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highly alkaline media, glass electrodes suffer from chemical deterioration and are subject to large alkaline errors[9, 10].

pH sensing using optical methods provides an attractive alternative for measuring pH due to the advantages offered in terms of immunity to electrical interference, enhanced safety and the possibility of remote sensing. Most optical pH sensors function through monitoring the changes in the absorbance or fluorescence properties of certain pH sensitive indicators which are immobilized on/in proton-permeable solid substrates/supports[11]. A number of pH indicators therefore have been designed or investigated for this application. However, the majority of them were demonstrated to work in the physiological/near neutral[12-16] or acidic pH region[5, 8, 11, 17, 18]. Materials that have been reported for use in the highly alkaline pH region are mainly non-immobilized forms that are not ready for use in certain sensing applications[19, 20] or restricted to the absorbance/transmission based group[6, 21-23], which has certain disadvantages compared to the fluorescence-based counterpart. In recent years, a few fluorescent sensors for the alkaline pH region have been prepared[24-27]. However, the number of reports is still very limited. Considering the importance of monitoring pH in highly alkaline media as occurring in numerous industrial processes[9], scrubbers, waste water treatment plants and concrete structures[28, 29], there is essentially a need for accurate, sensitive and reliable sensing materials suitable for measurement of high basicity.

In this paper, the synthesis and characterization of novel polymerizable coumarins bearing imidazolyl groups of which the deprotonation/protonation allows for the determination of pH in the alkaline region of the pH scale are described and their spectral and sensing properties are reported. Coumarins have been chosen because they have many advantages including high fluorescence quantum yield, large Stokes shift

and excellent photo-stability[30]. In addition to the development of appropriate pH indicators, the choice of solid supports and the effective immobilization of the indicators to the supports are also discussed in the paper as they are key factors in the development of an optimum optical pH sensor since they govern the lifetime and signal stability of the sensor. The fluorescence detection method was employed rather than the simpler and more commonly-used method based on absorbance/transmission measurements as fluorescent sensors are usually more precise, having less interference from the light source, and offer higher sensitivity than their absorbance counterparts[7, 31].

2. Experimental

2.1. General

All chemicals were of analytical grade, purchased from Sigma-Aldrich and were used without further purification. All solvents used were of HPLC grade from Fisher Scientific or Sigma-Aldrich. All aqueous solutions were prepared using distilled deionized water. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer. Mass spectra were run by electron ionisation (EI) mode on a Thermo Finnigan MAT900xp mass spectrometer. IR spectra were recorded on a Bruker Alpha Fourier Transform Infrared Spectrophotometer and were run neat. Melting points were recorded using a Stuart SMP30 melting point apparatus and were uncorrected. Elemental analyses were carried out at the Microanalytical Laboratory, Department of Chemistry at University College London. Absorption and fluorescence measurements of aqueous solutions containing fluorophores were carried out on a PerkinElmer Lambda 35 spectrophotometer and a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer system with FluorEssenceTM as driving software, respectively.

Refractive indices were measured on an Abbe refractometer. Quantum yields of fluorescence were determined using quinine sulfate as the standard ($\Phi = 0.55$)[32, 33].

2.2. Synthesis of the fluorescent dyes

3-N-(Carbethoxy)aminophenol (1): **1** was prepared similarly to the method reported in the literature[34]. To a two necked flask equipped with a condenser and a septum were added 3- aminophenol (10.9 g, 100 mmol) and EtOAc (40 mL). The mixture was heated to reflux for 30 min. Ethyl chloroformate (5.4 g, 4764 μ L, 50 mmol) was added via syringe over a 10 min period. The reaction mixture was left stirred for a further 20 min then allowed to cool to room temperature to form a white precipitate. The precipitate was removed by filtration and washed with EtOAc (50 mL) and petroleum ether (50 mL). The combined filtrate was concentrated to give **1** as an off-white solid which was further purified by recrystallization from toluene to afford **1** (8.4g, 93%) as white crystals, mp 92 °C [lit. mp 94-95 °C (benzene/cyclohexane)]; IR (neat) $\nu_{\max}$ (cm^{-1}) 3294 (NH), 1684 (C=O), 1556, 1449, 1249, 1061; $^1\text{H-NMR}$ (500 MHz, CDCl_3) $\delta(\text{ppm})$: 7.35 (s, 1H), 7.13 (t, 1H, $J = 8.08$), 6.69 (s, 1H), 6.64 (m, 1H), 6.57 (m, 1H), 6.33 (m, 1H), 4.23 (q, 2H, $-\text{CH}_2-$, $J=7.12$ Hz), 1.30 (t, 3H, $-\text{CH}_3$, $J = 7.12$); MS (EI): Calcd. $m/z = 181.07334$ ($\text{C}_9\text{H}_{11}\text{O}_3\text{N}$). Found $m/z = 181.07296$ (M^+).

4-Chloromethyl-7-N-(Carbethoxy)aminocoumarin (2): H_2SO_4 (80%, 40 mL) was pre-cooled in an ice bath. **1** (1.8 g, 10 mmol) was added, followed by ethyl 4-chloroacetoacetate (2.3 g, 1906 μ L, 14 mmol) in portions. The mixture was stirred at room temperature under Ar for 19 h, after which it was poured into ice-water (50 mL) and left stirred for a further 30 min. The white precipitate formed was filtered, washed with H_2O and recrystallized from EtOH to afford **2** (2.0 g, 71%) as fine needles, mp 242 °C (decomp.) [lit.[35] mp 247 °C (MeOH)]; IR (neat) $\nu_{\max}$ (cm^{-1}) 3280 (NH), 3084,

2992, 2929, 1704 (C=O), 1619, 1588, 1520, 1404, 1332, 1204, 1075; ¹H-NMR (500 MHz, CDCl₃) δ(ppm): 7.59 (d, 1H, H5, *J*_{5,6}=8.7 Hz), 7.50 (d, 1H, H8, *J*_{8,6}=2.14 Hz), 7.36 (dd, 1H, H6, *J*_{6,5}=8.7 Hz, *J*_{6,8}=2.14), 6.82 (s, 1H, NH), 6.45 (s, 1H, H3), 4.65 (s, 2H, -CH₂Cl), 4.27 (q, 2H, OCH₂-, *J* = 7.13), 1.34 (t, 3H, -CH₃, *J* = 7.13); MS (EI): Calcd. *m/z* = 281.04494 (C₁₃H₁₂O₄NCl). Found *m/z* = 281.04550 (M⁺).

4-Chloromethyl-7-aminocoumarin (3): **2** (563.4 mg, 2 mmol) was suspended in a mixture of concentrated H₂SO₄ (1.7 mL) and glacial acetic acid (1.7 mL). The mixture was heated to 125 °C for 2h. After cooling to room temperature, the yellow solution was poured into H₂O and a voluminous precipitate was formed. 4 M NaOH aqueous solution was added to the mixture to bring pH to ~ 9. The yellow precipitate was filtered, washed with H₂O, dried *in vacuo* to afford **3** (366 mg, 87%) as a pale yellow solid which was used for synthesis without further purification, mp 182 °C [lit.[35] mp 187 °C]; IR (neat) *ν*_{max} (cm⁻¹) 3445 (NH₂), 3352 (NH₂), 1686 (C=O), 1601 (NH₂), 1548, 1404, 1263, 1210, 1145; ¹H-NMR (500 MHz, DMSO) δ(ppm): 7.47 (d, 1H, H5, *J*_{5,6}=8.7 Hz), 6.58 (dd, 1H, H6, *J*_{6,5}=8.7 Hz, *J*_{6,8}=2.00), 6.43 (d, 1H, H8, *J*_{8,6}=2.00 Hz) 6.19 (s+s, nonres., 3H, -NH₂ + H3), 4.86 (s, 2H, -CH₂Cl); MS (EI): Calcd. *m/z* = 209.02381 (C₁₀H₈O₂NCl). Found *m/z* = 209.02417 (M⁺).

