

Synthesis of novel monoazo benzotriazole dyes specifically for surface enhanced resonance Raman scattering†

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Surface enhanced resonance Raman scattering (SERRS) has considerable potential as an ultra-sensitive analytical technique; for the first time the synthesis of specific dye ligands designed to overcome major problems in obtaining reliable SERRS is described.

Surface enhanced resonance Raman scattering (SERRS) has recently proven to be an extremely sensitive and selective technique for the detection and identification of suitable molecules.¹⁻⁴ It has significant advantages, being applicable to a wide range of chromophores and provides molecularly informative analysis with minimal separation procedures at attomolar concentrations or below. The main problem for analysis by SERRS is that the technique lacks reliability and reproducibility. We report for the first time the synthesis of four dyes specifically designed to provide effective and reliable SERRS. They are based on simple azo structures and utilise a surface complexing group to release the advantages of SERRS for accurate and reliable analysis.

The technique requires the adsorption of a suitable analyte onto an appropriate metal surface, excitation of the surface with light and collection of the scattered radiation. The Raman component of scattering, enhanced by contributions from molecular resonance and surface enhancement can rival fluorescence in efficiency and provide molecularly specific signals. In this study silver colloid is used to provide the metal surface⁵ and 457.9 nm excitation was provided by an argon ion laser. The main problem is to produce a chromophore for both resonance and surface attachment which will withstand changes in conditions of the analyte solution and will not desorb from the silver.

The dyes synthesised use benzotriazole as a surface attachment group. Benzotriazole dyes have been synthesised before but only as dyes and not metal complexing agents for improved spectroscopy.⁶ Benzotriazole is commonly used as an anti-tarnish agent on silver and is believed to adhere by complexing to more than one silver ion to form a polymeric species coated on the surface.⁷⁻⁹ Thus covalent attachment to the surface is possible and the orientation of the dye will be fixed. This eliminates changes in peak wavenumber owing to orientational changes or desorption from the surface commonly observed below monolayer coverage.¹⁰ The colloid used is stable for three to six months due to the high negative charge on the surface and has been routinely used for analysis previously.^{3,5} The main advantage of covalent attachment through benzotriazole is that the process is essentially irreversible and can displace surface charged species. Thus, once attached the dye remains in position over a wide range of experimental conditions and concentrations to provide reproducible analysis.

Four monoazo dyes containing benzotriazole were synthesised by diazotising 5-aminobenzotriazole§ and coupling¶ to phenylamine analogues (Fig. 1). The analogues used were aniline, anisidine, dimethoxyaniline and naphthalamine to produce the dyes 4-(5'-azobenzotriazolyl)phenylamine **1**, 3-methoxy-4-(5'-azobenzotriazolyl)phenylamine **2**, 3,5-dimethoxy-

4-(5'-azobenzotriazolyl)phenylamine **3** and 4-(5'-azobenzotriazolyl)naphthalen-1-ylamine **4**.|| The amino derivatised aromatics were chosen for two reasons. Firstly the amine group provides a suitable point for derivatisation once the dye has been synthesised and secondly the aromatic ring is highly activated towards electrophilic attack of the diazonium. The diazotised aminobenzotriazole is not a strong electrophile and requires an activated aromatic ring, *i.e.* aniline and the presence of a buffer. In this case sodium acetate buffered to a pH of 6.0 by the addition of glacial acetic acid was used. The buffer ensured the aromatic amine was not protonated thus maintaining the reactivity. Acetone was added to aid solubility and allow the reaction to proceed smoothly. The products precipitated and were purified by trituration using methanol and diethyl ether.

Each of the dyes produced distinct, strong SERRS at 1×10^{-7} M except 4-(5'-azobenzotriazolyl)phenylamine **1** which was used at a concentration of 1×10^{-5} M (Fig. 2). The enhancement obtained depends on the separation between the electronic maximum and the laser frequency. In the case of dye **1**, the spectrum is weak because the absorbance maximum is well away from the excitation frequency and hence from resonance. Major peaks which appear in all four are the quadrant stretch of the phenyl ring just above 1600 cm^{-1} , the azo stretch above 1400 cm^{-1} , and bands at around 1150 and 950 cm^{-1} which correspond to more complex phenyl and bridge stretching modes.

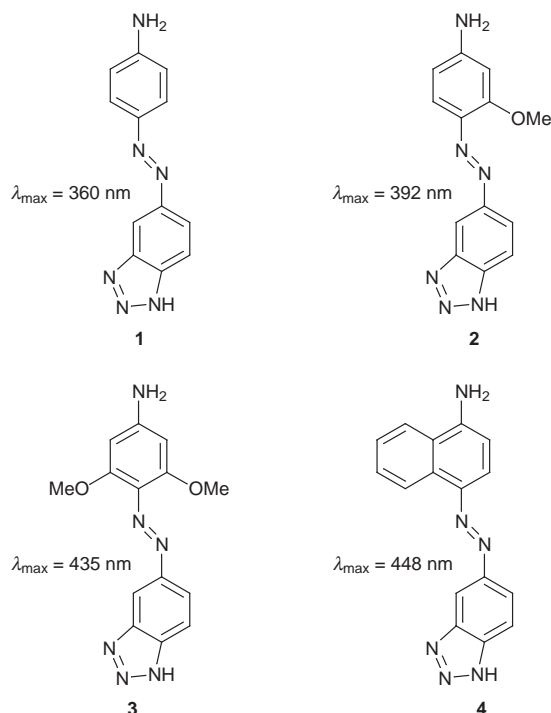


Fig. 1 The four benzotriazole dyes synthesised and their corresponding λ_{max} in methanol

