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1 **Novel coumarin-based pH sensitive fluorescent probes for the highly**
2 **alkaline pH region**

3 *T. Hien Nguyen*, Tong Sun and Kenneth T.V. Grattan*

4 *Photonics and Instrumentation Research Centre, City University of London, London*
5 *EC1V 0HB, UK*

6 *Email: hien.nguyen@city.ac.uk, Tel : +44(0)2070403257, Fax : +44(0)2070408568*

7
8 **Abstract:**

9 The design, synthesis and spectroscopic properties of novel polymerizable coumarin-
10 based pH indicators are reported. They show a fluorescence response to pH in the highly
11 alkaline pH region with calculated pK_a values in the range of 12.2 – 12.5. Covalent
12 immobilization of the indicators to polymer supports provides novel pH-sensitive
13 materials that possess excellent photostability. The materials are sensitive to pH in a
14 similar range and can be applied in optical pH sensors for measuring pH in various
15 alkaline media.

16 **Keywords:** Fluorescent sensor, fluorescent polymer, high pH sensor, coumarin dye.

17
18 **1. Introduction**

19 The development of reliable techniques to monitor pH has received significant attention
20 because of the importance of pH measurement in various areas of scientific research
21 and other practical applications[1-8]. The determination of pH is routinely performed
22 using glass electrodes. However, glass electrodes have several disadvantages and thus
23 are not suitable for certain applications. They are bulky, invasive and potentially create
24 the risk of electric shock during *in vivo* measurements. They show poor performance
25 when measuring solutions of low ionic strength and suffer from surface potentials. In

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

28 pH sensing using optical methods provides an attractive alternative for measuring pH
29 due to the advantages offered in terms of immunity to electrical interference, enhanced
30 safety and the possibility of remote sensing. Most optical pH sensors function through
31 monitoring the changes in the absorbance or fluorescence properties of certain pH
32 sensitive indicators which are immobilized on/in proton-permeable solid
33 substrates/supports[11]. A number of pH indicators therefore have been designed or
34 investigated for this application. However, the majority of them were demonstrated to
35 work in the physiological/near neutral[12-16] or acidic pH region[5, 8, 11, 17, 18].
36 Materials that have been reported for use in the highly alkaline pH region are mainly
37 non-immobilized forms that are not ready for use in certain sensing applications[19,
38 20] or restricted to the absorbance/transmission based group[6, 21-23], which has
39 certain disadvantages compared to the fluorescence-based counterpart. In recent years,
40 a few fluorescent sensors for the alkaline pH region have been prepared[24-27].
41 However, the number of reports is still very limited. Considering the importance of
42 monitoring pH in highly alkaline media as occurring in numerous industrial
43 processes[9], scrubbers, waste water treatment plants and concrete structures[28, 29],
44 there is essentially a need for accurate, sensitive and reliable sensing materials suitable
45 for measurement of high basicity.

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

51 and excellent photo-stability[30]. In addition to the development of appropriate pH
52 indicators, the choice of solid supports and the effective immobilization of the
53 indicators to the supports are also discussed in the paper as they are key factors in the
54 development of an optimum optical pH sensor since they govern the lifetime and signal
55 stability of the sensor. The fluorescence detection method was employed rather than the
56 simpler and more commonly-used method based on absorbance/transmission
57 measurements as fluorescent sensors are usually more precise, having less interference
58 from the light source, and offer higher sensitivity than their absorbance counterparts[7,
59 31].

60

61 **2. Experimental**

62 2.1. General

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

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

78

79 2.2. Synthesis of the fluorescent dyes

80 **3-N-(Carbethoxy)aminophenol (1):** **1** was prepared similarly to the method reported
81 in the literature[34]. To a two necked flask equipped with a condenser and a septum
82 were added 3- aminophenol (10.9 g, 100 mmol) and EtOAc (40 mL). The mixture was
83 heated to reflux for 30 min. Ethyl chloroformate (5.4 g, 4764 μ L, 50 mmol) was added
84 via syringe over a 10 min period. The reaction mixture was left stirred for a further 20
85 min then allowed to cool to room temperature to form a white precipitate. The
86 precipitate was removed by filtration and washed with EtOAc (50 mL) and petroleum
87 ether (50 mL). The combined filtrate was concentrated to give **1** as an off-white solid
88 which was further purified by recrystallization from toluene to afford **1** (8.4g, 93%) as
89 white crystals, mp 92 °C [lit. mp 94-95 °C (benzene/cyclohexane)]; IR (neat) ν_{\max} (cm^{-1})
90 3294 (NH), 1684 (C=O), 1556, 1449, 1249, 1061; $^1\text{H-NMR}$ (500 MHz, CDCl_3)
91 δ (ppm): 7.35 (s, 1H), 7.13 (t, 1H, $J = 8.08$), 6.69 (s, 1H), 6.64 (m, 1H), 6.57 (m, 1H),
92 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):
93 Calcd. $m/z = 181.07334$ ($\text{C}_9\text{H}_{11}\text{O}_3\text{N}$). Found $m/z = 181.07296$ (M^+).