7-amino-4-((2-methyl-4-nitro-1*H*-imidazol-1-yl)methyl)-coumarin (4): NaH in mineral oil (60%, 60 mg) was added to a solution of 2-methyl-4-nitroimidazole (203 mg, 1.6 mmol) in DMF (6 mL). The mixture was heated to 100 °C for 10 min and then cooled down to 60 °C, after which **3** (314 mg, 1.5 mmol) dissolved in DMF (6 mL) was added. The reaction mixture was left stirring at the same temperature for 18 h then poured into ice. The yellow precipitate formed was collected by filtration, washed with water and recrystallized from EtOH to afford **4** (390 mg, 87%) as yellow fine crystals, mp 298-300 °C (decomp.); IR (neat) *ν*_{max} (cm⁻¹) 3430 (NH₂), 3324 (NH₂), 3221, 3144,

126 1695 (C=O), 1595 (NH₂), 1542, 1498, 1402, 1328, 1290, 1132; ¹H-NMR (500 MHz,
127 DMSO) δ(ppm): 8.37 (s, 1H, 5-H-imidazole), 7.44 (d, 1H, H5, *J*_{5,6}=8.69 Hz), 6.61 (dd,
128 1H, H6, *J*_{6,5}=8.69 Hz, *J*_{6,8}=2.14), 6.45 (d, 1H, H8, *J*_{8,6}=2.14 Hz) 6.27 (s, 2H, -NH₂),
129 5.51 (s, 2H, -CH₂-), 5.05 (s, 1H, H3), 2.31 (s, 3H, -CH₃); ¹³C-NMR (DMSO) δ(ppm):
130 160.7 (C2), 155.9 (C9), 154.0 (C7), 151.1 (4-C-imidazole), 146.1 (C4), 125.7 (C5),
131 123.1 (5-C-imidazole), 111.7 (C6), 106.2 (C10), 104.5 (C3), 99.0 (C8), 46.6 (-CH₂-),
132 12.8 (-CH₃); MS (EI): Calcd. *m/z* = 300.08531 (C₁₄H₁₂O₄N₄). Found *m/z* = 300.08584
133 (M⁺); Elem. Anal. Calcd. for C₁₄H₁₂O₄N₄ (300.27): C 56.00, H 4.03, N 18.66. Found:
134 C 56.72, H 4.50, N 18.19.

135 **7-(4-vinylbenzylamino)-4-((2-methyl-4-nitro-1*H*-imidazol-1-yl)methyl)-coumarin**
136 **(5):** A mixture of **4** (300 mg, 1 mmol), vinylbenzylchloride (155 μL, 168 mg, 1.1 mmol,
137 1 mmol), potassium carbonate (401 mg, 2.9 mmol), potassium iodide (49 mg, 0.3
138 mmol) in dry MeCN (20 mL) was heated under argon at 80 °C for 2 days. After cooling
139 to room temperature, H₂O was added to dissolve the inorganic salts. The mixture was
140 filtered to remove insoluble materials. EtOAc was then added. The organic phase was
141 washed with H₂O (2 x 50 mL) and saturated aqueous NaCl (50 mL), dried over MgSO₄,
142 filtered, and concentrated *in vacuo*. The resulting orange-red residue was
143 chromatographed on silica gel using CH₂Cl₂-EtOAc (4:6, v/v) as eluent to give an
144 orange solid which was further purified by recrystallization from ethanol to afford **5**
145 (40 mg, 10 %) as a yellow-orange solid, mp 194 °C; IR (neat) *v*_{max} (cm⁻¹) 3335 (NH),
146 3144, 1694 (C=O), 1601, 1540, 1500, 1404, 1321, 1291, 1148; ¹H-NMR (500 MHz,
147 CDCl₃) δ(ppm): 7.69 (s, 1H, 5-H-imidazole), 7.41 (d, 2H, aromaticH. *J*=8.13 Hz), 7.30
148 (d, 2H, aromaticH *J*=8.15 Hz), 7.20 (d, 1H, H5, *J*_{5,6}=8.72 Hz), 6.71 (dd, 1H, CH=CH₂,
149 *J*=10.91 Hz, *J*=17.60 Hz), 6.57 (dd, 1H, H6, *J*_{6,5}=8.72 Hz, *J*_{6,8}=2.36 Hz), 6.53 (d, 1H,
150 H8, *J*_{8,6}=2.32 Hz), 5.75 (d, 1H, CH=CH_aH_b *J*=17.61 Hz), 5.43 (s, 1H, H3), 5.26 (d,

151 1H, CH=CH_a**H_b**, $J=10.90$ Hz), 5.17 (s, 2H, C4CH₂-), 4.85 (t, 1H, -NH-, $J_{\text{NH-CH}_2} = 5.52$
 152 Hz), 4.41 (d, 2H, CH₂NH-, $J_{\text{CH}_2\text{-NH}} = 5.55$ Hz), 2.44 (s, 3H, -CH₃); ¹³C-NMR (CDCl₃)
 153 $\delta(\text{ppm})$: 156.3 (C2), 155.0 (2-C-imidazole), 154.0 (C9), 147.7 (C7), 141.1 (C4), 140.0
 154 (4-C-imidazole), 137.3 (CCH₂NH-), 136.2 (CH=CH₂), 128.0 (CCH=CH₂), 127.6
 155 (aromaticC), 126.8 (aromaticC), 123.7 (C5), 119.9 (5-C-imidazole), 114.8 (CH=CH₂),
 156 107.6 (C3), 107.1 (C10), 99.1 (C8), 47.5 (-CH₂NH-), 46.9 (C4CH₂-), 13.1 (-CH₃); MS
 157 (EI): Calcd. $m/z = 416.14791$ (C₂₃H₂₀O₄N₄). Found $m/z = 416.14827$ (M⁺); Elem. Anal.
 158 Calcd. for C₂₃H₂₀O₄N₄ (416.43): C 66.34, H 4.89, N 13.45. Found: C 65.76, H 4.66,
 159 N 12.89.

160 **7-acrylamido-4-((2-methyl-4-nitro-1H-imidazol-1-yl)methyl)-coumarin (6):**

161 Acryloyl chloride (76 μL , 75 mg, 0.93 mmol) in dry THF (4 mL) was added slowly to
 162 a suspension of **4** (112 mg, 0.373 mmol) in dry THF (10 mL) under argon. The reaction
 163 mixture was left stirred at room temperature overnight. The resulting precipitate was
 164 collected by filtration, washed with THF, H₂O and ethyl acetate then dried *in vacuo* to
 165 afford **6** (124 mg, 94%) as a pale yellow solid, mp 291 °C (decomp.); IR (neat) ν_{max}
 166 (cm⁻¹) 3301, 1695 (C=O), 1608, 1501, 1414, 1332, 1285, 1211, 1188, 1141; ¹H-NMR
 167 (500 MHz, DMSO) $\delta(\text{ppm})$: 10.65 (s, 1H, -CONH-), 8.38 (s, 1H, 5-H-imidazole), 7.90
 168 (d, 1H, H8, $J_{8,6}=1.68$ Hz), 7.77 (d, 1H, H5, $J_{5,6}=8.70$ Hz), 7.59 (dd, 1H, H6, $J_{6,5}=8.72$
 169 Hz, $J_{6,8}=1.71$), 6.46 (dd, 1H, CH=CH₂, $J=10.08$ Hz, $J=16.91$ Hz), 6.33 (dd, 1H,
 170 CH=CH_a**H_b**, $J=16.95$ Hz, $J=1.54$ Hz), 5.85 (dd, 1H, CH=CH_a**H_b**, $J=10.19$ Hz, $J=1.43$
 171 Hz), 5.61 (s, 2H, -CH₂-), 5.48 (s, 1H, H3), 2.35 (s, 3H, -CH₃); ¹³C-NMR (DMSO)
 172 $\delta(\text{ppm})$: 163.8 (C=ONH-), 160.7 (C2), 153.8 (C9), 149.9 (C7), 145.8 (4-C-imidazole),
 173 142.8 (C4), 131.3 (CH=CH₂), 129.0 (CH=CH₂), 125.2 (C5), 122.7 (5-C-imidazole),
 174 115.5 (C6), 112.0 (C10), 110.1 (C3), 106.1 (C8), 46.3 (-CH₂-), 12.5 (-CH₃); MS (EI):
 175 Calcd. $m/z = 354.09587$ (C₁₇H₁₄O₅N₄). Found $m/z = 354.09630$ (M⁺); Elem. Anal.

