmol) of sodium nitrite dissolved in 4 ml, of water was slowly added to a solution of 2·2 g (7·6 mmol) of sodium 2-hydroxynaphthalene-6-sulphonate (80%), 0·34 g (8·5 mmol) of sodium hydroxide and 1·8 g s (17 mmol) of sodium carbonate in 40 ml of water. The mixture was stirred for 1 h and acidified to give 2·8 g of crude dye (77%). Recrystallization from methanol–water (2 : 1) gave orange crystals, m.p. >300°C.

1H-NMR (DMSO): δ (ppm) 8·44 (d, 1 H, =CH—), 8·1 (m, 6 H, =CH—), 7·83 (d, 1 H, =CH—), 6·78 (d, 1 H, =CH—), 4·93 (t, 1 H, —OH), 3·74 (q, 2 H, —CH2—), 3·51 (t, 2 H, —CH_2—). MS: *m/z* (%): 356 (1%, $M^{\cdot+}$ without —SO_3Na); 64 (100%, base peak). Calc'd for $C_{18}H_{15}NaN_2O_7S_2+H_2O$): C, 45·38; H, 3·59; N, 5·88; found: C, 45·25; H, 3·62; N, 5·84.

2.3.8 Disodium 2-hydroxy-1-[4-(2-hydroxyethylsulphonyl)-phenylazo]-naphthalene-3,6-disulphonate (**8**)[6]

This was obtained following the procedure described above for **7**; m.p. >300°C (methanol/water, 2 : 1).

^{1}H-NMR (DMSO): δ (ppm) 8·36 (d, 1 H, =CH—), 8·24 (s, 1 H, =CH—), 7·7–8·1 (m, 6 H, =CH—), 4·96 (t, 1 H, —OH), 3·76 (q, 2 H, —CH_2—), 3·53 (t, 2 H, —CH_2—). Calc'd for $C_{18}H_{14}Na_2N_2O_{10}S_3$+5 H_2O: C, 33·23; H, 3·71; N, 4·31; found: C, 33·18; H, 3·29; N, 4·30.

2.3.9 1-Amino-4-[4-(2-hydroxyethylsulphonyl)-phenylazo]-naphthalene (**9**)

An ice-cold diazonium chloride solution, prepared from 1·6 g (6·7 mmol) of GM1, 2·25 ml (13·5 mmol) of 6 N hydrochloric acid and 0·44 g (6·4 mmol) of sodium nitrite dissolved in 3 ml of water, was slowly added to a solution of 0·84 g (5·9 mmol) of l-aminonaphthalene in 40 ml of 96% ethanol. The mixture was stirred for 3 h and the crude dye filtered off and washed with 50 ml of water to yield 1·7 g (81%) of **9**. The crude material was dissolved in a hot mixture of methanol–water (1 : 1) and the red dye precipitated by adding a saturated solution of sodium carbonate in water; m.p. 179°C.

^{1}H-NMR (DMSO): δ (ppm) 8·93 (d, 1 H, =CH—), 8·26 (d, 1 H, =CH—), 8·02 (t, 5 H, =CH—), 7·5–7·75 (m, 2H, =CH—) 7·32 (s, 2 H, —NH_2), 6·82 (d, 1 H, =CH—), 4·93 (t, 1 H, —OH), 3·75 (q, 2 H, —CH_2—), 3·52 (t, H, —CH_2—). MS: *m/z* (%): 355 (23%, $M^{\cdot+}$); 142

(100%, base peak). Calc'd for $C_{18}H_{17}N_3O_3S$: C, 60·83; H, 4·82; N, 11·82; found: C, 60·62; H, 4·77; N, 11·62.

2.3.10 Sodium 1-amino-2-[4-(2-hydroxyethylsulphonyl)-phenylazo]-naphthalene-4-sulphonate (**10**)

An ice-cooled diazonium chloride solution was made in the usual way using 5·0 g (21 mmol) of GM1, 7·0 ml (42 mmol) of 6 N hydrochloric acid and 1·38 g (20 mmol) of sodium nitrite dissolved in 8 ml of water. The diazonium chloride solution was added to a cooled solution consisting of 5·8 g (21 mmol) of 1-aminonaphthalene-4-sulphonate and 6·0 g (73 mmol) of sodium acetate in 100 ml of water. After stirring for 1 h, the dye was precipitated with 6 N hydrochloric acid, filtered off and washed with 20 ml of cold water. Yield 8·7 g (93%). Recrystallization from water gave purple crystals, m.p. *c.* 235°C (decomp.).