94 **4-Chloromethyl-7-N-(Carbethoxy)aminocoumarin (2):** H_2SO_4 (80%, 40 mL) was
95 pre-cooled in an ice bath. **1** (1.8 g, 10 mmol) was added, followed by ethyl 4-
96 chloroacetoacetate (2.3 g, 1906 μ L, 14 mmol) in portions. The mixture was stirred at
97 room temperature under Ar for 19 h, after which it was poured into ice-water (50 mL)
98 and left stirred for a further 30 min. The white precipitate formed was filtered, washed
99 with H_2O and recrystallized from EtOH to afford **2** (2.0 g, 71%) as fine needles, mp
100 242 °C (decomp.) [lit.[35] mp 247 °C (MeOH)]; IR (neat) ν_{\max} (cm^{-1}) 3280 (NH), 3084,

101 2992, 2929, 1704 (C=O), 1619, 1588, 1520, 1404, 1332, 1204, 1075; ¹H-NMR (500
102 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),
103 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,
104 2H, -CH₂Cl), 4.27 (q, 2H, OCH₂-, *J* = 7.13), 1.34 (t, 3H, -CH₃, *J* = 7.13); MS (EI):
105 Calcd. *m/z* = 281.04494 (C₁₃H₁₂O₄NCl). Found *m/z* = 281.04550 (M⁺).

106 **4-Chloromethyl-7-aminocoumarin (3):** **2** (563.4 mg, 2 mmol) was suspended in a
107 mixture of concentrated H₂SO₄ (1.7 mL) and glacial acetic acid (1.7 mL). The mixture
108 was heated to 125 °C for 2h. After cooling to room temperature, the yellow solution
109 was poured into H₂O and a voluminous precipitate was formed. 4 M NaOH aqueous
110 solution was added to the mixture to bring pH to ~ 9. The yellow precipitate was
111 filtered, washed with H₂O, dried *in vacuo* to afford **3** (366 mg, 87%) as a pale yellow
112 solid which was used for synthesis without further purification, mp 182 °C [lit.[35] mp
113 187 °C]; IR (neat) *ν*_{max} (cm⁻¹) 3445 (NH₂), 3352 (NH₂), 1686 (C=O), 1601 (NH₂), 1548,
114 1404, 1263, 1210, 1145; ¹H-NMR (500 MHz, DMSO) δ(ppm): 7.47 (d, 1H, H5, *J*_{5,6}=8.7
115 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,
116 nonres., 3H, -NH₂ + H3), 4.86 (s, 2H, -CH₂Cl); MS (EI): Calcd. *m/z* = 209.02381
117 (C₁₀H₈O₂NCl). Found *m/z* = 209.02417 (M⁺).

118 **7-amino-4-((2-methyl-4-nitro-1*H*-imidazol-1-yl)methyl)-coumarin (4):** NaH in
119 mineral oil (60%, 60 mg) was added to a solution of 2-methyl-4-nitroimidazole (203
120 mg, 1.6 mmol) in DMF (6 mL). The mixture was heated to 100 °C for 10 min and then
121 cooled down to 60 °C, after which **3** (314 mg, 1.5 mmol) dissolved in DMF (6 mL) was
122 added. The reaction mixture was left stirring at the same temperature for 18 h then
123 poured into ice. The yellow precipitate formed was collected by filtration, washed with
124 water and recrystallized from EtOH to afford **4** (390 mg, 87%) as yellow fine crystals,
125 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_aH_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 δ (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 μ L, 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) δ (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_aH_b, $J=16.95$ Hz, $J=1.54$ Hz), 5.85 (dd, 1H, CH=CH_aH_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 δ (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 C₁₇H₁₄O₅N₄ (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):** H₂SO₄ (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 μL, 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 H₂O, dried
183 over phosphorus pentoxide and recrystallized from EtOH to afford **7** (1.87 g, 68%) as
184 a white solid, mp 211 °C; IR (neat) ν_{\max} (cm⁻¹) 3065, 1736 (C=O), 1598, 1395, 1271,
185 1243, 1173, 1151, 1081; ¹H-NMR (500 MHz, CDCl₃) 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, -CH₂Cl); ¹³C-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 (-CH₂-); MS (EI): Calcd. m/z = 271.89501 (C₁₀H₆O₂BrCl). Found m/z =
190 271.89465 (M⁺). Elem. Anal. Calcd. for C₁₀H₆O₂BrCl (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 °C for 10 min and then
195 cooled down to 60 °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 °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

226 (d, 1H, CH=CH_aH_b, *J*=17.70 Hz), 5.68 (s, 2H, -CH₂-), 5.63 (s, 1H, H₃), 5.34 (d, 1H,
 227 CH=CH_aH_b, *J*=11.00 Hz), 2.37 (s, 3H, -CH₃); ¹³C-NMR (DMSO) δ(ppm): 159.5 (C₂),
 228 153.6 (C₉), 149.8 (4-C-imidazole), 145.9 (C₄), 145.8 (C₇), 143.6 (2-C-imidazole),
 229 137.6 (C₇), 137.2 (CCH=CH₂), 136.0 (CH=CH₂), 127.3 (aromaticC.), 126.9
 230 (aromaticC.), 125.1 (C₅), 122.7 (C₆), 122.6 (5-C-imidazole), 116.1 (C₁₀), 115.3
 231 (CH=CH₂), 114.1 (C₈), 112.0 (C₃), 46.4 (-CH₂-), 12.5 (-CH₃); MS (EI): Calcd. m/z =
 232 387.12136 (C₂₂H₁₇O₄N₃). Found m/z = 387.12164 (M⁺); Elem. Anal. Calcd. for
 233 C₂₂H₁₇O₄N₃ (387.39): C 68.21, H 4.42, N 10.85. Found: C 66.92, H 4.14, N 10.15.
 234

235 2.3. Preparation of polymers

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

Polymer	Fluorescent dye		MAA	BAP	P84	DMF
	Type	/mmol				
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

237

238 **Table 1.** Composition of polymers prepared using different coumarin-based pH
 239 indicators. MAA: methacrylate, BAP: 1,4-bis(acryloyl)piperrazine, P84:
 240 Pluronic P84.