176 Calcd. for $C_{17}H_{14}O_5N_4$ (354.32): C 57.63, H 3.98, N 15.81. Found: C 56.14, H 3.61,
177 N 14.05.

178 **4-Chloromethyl-7-bromocoumarin (7):** H_2SO_4 (80%, 40 mL) was pre-cooled in an
179 ice bath. 3-bromophenol (1.73 g, 10 mmol) was added, followed by ethyl 4-
180 chloroacetoacetate (2.3 g, 1906 μ L, 14 mmol) in portions. The mixture was stirred at
181 r.t under Ar for 22 h, after which it was poured into ice-water (50 mL) and left stirred
182 for a further 30 min. The white precipitate formed was filtered, washed with H_2O , dried
183 over phosphorus pentoxide and recrystallized from EtOH to afford **7** (1.87 g, 68%) as
184 a white solid, mp 211 $^{\circ}C$; IR (neat) ν_{max} (cm^{-1}) 3065, 1736 (C=O), 1598, 1395, 1271,
185 1243, 1173, 1151, 1081; 1H -NMR (500 MHz, $CDCl_3$) δ (ppm): 7.56 (d, 1H, H8,
186 $J_{8,6}=1.84$ Hz), 7.54 (d, 1H, H5, $J_{5,6}=8.51$ Hz), 7.47 (dd, 1H, H6, $J_{6,5}=8.37$ Hz, $J_{6,8}=1.68$),
187 6.58 (s, 1H, H3), 4.64 (s, 2H, $-CH_2Cl$); ^{13}C -NMR (DMSO) δ (ppm): 159.1 (C2), 153.8
188 (C4), 150.2 (C9), 127.5 (C6), 126.9 (C8), 125.1 (C7), 119.8 (C5), 116.4 (C10), 115.8
189 (C3), 41.1 ($-CH_2-$); MS (EI): Calcd. m/z = 271.89501 ($C_{10}H_6O_2BrCl$). Found m/z =
190 271.89465 (M^+). Elem. Anal. Calcd. for $C_{10}H_6O_2BrCl$ (273.51): C 43.91, H 2.21.
191 Found: C 43.35, H 2.19.

192 **7-bromo-4-((2-methyl-4-nitro-1H-imidazol-1-yl)methyl)-coumarin (8):** NaH in
193 mineral oil (60%, 80 mg) was added to a solution of 2-methyl-4-nitroimidazole (254.2
194 mg, 2 mmol) in DMF (8 mL). The mixture was heated to 110 $^{\circ}C$ for 10 min and then
195 cooled down to 60 $^{\circ}C$, after which **7** (547.0 mg, 2 mmol) suspended in DMF (8 mL)
196 was added. The reaction mixture was left stirring at the same temperature for 20 h then
197 poured into ice. The precipitate formed was collected by filtration, washed with water,
198 dried and recrystallized from EtOH to afford **8** (538 mg, 74%) as an off-white solid, mp
199 289-291 $^{\circ}C$ (decomp.); IR (neat) ν_{max} (cm^{-1}) 3135, 1738 (C=O), 1597, 1541, 1498,
200 1398, 1342, 1299, 1244, 1171, 1147; 1H -NMR (500 MHz, DMSO) δ (ppm): 8.36 (s, 1H,

201 5-H-imidazole), 7.81 (d, 1H, H8, $J_{8,6}=1.90$ Hz), 7.73 (d, 1H, H5, $J_{5,6}=8.53$ Hz), 7.66
202 (dd, 1H, H6, $J_{6,5}=8.52$ Hz, $J_{6,8}=1.92$ Hz), 5.68 (s, 1H, H3), 5.63 (s, 2H, -CH₂-), 2.35 (s,
203 3H, -CH₃); ¹³C-NMR (DMSO) δ (ppm): 158.8 (C2), 153.4 (C9), 149.5 (4-C-imidazole),
204 145.9 (C4), 145.8 (2-C-imidazole), 127.6 (C6), 126.2 (C5), 125.4 (C7), 122.6 (5-C-
205 imidazole), 119.8 (C8), 116.4 (C10), 112.8 (C3), 46.3 (-CH₂-), 12.5 (-CH₃); MS (EI):
206 Calcd. m/z = 362.98492 (C₁₄H₁₀O₄N₃Br). Found m/z = 362.98521 (M⁺); Elem. Anal.
207 Calcd. for C₁₄H₁₀O₄N₃Br (364.15): C 46.18, H 2.77, N 11.54. Found: C 45.19, H 2.36,
208 N 10.88.

209 **7-vinylphenyl-4-((2-methyl-4-nitro-1H-imidazol-1-yl)methyl)-coumarin (9):** **9** was
210 prepared from **8** via a Suzuki coupling reaction[36-38] with vinylphenylboronic acid.
211 A mixture of **8** (400.6 mg, 1.1 mmol), 4-vinylphenylboronic acid (244.2 mg, 1.65
212 mmol, 1.5 mol equiv), potassium carbonate (570 mg, 4.125 mmol, 3.75 mol equiv) and
213 dioxane (11 mL) was stirred in a two necked flask at room temperature under argon for
214 0.5 h. Tetrakis(triphenylphosphine)palladium(0) (63.6 mg, 0.055 mmol, 5 mol %) was
215 added. A condenser was fitted and the flask was evacuated and filled with argon three
216 times before being heated to 80 °C. The reaction was left at 80 °C in the dark for 41 h.
217 After cooling to room temperature, EtOAc was added. The organic phase was washed
218 with water (3 × 50 mL), saturated aqueous NaCl (50 mL), dried over MgSO₄, filtered,
219 concentrated *in vacuo* to give the crude product which was purified by flash
220 chromatography on silica gel with EtOAc-petroleum ether (95:5, v/v) as eluent to give
221 **9** as an orange solid, which was further purified by recrystallization from EtOH-EtOAc
222 (5:1, v/v) to give orange crystals (176 mg, 41%), mp 215 °C; IR (neat) ν_{max} (cm⁻¹) 3147,
223 1716 (C=O), 1612, 1538, 1496, 1395, 1322, 1290, 1160; ¹H-NMR (500 MHz, DMSO)
224 δ (ppm): 8.40 (s, 1H, 5-H-imidazole), 7.89-7.78 (m, 5H, H5, H6, H8, aromaticH), 7.62
225 (d, 2H, aromaticH $J=8.34$ Hz), 6.80 (dd, 1H, CH=CH₂, $J=10.98$ Hz, $J=17.67$ Hz), 5.94

(d, 1H, CH=CH_aH_b, $J=17.70$ Hz), 5.68 (s, 2H, -CH₂-), 5.63 (s, 1H, H₃), 5.34 (d, 1H, CH=CH_aH_b, $J=11.00$ Hz), 2.37 (s, 3H, -CH₃); ¹³C-NMR (DMSO) δ (ppm): 159.5 (C2), 153.6 (C9), 149.8 (4-C-imidazole), 145.9 (C4), 145.8 (C7), 143.6 (2-C-imidazole), 137.6 (CC7), 137.2 (CCH=CH₂), 136.0 (CH=CH₂), 127.3 (aromaticC.), 126.9 (aromaticC.), 125.1 (C5), 122.7 (C6), 122.6 (5-C-imidazole), 116.1 (C10), 115.3 (CH=CH₂), 114.1 (C8), 112.0 (C3), 46.4 (-CH₂-), 12.5 (-CH₃); MS (EI): Calcd. m/z = 387.12136 (C₂₂H₁₇O₄N₃). Found m/z = 387.12164 (M⁺); Elem. Anal. Calcd. for C₂₂H₁₇O₄N₃ (387.39): C 68.21, H 4.42, N 10.85. Found: C 66.92, H 4.14, N 10.15.

2.3. Preparation of polymers

The compositions of the different polymers are given in Table 1.

Polymer	Fluorescent dye		MAA	BAP	P84	DMF
	Type	/mmol	/mmol	/mmol	/mmol	/μL
PVIC	VIC (5)	0.05	0.1	0.5	0.02	500
PAIC	AIC (6)	0.05	0.1	0.5	0.02	500
PSIC	SIC (9)	0.05	0.1	0.5	0.02	500

Table 1. Composition of polymers prepared using different coumarin-based pH indicators. MAA: methylmethacrylate, BAP: 1,4-bis(acryloyl)piperazine, P84: Pluronic P84.

