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pH Indicating resins

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pH indicating resins were prepared by covalently attaching carboxylic acid derivatives of sulfthalein dyes, synthesized using a Suzuki cross-coupling, onto resin beads. The resin-bound indicators showed the expected colour changes according to pH and their behaviour was analysed using a micro UV/Vis spectrometer.

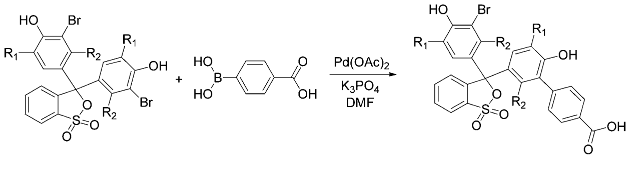
Over the last decade many efforts have been made to attach a variety of components to a wide number of solid supports. Such supported components facilitate, amongst other things, economic and automated chemistry. In particular, the binding of a chemically active probe to solid supports can eliminate the possibility of leaching when in contact with the intended environment and removes the need for a purification step. In this regard, many studies on the binding of fluorescent compounds to solid supports have been carried out in the field of analytical, biological and combinatorial chemistry.[1]

Some fluorescent compounds such as fluorescein and rhodamine permit covalent attachment to solid supports because they contain accessible handles (*e.g.*, amine, hydroxy, isothiocyanate, or carboxylic acid groups). However, fluorescent methods have major problems with absorption, quenching and photo-degradation phenomena and, in addition, binding to a solid support can result in significant changes in chemical and optical properties.[2]

Sulfthalein type dyes are chemically very robust and detectable in the visible range and are particularly useful when looking to measure pH values because of the exact, well defined and conspicuous colour change following protonation. However, sulfthalein dyes have, to date, been immobilised by deposition and coating due to a lack of suitable functionality for covalent binding and difficulties in derivatisation.[3]

Here, we describe the modification of three sulfthalein dyes to generate coupling derivatives without unduly interfering with pH sensitivity. These dyes were attached to resins and the beads used as pH indicators. In addition these dyes provide a range of potential quenchers for use in FRET pairing.

To introduce functionality without loss of conjugation, Suzuki cross-coupling reactions were employed on bromophenol blue (BPB), bromocresol purple (BCP), and bromothymol blue (BTB). Thus carboxyphenylboronic acid (1.2 equiv.) was coupled to the dyes to give the desired corresponding carboxy-functionalised dyes in 33%,[4] 43%†, and 21%‡ yield respectively. Unreacted substrate and disubstituted forms were the other major materials recovered (Scheme 1). The λ_{max} of each modified dye showed similar values compared to the original dyes, and the ε_{max} values, although slightly reduced, were still sufficiently high to act as indicators (Table).



Scheme 1 Suzuki reactions of sulfthalein dyes with carboxyphenylboronic acid: (a) bromophenol blue: R_1: Br, R_2: H; (b) bromocresol purple: R_1: Me, R_2: H; (c) bromothylmol blue: R_1: *t*-Bu, R_2: Me.

The modified sulfthalein dyes, along with methyl red (MR) (in order to extend the pH range) were attached to 5% of the sites on aminomethylated TentaGel resin (0.44 mmol of NH_2 per g)[5] with the ε_{max} value dictating the ratios of methyl red and the sulfthalein dyes used (the remaining amino groups were capped by acetylation (Scheme 2)).§

The colours of the pH-indicating resins were observed visually in five different buffers and all resins exhibited the expected colour pattern according to pH. Colour changes from yellowish orange to blue of each resin took place at pH 3 (MR + BPB resin), pH 5 (MR + BCP resin), pH 9 (MR + BTB resin) due to the different pK_a values of the modified sulfthalein dyes.