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

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

253

254 2.4. Fluorescence measurements

255 Emission spectra of fluorescent indicators and polymers were recorded using a 4 mL
256 (1×1×4 cm) quartz cuvette. Slit widths were adjusted to give emission below the
257 saturating limit (1000000 cps) of the detector, and then the same settings were used for
258 a complete set of experiments.

259 0.8 mM indicator stock solutions were prepared by dissolving 2×10^{-3} mmol of each
260 indicator in 2.5 mL EtOH. PVIC (6.3 mg/mL, containing 2.5 μmol coumarin/mL),
261 PAIC (5.7 mg/mL) and PSIC (5.8 mg/mL) stock solutions were prepared in deionized
262 distilled water.

263 50 mM phosphate at various pH values ranging from 10 to 13.6 was prepared by
264 dissolving Na₂HPO₄ (1.77 g, 12.5 mmol) in distilled deionized water (240 mL) in a
265 conical flask. The buffer was adjusted to the desired pH with aqueous NaOH. The
266 solution was then transferred to a 250 mL volumetric flask and made up to the volume
267 with distilled water.

268 20 μL of each coumarin stock solution or 50 μL of each polymer stock solution was
269 added to 3 mL of buffer in a cuvette, followed by measurement of emission spectra.

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

272 It should be noted that phosphate does not act as a good buffer at pH values higher than
273 12 -12.5. However, phosphate was used for all pH to avoid any differences in
274 fluorescence caused by the difference in buffer composition. It has also been confirmed
275 by measuring pH of the solutions before and after adding the dyes/polymers that a small
276 amount of either the free dyes or the polymers does not cause any change in the pH
277 values of the solutions.

278

279 **3. Results and discussion**

280 3.1. Design and synthesis of fluorescent pH indicators

281 Although there has been a variety of pH indicators, both commercially available
282 including azo dyes[6, 21, 23, 39, 40], trinitrobenzenesulfonic acid[28], thymol blue[29,
283 41-43], cresol red[41], phenolphthalein[44], aniline blue[45], rhodamine 19[46],
284 Victoria blue[47] etc. as well as reported in the literature such as fluorescent Schiff
285 bases[48], porpholactones[49] or a benzo[de]anthracen-7-one derivative[31] , that are
286 known to be useful for the spectroscopic determination of high pHs, the majority of
287 them are absorptive dyes rather than fluorescent dyes and only a few meet the
288 requirements of being stable for a long period of time in highly alkaline media,
289 possessing a pK_a in the range between 11-12.5, having reasonable fluorescence
290 quantum yield and good photostability and bearing one or more functional chemical
291 groups suitable for covalent immobilization of the indicator. Coumarins have been
292 employed and investigated in this work as they are widely used as laser dyes for single-
293 molecule fluorescence and so they are 'tried and tested' in terms of the key property of
294 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.

298

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

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

323

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

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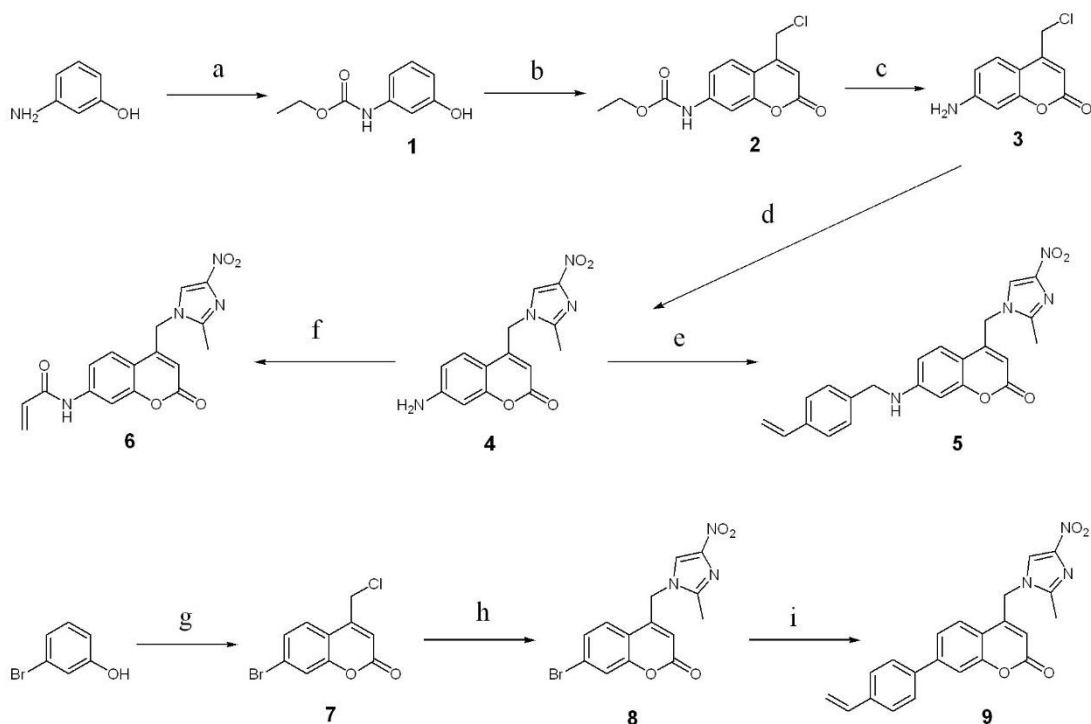
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356 **Scheme 1.** Preparation of fluorescent monomers. (a) ClCOOEt, EtOAc, 100 °C, 93%;357 (b) ethyl 4-chloroacetoacetate, H₂SO₄, H₂O, r.t, 19 h, 71%: (c) conc.H₂SO₄, glacial

