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Immobilisation and assessment of aniline dyes for non-fluorescent pH sensing applications

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Abstract—The attachment of two aniline-based chromophores, Disperse Black 3 and Pararosaniline, to beaded controlled pore glass (CPG) and their testing as fibre optic based pH sensors is described. Synthetic methods for their attachment at specified loadings to CPG were developed. The Disperse Black 3 sensor displayed a rapid response time and a dynamic range between pH 1.0 and 2.5, while the Pararosaniline-bearing sensor gave an extremely slow response time but a large sensing range from pH 1 to 11.
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The development of solid-state spectrometric pH sensors is the subject of intense investigation in many laboratories.[1–4] In the case of UV–vis sensors, organic indicator dyes supported on an insoluble macromolecular matrix interfaced to a UV–vis spectrometer to allow colorimetric changes with pH to be determined. As many indicators are aniline derivatives the development of procedures that allow the covalent attachment of these moieties at specified loadings would be useful. Here, the attachment of Disperse Black 3 **1** and Pararosaniline base **2** to macroporous controlled pore glass (CPG) beads is described, allowing their integration into a sensor assembly[5] and their UV–vis spectrometric responses with pH changes to be investigated. The azo-dye **1** was compared to the structurally related *para*-Methyl Red,[5] while the triphenylmethane dye **2** displayed two colorimetric changes over the pH range (at pH 1–3 and 11–14)[6] and offered the opportunity to extend the dynamic range of dye-based sensors, hitherto a major limitation of these sensors, which usually operate only within ±2 units of the pK_i of the dyes used.[7]

For the synthesis of the CPG-supported indicators, (Scheme 1) aminopropyl-CPG conjugated to two 6-aminohexanoic acid spacers[5] **3** was coupled with a mixture of *N*-Fmoc- and Cbz-protected glycine at various ratios to achieve a series of CPG beads with specified loadings (Table 1). Selective Fmoc deprotection and coupling with adipoyl dichloride followed by quenching of the supported acyl chloride (to avoid intrasite cyclisation or crosslinking) provided the carboxy-functionalised beads. These were coupled to the dyes using PyBOP since this reagent would not undergo guanidine formation[8] with the excess aniline used. Other attachment methods for anilines such as 2,4,6-trichlorotriazine[9,10] or isocyanates[11] proved unsuccessful for **2**.

After coupling, the dyes continued to respond to pH variations (Fig. 1). For **4**, a single transition was observed as expected while with **5**, three transitions were seen, with decolourisation occurring at the extremes of the pH range.

Under a microscope, a single 'sensor bead' of appropriate size was inserted in the sensor assembly[5] between the ends of the optical fibre and the UV–vis spectra, pH profile and response times were examined in a 1 M HCl/NaOH system. In both cases, beads with a loading of 5 μmol/g were found to be sufficient for sensing purposes. For **4**, the absorbance at λ_{max} (546 nm), corresponding to the protonated indicator, was plotted against pH and the best-fit sigmoid curve gave a pK_i of 1.71 (Figs. 2 and 3), similar to that of the *para*-Methyl

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Keywords: pH sensor; Pararosaniline; Disperse Black 3; UV–vis; Controlled pore glass; Solid phase.

