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

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# Author statement

**Thu Hien Nguyen:** conceptualisation, planning of experiments, data analysis, preparation of the manuscript.

Tong Sun: insightful comments and suggestions, preparation of the manuscript.

**Kenneth T. V. Grattan:** insightful comments and suggestions, preparation of the manuscript.

Journal Pre-proof

	journal i re-proor
1	Novel coumarin-based pH sensitive fluorescent probes for the highly
2	alkaline pH region
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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
11	highly alkaline pH region with calculated $pK_a$ values in the range of 12.2 – 12.5.
12	Covalent immobilization of the indicators to polymer supports provides novel pH-
13	sensitive materials that possess excellent photostability. The materials are sensitive to
14	pH in a similar range and can be applied in optical pH sensors for measuring pH in
15	various 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
20	attention because of the importance of pH measurement in various areas of scientific
21	research and other practical applications[1-8]. The determination of pH is routinely
22	performed using glass electrodes. However, glass electrodes have several
23	disadvantages and thus are not suitable for certain applications. They are bulky,
24	invasive and potentially create the risk of electric shock during in vivo measurements.

25 They show poor performance when measuring solutions of low ionic strength and

suffer from surface potentials. In highly alkaline media, glass electrodes suffer from
chemical deterioration and are 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, 30 enhanced safety and the possibility of remote sensing. Most optical pH sensors 31 function through monitoring the changes in the absorbance or fluorescence properties of certain pH sensitive indicators which are immobilized on/in proton-permeable solid 32 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 40 years, 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 45 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

51 shift and excellent photo-stability[30]. In addition to the development of appropriate 52 pH 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 stability of the sensor. The fluorescence detection method was employed rather 55 56 than the simpler and more commonly-used method based on absorbance/transmission measurements as fluorescent sensors are usually more precise, having less 57 58 interference from the light source, and offer higher sensitivity than their absorbance 59 counterparts[7, 31].

10-Q1

60

## 61 **2. Experimental**

62 2.1. General

63 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 64 Scientific or Sigma-Aldrich. All aqueous solutions were prepared using distilled 65 deionized water. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker Avance 500 66 spectrometer. Mass spectra were run by electron ionisation (EI) mode on a Thermo 67 68 Finnigan MAT900xp mass spectrometer. IR spectra were recorded on a Bruker Alpha 69 Fourier Transform Infrared Spectrophotometer and were run neat. Melting points 70 were 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 of aqueous solutions containing fluorophores were carried out on a PerkinElmer 73 74 Lambda 35 spectrophotometer and a Horiba Jobin Yvon Fluoromax-4 spetrofluorometer system with FluorEssence<sup>TM</sup> as driving software, respectively. 75

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].

79

80 2.2. Synthesis of the fluorescent dyes

81 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 82 83 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 µL, 50 mmol) was 84 85 added via syringe over a 10 min period. The reaction mixture was left stirred for a 86 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 87 88 petroleum ether (50 mL). The combined filtrate was concentrated to give 1 as an off-89 white solid which was further purified by recrystallization from toluene to afford 1 90 (8.4g, 93%) as white crystals, mp 92 °C [lit. mp 94-95 °C (benzene/cyclohexane)]; IR (neat)  $v_{\text{max}}$  (cm<sup>-1</sup>) 3294 (NH), 1684 (C=O), 1556, 1449, 1249, 1061; <sup>1</sup>H-NMR (500 91 92 MHz, CDCl<sub>3</sub>)  $\delta$ (ppm): 7.35 (s, 1H), 7.13 (t, 1H, J = 8.08), 6.69 (s, 1H), 6.64 (m, 1H), 93 6.57 (m, 1H), 6.33 (m, 1H), 4.23 (q, 2H, -CH<sub>2</sub>-, J=7.12 Hz), 1.30 (t, 3H, -CH<sub>3</sub>, J = 94 7.12); MS (EI): Calcd. m/z = 181.07334 (C<sub>9</sub>H<sub>11</sub>O<sub>3</sub>N). Found m/z = 181.07296 (M<sup>+</sup>). 95 4-Chloromethyl-7-N-(Carbethoxy)aminocoumarin (2): H<sub>2</sub>SO<sub>4</sub> (80%, 40 mL) was 96 pre-cooled in an ice bath. 1 (1.8 g, 10 mmol) was added, followed by ethyl 4-97 chloroacetoacetate (2.3 g, 1906 µL, 14 mmol) in portions. The mixture was stirred at 98 room temperature under Ar for 19 h, after which it was poured into ice-water (50 mL) 99 and left stirred for a further 30 min. The white precipitate formed was filtered, washed 100 with H<sub>2</sub>O 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) v<sub>max</sub> (cm<sup>-1</sup>) 3280 (NH), 101 102 3084, 2992, 2929, 1704 (C=O), 1619, 1588, 1520, 1404, 1332, 1204, 1075; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ(ppm): 7.59 (d, 1H, H5, J<sub>5.6</sub>=8.7 Hz), 7.50 (d, 1H, H8, J<sub>8.6</sub>=2.14 103 Hz), 7.36 (dd, 1H, H6, J<sub>65</sub>=8.7 Hz, J<sub>68</sub>=2.14), 6.82 (s, 1H, NH), 6.45 (s, 1H, H3), 104 105 4.65 (s, 2H, -CH<sub>2</sub>Cl), 4.27 (q, 2H, OCH<sub>2</sub>-, *J* = 7.13), 1.34 (t, 3H, -CH<sub>3</sub>, *J* = 7.13); MS (EI): Calcd. m/z = 281.04494 (C<sub>13</sub>H<sub>12</sub>O<sub>4</sub>NCl). Found m/z = 281.04550 (M<sup>+</sup>). 106 107 4-Chloromethyl-7-aminocoumarin (3): 2 (563.4 mg, 2 mmol) was suspended in a 108 mixture of concentrated H<sub>2</sub>SO<sub>4</sub> (1.7 mL) and glacial acetic acid (1.7 mL). The mixture 109 was heated to 125 °C for 2h. After cooling to room temperature, the yellow solution 110 was poured into H<sub>2</sub>O and a voluminous precipitate was formed. 4 M NaOH aqueous solution was added to the mixture to bring pH to  $\sim 9$ . The yellow precipitate was 111 filtered, washed with H<sub>2</sub>O, dried *in vacuo* to afford **3** (366 mg, 87%) as a pale yellow 112 113 solid which was used for synthesis without further purification, mp 182 °C [lit.[35] mp 187 °C]; IR (neat) v<sub>max</sub> (cm<sup>-1</sup>) 3445 (NH<sub>2</sub>), 3352 (NH<sub>2</sub>), 1686 (C=O), 1601 (NH<sub>2</sub>), 114 1548, 1404, 1263, 1210, 1145; <sup>1</sup>H-NMR (500 MHz, DMSO) δ(ppm): 7.47 (d, 1H, H5, 115  $J_{5.6}$ =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) 116 6.19 (s+s, nonres., 3H,  $-NH_2 + H3$ ), 4.86 (s, 2H,  $-CH_2Cl$ ); MS (EI): Calcd. m/z = 117 118 209.02381 ( $C_{10}H_8O_2NCl$ ). Found m/z = 209.02417 ( $M^+$ ). 119 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