^{1}H-NMR (DMSO): δ (ppm) 8·77 (d, 1 H, =CH—), 8·52 (d, 1 H, =CH—), 8·0–8·3 (m, 5 H, =CH—), 7·5–7·7 (m, 2 H, =CH—), 4·93 (t, 1 H, —OH), 3·74 (q, 2 H, —CH_2—), 3·52 (t, 2 H, —CH_2—). MS: *m/z*(%): 355 (26%, $M^{\cdot+}$ without —SO_3Na); 143 (100%, base peak). Calc'd for $C_{18}H_{16}NaN_3O_6S_2$ + 1/2 H_2O: C, 46·35; H, 3·68; N, 9·01; found: C,46·86; H, 4·24; N, 9·13.

2.3.11 4-N,N-Dimethylamino-4'-(2-hydroxyethylsulphonyl)-azobenzene (**11**)

2 g (8·4 mmol) of GM1 and 2·8 ml (16·8 mol) of 6 N hydrochloric acid were cooled below 5°C in an ice bath. To the mixture was slowly added 0·55 g (8 mmol) of sodium nitrite in 3 ml of water. The solution was then added to a stirred ice-cold solution of 1·0 g (8·4 mmol) of *N,N*-dimethylaniline in 8·4 ml (8·4 mmol) of 1 N hydrochloric acid. After stirring for 1 h, a saturated aqueous solution of sodium acetate was added to precipitate the dye. The crude dye was filtered off and washed with 20 ml of cold water and dried at 60°C to yield 1·9 g (68%). Recrystallization from methanol–ethyl acetate (1 : 1) gave red needles, m.p. 170°C.

^{1}H-NMR (DMSO): delta (ppm) 7·8–8·1 (m, 6 H, =CH—); 6·88 (d, 2 H, =CH—), 4·92 (t, 1 H, —OH); 3·74 (q, 2 H, —CH_2—); 3·52 (t, 2 H, —CH_2—); 3·13 (s, 6 H, —CH_3). MS: *m/z* (%): 333 (30%, $M^{\cdot+}$); 120 (100%, base peak). Calc'd for $C_{16}H_{19}N_3O_3S$: C, 57·64; H, 5·74; N, 12·60; found: C, 57·39; H, 5·64; N, 12·43.

2.4 General protocol for dye immobilization on cellulose

The method of immobilization of vinylsulphonyl dyes has been described.[11,12] In essence, the 2-hydroxyethylsulphonyl group of the dye is

activated by treatment with sulphuric acid to give the ester of general formula (dye-SO_2—CH_2—CH_2—O—SO_3H), which, after reaction with sodium carbonate, forms the vinylsulphonyl derivative (dye—SO_2—$CH{=}CH_2$). In strongly alkaline solution, the vinylsulphonyl group reacts with cellulose (Cell-OH) to give the dye/cellulose conjugate (dye-SO_2—CH_2—CH_2—O-Cell).

2.5 Spectroscopic measurements

Absorption spectra of dyes were obtained as follows: 100 μmol solutions in water (or in methanol if the dye was only slightly water-soluble) were diluted to 10 μM solutions by the addition of buffer solutions of different pH. Absorption spectra of the membranes were obtained by fixing the membranes in cuvettes and adding the buffer solutions.