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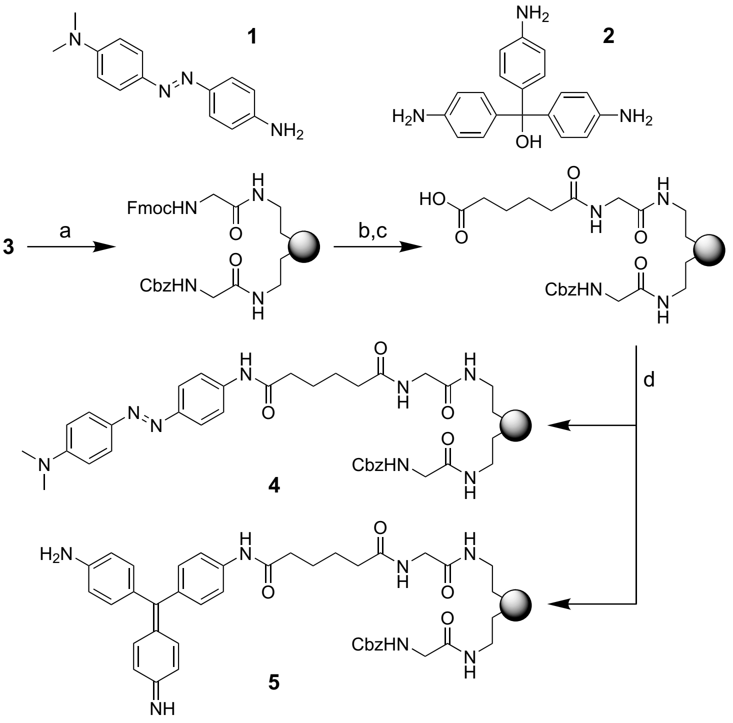


Scheme 1. Attachment of indicators to CPG. Reagents and conditions: (a) Fmoc/Cbz-Gly-OH, DIC, HOBt, DMF, 4 h; (b) 20% v/v piperidine, DMF, 30 min; (c) adipoyl dichloride, DIPEA, DCM, N_2, 3 h; then 50% v/v H_2O, THF; (d) **1** or **2**, PyBOP, DIPEA, NMP, 16 h.

Table 1. Loading of CPG after Cbz- and Fmoc-Gly-OH co-acylation[a]

Molar ratio of Cbz-:Fmoc-Gly-OH	Fmoc loading post reaction	
	μmol/g	% relative to control
99:1	0.7	0.9
97:3	2.8	3.7
94:6	5.1	6.8
90:10	16	21
75:25	25	33
50:50	56	75
0:100 (Control)	75	100

[a] 10 mol equiv of acid mixture relative to CPG loading.

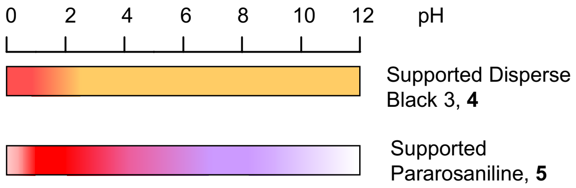


Figure 1. Visually observed colour change for beads in aqueous buffer.

Red sensor, which had a λ_{max} of ~540 nm and a pK_i of 2.07[5] and to a solution of **1**, which had a pK_i of ~1.78.[12]

For Pararosaniline, the single peak in the spectra of **2** in aqueous solution,[6] was resolved into two peaks for the supported dye **5** (Fig. 4) and could be related to the microenvironment around the dye[13] afforded by the glass support.

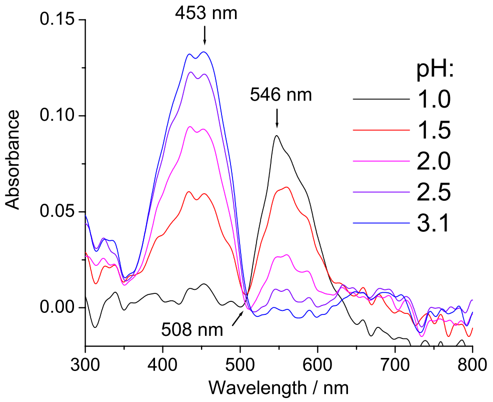


Figure 2. UV–vis absorbance spectra of **4**.

Plots of absorbance intensities of the two major peaks in the UV–vis spectra (460 and 581 nm) and the depression between the peaks (512 nm) did not indicate any obvious pK_i transition points (Fig. 5). Nevertheless, the sensor had a large response range from pH 1 to 11 although there was a completely unresponsive region between pH 7 and 8 while at both extremes of the pH range the absorbance dropped as the dye became de- colourised presumably due to the addition of the counter ion to the chromophore[6] (**5a** and **5d**, Scheme 2).