358 AcOH, 125 °C, 2 h, 87%; (d) NaH (60%), 2-methyl-4-nitroimidazole, DMF, 100 °C to

359 60 °C, 18 h, 87%; (e) vinylbenzylchloride, K₂CO₃, KI, MeCN, 80 °C, 2 days, 10%; (f)360 CH₂=CHCOCl, dry THF, r.t., 12 h, 94%; (g) ethyl 4-chloroacetoacetate, H₂SO₄, H₂O,

361 r.t, 22 h, 68%; (h) NaH (60%), 2-methyl-4-nitroimidazole, DMF, 110 °C to 60 °C, 20

362 h, 74%; (i) CH₂=CHC₆H₄B(OH)₂, Pd(PPh₃)₄, K₂CO₃, dioxane, 80 °C, 41 h, 41%.

363

364 3.2. Spectroscopic properties and pK_a values of the free indicators

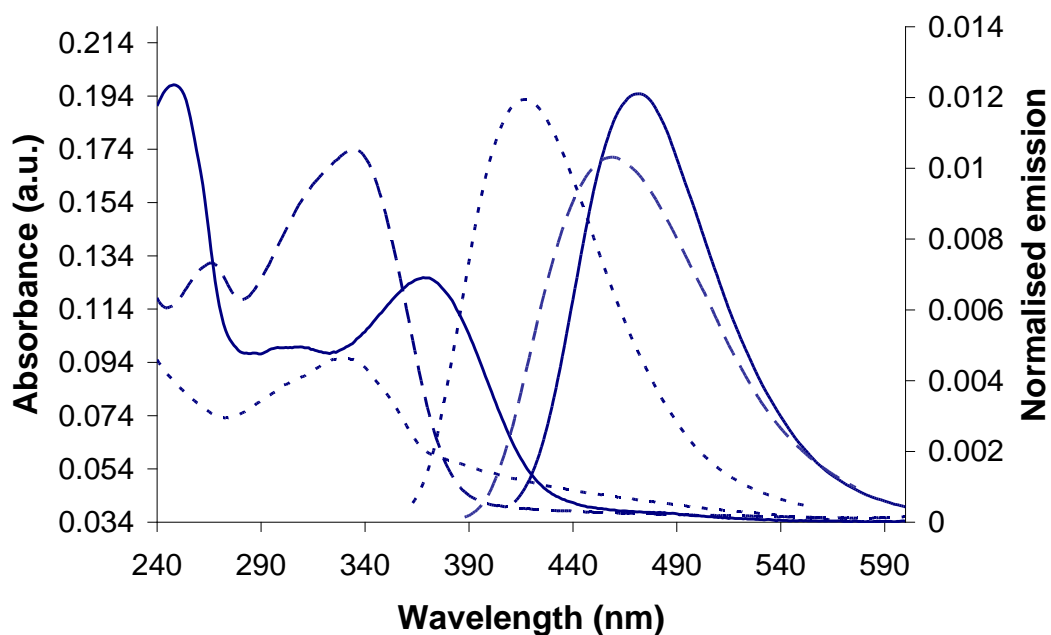
365 In neutral aqueous solution (with the addition of 1.5% EtOH to solubilize the

366 compounds), the absorption spectra of VIC (**5**) and AIC (**6**) show only one main

367 absorption band of each dye in the UV region, at 370 nm and 330 nm, respectively,

368 (Fig. 1) whereas that of SIC (**9**) shows two main bands, at 266 nm with weaker

369 absorbance and 334 nm with higher absorbance.



370

371 **Fig.1.** Absorption (left) and emission (right) spectra of **VIC (5)** (solid lines), **SIC (9)**
 372 (broken lines) and **AIC (6)** (dotted lines) (5 μM) in H_2O . Emission spectra recorded
 373 with $\lambda_{\text{ex}} = 330$ nm for **SIC** and **AIC** and 370 nm for **VIC**.