The pK_a determinations were performed by measuring the pH-dependent absorption of the dye at a fixed wavelength chosen such that the difference in the absorption of acid and conjugate base form was maximal. Plots of absorbance versus pH give a sigmoidal curve and the pH of the turning point is the pK_a. The pK_a values were calculated via the following equation:

$$pK_a = pH + \log (A_x - A_b)/(A_a - A_x)$$

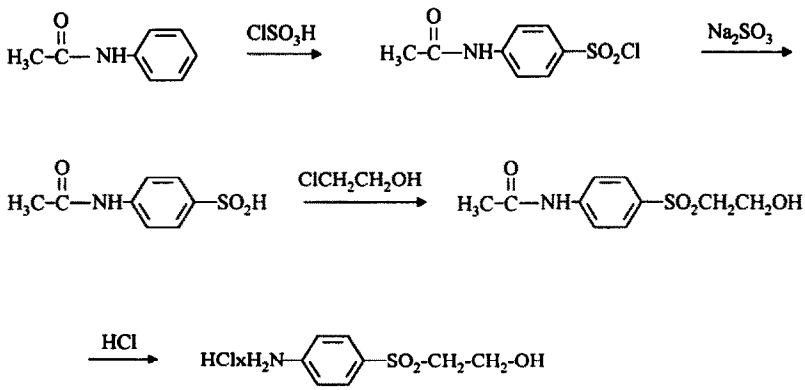
where A_x, A_b and A_a are the absorbances of the (immobilized) dye at a defined pH near the pK_a the base form and the acid form, respectively.

3 RESULTS AND DISCUSSION

3.1 Synthetic strategy

It appeared desirable to synthesize a variety of dyes via a single synthon, which, in combination with various coupling components, is capable of forming a large variety of different dyes possessing different properties. We considered 4-(2-hydroxyethylsulphonyl)anilinium chloride (GM1) to be a good candidate. The phenyl group of GM1 is substituted by one amino group (later to be converted into a diazonium salt) and a 2-hydroxyethylsulphonyl group in the *para* position. The latter is the reactive part which, after being converted into a vinylic group, can add to amino, hydroxy and thiol groups of a solid support, thereby enabling covalent immobilization.

The synthesis of GM1 imposes no problems: starting from acetanilide via 4-acetylaminophenyl sulphonyl chloride,[7] 4-acetylaminophenyl sul-



GM1

Scheme 1

phinic acid,[7] 4-acetylaminophenyl-(2-hydroxyethyl)-sulphone,[8] the 4-(2-hydroxyethylsulphonyl)-anilinium chloride GM1[8] was obtained in fair yield (Scheme 1).

In the next step, GM1 was diazotized and reacted with various substituted 1-hydroxynaphthalenes, 2-hydroxynaphthalenes, 1-aminonaphthalenes and N,N-dimethylaniline to give 11 reactive azo dyes whose structures are presented in Fig. 1.

3.2 Separation of dye isomers

Unfortunately, positional isomers are formed when GM1 is coupled with 1-hydroxynaphthalenes or 1-aminonaphthalenes which are unsubstituted in the 2- or 4-positions. 1-Hydroxynaphthalene is a typical example of this, i.e. with respect to the synthesis and purification of each isomeric dye in pure form and its structural identification. When the synthesis was performed according to the usual procedure,[13,14] i.e. dissolving 1-hydroxynaphthalene by addition of sodium hydroxide and using sufficient sodium hydroxide to keep the solution alkaline during the diazotization, all possible isomers were formed (**1**, **2** and the bis-azo dye). The bis-azo dye was formed in traces only, and therefore not isolated. The *para*-azo dye **1** and the *ortho*-azo dye **2** were isolated by column chromatography and characterized by mass spectra, elemental analysis and ^{1}H-NMR. Both isomers were also identified by colour change and pK_a values. Indicator **2** exists in a more planar conformation because of intramolecular hydrogen bonding. Therefore, it changes its colour from purple–red to orange on acidification (similar to **4**), while **1** changes from purple to yellow (similar to **3**). Furthermore, the intramolecular hydrogen bond is