126	yellow fine crystals, mp 298-300 °C (decomp.); IR (neat) $v_{max}$ (cm <sup>-1</sup> ) 3430 (NH <sub>2</sub> ),
127	3324 (NH <sub>2</sub> ), 3221, 3144, 1695 (C=O), 1595 (NH <sub>2</sub> ), 1542, 1498, 1402, 1328, 1290,
128	1132; <sup>1</sup> H-NMR (500 MHz, DMSO) δ(ppm): 8.37 (s, 1H, 5-H-imidazole), 7.44 (d, 1H,
129	H5, $J_{5,6}$ =8.69 Hz), 6.61 (dd, 1H, H6, $J_{6,5}$ =8.69 Hz, $J_{6,8}$ =2.14), 6.45 (d, 1H, H8,
130	J <sub>8,6</sub> =2.14 Hz) 6.27 (s, 2H, -NH <sub>2</sub> ), 5.51 (s, 2H, -CH <sub>2</sub> -), 5.05 (s, 1H, H3), 2.31 (s, 3H, -
131	CH <sub>3</sub> ); <sup>13</sup> C-NMR (DMSO) δ(ppm): 160.7 (C2), 155.9 (C9), 154.0 (C7), 151.1 (4-C-
132	imidazole), 146.1 (C4), 125.7 (C5), 123.1 (5-C-imidazole), 111.7 (C6), 106.2 (C10),
133	104.5 (C3), 99.0 (C8), 46.6 (-CH <sub>2</sub> - ), 12.8 (-CH <sub>3</sub> ); MS (EI): Calcd. $m/z = 300.08531$
134	$(C_{14}H_{12}O_4N_4)$ . Found m/z = 300.08584 (M <sup>+</sup> ); Elem. Anal. Calcd. for $C_{14}H_{12}O_4N_4$
135	(300.27): C 56.00, H 4.03, N 18.66. Found: C 56.72, H 4.50, N 18.19.
136	7-(4-vinylbenzylamino)-4-((2-methyl-4-nitro-1 <i>H</i> -imidazol-1-yl)methyl)-coumarin
137	(5): A mixture of 4 (300 mg, 1 mmol), vinylbenzylchloride (155 $\mu$ L, 168 mg, 1.1
138	mmol, 1 mmol), potassium carbonate (401 mg, 2.9 mmol), posstasium iodide (49 mg,
139	0.3 mmol) in dry MeCN (20 mL) was heated under argon at 80 $^{\circ}$ C for 2 days. After
140	cooling to room temperature, H <sub>2</sub> O was added to dissolve the inorganic salts. The
141	mixture was filtered to remove insoluble materials. EtOAc was then added. The
142	organic phase was washed with $H_2O$ (2 x 50 mL) and saturated aqueous NaCl (50
143	mL), dried over MgSO <sub>4</sub> , filtered, and concentrated <i>in vacuo</i> . The resulting orange-red
144	residue was chromatographed on silica gel using $CH_2Cl_2$ -EtOAc (4:6, v/v) as eluent to
145	give an orange solid which was further purified by recrystallization from ethanol to
146	afford 5 (40 mg, 10 %) as a yellow-orange solid, mp 194 °C; IR (neat) $v_{max}$ (cm <sup>-1</sup> )
147	3335 (NH), 3144, 1694 (C=O), 1601, 1540, 1500, 1404, 1321, 1291, 1148; <sup>1</sup> H-NMR
148	(500 MHz, CDCl <sub>3</sub> ) δ(ppm): 7.69 (s, 1H, 5-H-imidazole), 7.41 (d, 2H, aromaticH <sub>1</sub>
149	J=8.13 Hz), 7.30 (d, 2H, aromaticH J=8.15 Hz), 7.20 (d, 1H, H5, J <sub>5,6</sub> =8.72 Hz), 6.71
150	(dd, 1H, CH=CH <sub>2</sub> , J=10.91 Hz, J=17.60 Hz), 6.57 (dd, 1H, H6, J <sub>6,5</sub> =8.72 Hz,