Coumarin, methacrylic acid, 1,4-bis(acryloyl)piperazine and pluronic P84 were weighed into a borosilicate glass vial and dissolved in dimethylformamide. Azobis(isobutyronitrile) (AIBN) (5 mg) was then added. The vial was placed in a sonicating water bath until AIBN was fully dissolved, then purged thoroughly with argon for about 2 min before being tightly capped and sealed. Polymerization was

carried out at 80 °C in the dark for approximately 20 h. The resulting hard bulk polymer was then hand ground with a mortar and pestle until fine particles were obtained. The polymer particles were washed to remove unreacted materials by repeated incubation in MeOH (50 mL solvent each), centrifugation and re-suspension (4×0.5h incubations), followed by the same procedure with H₂O (2×0.5 h incubations) and finally on a sintered filter with MeOH (50 mL). After washing, polymer particles were dried in vacuum and stored in the dark until use.

2.4. Fluorescence measurements

Emission spectra of fluorescent indicators and polymers were recorded using a 4 mL (1×1×4 cm) quartz cuvette. Slit widths were adjusted to give emission below the saturating limit (1000000 cps) of the detector, and then the same settings were used for a complete set of experiments.

0.8 mM indicator stock solutions were prepared by dissolving 2×10^{-3} mmol of each indicator in 2.5 mL EtOH. PVIC (6.3 mg/mL, containing 2.5 μ mol coumarin/mL), PAIC (5.7 mg/mL) and PSIC (5.8 mg/mL) stock solutions were prepared in deionized distilled water.

50 mM phosphate at various pH values ranging from 10 to 13.6 was prepared by dissolving Na₂HPO₄ (1.77 g, 12.5 mmol) in distilled deionized water (240 mL) in a conical flask. The buffer was adjusted to the desired pH with aqueous NaOH. The solution was then transferred to a 250 mL volumetric flask and made up to the volume with distilled water.

20 μ L of each coumarin stock solution or 50 μ L of each polymer stock solution was added to 3 mL of buffer in a cuvette, followed by measurement of emission spectra.

The cuvette was shaken vigorously before each measurement. All samples were prepared in duplicate or triplicate and the data presented were average values.

It should be noted that phosphate does not act as a good buffer at pH values higher than 12 -12.5. However, phosphate was used for all pH to avoid any differences in fluorescence caused by the difference in buffer composition. It has also been confirmed by measuring pH of the solutions before and after adding the dyes/polymers that a small amount of either the free dyes or the polymers does not cause any change in the pH values of the solutions.

3. Results and discussion

3.1. Design and synthesis of fluorescent pH indicators

Although there has been a variety of pH indicators, both commercially available including azo dyes[6, 21, 23, 39, 40], trinitrobenzensulfonic acid[28], thymol blue[29, 41-43], cresol red[41], phenolphthalein[44], aniline blue[45], rhodamine 19[46], Victoria blue[47] etc. as well as reported in the literature such as fluorescent Schiff bases[48], porpholactones[49] or a benzo[de]anthracen-7-one derivative[31] , that are known to be useful for the spectroscopic determination of high pHs, the majority of them are absorptive dyes rather than fluorescent dyes and only a few meet the requirements of being stable for a long period of time in highly alkaline media, possessing a pK_a in the range between 11-12.5, having reasonable fluorescence quantum yield and good photostability and bearing one or more functional chemical groups suitable for covalent immobilization of the indicator. Coumarins have been employed and investigated in this work as they are widely used as laser dyes for single-molecule fluorescence and so they are ‘tried and tested’ in terms of the key property of being photo-stable[50, 51]. It has also been reported that a coumarin with an imidazole

295 substituent shows response to pH in the alkaline region [19]. The design of the indicator
296 dyes was tailored based on this core structure and suitable functional group(s) for
297 immobilization of the indicator to a substrate.

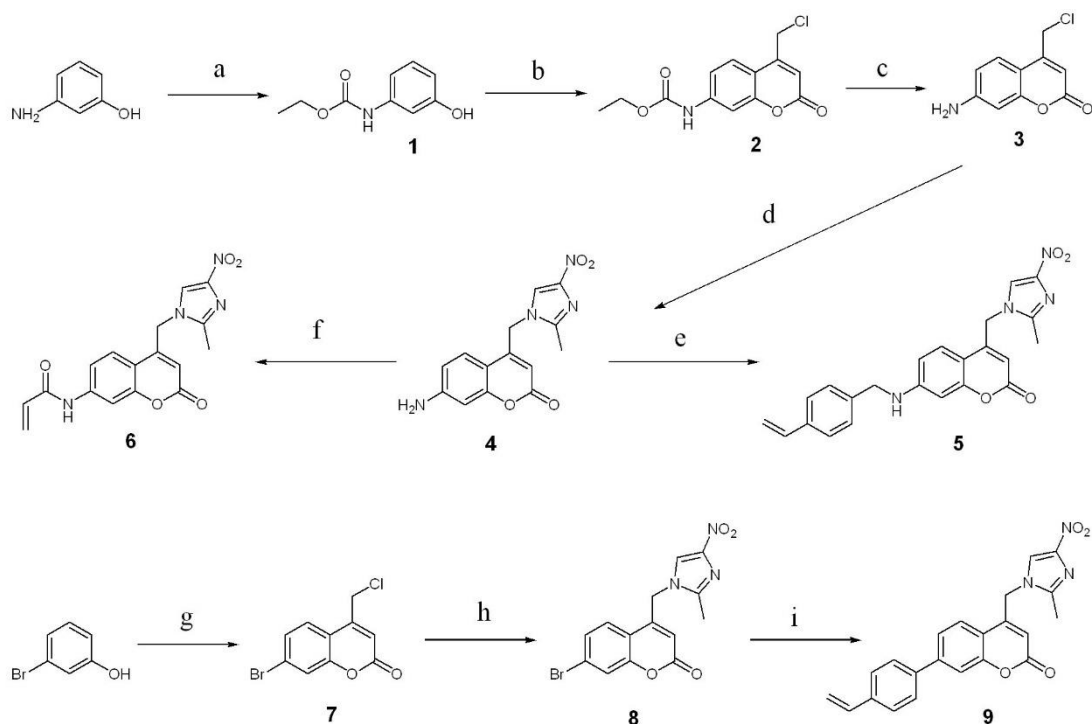
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299 Having created appropriate indicator dyes, it is equally important to perform an
300 effective immobilization of the dyes to a suitable solid support/substrate. Prior work
301 has shown that poor immobilization results in dye leaching and consequently a drifting
302 of the calibration of the probe, which leads to the gradual breakdown of its useful
303 sensing ability[11]. Among several widely used immobilization methods such as
304 absorption or entrapment[52-54], layer-by-layer (LbL) electrostatic self-assembly [55,
305 56] and covalent binding[21, 57-60], the covalent binding method is believed to
306 produce more reliable and durable sensors, as the indicators are virtually bonded to the
307 substrate therefore they are unlikely to leach out under normal conditions, although the
308 fabrication process is relatively complicated and time-consuming.

309 Regarding solid supports, various materials have been employed as the supports for dye
310 immobilization including cellulose membrane[21, 23, 57, 61], silica sol-gels[15, 62]
311 and synthetic organic polymers[39, 60] with cellulose and sol-gels being more
312 commonly used than the other due to the optical transparency of their matrices and in
313 many cases, the convenience of attachment process. However, cellulose degrades easily
314 in highly alkaline media[22, 39] and sol-gels are completely deteriorated at pH higher
315 than 10 within relatively short time. Therefore, they are not suitable for this study,
316 leaving synthetic polymers the favourable option. The indicator dye can be connected
317 into polymer matrix by either co-polymerisation of the dye monomer and appropriate
318 co-monomer(s) or by attaching the reactive dye to a readily prepared polymer. In this
319 work, the former method was used because it is easier to design different polymer

matrices and control their properties to meet the requirements of the sensor in terms of polymer rigidity, hydrophilicity and optical density by using different mixtures of co-monomers.