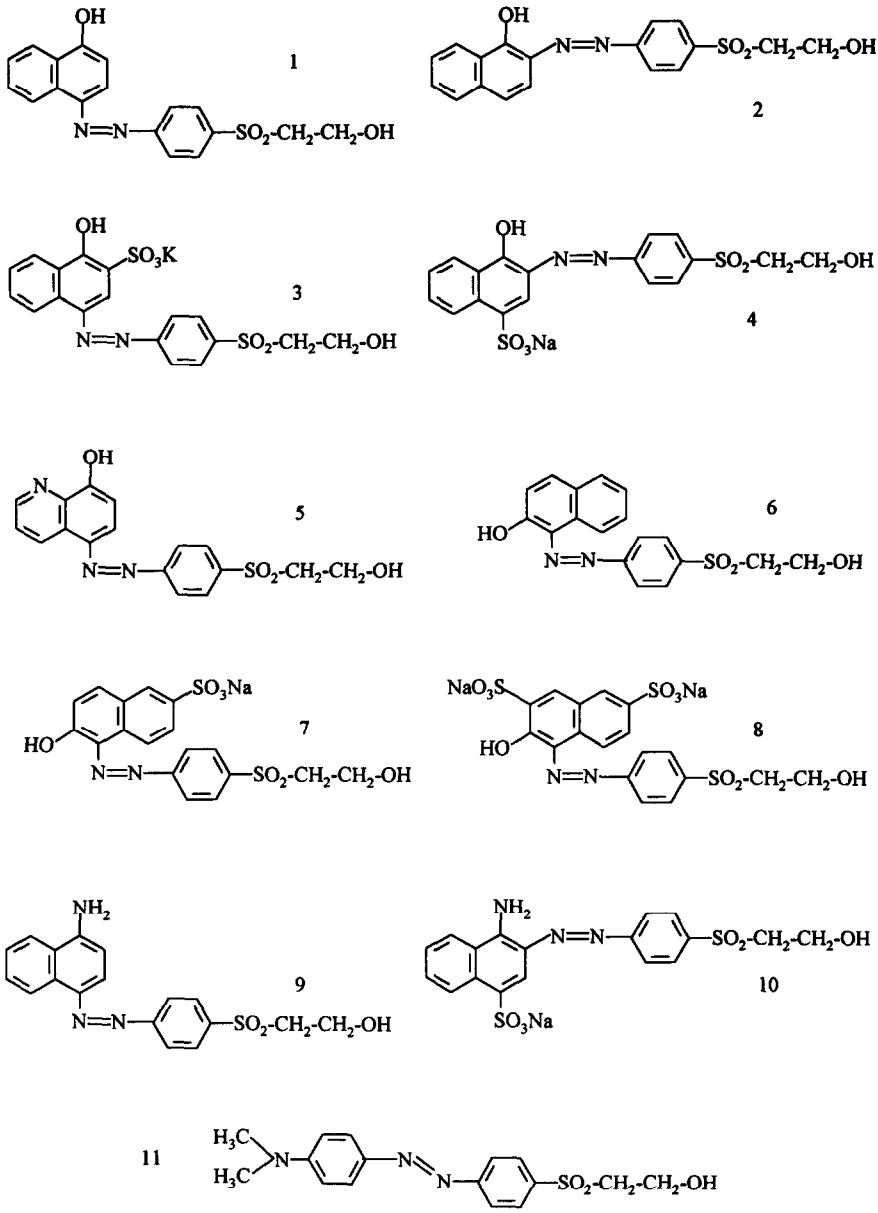


Fig. 1. Structures of reactive azo dyes **1**—**11**.

the reason why **2** shows a pK_a of 9·05 a value which is distinctly higher than that of **1** (8·32).

Column chromatography was used to separate the compounds, but yields were not satisfactory and the procedure was tedious. Therefore, an attempt was made to obtain pure isomers by modification of the synthesis. It was found that by increasing the pH, more *bis*-azo and *ortho*-azo dye were formed. However, there was always a mixture of all three isomers. Therefore, diazotation of 1-hydroxynaphthalene was performed in acetic acid. In this case, the product was almost pure *para*-azo dye (**1**) which could be purified by repeated recrystallization. When 8-hydroxyquinoline was diazotized in acetic acid with GM1, *para* coupling was exclusively, observed (dye **5**). The other couplings were performed using 1-hydroxynaphthalenes, which only allowed one possible isomer to be formed. Thus, potassium 1-hydroxynaphthalene-2-sulphonate gave **3**, and sodium 1-hydroxynapthalene-4-sulphonate gave **4**.

In case of all 2-hydroxynaphthalenes, coupling occurs only in the 1-position (**6**, **7**, **8**), and hence there are no problems with respect to isomers and their separation.