161	7-acrylamido-4-((2-methyl-4-nitro-1 <i>H</i> -imidazol-1-yl)methyl)-coumarin (6):
160	(416.43): C 66.34, H 4.89, N 13.45. Found: C 65.76, H 4.66, N 12.89.
159	$(C_{23}H_{20}O_4N_4)$ . Found m/z = 416.14827 (M <sup>+</sup> ); Elem. Anal. Calcd. for $C_{23}H_{20}O_4N_4$
158	$CH_2NH_2$ ), 46.9 (C4 $CH_2$ -), 13.1 (-CH <sub>3</sub> ); MS (EI): Calcd. m/z = 416.14791
157	119.9 (5-C-imidazole), 114.8 (CH=CH <sub>2</sub> ), 107.6 (C3), 107.1 (C10), 99.1 (C8), 47.5 (-
156	(CH=CH <sub>2</sub> ), 128.0 ((CCH=CH <sub>2</sub> ), 127.6 (aromaticC), 126.8 (aromaticC), 123.7 (C5),
155	(C9), 147.7 (C7), 141.1 (C4), 140.0 (4-C-imidazole), 137.3 (CCH <sub>2</sub> NH-), 136.2
154	3H, -CH <sub>3</sub> ); <sup>13</sup> C-NMR (CDCl <sub>3</sub> ) δ(ppm): 156.3 (C2), 155.0 (2-C- imidazole), 154.0
153	(t, 1H, -NH-, $J_{\text{NH-CH2}} = 5.52$ Hz), 4.41 (d, 2H, CH <sub>2</sub> NH-, $J_{\text{CH2-NH}} = 5.55$ Hz), 2.44 (s,
152	5.43 (s, 1H, H3), 5.26 (d, 1H, CH=CH <sub>a</sub> $H_b$ , J=10.90 Hz), 5.17 (s, 2H, C4CH <sub>2</sub> -), 4.85
151	$J_{6,8}$ =2.36 Hz), 6.53 (d, 1H, H8, $J_{8,6}$ =2.32 Hz), 5.75 (d, 1H, CH=C $H_a$ H <sub>b</sub> J=17.61 Hz),

Acryloyl chloride (76 µL, 75 mg, 0.93 mmol) in dry THF (4 mL) was added slowly to 162 163 a suspension of 4 (112 mg, 0.373 mmol) in dry THF (10 mL) under argon. The reaction mixture was left stirred at room temperature overnight. The resulting 164 precipitate was collected by filtration, washed with THF, H<sub>2</sub>O and ethyl acetate then 165 dried in vacuo to afford 6 (124 mg, 94%) as a pale yellow solid, mp 291 °C 166 (decomp.); IR (neat)  $v_{\text{max}}$  (cm<sup>-1</sup>) 3301, 1695 (C=O), 1608, 1501, 1414, 1332, 1285, 167 1211, 1188, 1141; <sup>1</sup>H-NMR (500 MHz, DMSO) δ(ppm): 10.65 (s, 1H, -CONH-), 8.38 168 169 (s, 1H, 5-H-imidazole), 7.90 (d, 1H, H8, J<sub>8.6</sub>=1.68 Hz), 7.77 (d, 1H, H5, J<sub>5.6</sub>=8.70 Hz), 7.59 (dd, 1H, H6, J<sub>6.5</sub>=8.72 Hz, J<sub>6.8</sub>=1.71), 6.46 (dd, 1H, CH=CH<sub>2</sub>, J=10.08 Hz, 170 171 J=16.91 Hz), 6.33 (dd, 1H, CH=CH<sub>a</sub>H<sub>b</sub>, J=16.95 Hz, J=1.54 Hz), 5.85 (dd, 1H, 172 CH=CH<sub>a</sub>H<sub>b</sub>, J=10.19 Hz, J=1.43 Hz), 5.61 (s, 2H, -CH<sub>2</sub>-), 5.48 (s, 1H, H3), 2.35 (s, 3H, -CH<sub>3</sub>); <sup>13</sup>C-NMR (DMSO) δ(ppm): 163.8 (*C*=ONH-), 160.7 (C2), 153.8 (C9), 173 174 149.9 (C7), 145.8 (4-C-imidazole), 142.8 (C4), 131.3 (CH=CH<sub>2</sub>), 129.0 (CH=CH<sub>2</sub>), 175 125.2 (C5), 122.7 (5-C-imidazole), 115.5 (C6), 112.0 (C10), 110.1 (C3), 106.1 (C8),

- 176 46.3 (-CH<sub>2</sub>- ), 12.5 (-CH<sub>3</sub>); MS (EI): Calcd. m/z = 354.09587 (C<sub>17</sub>H<sub>14</sub>O<sub>5</sub>N<sub>4</sub>). Found
- 177  $m/z = 354.09630 (M^+)$ ; Elem. Anal. Calcd. for  $C_{17}H_{14}O_5N_4$  (354.32): C 57.63 , H
- 178 3.98, N 15.81. Found: C 56.14, H 3.61, N 14.05.
- 179 4-Chloromethyl-7-bromocoumarin (7): H<sub>2</sub>SO<sub>4</sub> (80%, 40 mL) was pre-cooled in an 180 ice bath. 3-bromophenol (1.73 g, 10 mmol) was added, followed by ethyl 4chloroacetoacetate (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 182 183 for a further 30 min. The white precipitate formed was filtered, washed with H<sub>2</sub>O, dried over phosphorus pentoxide and recrystallized from EtOH to afford 7 (1.87 g, 184 68%) as a white solid, mp 211 °C; IR (neat)  $v_{max}$  (cm<sup>-1</sup>) 3065, 1736 (C=O), 1598, 185 1395, 1271, 1243, 1173, 1151, 1081; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ(ppm): 7.56 (d, 186 1H, H8, J<sub>8.6</sub>=1.84 Hz), 7.54 (d, 1H, H5, J<sub>5.6</sub>=8.51 Hz), 7.47 (dd, 1H, H6, J<sub>6.5</sub>=8.37 Hz, 187 188  $J_{6.8}=1.68$ ), 6.58 (s, 1H, H3), 4.64 (s, 2H, -CH<sub>2</sub>Cl); <sup>13</sup>C-NMR (DMSO)  $\delta$ (ppm): 159.1 (C2), 153.8 (C4), 150.2 (C9), 127.5 (C6), 126.9 (C8), 125.1 (C7), 119.8 (C5), 116.4 189 (C10), 115.8 (C3), 41.1 (-CH<sub>2</sub>-); MS (EI): Calcd. m/z = 271.89501 (C<sub>10</sub>H<sub>6</sub>O<sub>2</sub>BrCl). 190 191 Found m/z = 271.89465 (M<sup>+</sup>). Elem. Anal. Calcd. for C<sub>10</sub>H<sub>6</sub>O<sub>2</sub>BrCl (273.51): C 43.91, 192 H 2.21. Found: C 43.35, H 2.19.
- 193 7-bromo-4-((2-methyl-4-nitro-1*H*-imidazol-1-yl)methyl)-coumarin (8): NaH in 194 mineral oil (60%, 80 mg) was added to a solution of 2-methyl-4-nitroimidazole (254.2 mg, 2 mmol) in DMF (8 mL). The mixture was heated to 110 °C for 10 min and then 195 196 cooled down to 60 °C, after which 7 (547.0 mg, 2 mmol) suspended in DMF (8 mL) 197 was added. The reaction mixture was left stirring at the same temperature for 20 h then poured into ice. The precipitate formed was collected by filtration, washed with 198 199 water, dried and recrystallized from EtOH to afford 8 (538 mg, 74%) as an off-white solid, mp 289-291 °C (decomp.); IR (neat) v<sub>max</sub> (cm<sup>-1</sup>) 3135, 1738 (C=O), 1597, 1541, 200