To meet the desired requirements discussed above, the fluorescent dyes used in this work were designed to include a polymerizable group and the coumarin core structure with an imidazole substituent. The linkage between the polymerizable group and the coumarin should be stable enough to sustain strongly alkaline media. Three novel polymerisable coumarins: vinylbenzylamino imidazolylmethyl coumarin (VIC, **5**), acrylamido imidazolylmethyl coumarin (AIC, **6**) and styryl imidazolylmethyl coumarin (SIC, **9**) were prepared in multiple steps starting from commercially available phenolic compounds as outlined in Scheme 1. The Pechmann reaction of phenols with a β -carbonyl ester is a versatile approach for the synthesis of 4-substituted coumarins. **2** and **7** were prepared similarly to the method reported in the literature[35]. The substitution of chlorine for an imidazolyl group was achieved by performing a reaction with 2-methyl-4-nitroimidazole, following the method described by Al-Soud *et al.* [63]. Polymerizable groups were introduced into the coumarin structures either *via* a Suzuki coupling[36-38] of the Br-substituted coumarin **8** with vinylphenylboronic acid using K_2CO_3 in dioxane as a base/solvent mixture or by reacting the amine substituted coumarin **4** with acryloyl chloride to give the amide **6** or with 4-vinylbenzylchloride to give the secondary amine **5**.



Scheme 1. Preparation of fluorescent monomers. (a) ClCOOEt, EtOAc, 100 °C, 93%; (b) ethyl 4-chloroacetoacetate, H₂SO₄, H₂O, r.t, 19 h, 71%; (c) conc.H₂SO₄, glacial AcOH, 125 °C, 2 h, 87%; (d) NaH (60%), 2-methyl-4-nitroimidazole, DMF, 100 °C to 60 °C, 18 h, 87%; (e) vinylbenzylchloride, K₂CO₃, KI, MeCN, 80 °C, 2 days, 10%; (f) CH₂=CHCOCl, dry THF, r.t., 12 h, 94%; (g) ethyl 4-chloroacetoacetate, H₂SO₄, H₂O, r.t, 22 h, 68%; (h) NaH (60%), 2-methyl-4-nitroimidazole, DMF, 110 °C to 60 °C, 20 h, 74%; (i) CH₂=CHC₆H₄B(OH)₂, Pd(PPh₃)₄, K₂CO₃, dioxane, 80 °C, 41 h, 41%.

3.2. Spectroscopic properties and pK_a values of the free indicators

In neutral aqueous solution (with the addition of 1.5% EtOH to solubilize the compounds), the absorption spectra of VIC (5) and AIC (6) show only one main absorption band of each dye in the UV region, at 370 nm and 330 nm, respectively, (Fig. 1) whereas that of SIC (9) shows two main bands, at 266 nm with weaker absorbance and 334 nm with higher absorbance.

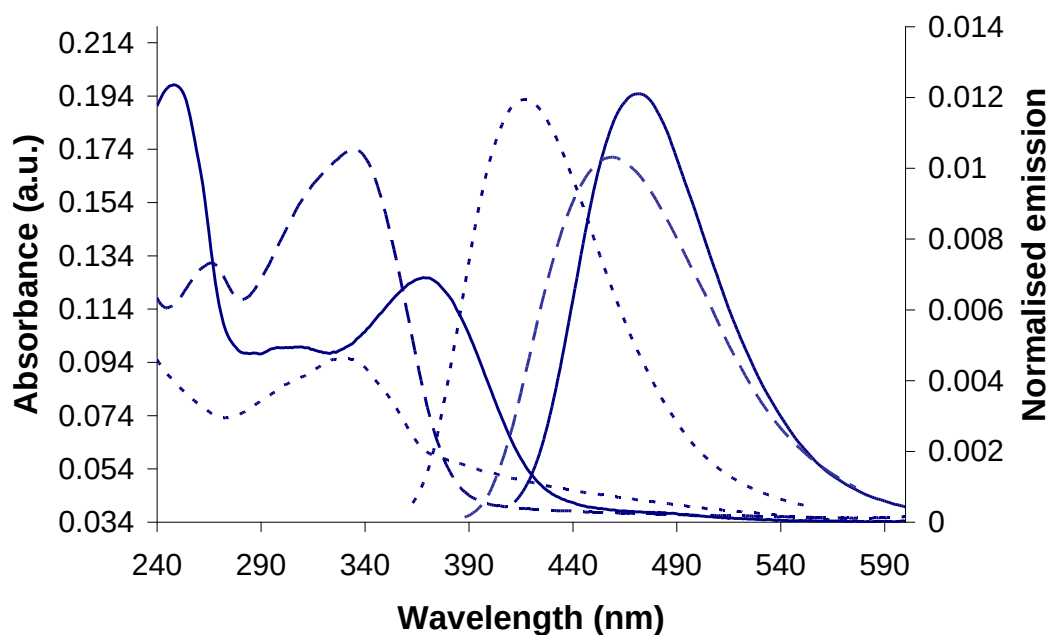


Fig.1. Absorption (left) and emission (right) spectra of **VIC (5)** (solid lines), **SIC (9)** (broken lines) and **AIC (6)** (dotted lines) (5 μ M) in H₂O. Emission spectra recorded with λ_{ex} = 330 nm for **SIC** and **AIC** and 370 nm for **VIC**.

Dye	Solvent	UV max (nm)	Emission max (nm)	pK _a	Φ (%)
VIC (5)	EtOH	371	456	-	2.6
VIC (5)	H ₂ O	370	470	12.48 $\pm$ 0.06	0.8
AIC (6)	EtOH	334	407	-	1.7
AIC (6)	H ₂ O	330	416	12.22 $\pm$ 0.07	0.6
SIC (9)	EtOH	335	448	-	25.0
SIC (9)	H ₂ O	334	458	12.40 $\pm$ 0.24	2.0

Table 2 Spectral data and pK_a values of the fluorescent dyes.

Emission spectra for each compound recorded in the same solvent using excitation at the absorbance maxima include only one band in the 415-470 nm region. VIC absorbs and emits at longer wavelengths compared to AIC and SIC but its fluorescence quantum yield is fairly similar to that of AIC and much lower than that of SIC (Table 2). This difference in fluorescence can be explained by the fact that coumarin molecules have close lying π, π^* and n, π^* states which are easily perturbed by changes in solvents, substituents and other factors[64-66], and hence the excited state S_1 probably has both π, π^* and n, π^* character. It is proposed that in VIC, a n, π^* state may lie below the π, π^* state and at a low energy level, due to the great conjugation of the lone-pair electrons of the amine nitrogen at the 7 position with the π system. This degree of conjugation is decreased in AIC where the amine nitrogen is converted to an amide counterpart, which may result in the low lying excited state shifting to higher energy. In SIC where the π -electron system of coumarin is enhanced by the substituted styryl group, the π, π^* state may lie below the n, π^* state, as shown in Fig. 2. This could account for the lower quantum yields of VIC and AIC compared to SIC, $n \leftarrow \pi^*$ transitions being generally less intense than $\pi \leftarrow \pi^*$ transitions because they characteristically have longer lifetimes which enhance intersystem crossing[67, 68]. The red-shifted fluorescence of VIC with respect to AIC and SIC may be due to the smaller HOMO-LUMO gap.

It is also noted that all dyes exhibit very large Stokes shifts (the difference in wavelength between the absorption and the fluorescence spectral peaks) of 100 nm for VIC, 86 nm for AIC and 124 nm for SIC, which is very important for sensing applications since this minimizes the interference of the excitation light with the fluorescence emission.