With 1-aminonaphthalene, coupling was performed in ethanol to give the pure *para*-azo compound (**9**). Similarly, 1-aminonaphthalene-4-sulphonate and *N*,*N*-dimethylaniline gave only one product: dye **10** was exclusively obtained from 1-aminonaphthalene-4-sulphonate, and **11** from *N*,*N*-dimethylaniline.

3.3 Covalent immobilization

The reactive 2-hydroxyethyl sulphonyl group of the dyes can be activated by esterification with concentrated sulphuric acid, followed by elimination of the sulphate with alkali to give the vinylsulphonyl group, which is covalently linked (*in situ*) to the hydroxy groups of the cellulose (Scheme 2). We used a new type of transparent membranes for dye immobilization, consisting of a 10 μm layer of cellulose acetate on an optically transparent polyester support (100 μm). The cellulose acetate is converted into free cellulose under the experimental conditions of the dye immobilization,[11,12]

Cellulose has a proton-permeability much higher than that of other polymers such as PVC, polyesters and even hydrogels. Cellulose is highly hydrophilic and, therefore, the membrane exhibits fast response during pH monitoring. The high homogeneity of the manufactured membranes[11] allows production of membranes with very reproducible properties. The membrane preparation does not require any toxic solvents (such as chloroform or tetrahydrofuran) to be used.

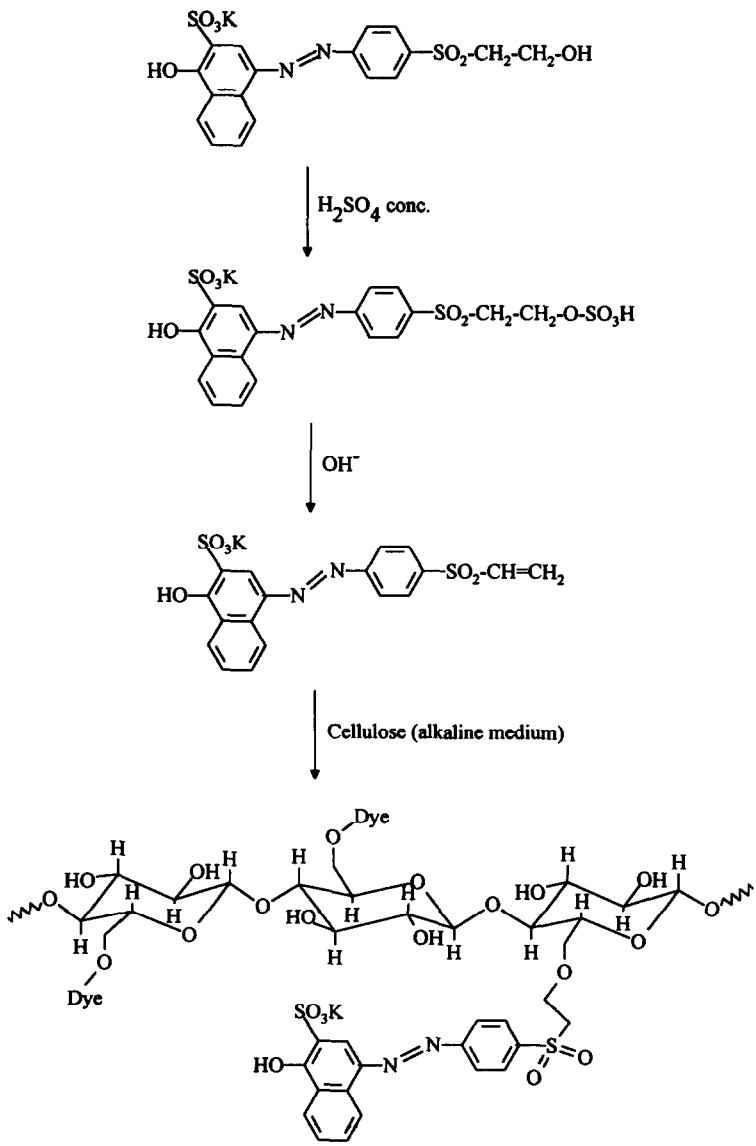
TABLE 1
Absorption Maxima (in nm), Molar Absorbances (ε), and pK_a Values of Dissolved (**1–11**) and Membrane-immobilized Dyes (**M1–M11**) at 21°C