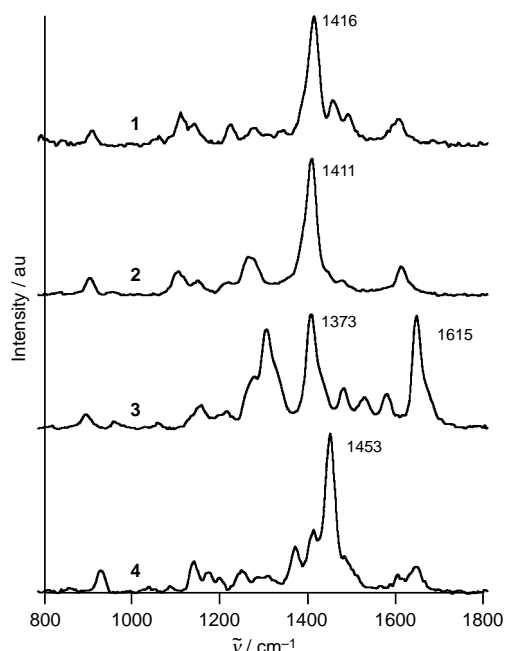


Fig. 2 SERS spectra of the dyes at 457.9 nm at 1×10^{-7} M except for **1** which was at 1×10^{-5} M

Three dyes produce similar spectra and exact peak positions can be used to discriminate between them. However, when a second methoxy group is added to anisidine analogue, the resulting dye produces a spectrum which is quite different. The extra electron donation of the methoxy group has probably twisted the ring and also created sufficient electron donation to produce partial bonding to the C–N and N=N bonds.

Surface enhancement of vibrations is dependent upon the orientation of the molecule on the surface.¹⁰ Hence different vibrations are enhanced as the molecular orientation changes producing a variation in spectra. Changes in surface orientation are related to the concentration of the analyte on the surface. However by making use of the strong complexing nature of benzotriazole on the silver a structure locked in one conformation can be obtained. The vibrations enhanced will always be the same at any concentration therefore increasing reliability. Dye **3** was studied in more detail between 5×10^{-9} and 1×10^{-6} M and unlike previous studies the relative intensities and position of the signals remained constant. The signal intensity obtained was proportional to concentration which will allow development of quantitative analysis by SERS.

The technique has now been shown to be reliable and since it is sensitive to changes in the environment and structure of the chromophore, there is considerable scope for the synthesis of

designed dyes to permit specific analytical procedures to be developed for ultra-sensitive analysis. For example the ligands require chemical functionalisation to allow addition of groups such as metal complexing functions or attachment to target analyte molecules such as DNA. This is a significant breakthrough which demonstrates that with the correct attention to surface chemistry SERS can be used as a reliable analytical method.

Notes and References

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‡ This work arose from initial studies with a different focus by Heather Wilson, PhD, University of Strathclyde, 1994.

§ *Diazotisation*: 5-aminobenzotriazole (1.0 g, 1.1 equiv., 7.63 mmol) was dissolved in HCl (5 ml, 50% v/v) and diazotised by dropwise addition of sodium nitrite (0.578 g, 1.2 equiv., in 5 ml H₂O) at 0 °C. An excess of sodium nitrite was detected using starch iodide paper. A dark blue colour indicated excess nitrous acid which inferred the formation of the diazonium salt.

¶ *Coupling*: the amine (1 equiv.) was dissolved in sodium acetate buffer (1.0 M, 5 ml, pH 6.0) and acetone (5 ml). Diazotised aminobenzotriazole (1.1 equiv.) was added to this solution dropwise at 0 °C with stirring over 1 h after which the solution was neutralised by addition of sodium hydroxide (2 M). The solid produced was isolated by filtration and washed with saturated KCl (3 × 50 ml) prior to purification by trituration using methanol and diethyl ether.

|| All novel compounds were characterized by UV spectrometry, proton NMR and FAB mass spectrometry. The purity of the compounds was confirmed by HPLC [retention time for **2** = 4.9 min at 430 nm from methanol–water (60:40)] owing to the difficulty in crystallisation. **2**: 40%, *R_f* [CH₂Cl₂–CH₃OH (9/1)] 0.34; δ_H[400 MHz, (CD₃)₂SO] 3.96 (3 H, s, OCH₃), 6.46 (1 H, s, H-2), 6.61 (1 H, d, *J* 8.8, H-5), 7.10 (2 H, br s, NH₂), 7.85 (1 H, dd, *J* 6.0 H-6), 7.98 (2 H, d, *J* 10.2, H-6',7'), 8.18 (1 H, s, H-4'); λ_{max}(MeOH) 392 nm; FAB MS: *m/z* 269.1144 [C₁₃H₁₂ON₆ (*M* + 1) <0.1 ppm].

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