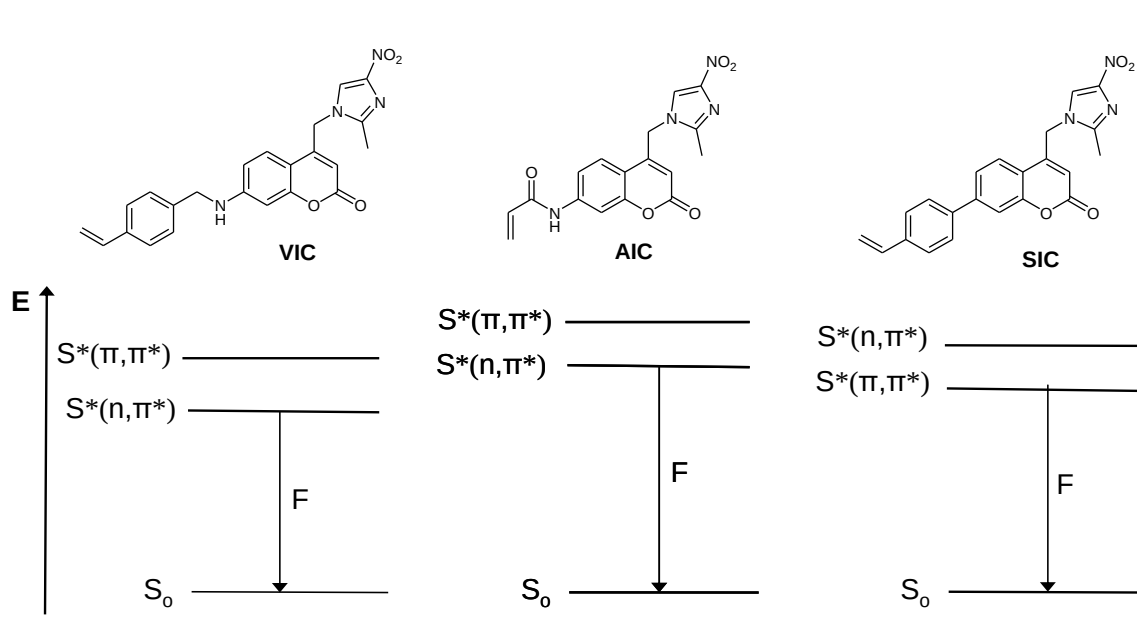
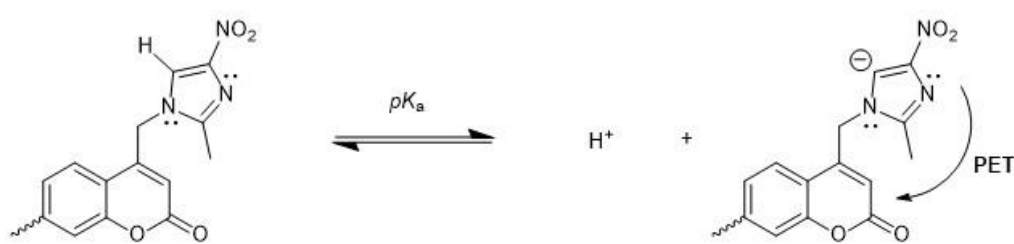


Fig. 2 Possible energy diagrams for VIC, AIC and SIC. S_0 : ground state, S^* : excited single state, F: fluorescence.

In aqueous solution, all the dyes show no change in the fluorescence properties in the neutral and near neutral pH region (data not shown) and a decrease in fluorescence intensity with increasing pH in the alkaline region (Fig.3). This can be attributed to the equilibrium between the protonated and deprotonated forms of the imidazolyl group as shown in Scheme 2. The protonated form is fluorescent and the deprotonated form is much less so. Therefore, the fluorescence intensities of the dyes are lower at higher pH values.

The deprotonation of the imidazolyl group at high pH values was further confirmed by ^{13}C NMR titrations of VIC, AIC and SIC with sodium methoxide (MeONa), a very strong base, in $\text{DMSO-}d_6$. Initially, the ^{13}C spectrum of each fluorophore in $\text{DMSO-}d_6$ (0.1 M concentration) was recorded. One mole equivalent of sodium methoxide in $\text{DMSO-}d_6$ was then added to each solution and ^{13}C NMR spectra were recorded again. The addition of MeONa resulted in chemical shifts of all carbon nuclei. However, the largest changes in shift were for the carbon nuclei attached to the only proton of the

imidazolyl group (C5-imidazole) with changes of up to 1.71 ppm for the titration of VIC and 1.63 ppm and 1.47 ppm respectively for the titrations of AIC and SIC. These results confirm that the carbon nuclei C5 of the imidazolyl group is involved directly in the interactions with the base through deprotonation. It has been reported that imidazole is a very weak acid with $pK_a = 14.5$ [69]. However, in the presence of a strong electron acceptor – NO₂ group, its acidity increases and pK_a can be shifted to a lower value.



Scheme 2. Protonation-deprotonation equilibria of the fluorescent indicators in aqueous solution.

It has been proposed that the difference in fluorescence intensity between two forms is due to photoinduced electron transfer (PET) between the imidazole anion (proton receptor) and coumarin (fluorophore)[19, 70]. At high pH, the electron-enriched imidazole anion quenches the fluorescence of the coumarin through PET. At lower pH, the presence of low concentration of hydrogen ions causes protonation of C5-imidazole, resulting in PET from the imidazole to coumarin being hindered and hence the fluorescence intensity increases.

The equilibrium between the protonated (HA) and deprotonated (A⁻) forms of the indicators can be written in short as:



The relationship between the protonation state of the indicator and the pH is governed by the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad (1)$$

where $[A^-]$ and $[HA]$ are the concentrations of the deprotonated and protonated forms of the indicator and pK_a is the acid-base constant. $[A^-]$ and $[HA]$ are related to fluorescence intensities by $[A^-] = F - F_{\max}$ and $[HA] = F_{\min} - F$ where F is a measured fluorescence intensity of the system, $F_{\max}$ is the fluorescence intensity of the fully protonated system and $F_{\min}$ is the fluorescence intensity of the deprotonated system. The expressions are then substituted into Equation 1 to provide Equation 2.

$$pH = pK_a + \log \frac{F - F_{\max}}{F_{\min} - F} \quad (2)$$

Equation 2 can be re-written in terms of F to give

$$F = \frac{F_{\max} + F_{\min} \times 10^{(pH - pK_a)}}{10^{(pH - pK_a)} + 1} \quad (3)$$

This results in an ‘S-shaped’ relation of the fluorescence intensity versus pH graph, centered on the pK_a value, which is the pH where 50% of the dye population in solution is protonated.

The calculation of the pK_a values for the indicators was performed based on the fluorescence intensities at a fixed maximum emission wavelength at different pH values using the model for nonlinear fitting method according to Equation 3. The data obtained are summarized in Table 2. The pK_a values for the three dyes are very similar, suggesting that the substitution at the 7 position of the coumarin has no significant effect on the protonation-deprotonation of the imidazolyl group.