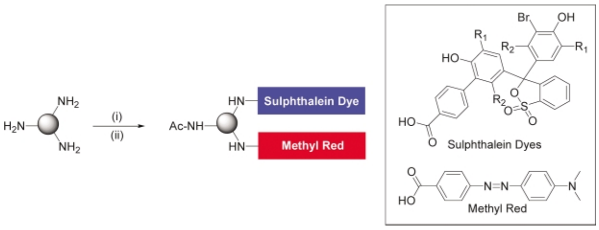
Finally the photometric properties of the pH-indicating resins were evaluated using a single bead UV/Vis spectrometer.[6] The λ_{max} of MR + BCP resin was 539 nm at pH 1 and 481 nm at pH 5, which corresponds to that of protonated and deprotonated form of MR. Compared with the λ_{max} of free MR in solution, both forms were shifted to higher wavelength by approximately 45 nm. The peak at pH 9 gave the λ_{max} of BCP as 603 nm. In the case of the MR + BTB resin, a peak corresponding to deprotonated MR survived until pH 9 although the peak at 608 nm corresponding to BTB began to increase. The spectra of only BTB was finally detected at a pH > 11.

In conclusion, pH-indicating resins offer an easily separated indicator, ideal for array synthesis or immobilisation within a "tea-bag" device for large scale pH monitoring without contamination and the potential for reuse. These supported indicators have the

Table 1 Photometric properties of sulfthalein dye derivatives[a]

	λ_{max}/nm	ε_{max}/cm^{-1}
Bromophenol blue	603	110,000
Carboxyphenyl bromphenol blue	601	73,000
Bromcresol purple	589	63,100
Carboxyphenyl bromocresol purple	588	41,400
Bromothymol blue	616	37,400
Carboxyphenyl bromothymol blue	608	17,500

[a] All values were determined under 20% piperidine in DMF.



Scheme 2 Preparation of pH-indicating resins: (i) sulfthalein dye derivatives, methyl red, TFFH, TEA, DMF; (ii) acetic anhydride, TEA.

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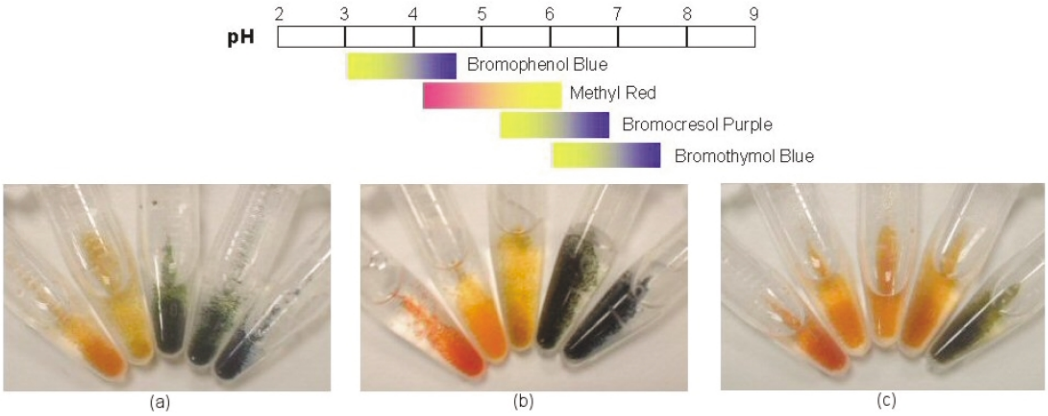

Fig. 1 Colour change of MR + (a) BPB, (b) BCP, (c) BPB resin in buffer solutions: From left tube pH 1, 3, 5, 7, 9 in each picture (The top figure about pH indicator transitions is based on Aldrich catalogue for dyes, indicators & intermediates).