The response time of sensor **4** around its pK_i was comparable to that of the previously cited CPG-based sensors,[5] with a $t_{95\%}$ of 8.7 s and a k of 0.348 s^{-1} (Fig. 6). The response speed measurements of **5** were more

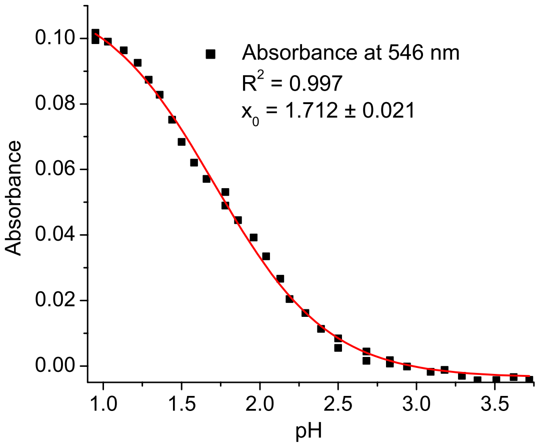


Figure 3. UV–vis absorbance against pH for supported Disperse Black 3, **4**.

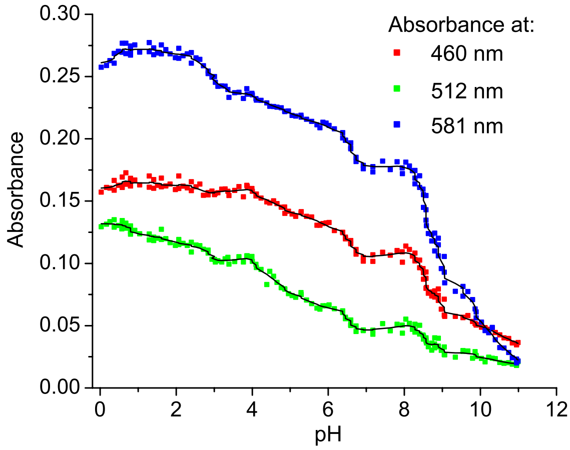


Figure 5. UV–vis absorbance against pH of supported Pararosaniline, **5**.

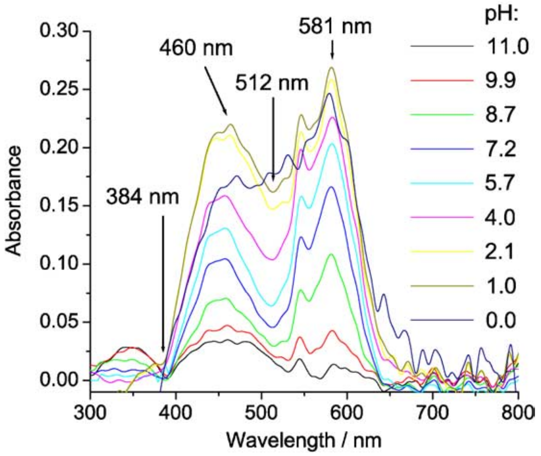
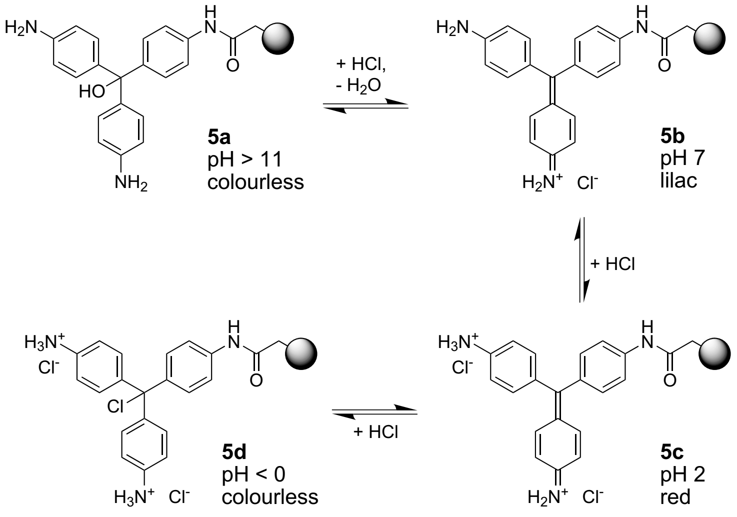