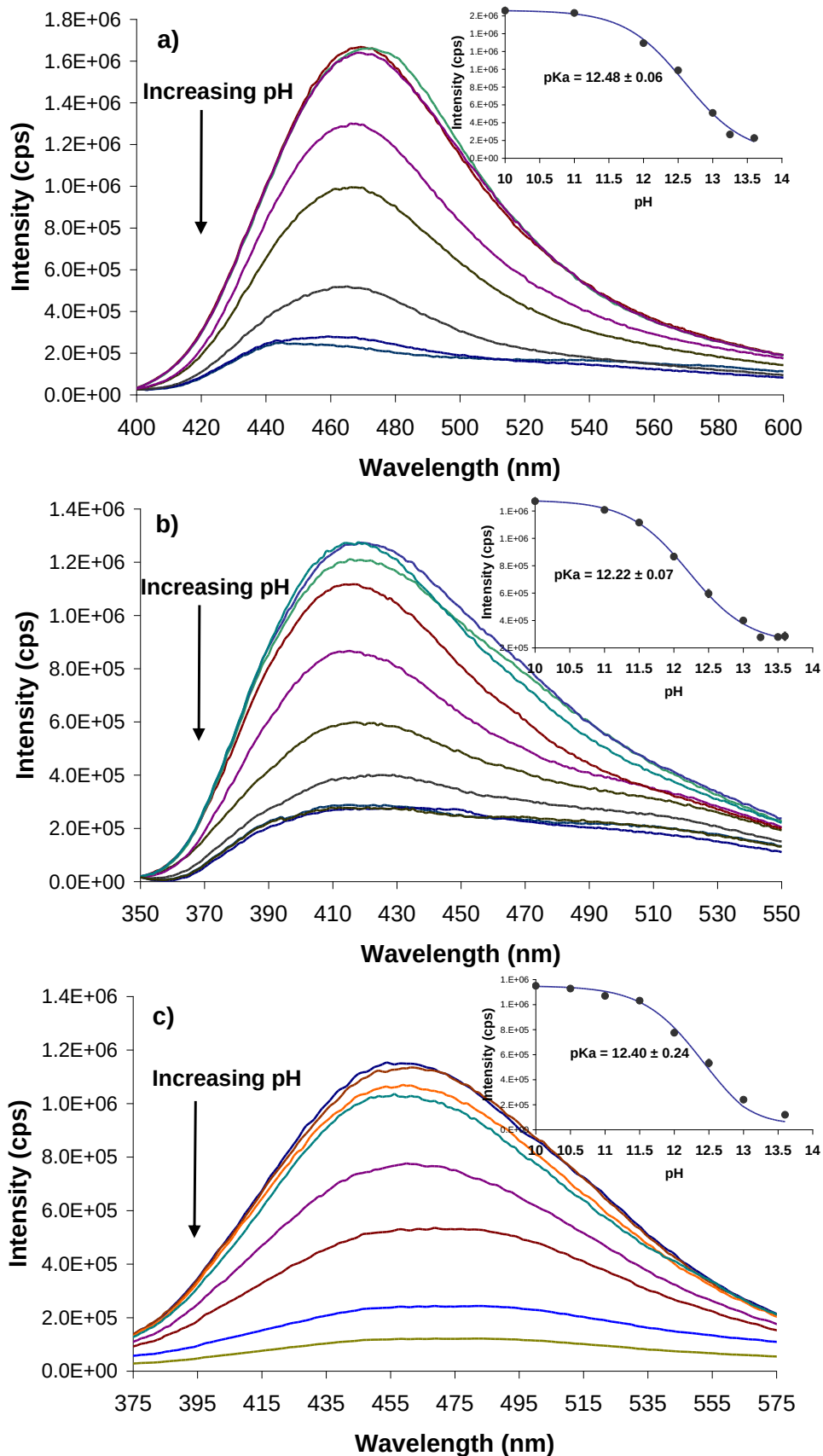


Fig 3. Emission spectra of VIC (a), AIC (b) and SIC (c) at pH from 10.0 to 13.6. The insets show the titration plots at emission maxima. $\lambda_{\text{ex}} = 370$ nm (a), 330 nm (b and c).

3.3. Polymer preparation

Polymers were prepared in dimethylformamide (DMF) by free-radical polymerization using azobis(isobutyronitrile) as initiator (refer to Table 1 for the compositions of the different polymers). These were prepared as monolithic polymers and ground into small particles, since these are easier to produce than spherical beads which can be made by precipitation polymerisation and since a minimal amount of solvent is required. There is no problem with scattering from the irregular-shaped particles because with the coumarins used here the Stokes shift is sufficient that emission can be measured well above the scattered wavelengths. Since the materials were designed to work in aqueous environments, 1,4-bis(acryloyl)piperazine was used as the cross-linker as it is water soluble, hence producing polymers with a more hydrophilic backbone. Methacrylic acid was included as a co-monomer so that the polymers should not be too rigid and it can also increase the hydrophilicity of the polymers. Pluronic P84 was added to increase the surface area and porosity. It is important that the polymer used has the right molar ratio of cross-linker to monomers since lower cross-linking gives insufficiently robust polymer and higher cross-linking gives too rigid polymer, hence preventing the diffusion of hydrogen ions from surrounding environments to the fluorescent receptor. After trying and testing different ratios in terms, it was found that a molar ratio of cross-linker to monomers of about 10:3-4 produced good polymers for this application. The molar amount of fluorescent monomer used was fixed at 1:10 of the cross-linker. More fluorescent monomer would be expected to give a stronger fluorescent signal but too high a concentration of fluorophore could also result in fluorescence quenching by the inner filter effect.

3.4. Photostability

Photostability is one of the critical properties of fluorescent materials used for sensor applications. It is proposed that the substitution at the 7 position of the coumarin has no significant effect on the photostability of the coumarin core structure. Photostability was tested with one of the polymers PVIC by holding a layer consisting of 4 mg of the polymer particles in between a quartz disc (6 mm dia.) and a nylon membrane (20 μ m pores, GE), which was kept in place by an O-ring and coupled into the fluorimeter through a dichroic mirror using a fiber bundle. The excitation light (at a wavelength of 375 nm) was launched to the distal end of the probe illuminating the polymer with light from the intense, high power Xe lamp of the fluorimeter continuously for 1 h. The fluorescence intensity data from the polymer were collected over that period and displayed. Fig.4 shows the fluorescence intensities of the polymer in dry state and in a pH 10 buffer solution as function of time during 60 min of continuous illumination by light from a high power Xe lamp. It was interesting to observe that the intensity of fluorescence was reduced by 7-8% for the dry state over the time investigated and with the high flux of photons onto the polymer. However, under the same conditions, no photobleaching was seen when the polymer was immersed in a pH 10 buffer solution. The reason for the difference in photostability between the dry state and wet state is unclear. It could be because the excited single state of the fluorophore is stabilised by solvation. When compared to the results of other materials, this still offers excellent performance: the decrease observed in the fluorescence intensity was 65% for carboxyfluorescein and 10-13% for iminocoumarin derivatives, again after 60 min of continuous illumination using a mercury lamp[7]. Thus an important conclusion is that the material prepared in this work possesses superior photostability, a feature that is

critically important with excitation of sensor probes by high intensity solid state sources.

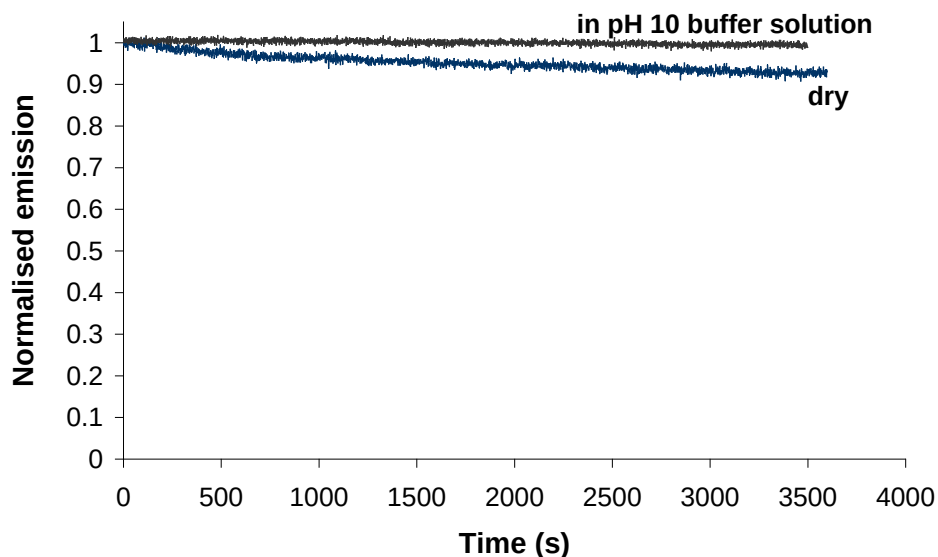


Fig.4. Fluorescence intensity of PVIC (in dry state and in pH 10 buffer solution) as function of time during 60 min of continuous illumination by light from a high power Xe lamp ($\lambda_{\text{ex}} = 375 \text{ nm}$, $\lambda_{\text{em}} = 500 \text{ nm}$). Fluorescence intensities are normalised to the initial emission of the polymer for each data set.

3.5. Fluorescence studies and response of polymers to pH

Fluorescence measurements of the polymers were performed in 50 mM phosphate buffer at various pHs as used for the free dyes. The cuvette was shaken vigorously before each measurement to enable the uniform dispersion of the polymer. No significant settling out was observed on the timescale of the measurements and the results were found to be reproducible.

It should be noted that incubation was not required before the measurements as the response time of the material is very fast (within 10s). This was confirmed by comparing the data obtained from immediate measurements with those obtained for the

574 samples that were allowed to incubate for a couple of hours before measurements.

575 There was essentially no difference between two sets of data.

576 Fig. 5 shows emission spectra of different polymers at different pHs. It can be seen that

577 the fluorophores emit at lower wavelengths in the polymers than in their free forms.

578 The blue shift could be because when fixed in a rigid polymer network the excited states

579 of the fluorophores undergo less stabilization from solvent rearrangement.

580 All polymers exhibited a decrease in fluorescence intensity with increasing pH in the

581 range from 10 to 13.6, which is similar to the dynamic response range of the free dyes.