Dye/membrane	λ_{max} (nm)		*ε litre (mol cm) base/acid*	pK_a
	Base form	*Acidic form*		
1	541	466	38 700/36 700	8·32
2	528	497	23 300/17 400	9·05
3	527	479	34 600/36 800	7·14
4	507	488	25 600/27 500	7·66
5	519	463	38 700/20 500	7·54
6	478	481	15 300/21 900	11·26
7	466	476	14 400/25 000	10·18
8	480	483	17 700/29 000	10·38
9	497	512	22 400/44 700	4·57
10	482	524	16 000/26 400	~1·0
11	481	500	32 300/36 800	3·18
M1	553	460		9·37
M2	535	501		9·26
M3	541	473		7·55
M4	517	491		7·83
M5	518	455		7·34
M6	476	487		11·28
M7	474	479		10·68
M8	481	488		10·64
M9	507	509		3·68
M10	492	518		~0·5
M11	486	503		2·24

Table 1 summarizes the absorption maxima of the dyes in their acidic and conjugate base forms, the molar absorbances, and the pK_a values of both the dissolved and the cellulose-immobilized dyes. The data show that the series of dyes presented in this work is suitable for sensing pH over a wide range, but it should be kept in mind that the dynamic range of a single dye (i.e. the pH range over which a measurable colour change occurs) does not exceed around 3 pH units.

3.4 Structure–colour relationship of phenols 1–8

The phenol PC forms of dyes **1–8** are usually yellow or orange, and the deprotonated forms red or purple. A change from the conjugate acid to the base form of *para* azo dyes (**1,3,5**) results in a significant colour change from yellow to purple. There is little overlap in the absorption spectra (Fig. 2).



Scheme 2

Dyes derived from 2-hydroxynaphthalene (**6,7,8**) do not display such an intense colour change because of their different structure (Fig. 3). A quinoidic form, comparable to dyes made from 1-hydroxynaphthalene, is not possible. In addition, the smaller colour change from acid to base form may be attributed to the hydrogen bond between the proton of the hydroxy group and the nitrogen of the azo group. Dyes being fixed to a

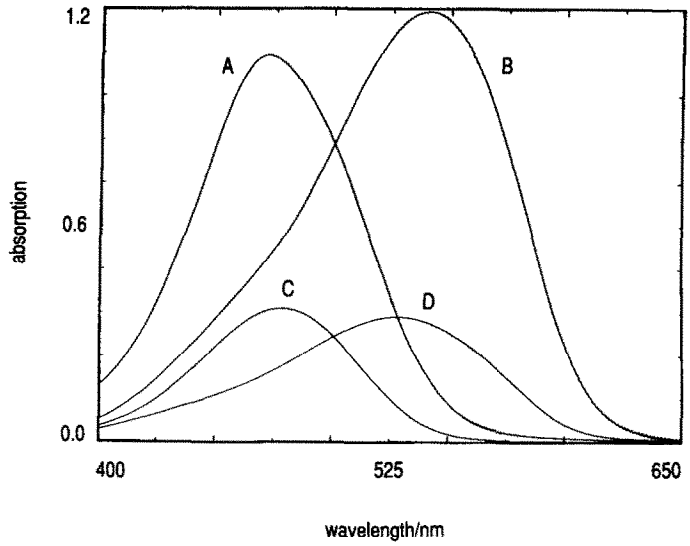


Fig. 2. Absorption spectra of dissolved and immobilized **3** in the acid and conjugate base forms, respectively, showing the conjugate base to be the one with the longest wavelength absorption: A, membrane **M3** in acidic form; B, membrane **M3** in conjugate base form; C, dye **3** in acidic form; D, dye **3** in base form.