Dye	Solvent	UV max (nm)	Emission max (nm)	$\text{p}K_{\text{a}}$	Φ (%)
VIC (5)	EtOH	371	456	-	2.6
VIC (5)	H_2O	370	470	12.48 ± 0.06	0.8
AIC (6)	EtOH	334	407	-	1.7
AIC (6)	H_2O	330	416	12.22 ± 0.07	0.6
SIC (9)	EtOH	335	448	-	25.0
SIC (9)	H_2O	334	458	12.40 ± 0.24	2.0

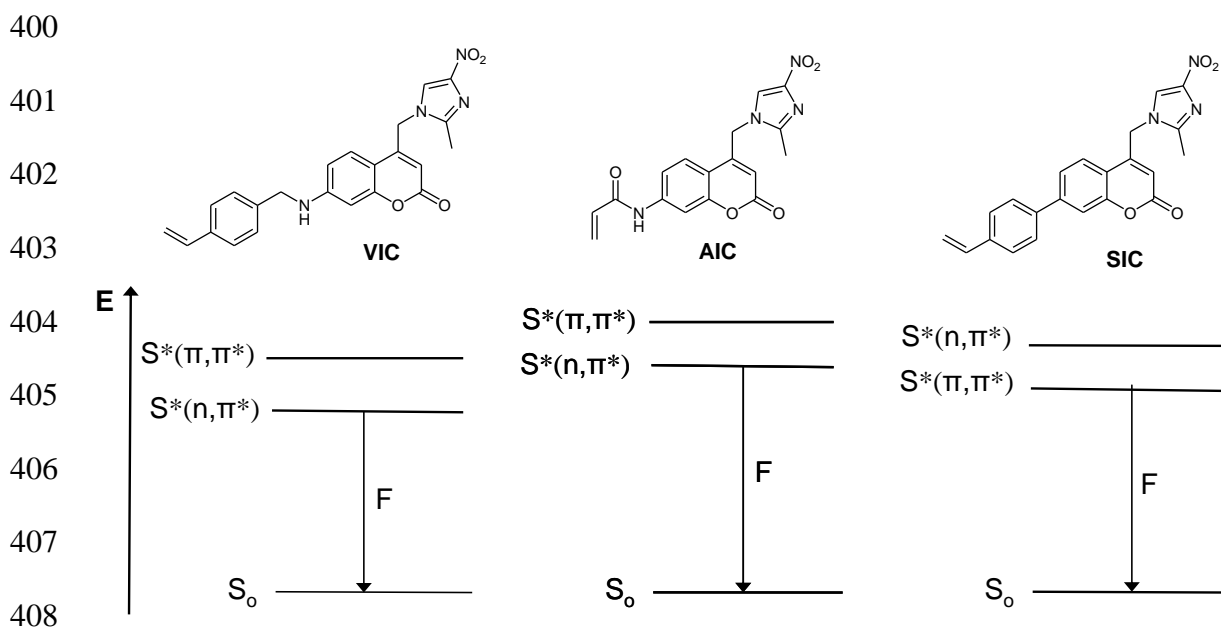
374 **Table 2** Spectral data and $\text{p}K_{\text{a}}$ values of the fluorescent dyes.

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

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

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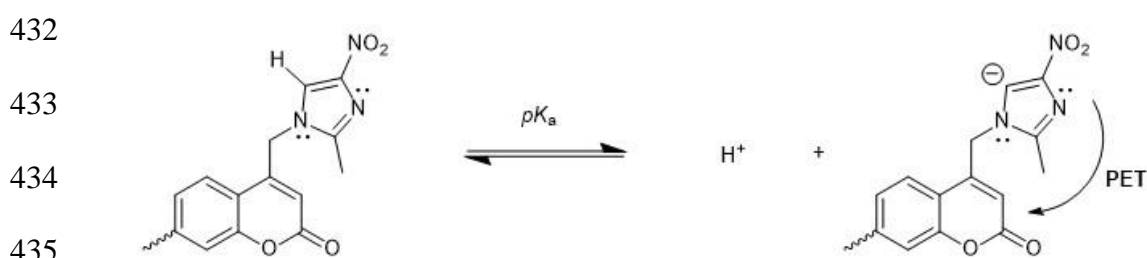


409 **Fig. 2** Possible energy diagrams for VIC, AIC and SIC. S_0 : ground state, S^* : excited
410 single state, F: fluorescence.

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

418 The deprotonation of the imidazolyl group at high pH values was further confirmed by
419 ^{13}C NMR titrations of VIC, AIC and SIC with sodium methoxide (MeONa), a very
420 strong base, in $\text{DMSO-}d_6$. Initially, the ^{13}C spectrum of each fluorophore in $\text{DMSO-}d_6$
421 (0.1 M concentration) was recorded. One mole equivalent of sodium methoxide in
422 $\text{DMSO-}d_6$ was then added to each solution and ^{13}C NMR spectra were recorded again.
423 The addition of MeONa resulted in chemical shifts of all carbon nuclei. However, the
424 largest changes in shift were for the carbon nuclei attached to the only proton of the

425 imidazolyl group (C5-imidazole) with changes of up to 1.71 ppm for the titration of
 426 VIC and 1.63 ppm and 1.47 ppm respectively for the titrations of AIC and SIC. These
 427 results confirm that the carbon nuclei C5 of the imidazolyl group is involved directly
 428 in the interactions with the base through deprotonation. It has been reported that
 429 imidazole is a very weak acid with $pK_a = 14.5$ [69]. However, in the presence of a strong
 430 electron acceptor – NO_2 group, its acidity increases and pK_a can be shifted to a lower
 431 value.