201	1498, 1398, 1342, 1299, 1244, 1171, 1147; <sup>1</sup> H-NMR (500 MHz, DMSO) δ(ppm):
202	8.36 (s, 1H, 5-H-imidazole), 7.81 (d, 1H, H8, <i>J</i> <sub>8,6</sub> =1.90 Hz), 7.73 (d, 1H, H5, <i>J</i> <sub>5,6</sub> =8.53
203	Hz), 7.66 (dd, 1H, H6, J <sub>6,5</sub> =8.52 Hz, J <sub>6,8</sub> =1.92 Hz), 5.68 (s, 1H, H3), 5.63 (s, 2H, -
204	CH <sub>2</sub> -), 2.35 (s, 3H, -CH <sub>3</sub> ); <sup>13</sup> C-NMR (DMSO) δ(ppm): 158.8 (C2), 153.4 (C9), 149.5
205	(4-C-imidazole), 145.9 (C4), 145.8 (2-C-imidazole), 127.6 (C6), 126.2 (C5), 125.4
206	(C7), 122.6 (5-C-imidazole), 119.8 (C8), 116.4 (C10), 112.8 (C3), 46.3 (-CH <sub>2</sub> -), 12.5
207	(-CH <sub>3</sub> ); MS (EI): Calcd. $m/z = 362.98492$ (C <sub>14</sub> H <sub>10</sub> O <sub>4</sub> N <sub>3</sub> Br). Found $m/z = 362.98521$
208	(M <sup>+</sup> ); Elem. Anal. Calcd. for $C_{14}H_{10}O_4N_3Br$ (364.15): C 46.18 , H 2.77, N 11.54.
209	Found: C 45.19, H 2.36, N 10.88.
210	7-vinylphenyl-4-((2-methyl-4-nitro-1 <i>H</i> -imidazol-1-yl)methyl)-coumarin (9): 9
210 211	7-vinylphenyl-4-((2-methyl-4-nitro-1 <i>H</i> -imidazol-1-yl)methyl)-coumarin (9): 9 was prepared from 8 <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic
<ul><li>210</li><li>211</li><li>212</li></ul>	7-vinylphenyl-4-((2-methyl-4-nitro-1 <i>H</i> -imidazol-1-yl)methyl)-coumarin (9): 9 was prepared from 8 <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic acid. A mixture of 8 (400.6 mg, 1.1 mmol), 4-vinylphenylboronic acid (244.2 mg,
<ul><li>210</li><li>211</li><li>212</li><li>213</li></ul>	<ul> <li>7-vinylphenyl-4-((2-methyl-4-nitro-1<i>H</i>-imidazol-1-yl)methyl)-coumarin (9): 9</li> <li>was prepared from 8 <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic acid. A mixture of 8 (400.6 mg, 1.1 mmol), 4-vinylphenylboronic acid (244.2 mg, 1.65 mmol, 1.5 mol equiv), potassium carbonate (570 mg, 4.125 mmol, 3.75 mol</li> </ul>
<ul> <li>210</li> <li>211</li> <li>212</li> <li>213</li> <li>214</li> </ul>	<b>7-vinylphenyl-4-((2-methyl-4-nitro-1<i>H</i>-imidazol-1-yl)methyl)-coumarin (9): 9</b> was prepared from <b>8</b> <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic acid. A mixture of <b>8</b> (400.6 mg, 1.1 mmol), 4-vinylphenylboronic acid (244.2 mg, 1.65 mmol, 1.5 mol equiv), potassium carbonate (570 mg, 4.125 mmol, 3.75 mol equiv) and dioxane (11 mL) was stirred in a two necked flask at room temperature
<ul> <li>210</li> <li>211</li> <li>212</li> <li>213</li> <li>214</li> <li>215</li> </ul>	<b>7-vinylphenyl-4-((2-methyl-4-nitro-1<i>H</i>-imidazol-1-yl)methyl)-coumarin (9): 9</b> was prepared from <b>8</b> <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic acid. A mixture of <b>8</b> (400.6 mg, 1.1 mmol), 4-vinylphenylboronic acid (244.2 mg, 1.65 mmol, 1.5 mol equiv), potassium carbonate (570 mg, 4.125 mmol, 3.75 mol equiv) and dioxane (11 mL) was stirred in a two necked flask at room temperature under argon for 0.5 h. Tetrakis(triphenylphosphine)palladium(0) (63.6 mg, 0.055