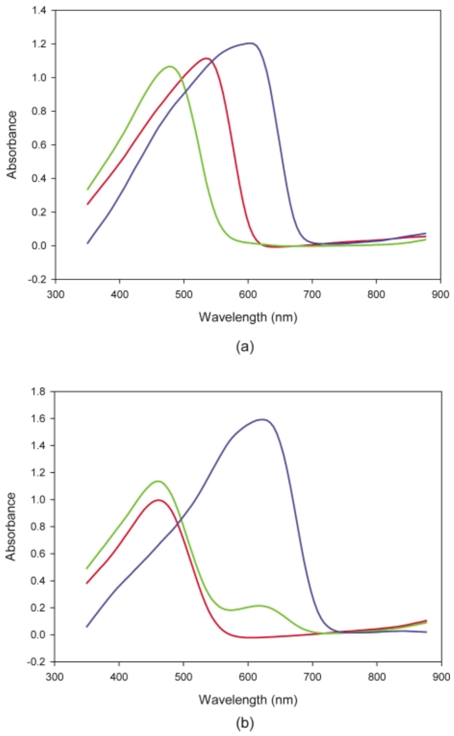

Fig. 2 Single bead UV/Vis spectra of pH-indicating resins: (a) MR+BCP at pH 1 (red), pH 5 (green), pH 9 (blue); (b) MR+BTB at pH 5 (red), pH 9 (green), pH 11 (blue).

potential to be developed much further for more advanced monitoring devices for accurate and automated detection of pH and array immobilisaton.

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Notes and references

† *Synthesis of BCP derivative*: Bromocresol purple (2.0 g, 3.0 mmol), 4-carboxyphenylboronic acid (3.6 mmol, 1.2 equiv.), potassium phosphate (9.0 mmol, 3.0 equiv.), tetrabutylammonium bromide (3.0 mmol, 1.0 equiv.), and palladium(II) acetate (2.0 mol%) were placed in a reactor capped with a septum and gently sparged with argon for 10 min. DMF (10 mL) was added and the reaction mixture was stirred under argon at 80 °C for 18 h. The resulting mixture was treated with conc. HCl, extracted with EtOAc, dried over $MgSO_4$, and evaporated under reduced pressure to afford a reddish oil. The product was isolated by column chromatography on silica gel (chloroform : methanol = 5 : 1) to give the desired bromocresol purple derivative in 43% yield: ^{1}H NMR (400 MHz, 5% CF_3CO_2D in CD_3CN): δ = 8.03 (d, 2H, J = 8.0 Hz), 7.91 (d, 1H, J = 8.0 Hz), 7.78 (t, 1H, J = 7.6 Hz), 7.71 (t, 1H, J = 7.6 Hz), 7.51–7.54 (m, 3H), 7.27 (d, 1H, J = 2.4 Hz), 7.11 (d, 1H, J = 2.4 Hz), 7.09 (s, 2H, J = 2.8 Hz), 6.99 (d, 1H, J = 2.8 Hz), 2.23 (s, 3H), 2.21 (s, 3H); a calculated mass for $C_{28}H_{21}BrO_7S$: 581.4, found: ESI-MS, *m*/*z*, negative $[M - H]^-$: 579.3, 581.3.

‡ *Synthesis of BTB derivative*: The BTB derivative was prepared following the procedure outlined above. The crude product was purified by column chromatography on silica gel (chloroform : methanol = 5 : 1) to give the desired bromothylmol blue derivative in 21% yield: ^{1}H NMR (400 MHz, 5% CH_3CO_2D in $(CD_3)_2SO$, 100 °C): δ = 7.94–8.01 (m, 3H), 7.16–7.36 (m, 6H), 6.93 (t, 1H, J = 7.6 Hz), 2.96–3.12 (br, 2H), 2.19 (s, 1,5H, rotamer), 2.11 (s, 1.5H, rotamer), 1.63 (s, 1.5H, rotamer), 1.54 (s, 1.5H, rotamer), 0.97–1.03 (m, 12H); a calculated mass for $C_{34}H_{33}BrO_7S$: 665.6, found: ESI-MS, *m*/*z*, negative $[M - H]^-$: 664.6, 662.7.

§ *Preparation of pH-indicating resins*: Aminomethylated TentaGel resin (100 mg, 44 μmol of NH_2) was added to a mixture of MR and each sulfthalein dye (2.2 μmol, 5% of total NH_2 loading, MR : BPB, MR : BCP, MR : BTB = 1 : 2, 1 : 5, 1 : 10, respectively), TFFH (6.6 μmol, 3 equiv), triethylamine (6.6 μmol, 3 equiv.) in DMF and the suspension was shaken for 4 h. The resin was filtered and washed with 25% trifluoroacetic acid in DCM, 5% triethylamine in DCM, and DMF by turns until no colour was present in the washings.

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