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

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

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



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

450

451

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

452

453 where $[A^-]$ and $[HA]$ are the concentrations of the deprotonated and protonated forms

454 of the indicator and pK_a is the acid-base constant. $[A^-]$ and $[HA]$ are related to

455 fluorescence intensities by $[A^-] = F - F_{\max}$ and $[HA] = F_{\min} - F$ where F is a measured

456 fluorescence intensity of the system, F_{\max} is the fluorescence intensity of the fully

457 protonated system and F_{\min} is the fluorescence intensity of the deprotonated system.

458 The expressions are then substituted into Equation 1 to provide Equation 2.

459

460

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

461

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

463

464

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

465

466 This results in an ‘S-shaped’ relation of the fluorescence intensity versus pH graph,

467 centered on the pK_a value, which is the pH where 50% of the dye population in solution

468 is protonated.

469 The calculation of the pK_a values for the indicators was performed based on the

470 fluorescence intensities at a fixed maximum emission wavelength at different pH values

471 using the model for nonlinear fitting method according to Equation 3. The data obtained

472 are summarized in Table 2. The pK_a values for the three dyes are very similar,

473 suggesting that the substitution at the 7 position of the coumarin has no significant

474 effect on the protonation-deprotonation of the imidazolyl group.

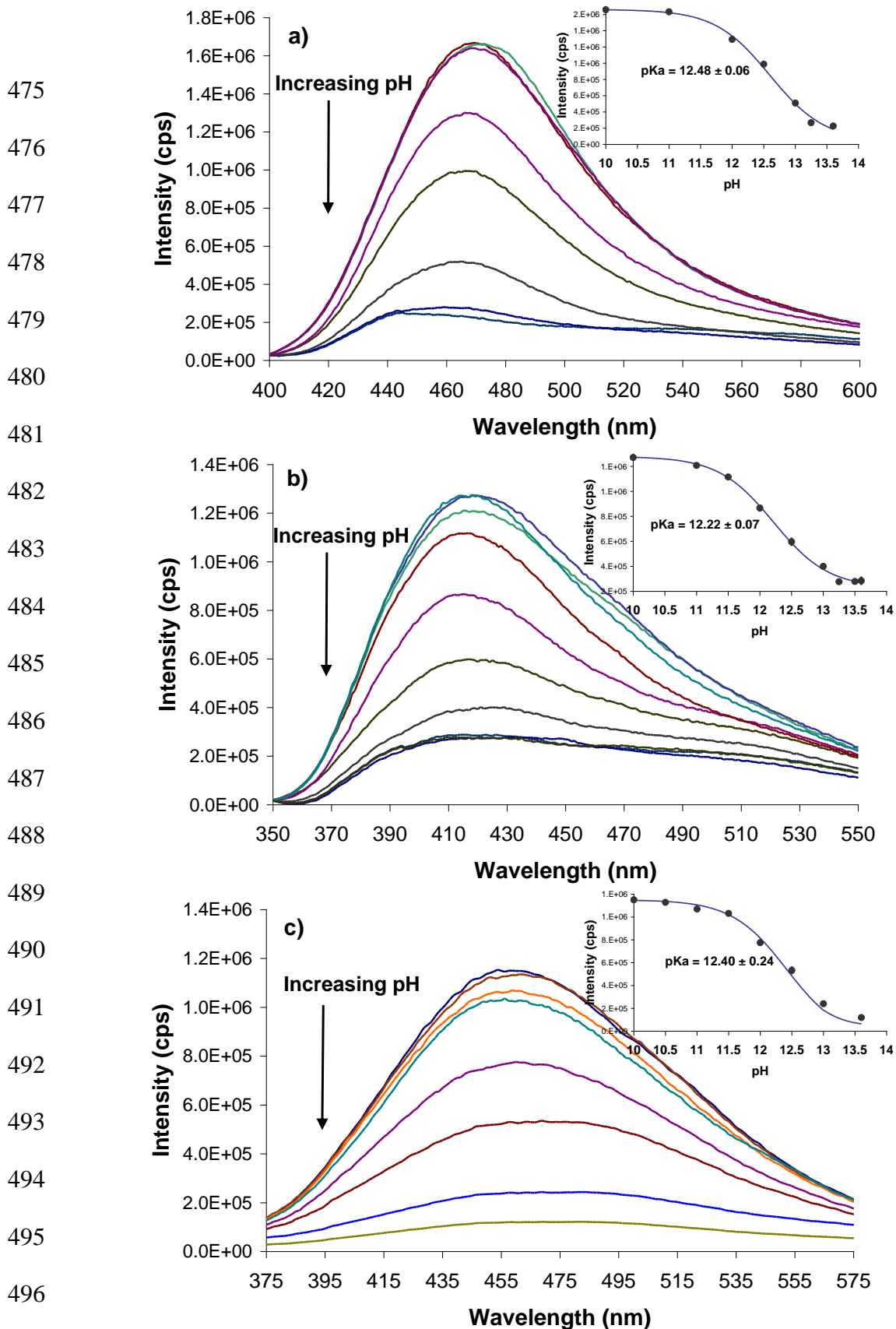


Fig 3. Emission spectra of VIC (a), AIC (b) and SIC (c) at pH from 10.0 to 13.6. The insets show the titration plots at emission maxima. $\lambda_{\text{ex}} = 370 \text{ nm}$ (a), 330 nm (b and c).