<ul> <li>210</li> <li>211</li> <li>212</li> <li>213</li> <li>214</li> <li>215</li> <li>216</li> </ul>	<b>7-vinylphenyl-4-((2-methyl-4-nitro-1<i>H</i>-imidazol-1-yl)methyl)-coumarin (9): 9</b> was prepared from <b>8</b> <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic acid. A mixture of <b>8</b> (400.6 mg, 1.1 mmol), 4-vinylphenylboronic acid (244.2 mg, 1.65 mmol, 1.5 mol equiv), potassium carbonate (570 mg, 4.125 mmol, 3.75 mol equiv) and dioxane (11 mL) was stirred in a two necked flask at room temperature under argon for 0.5 h. Tetrakis(triphenylphosphine)palladium(0) (63.6 mg, 0.055 mmol, 5 mol %) was added. A condenser was fitted and the flask was evacuated and
210 211	<b>7-vinylphenyl-4-((2-methyl-4-nitro-1<i>H</i>-imidazol-1-yl)methyl)-coumarin (9):</b> 9 was prepared from <b>8</b> <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic
<ul><li>210</li><li>211</li><li>212</li><li>213</li></ul>	<ul> <li>7-vinylphenyl-4-((2-methyl-4-nitro-1<i>H</i>-imidazol-1-yl)methyl)-coumarin (9): 9</li> <li>was prepared from 8 <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic acid. A mixture of 8 (400.6 mg, 1.1 mmol), 4-vinylphenylboronic acid (244.2 mg, 1.65 mmol, 1.5 mol equiv), potassium carbonate (570 mg, 4.125 mmol, 3.75 mol</li> </ul>
<ul> <li>210</li> <li>211</li> <li>212</li> <li>213</li> <li>214</li> </ul>	<b>7-vinylphenyl-4-((2-methyl-4-nitro-1<i>H</i>-imidazol-1-yl)methyl)-coumarin (9): 9</b> was prepared from <b>8</b> <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic acid. A mixture of <b>8</b> (400.6 mg, 1.1 mmol), 4-vinylphenylboronic acid (244.2 mg, 1.65 mmol, 1.5 mol equiv), potassium carbonate (570 mg, 4.125 mmol, 3.75 mol equiv) and dioxane (11 mL) was stirred in a two necked flask at room temperature
<ul> <li>210</li> <li>211</li> <li>212</li> <li>213</li> <li>214</li> <li>215</li> </ul>	<b>7-vinylphenyl-4-((2-methyl-4-nitro-1<i>H</i>-imidazol-1-yl)methyl)-coumarin (9): 9</b> was prepared from <b>8</b> <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic acid. A mixture of <b>8</b> (400.6 mg, 1.1 mmol), 4-vinylphenylboronic acid (244.2 mg, 1.65 mmol, 1.5 mol equiv), potassium carbonate (570 mg, 4.125 mmol, 3.75 mol equiv) and dioxane (11 mL) was stirred in a two necked flask at room temperature under argon for 0.5 h. Tetrakis(triphenylphosphine)palladium(0) (63.6 mg, 0.055
<ul> <li>210</li> <li>211</li> <li>212</li> <li>213</li> <li>214</li> <li>215</li> <li>216</li> </ul>	<b>7-vinylphenyl-4-((2-methyl-4-nitro-1<i>H</i>-imidazol-1-yl)methyl)-coumarin (9): 9</b> was prepared from <b>8</b> <i>via</i> a Suzuki coupling reaction[36-38] with vinylphenylboronic acid. A mixture of <b>8</b> (400.6 mg, 1.1 mmol), 4-vinylphenylboronic acid (244.2 mg, 1.65 mmol, 1.5 mol equiv), potassium carbonate (570 mg, 4.125 mmol, 3.75 mol equiv) and dioxane (11 mL) was stirred in a two necked flask at room temperature under argon for 0.5 h. Tetrakis(triphenylphosphine)palladium(0) (63.6 mg, 0.055 mmol, 5 mol %) was added. A condenser was fitted and the flask was evacuated and

