

## Covalent Immobilisation of Solvatochromic Dyes

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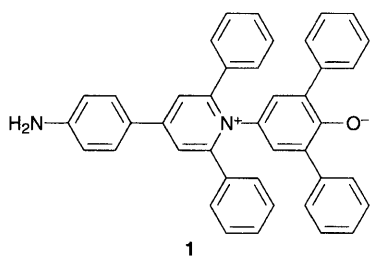
Solvatochromic dyes of the pyridinium-*N*-phenoxide family (e.g. Reichardt's dye) have been covalently immobilized on silica substrates with retention of solvatochromic behaviour, allowing construction of optical chemical sensors using these dyes.

Solvatochromic dyes show promise as transducer elements in optical chemical sensors. The family of compounds based on the pyridinium-*N*-phenoxide structure, exemplified by Reichardt's dye [2,6-diphenyl-4-(2,4,6-triphenyl-*N*-pyridinio)phenolate], show very strong solvatochromism<sup>1</sup> and their response can be made more selective by placing appropriate molecular constructs at the phenoxide end of the molecule (e.g. crown-6 ethers for potassium ions<sup>2</sup>). In order to construct a practical chemical sensor, however, the dye must be firmly bound to a solid substrate. For small molecules such as dyes this ideally means covalent attachment to reduce leaching problems. We report here the first covalent attachment of a solvatochromic dye to a solid support with retention of solvatochromic behaviour.

The preparation method for Reichardt's dye published by Johnson *et al.*<sup>3</sup> has been modified to produce an amino derivative of the dye, 2,6-diphenyl-4-[[4-(4-aminophenyl)-2,6-diphenyl]-*N*-pyridinio] phenolate **1**. The compound **1** was synthesised either from 4-nitrochalcone and acetophenone,<sup>4</sup> or by reaction of 4-nitrobenzaldehyde with acetophenone.<sup>5</sup> The pyrylium was then condensed with 2,6-diphenyl-4-aminophenol to produce the salt of the dye. The nitro group was reduced to an amino moiety in formic acid over palladium-charcoal catalyst before conversion of the salt into active solvatochromic dye.

The presence of the amino group causes a blue shift of the charge transfer band which gives rise to the solvatochromic response of the dye by about 80 nm in most solvents, as shown in Table 1. The magnitude of the solvatochromic shift between solvents is, however, retained.

Choice of a solid substrate in a chemical sensor is governed primarily by chemical compatibility of the substrate with the



**Table 1** The  $\lambda_{\max}$  values (nm) for the solvatochromic charge transfer bands of unmodified, amino-derivatized and immobilised [(i) thiourea linkage or (ii) amide linkage] Reichardt's dye in various solvents<sup>a</sup>

	Reichardt's dye (from ref. 7)	Amino derivative	Immobilised dye	
			(i)	(ii)
Methanol	515	428	460	460
Ethanol	551	432	470	470
Acetonitrile	622	588	510	510
Chloroform	731	662	560	540
Tetrahydrofuran	764	715	570	550

<sup>a</sup> Values for free dye were obtained in solution by transmission spectroscopy, while data for the immobilised dyes were obtained by measurement of diffuse reflectance spectra.

environment in which it is to be used. Our sensors will be used in organic solvents and therefore silica was chosen as a substrate. Activation of the silica surface is necessary in order to attach the dye but for particulate silica, as used here, preactivated supports are available in the form of chromatographic packing materials. Aminopropylated silica is the most readily available and has been used extensively for immobilisation of enzymes and antibodies *via* established methods.<sup>6</sup>

Attempts at coupling using glutaraldehyde as a homobifunctional linker were unsuccessful because the tan or red colour which formed on glutaraldehyde treatment of the silica persisted after reaction with the dye, masking the solvatochromic response of the dye.

Modification of the aminopropyl group with thiophosgene (80 cm<sup>3</sup> of 10% thiophosgene solution in chloroform added to 1 g of aminopropyl silica, refluxed overnight then filtered off and washed thoroughly with chloroform) yielded an isothiocyanatopropyl modified silica. This was used rapidly for reaction with the dye (0.5 g modified silica added to 2 mg dye in 5 cm<sup>3</sup> chloroform, stirred at room temperature for 2 d then filtered off and washed) to generate a thiourea-linked immobilised dye.

Successful immobilisation was also achieved through an amide linkage. A mixture of 1 g of succinic anhydride and 1 g of aminopropyl silica was stirred in 25 cm<sup>3</sup> of 0.05 mol dm<sup>-3</sup> phosphate buffer, pH 6.0, for 24 h to generate a carboxyl termination. The product was filtered off and added to 0.1 g 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide in distilled water at pH 10.0. After 2 h the pseudourea product was filtered off, washed with water and dried. A 0.5 g portion of this product was added to a solution of 5 mg of dye in 10 cm<sup>3</sup> acetone, stirred for 2 d then filtered off, washed with acetone and dried. This method uses less toxic reagents than the first. Additionally, the use of a carboxypropyl-modified silica as starting material would eliminate the need for conversion of the active group.

Immobilisation of the dyes was confirmed by washing with acetone (a good solvent for the dye). Dye simply adsorbed to aminopropyl silica was removed easily, whereas the immobilised dye was not removed.

The immobilised dyes show reduced solvatochromism compared to the free dye (Table 1), the shift  $\lambda_{\max}$  in going from methanol to tetrahydrofuran being decreased from 250 to about 100 nm, but the wavelength shifts observed are still significant. Work is in progress to improve the coupling chemistry, with twin aims of simplifying the immobilisation and improving the solvatochromic response of the bound dye.

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