500 3.3. Polymer preparation

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

523

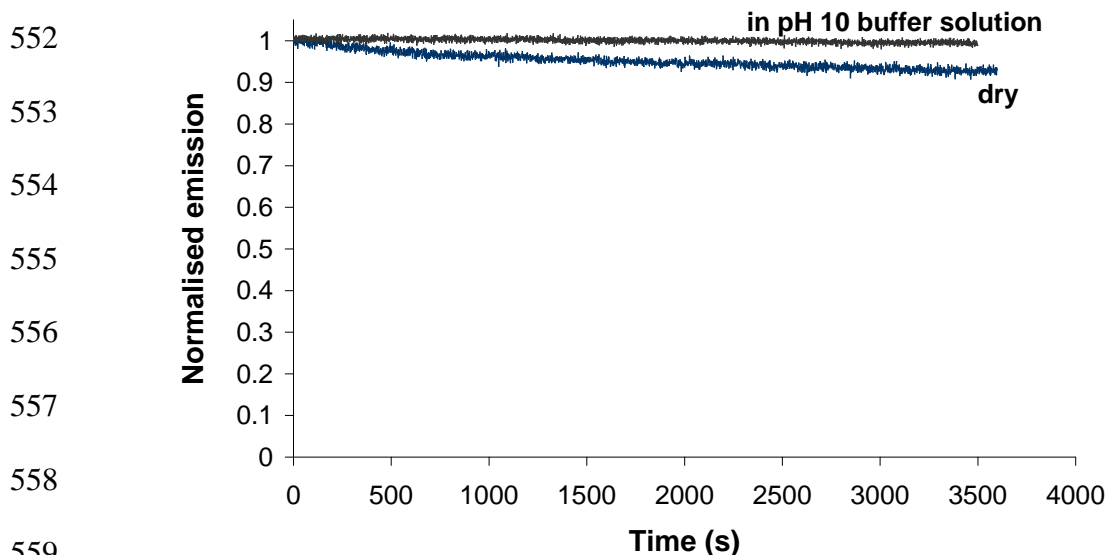
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525 3.4. Photostability

526 Photostability is one of the critical properties of fluorescent materials used for sensor
527 applications. It is proposed that the substitution at the 7 position of the coumarin has no
528 significant effect on the photostability of the coumarin core structure. Photostability
529 was tested with one of the polymers PVIC by holding a layer consisting of 4 mg of the
530 polymer particles in between a quartz disc (6 mm dia.) and a nylon membrane (20 μ m
531 pores, GE), which was kept in place by an O-ring and coupled into the fluorimeter
532 through a dichroic mirror using a fiber bundle. The excitation light (at a wavelength of
533 375 nm) was launched to the distal end of the probe illuminating the polymer with light
534 from the intense, high power Xe lamp of the fluorimeter continuously for 1 h. The
535 fluorescence intensity data from the polymer were collected over that period and
536 displayed. Fig.4 shows the fluorescence intensities of the polymer in dry state and in a
537 pH 10 buffer solution as function of time during 60 min of continuous illumination by
538 light from a high power Xe lamp. It was interesting to observe that the intensity of
539 fluorescence was reduced by 7-8% for the dry state over the time investigated and with
540 the high flux of photons onto the polymer. However, under the same conditions, no
541 photobleaching was seen when the polymer was immersed in a pH 10 buffer solution.
542 The reason for the difference in photostability between the dry state and wet state is
543 unclear. It could be because the excited single state of the fluorophore is stabilised by
544 solvation. When compared to the results of other materials, this still offers excellent
545 performance: the decrease observed in the fluorescence intensity was 65% for
546 carboxyfluorescein and 10-13% for iminocoumarin derivatives, again after 60 min of
547 continuous illumination using a mercury lamp[7]. Thus an important conclusion is that
548 the material prepared in this work possesses superior photostability, a feature that is

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

551



558

559 **Fig.4.** Fluorescence intensity of PVIC (in dry state and in pH 10 buffer solution) as
560 function of time during 60 min of continuous illumination by light from a high power
561 Xe lamp ($\lambda_{\text{ex}} = 375 \text{ nm}$, $\lambda_{\text{em}} = 500 \text{ nm}$). Fluorescence intensities are normalised to the
562 initial emission of the polymer for each data set.
563

564

565 3.5. Fluorescence studies and response of polymers to pH

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

571 It should be noted that incubation was not required before the measurements as the
572 response time of the material is very fast (within 10s). This was confirmed by
573 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.