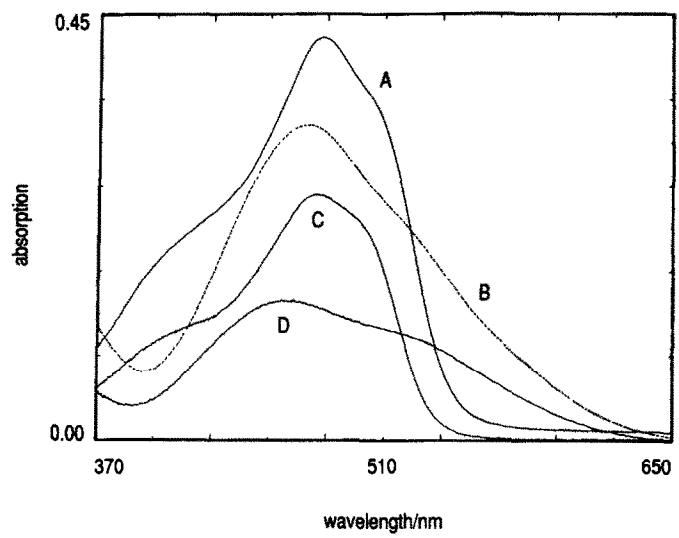


Fig. 3. Absorption spectra of dissolved and immobilized **7** in the acid and conjugate base form, respectively: A, membrane **M7** in acidic form; B, membrane **M7** in conjugate base form; C, dye **7** in acidic form; D dye **7** in base form.

more planar structure (in the protonated form) via hydrogen bonds (**2**,**4**,**6**,**7**,**8**,**10**) show absorptions that are shifted to longer wavelength. Therefore, the colour change is from orange to purple–red.

3.5 Structure–colour relationship of amines 9–11

Dyes 9–11 show an entirely different behaviour in that the absorption maxima of the amine (base) form are at shorter wavelengths than those of the protonated (cationic) form, a fact that is in agreement with existing models of structure–colour relationships and has not been studied in greater detail.

3.6 Effect of structure on pK_a values

Intramolecular hydrogen bonds exert a significant influence on pK_a values. The presence of nitrogen atoms *ortho* to a hydroxy group prevents deprotonation (dissociation). Therefore, the pK_a is higher by around 0·6 unit, as can be seen by comparing dye **1** (pK_a 8·32) with **2** (pK_a 9·05), and **3** (pK_a 7·14) with **4** (pK_a 7·66). Aside from hydrogen bonds, the different electronic structures of dyes derived from 1-hydroxynaphthalene and 2-hydroxynaphthalene causes the pK_a to increase by around 2·2 units: **2** has a pK_a of 9·05 whereas **6** has a pK_a of 11·26.

Predictably, a sulpho group lowers the pK_a and this can be seen when comparing dyes **1** and **3**, or **6** and **7**. In both cases, the pK_a decreases by one unit. In contrast, dye **7** (possessing one sulpho group only) and dye **8** (two sulpho groups) do not show significant differences in their pK_a values.

3.7 Quantum chemical calculations

The pK_a values of **1** and **2** are quite similar (8·32 and 9·05, respectively) whereas that of **6** is considerably higher (11·26). This is surprising, because the similarity in the structures of **2** and **6** suggests similar pK_a values (Fig. 4). In order to elucidate this observation, deprotonation energies of model compounds **1***, **2*** and **3*** were calculated. In contrast to the respective dyes (**1**, **2** and **6**), they lack the 2-hydroxyethylsulphonyl group. This is acceptable because such groups are known not to significantly affect the pK_a Hence, calculations were greatly simplified. Figure 4 gives the structures of model dyes **1***, **2***, **6*** and the respective anions ($\mathbf{1}^-$, $\mathbf{2}^-$, $\mathbf{3}^-$). AM1 calculations[15] were conducted using the MOPAC6 program[16] on a STARDENT Titan 3040 computer. The structures were optimized in a planar arrangement of the nuclei (C_s symmetry). Different conform-