Figure 4. UV–vis absorbance spectra of **5**.

complicated due to the lack of obvious transition points. Initial measurements covering a large part of the dynamic range did not give the classical exponential curve, the initial part of the plot was somewhat uneven followed by an apparently well defined curve (Fig. 7). The overall $t_{95\%}$ was ~2870 s and a best-fit exponential curve of this latter stage provided a k value of $3.47 \times 10^{-3}\ \mathrm{s}^{-1}$. The initial phase could be attributed mainly to formation of the cation to give **5b**, while the second phase was the protonation of the second *N*-aniline to give **5c**. The conversion from **5b** to **5c** was examined in more detail and unexpectedly, gave a $t_{95\%}$ of ~7560 s and a k of $6.50 \times 10^{-4}\ \mathrm{s}^{-1}$, significantly slower than the response over the whole range (Fig. 8). In either case, the response rates for sensor **5** were much slower than the other CPG- or sol–gel glass-based sensors.

Overall, the attachment of two aniline-functionalised chromophores to CPG and their subsequent testing as pH sensors is described. Synthetic methods for their attachment at specified loadings were developed, in particular for Pararosaniline. The assessment of the



Scheme 2. Ionisation pattern of sensor **5** in an HCl/NaOH aq system.

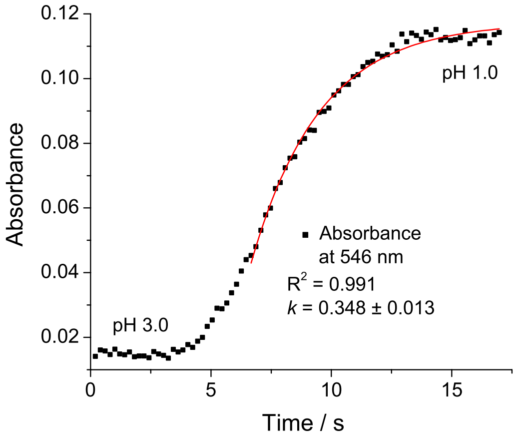


Figure 6. Graph of UV–vis absorbance against time for sensor **4** changing from pH 3.0 to 1.0.

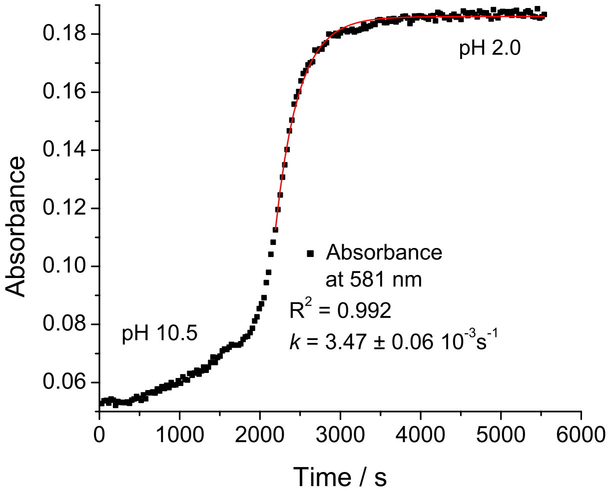


Figure 7. Graph of UV–vis absorbance against time for sensor **5** changing from pH 10.5 to 2.0.

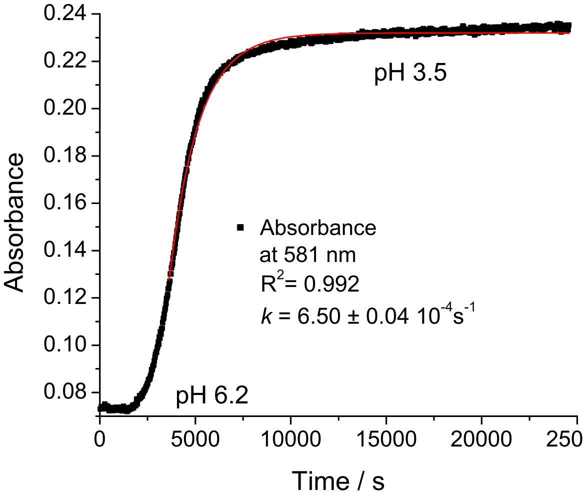


Figure 8. Graph of UV–vis absorbance against time for sensor **5** changing from pH 6.2 to 3.5.