582 The pK_a value calculation for the polymers was performed using the Boltzmann model

583 – Equation (4) where dpH is the slope of the curve within its linear zone.

584

585

$$F = \frac{F_{\max} + F_{\min} \times e^{(pH-pK_a)/dpH}}{e^{(pH-pK_a)/dpH} + 1} \quad (4)$$

586

587

588 pK_a values were found to be 12.65 ± 0.12 , 12.75 ± 0.10 and 12.76 ± 0.14 for PVIC,

589 PAIC and PSIC, respectively. These values for the immobilized forms of the dyes are

590 slightly higher than that for their free forms in solution and this arises probably because

591 of the change in the polarity of the microenvironment.

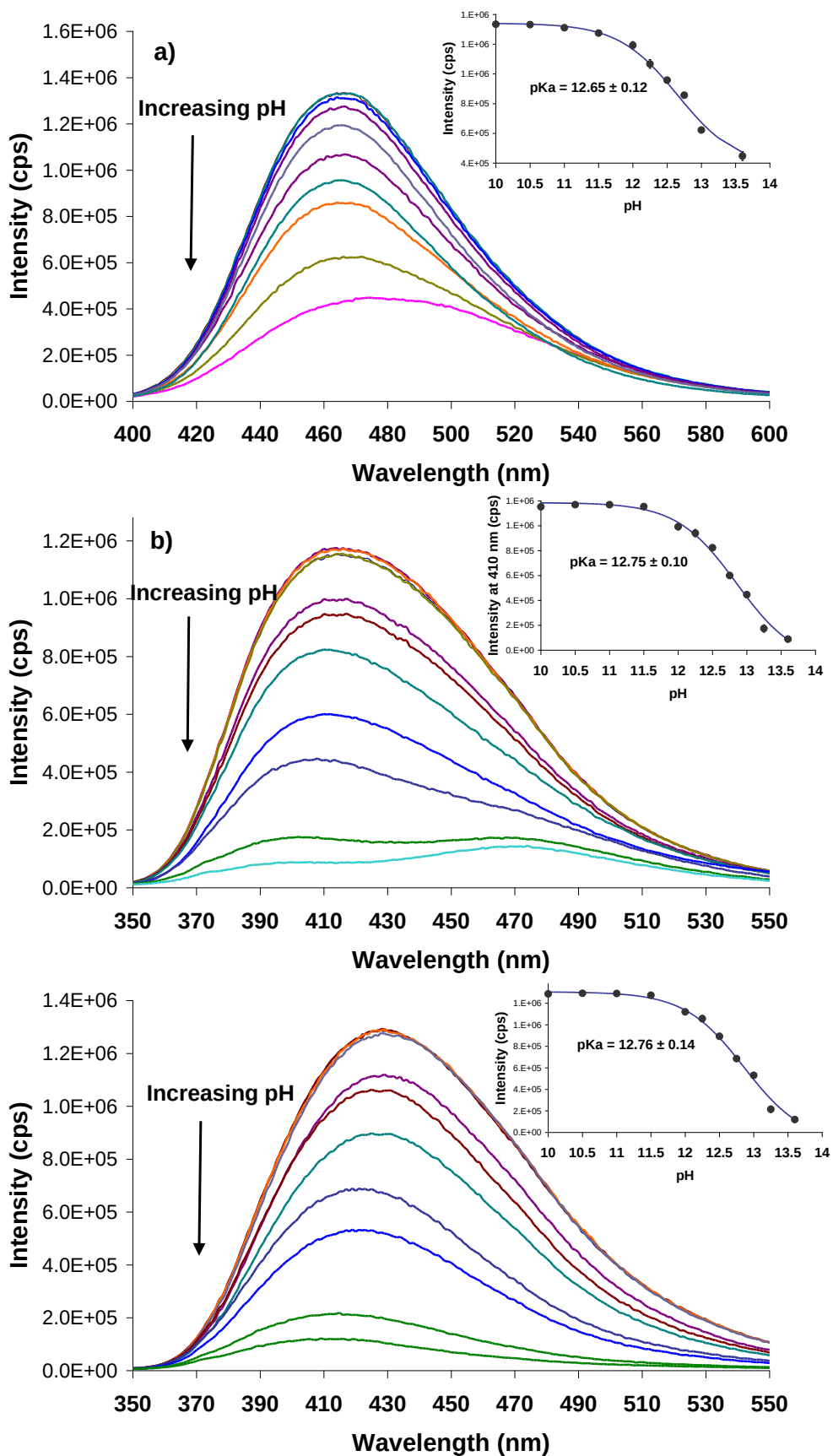


Fig 5. Emission spectra of PVIC (a), PAIC (b) and PSIC (c) at pH from 10.0 to 13.6. The insets show the titration plots at emission maxima. $\lambda_{\text{ex}} = 370$ nm (a), 330 nm (b and c).

3.6. Effect of ionic strength (IS)

Sensitivity to IS can be a serious problem in the cases of pH sensors as it affects pK_a values, thus resulting in errors in pH determination. The effect of IS was investigated with one of the polymers PVIC in the prepared pH 10 phosphate buffer solution adjusted with NaCl to different ionic strengths ranging from 10 mM to 2000 mM. The fluorescence intensity obtained for each solution was converted to a pH value using the calibration curve and the error was calculated. As can be seen from Fig.6, there appears to be no sensitivity to IS for the sensing polymer at pH 10, even at very high concentrations of NaCl. The insignificant errors caused are probably due to the system error rather than the change in IS.

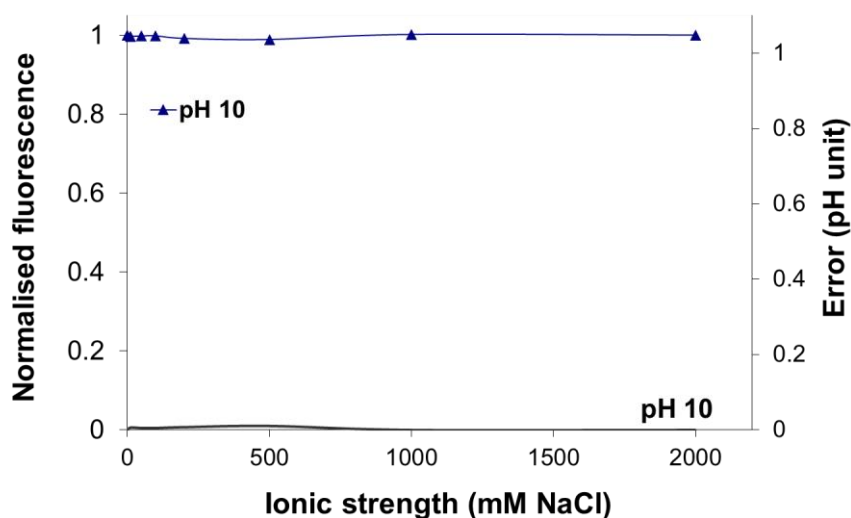


Fig 6. Effect of ionic strength (IS) on fluorescence intensity and calculated pH value.

Fluorescence intensities are normalised to the maximum emission at 470 nm of the polymer at pH 10 with IS = 0.

4. Conclusions

Novel coumarin-based fluorescent pH indicators vinylbenzylamino imidazolylmethyl coumarin (VIC), acrylamido imidazolylmethyl coumarin (AIC) and styryl imidazolylmethyl coumarin (SIC) have been synthesized. Their fluorescence behaviours have been investigated in aqueous solution at different pHs. All coumarins exhibited a significant decrease in fluorescence intensity with increasing pH in the highly alkaline region. Photophysical properties of the indicators have been postulated to explain their fluorescence properties and behaviours.

The indicators were covalently immobilized to polymer supports by co-polymerising with methacrylic acid co-monomer and 1,4-bis(acryloyl)piperazine cross-linker. These polymers are sensitive to pH in the similar response range of the free dyes and were highly photo-stable, showing no photo-bleaching, when immersed in a pH 10 buffer solution, after 60 minutes of continuous illumination using a high power Xe lamp.

The sensing materials developed in this study have been designed for use with optical fibre sensors and their characteristics shown indicate how well they are suited for use in optical pH sensors for measuring pH in various alkaline media. Discussion of the design and performance of such sensors will be the subject of future publications.

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