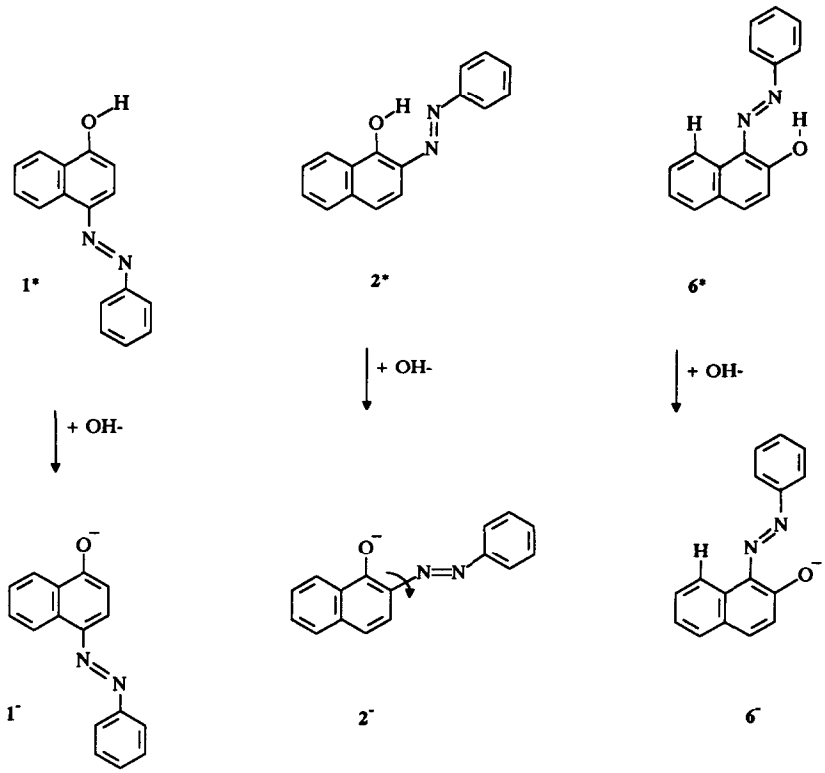


Fig. 4. Model structures **1***, **2*** and **6*** (derived from **1**, **2** and **6**, but without the 2-hydroxyethylsulphonyl groups) and their conjugated bases (anions) $\mathbf{1}^-$, $\mathbf{2}^-$ and $\mathbf{3}^-$, respectively.

TABLE 2

Calculated Heats of Formation (ΔH_f, in kcal/mol), Deprotonation Energies (ΔE_p, in kcal/mol) of Model Dyes **1***, **3*** and **6***, and of Respective Anions,[a] and pK_a Values of Dyes **1**, **2** and **6**

Dye	ΔH_f	ΔE_p	pK_a	
1*	78·8	272·6	**1**	8·32
2*	77·8	274·6	**2**	9·05
6*	79·4	278·6	**6**	11·26
$\mathbf{1}^-$	37·7			
$\mathbf{2}^-$	38·7			
$\mathbf{6}^-$	44·3			

[a] Model structures of **1**, **2** and **6**, but without the 2-hydroxyethylsulphonyl substituent, are named **1***, **2*** and **6***.

$\mathbf{1}^-$, $\mathbf{2}^-$ and $\mathbf{3}^-$ are the respective deprotonated forms (for structures, see Fig. 4).

ers, characterized by dihedral angles such as N=N—C(2)=C(I) and H—O—C(1)=C(2) in **2***, have been examined for each species. The resulting most stable structures are depicted in Fig. 4. Compounds **2*** and **6*** indicate intramolecular OH...N hydrogen bonding. Their bond strength was calculated to be 3 kcal/mol. The calculated deprotonation energies are listed in Table 2.

The significant difference in the deprotonation energies of **2*** and **6*** can be explained as follows: deprotonation of **2*** causes a change in the dihedral angle N=N—C(2)=C(1) from 0 to 180° in degrees in order to relieve repulsive N...O$^-$ interaction in **2**$^-$. A corresponding rearrangement in **6**$^-$ is not effective due to steric hindrance by H—C(8). Thus, the comparably high value of the heat of formation of **6**$^-$ is considered to be the main reason for the exceptionally high deprotonation energy of **6***.

4 CONCLUSION

Dyes prepared from synthon GM 1 and 1-hydroxynaphthalene (or its derivatives) are considered to display the best optical properties for application in pH sensing. They show large changes in the absorption spectra in changing the pH and are compatible with green LEDs. In later work, we will show that cellulose membranes coloured with such dyes are most suitable for precise optical fibre pH sensing.

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