sensors demonstrated that supported Disperse Black 3 gave a similar performance to *para*-Methyl Red in terms of response time and dynamic range. On the other hand, the Pararosaniline-bearing sensor gave extremely slow response times but a broad sensing range. In this case a pH value could be measured by using several wavelengths to improve accuracy.

The relationships between the structure, UV–vis spectra and pK_i have been well studied for both triarylmethane[13–15] and azo dyes[16,17] and allows the potential tuning of these sensors' structures to achieve the desired colour and dynamic sensing range. This tied in with new strategies for the synthesis of diverse ranges of dyes[18] opens up the door on the next generation of broad range pH sensors with rapid response times.

Supplementary data

Full procedures for the synthesis of sensors **4** and **5** and sensor analysis. Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.tetlet.2005.06.081.

References and notes

1. Leiner, M. J. P.; Wolfbeis, O. S. In *Fibre Optic Chemical Sensors and Biosensors*; Wolfbeis, O. S., Ed.; CRC Press: Boca Raton, 1991; Vol. 1, pp 359–384.
2. Leiner, M. J. P.; Hartmann, P. *Sens. Actuators, B* **1993**, *11*, 281–289.
3. Lin, J.; Brown, C. W. *Trends Anal. Chem.* **1997**, *16*, 200–211.
4. Mehrvar, M.; Bis, C.; Scharer, J. M.; Moo-Young, M.; Luong, J. H. *Anal. Sci.* **2000**, *16*, 677–692.
5. Wong, L. S.; Brocklesby, W. S.; Bradley, M. *Sens. Actuators, B* **2005**, *107*, 957–962.
6. Heinemann, R. L. *Stain Technol.* **1970**, *45*, 165–172.
7. Wolfbeis, O. S.; Huber, C.; Werner, T. In *Chemosensors of Ion and Molecule Recognition*; Desvergne, J. P., Czarnik, A. W., Eds.; Kluwer: Dordrecht, 1997, pp 61–74.
8. Albericio, F.; Cases, M.; Alsina, J.; Triolo, S. A.; Carpino, L. A.; Kates, S. A. *Tetrahedron Lett.* **1997**, *38*, 4853–4856.
9. Wolthuis, R.; McCrae, D.; Saaski, E.; Hartl, J.; Mitchell, G. *IEEE Trans. Biomed. Eng.* **1992**, *39*, 531–537.
10. Harper, G. B. *Anal. Chem.* **1975**, *47*, 348–351.
11. Lobnik, A.; Oehme, I.; Murkovic, I.; Wolfbeis, O. S. *Anal. Chim. Acta* **1998**, *367*, 159–165.
12. Şeclăman, E.; Salló, A.; Eleneş, F.; Crăşmăreanu, C.; Wikete, C.; Timofei, S.; Simon, Z. *Dyes Pigments* **2002**, *55*, 69–77.
13. Lueck, H. B.; McHale, J. L.; Edwards, W. D. *J. Am. Chem. Soc.* **1992**, *114*, 2342–2348.
14. Deno, N. C.; Jaruzelski, J. J.; Schriesheim, A. *J. Org. Chem.* **1957**, *19*, 155–167.
15. Gorman, S. A.; Hepworth, J. D.; Mason, D. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1889–1895.
16. Sawicki, E. *J. Org. Chem.* **1957**, *22*, 1084–1088.
17. Stoyanov, S.; Antonov, L.; Stoyanova, T.; Petrova, V. *Dyes Pigments* **1996**, *32*, 171–185.
18. Noack, A.; Schröder, A.; Hartmann, H. *Dyes Pigments* **2002**, *57*, 131–147.