592

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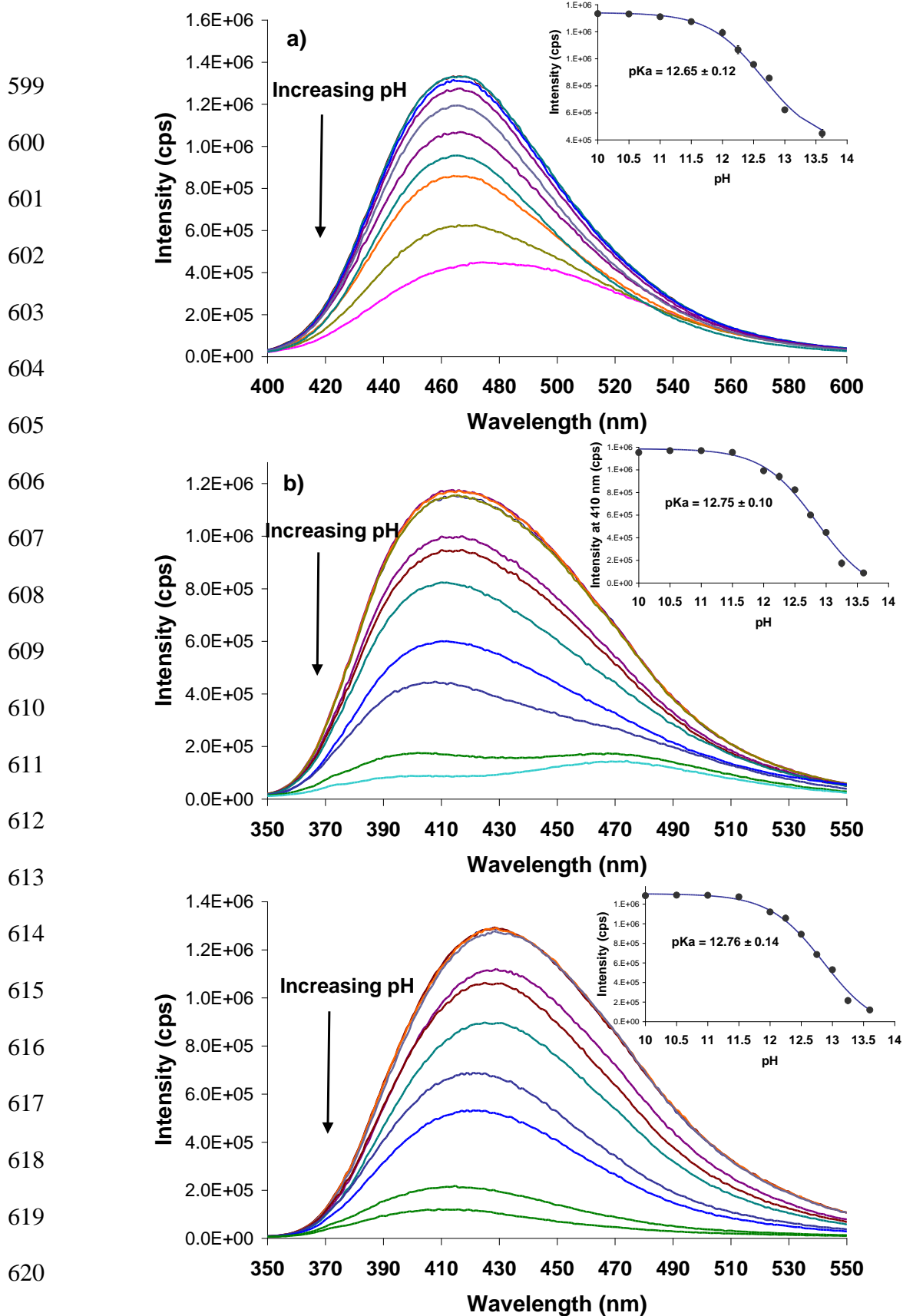
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621 **Fig 5.** Emission spectra of PVIC (a), PAIC (b) and PSIC (c) at pH from 10.0 to 13.6.
 622 The insets show the titration plots at emission maxima. $\lambda_{ex} = 370$ nm (a), 330 nm (b)
 623 and c).

624 3.6. Effect of ionic strength (IS)

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

634

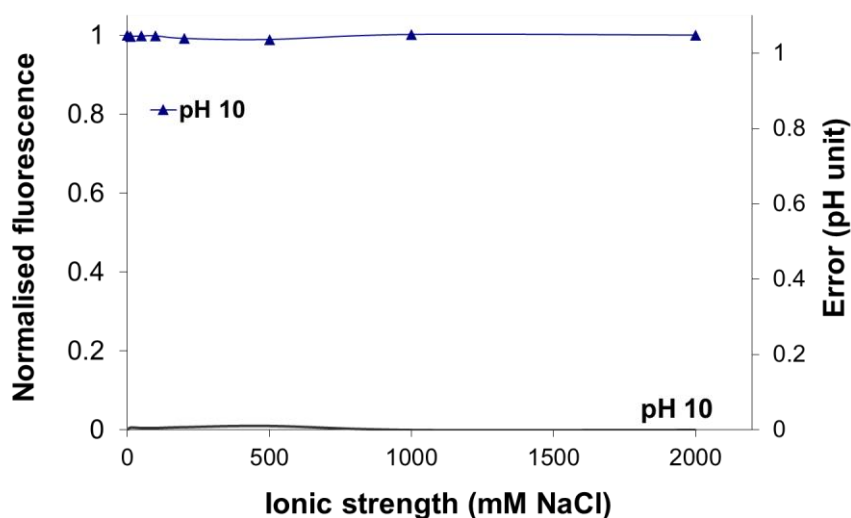
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641 **Fig 6.** Effect of ionic strength (IS) on fluorescence intensity and calculated pH value.

642 Fluorescence intensities are normalised to the maximum emission at 470 nm of the

643 polymer at pH 10 with IS = 0.

644

645

646 **4. Conclusions**

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

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

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

663

664 **Acknowledgments**

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669

670

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