219 organic phase was washed with water  $(3 \times 50 \text{ mL})$ , saturated aqueous NaCl (50 mL),

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222

<sup>o</sup>C in the dark for 41 h. After cooling to room temperature, EtOAc was added. The

220 dried over MgSO<sub>4</sub>, filtered, concentrated in vacuo to give the crude product which 221 was purified by flash chromatography on silica gel with EtOAc-petroleum ether (95:5,

v/v) as eluent to give 9 as an orange solid, which was further purified by recrystallization from EtOH-EtOAc (5:1, v/v) to give orange crystals (176 mg, 41%), 223

mp 215 °C; IR (neat) v<sub>max</sub> (cm<sup>-1</sup>) 3147, 1716 (C=O), 1612, 1538, 1496, 1395, 1322, 224

1290, 1160; <sup>1</sup>H-NMR (500 MHz, DMSO) δ(ppm): 8.40 (s, 1H, 5-H-imidazole), 7.89-225

226	7.78 (m, 5H, H5, H6, H8, aromaticH), 7.62 (d, 2H, aromaticH J=8.34 Hz), 6.80 (dd,
227	1H, C <b>H</b> =CH <sub>2</sub> , J=10.98 Hz, J=17.67 Hz), 5.94 (d, 1H, CH=C <b>H</b> <sub>a</sub> H <sub>b</sub> , J=17.70 Hz), 5.68
228	(s, 2H, -CH <sub>2</sub> -), 5.63 (s, 1H, H3), 5.34 (d, 1H, CH=CH <sub>a</sub> H <sub>b</sub> , J=11.00 Hz), 2.37 (s, 3H, -
229	CH <sub>3</sub> ); <sup>13</sup> C-NMR (DMSO) δ(ppm): 159.5 (C2), 153.6 (C9), 149.8 (4-C-imidazole),
230	145.9 (C4), 145.8 (C7), 143.6 (2-C-imidazole), 137.6 (CC7), 137.2 (CCH=CH <sub>2</sub> ),
231	136.0 (CH=CH <sub>2</sub> ), 127.3 (aromaticC <sub>1</sub> ), 126.9 (aromaticC <sub>1</sub> ), 125.1 (C5), 122.7 (C6),
232	122.6 (5-C-imidazole), 116.1 (C10), 115.3 (CH=CH <sub>2</sub> ), 114.1 (C8), 112.0 (C3), 46.4 (-
233	CH <sub>2</sub> -), 12.5 (-CH <sub>3</sub> ); MS (EI): Calcd. $m/z = 387.12136$ (C <sub>22</sub> H <sub>17</sub> O <sub>4</sub> N <sub>3</sub> ). Found $m/z =$
234	387.12164 (M <sup>+</sup> ); Elem. Anal. Calcd. for $C_{22}H_{17}O_4N_3$ (387.39): C 68.21 , H 4.42, N

- 235 10.85. Found: C 66.92 , H 4.14, N 10.15.
- 236

237 2.3. Preparation of polymers

Polymer	Fluorescent dye		MAA	BAP	P84	DMF
	Туре	/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

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

239

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

Coumarin, methacrylic acid, 1,4-bis(acryloyl)piperrazine 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 246 247 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 248 249 polymer was then hand ground with a mortar and pestle until fine particles were 250 obtained. The polymer particles were washed to remove unreacted materials by 251 repeated incubation in MeOH (50 mL solvent each), centrifugation and re-suspension (4×0.5h incubations), followed by the same procedure with  $H_2O$  (2×0.5 h incubations) 252 253 and finally on a sintered filter with MeOH (50 mL). After washing, polymer particles 254 were dried in vacuum and stored in the dark until use.

255

256 2.4. Fluorescence measurements

Emission spectra of fluorescent indicators and polymers were recorded using a 4 mL  $(1 \times 1 \times 4 \text{ cm})$  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 2x10<sup>-3</sup> mmol of each
indicator in 2.5 mL EtOH. PVIC (6.3 mg/mL, containing 2.5 µmol 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 Na<sub>2</sub>HPO<sub>4</sub> (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 µL of each coumarin stock solution or 50 µL 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.

280

# 281 **3. Results and discussion**

282 3.1. Design and synthesis of fluorescent pH indicators

Although there has been a variety of pH indicators, both commercially available 283 including azo dyes[6, 21, 23, 39, 40], trinitrobenzensulfonic acid[28], thymol blue[29, 284 41-43], cresol red[41], phenolphthalein[44], aniline blue[45], rhodamine 19[46], 285 Victoria blue<sup>[47]</sup> etc. as well as reported in the literature such as fluorescent Schiff 286 287 bases[48], porpholactones[49] or a benzo[de]anthracen-7-one derivative[31], that are 288 known to be useful for the spectroscopic determination of high pHs, the majority of 289 them are absorptive dyes rather than fluorescent dyes and only a few meet the 290 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 291 292 quantum yield and good photostability and bearing one or more functional chemical 293 groups suitable for covalent immobilization of the indicator. Coumarins have been 294 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 substituent shows response to pH in the alkaline region [19]. The design of the indicator dyes was tailored based on this core structure and suitable functional group(s) for immobilization of the indicator to a substrate.

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Having created appropriate indicator dyes, it is equally important to perform an 301 302 effective immobilization of the dyes to a suitable solid support/substrate. Prior work 303 has shown that poor immobilization results in dye leaching and consequently a 304 drifting of the calibration of the probe, which leads to the gradual breakdown of its 305 useful sensing ability[11]. Among several widely used immobilization methods such as absorption or entrapment[52-54], layer-by-layer (LbL) electrostatic self-assembly 306 307 [55, 56] and covalent binding[21, 57-60], the covalent binding method is believed to produce more reliable and durable sensors, as the indicators are virtually bonded to 308 the substrate therefore they are unlikely to leach out under normal conditions, 309 310 although the fabrication process is relatively complicated and time-consuming.

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

appropriate co-monomer(s) or by attaching the reactive dye to a readily prepared polymer. In this 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.

325

To meet the desired requirements discussed above, the fluorescent dyes used in this 326 327 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 328 329 coumarin should be stable enough to sustain strongly alkaline media. Three novel 330 polymerisable coumarins: vinylbenzylamino imidazolylmethyl coumarin (VIC, 5), acrylamido imidazolylmethyl coumarin (AIC, 6) and styryl imidazolylmethyl 331 332 coumarin (SIC, 9) were prepared in multiple steps starting from commercially 333 available phenolic compounds as outlined in Scheme 1. The Pechmann reaction of 334 phenols with a  $\beta$ -carbonyl ester is a versatile approach for the synthesis of 4-335 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 336 337 performing a reaction with 2-methyl-4-nitroimidazole, following the method 338 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 339 340 coumarin 8 with vinylphenylboronic acid using  $K_2CO_3$  in dioxane as a base/solvent 341 mixture or by reacting the amine substituted coumarin 4 with acryloyl chloride to give 342 the amide **6** or with 4-vinylbenzylchloride to give the secondary amine **5**.

343



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

- 370 (Fig. 1) whereas that of SIC (9) shows two main bands, at 266 nm with weaker
- absorbance and 334 nm with higher absorbance.



373 Fig.1. Absorption (left) and emission (right) spectra of VIC (5) (solid lines), SIC (9)

- 374 (broken lines) and AIC (6) (dotted lines) (5  $\mu$ M) in H<sub>2</sub>O. Emission spectra recorded
- 375 with  $\lambda_{ex} = 330$  nm for **SIC** and **AIC** and 370 nm for **VIC**.

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

377 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 378 379 and emits at longer wavelengths compared to AIC and SIC but its fluorescence 380 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 381 molecules have close lying  $\pi,\pi^*$  and  $n,\pi^*$  states which are easily perturbed by changes 382 in solvents, substituents and other factors [64-66], and hence the excited state  $S_1$ 383 probably has both  $\pi,\pi^*$  and  $n,\pi^*$  character. It is proposed that in VIC, a  $n,\pi^*$  state may 384 385 lie below the  $\pi,\pi^*$  state and at a low energy level, due to the great conjugation of the 386 lone-pair electrons of the amine nitrogen at the 7 position with the  $\pi$  system. This degree of conjugation is decreased in AIC where the amine nitrogen is converted to an 387 388 amide counterpart, which may result in the low lying excited state shifting to higher

Dye	Solvent <b>U</b>	JV max	Emission	pK <sub>a</sub>	Φ(%)
		nm)	max (nm)		
VIC (5)	EtOH	371	456	-	2.6
VIC (5)	H <sub>2</sub> O	370	470	$12.48\pm0.06$	0.8
AIC (6)	EtOH	334	407	-	1.7
AIC (6)	$H_2O$	330	416	$12.22\pm0.07$	0.6
SIC ( <b>9</b> )	EtOH	335	448	-	25.0
SIC (9)	H <sub>2</sub> O	334	458	$12.40\pm0.24$	2.0

389 energy. In SIC where the  $\pi$ -electron system of coumarin is enhanced by the 390 substituted styryl group, the  $\pi,\pi^*$  state may lie below the  $n,\pi^*$  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.

396 It is also noted that all dyes exhibit very large Stokes shifts (the difference in 397 wavelength between the absorption and the fluorescence spectral peaks) of 100 nm for 398 VIC, 86 nm for AIC and 124 nm for SIC, which is very important for sensing 399 applications since this minimizes the interference of the excitation light with the 400 fluorescence emission.

401

402



412 Fig. 2 Possible energy diagrams for VIC, AIC and SIC. S<sub>o</sub>: ground state, S\*: excited
413 single state, F: fluorescence.

414 In aqueous solution, all the dyes show no change in the fluorescence properties in the 415 neutral and near neutral pH region (data not shown) and a decrease in fluorescence

416 intensity with increasing pH in the alkaline region (Fig.3). This can be attributed to 417 the equilibrium between the protonated and deprotonated forms of the imidazolyl 418 group as shown in Scheme 2. The protonated form is fluorescent and the deprotonated 419 form is much less so. Therefore, the fluorescence intensities of the dyes are lower at 420 higher pH values.

421 The deprotonation of the imidazolyl group at high pH values was further confirmed
422 by <sup>13</sup>C NMR titrations of VIC, AIC and SIC with sodium methoxide (MeONa), a very



427 largest changes in shift were for the carbon nuclei attached to the only proton of the 428 imidazolyl group (C5-imidazole) with changes of up to 1.71 ppm for the titration of 429 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 430 431 in the interactions with the base through deprotonation. It has been reported that 432 imidazole is a very weak acid with  $pK_a = 14.5[69]$ . However, in the presence of a 433 strong electron acceptor – NO<sub>2</sub> group, its acidity increases and  $pK_a$  can be shifted to a 434 lower value.

435

436

437

438

439 Scheme 2. Protonation-deprotonation equilibria of the fluorescent indicators in440 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 C5imidazole, resulting in PET from the imidazole to coumarin being hindered and hence the fluorescence intensity increases.

- 448 The equilibrium between the protonated (HA) and deprotonated (A<sup>-</sup>) forms of the
- 449 indicators can be written in short as:

$$450 HA \leftrightarrow H^+ + A$$

451 The relationship between the protonation state of the indicator and the pH is governed452 by the Henderson-Hasselbalch equation:

452 by the Henderson-Hasselbalch e

453

454 
$$pH = pK_a + \log \frac{\left[A^{-}\right]}{\left[HA\right]} \quad (1)$$

455

where [A<sup>-</sup>] and [HA] are the concentrations of the deprotonated and protonated forms of the indicator and  $pK_a$  is the acid-base constant. [A<sup>-</sup>] and [HA] are related to fluorescence intensities by [A<sup>-</sup>] =  $F - F_{max}$  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.

462

463 
$$pH = pK_a + \log \frac{F - F_{\text{max}}}{F_{\text{min}} - F} \quad (2)$$

464

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

466

467

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

468

469 This results in an 'S-shaped' relation of the fluorescence intensity versus pH graph, 470 centered on the  $pK_a$  value, which is the pH where 50% of the dye population in 471 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



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499	
500	Fig 3. Emission spectra of VIC (a), AIC (b) and SIC (c) at pH from 10.0 to 13.6. The
501	insets show the titration plots at emission maxima . $\lambda_{ex} = 370$ nm (a), 330 nm (b and
502	c).
503	3.3. Polymer preparation
504	Polymers were prepared in dimethylformamide (DMF) by free-radical polymerization
505	using azobis(isobutyronitrile) as initiator (refer to Table 1 for the compositions of the
506	different polymers). These were prepared as monolithic polymers and ground into
507	small particles, since these are easier to produce than spherical beads which can be
508	made by precipitation polymerisation and since a minimal amount of solvent is
509	required. There is no problem with scattering from the irregular-shaped particles
510	because with the coumarins used here the Stokes shift is sufficient that emission can
511	be measured well above the scattered wavelengths. Since the materials were designed
512	to work in aqueous environments, 1,4-bis(acryloyl)piperrazine was used as the cross-
513	linker as it is water soluble, hence producing polymers with a more hydrophilic
514	backbone. Methacrylic acid was included as a co-monomer so that the polymers
515	should not be too rigid and it can also increase the hydrophilicity of the polymers.
	22

516 Pluronic P84 was added to increase the surface area and porosity. It is important that 517 the polymer used has the right molar ratio of cross-linker to monomers since lower 518 cross-linking gives insufficiently robust polymer and higher cross-linking gives too 519 rigid polymer, hence preventing the diffusion of hydrogen ions from surrounding 520 environments to the fluorescent receptor. After trying and testing different ratios in 521 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 522 523 monomer used was fixed at 1:10 of the cross-linker. More fluorescent monomer 524 would be expected to give a stronger fluorescent signal but too high a concentration of 525 fluorophore could also result in fluorescence quenching by the inner filter effect.

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# 528 3.4. Photostability

Photostability is one of the critical properties of fluorescent materials used for sensor 529 530 applications. It is proposed that the substitution at the 7 position of the coumarin has 531 no significant effect on the photostability of the coumarin core structure. 532 Photostability was tested with one of the polymers PVIC by holding a layer consisting 533 of 4 mg of the polymer particles in between a quartz disc (6 mm dia.) and a nylon 534 membrane (20 µm pores, GE), which was kept in place by an O-ring and coupled into 535 the fluorimeter through a dichroic mirror using a fiber bundle. The excitation light (at 536 a wavelength of 375 nm) was launched to the distal end of the probe illuminating the 537 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 538 539 over that period and displayed. Fig.4 shows the fluorescence intensities of the 540 polymer in dry state and in a pH 10 buffer solution as function of time during 60 min

541 of continuous illumination by light from a high power Xe lamp. It was interesting to 542 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, 543 544 under the same conditions, no photobleaching was seen when the polymer was 545 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 546 state of the fluorophore is stabilised by solvation. When compared to the results of 547 548 other materials, this still offers excellent performance: the decrease observed in the 549 fluorescence intensity was 65% for carboxyfluorescein and 10-13% for 550 iminocoumarin derivatives, again after 60 min of continuous illumination using a 551 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 552 553 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) asfunction of time during 60 min of continuous illumination by light from a high power

565 Xe lamp ( $\lambda_{ex} = 375$  nm,  $\lambda_{em} = 500$  nm). Fluorescence intensities are normalised to the

566 initial emission of the polymer for each data set.

567

568 3.5. Fluorescence studies and response of polymers to pH

569 Fluorescence measurements of the polymers were performed in 50 mM phosphate 570 buffer at various pHs as used for the free dyes. The cuvette was shaken vigorously 571 before each measurement to enable the uniform dispersion of the polymer. No 572 significant settling out was observed on the timescale of the measurements and the 573 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 samples that were allowed to incubate for a couple of hours before measurements. There was essentially no difference between two sets of data.

579 Fig. 5 shows emission spectra of different polymers at different pHs. It can be seen 580 that the fluorophores emit at lower wavelengths in the polymers than in their free

581 forms. The blue shift could be because when fixed in a rigid polymer network the 582 excited states of the fluorophores undergo less stabilization from solvent 583 rearrangement.

All polymers exhibited a decrease in fluorescence intensity with increasing pH in the range from 10 to 13.6, which is similar to the dynamic response range of the free dyes. The p $K_a$  value calculation for the polymers was performed using the Boltzmann model – Equation (4) where dpH is the slope of the curve within its linear zone.

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



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625	Fig 5. Emission spectra of PVIC (a), PAIC (b) and PSIC (c) at pH from 10.0 to 13.6.
626	The insets show the titration plots at emission maxima . $\lambda_{ex} = 370$ nm (a), 330 nm (b
627	and c).
628	3.6. Effect of ionic strength (IS)
629	Sensitivity to IS can be a serious problem in the cases of pH sensors as it affects $pK_a$
630	values, thus resulting in errors in pH determination. The effect of IS was investigated
631	with one of the polymers PVIC in the prepared pH 10 phosphate buffer solution
632	adjusted with NaCl to different ionic strengths ranging from 10 mM to 2000 mM. The
633	fluorescence intensity obtained for each solution was converted to a pH value using
634	the calibration curve and the error was calculated. As can be seen from Fig.6, there
635	appears to be no sensitivity to IS for the sensing polymer at pH 10, even at very high
636	concentrations of NaCl. The insignificant errors caused are probably due to the
637	system error rather than the change in IS.
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645	Fig 6. Effect of ionic strength (IS) on fluorescence intensity and calculated pH value.
646	Fluorescence intensities are normalised to the maximum emission at 470 nm of the
647	polymer at pH 10 with $IS = 0$ .
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650	4. Conclusions
651	Novel coumarin-based fluorescent pH indicators vinylbenzylamino imidazolylmethyl
652	coumarin (VIC), acrylamido imidazolylmethyl coumarin (AIC) and styryl
653	imidazolylmethyl coumarin (SIC) have been synthesized. Their fluorescence
654	behaviours have been investigated in aqueous solution at different pHs. All coumarins
655	exhibited a significant decrease in fluorescence intensity with increasing pH in the
656	highly alkaline region. Photophysical properties of the indicators have been postulated
657	to explain their fluorescence properties and behaviours.
658	The indicators were covalently immobilized to polymer supports by co-polymerising
659	with methacrylic acid co-monomer and 1,4-bis(acryloyl)piperrazine cross-linker.

660 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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# **Research Highlights**

- The design, synthesis and spectroscopic properties of novel polymerizable coumarin-based pH indicators for the highly alkaline pH region are reported.
- Polymers prepared using the synthesized dyes possess excellent photostability and show a response to pH in the range of 10.5 13.5.
- The materials can be applied in optical pH sensors for measuring pH in various alkaline media.

Journal Proproof

Conflicts of Interest Statement	-
Manuscript title: Novel coumarin-based pH sensitive gluorescent prob	es
for the highly alkaline pt region.	

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