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Analysis of the conversion of indigo into indigo carmine dye using SERRS

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In a novel application SERRS has been used, by employing a silver sol, to monitor and analyse the conversion of indigo into the indigo carmine dye.

Surface enhanced resonance Raman spectroscopy (SERRS) has found wide utility as a sensitive analytical tool in a multitude of interdisciplinary scientific investigations, including *e.g.* clinical and drug analysis, forensic, environmental and biological sciences and archaeological/historical applications. In fact there has been a growing interest in the uses of SERRS for highly sensitive chemical analyses, in a wide range of matrices, down to the single molecule level.[1,2] The inability to interrogate intrinsically highly fluorescent molecules, in solution, by dispersive (visible) Raman spectroscopy can be overcome by using several alternative Raman analytical techniques. These include *e.g.* FT-Raman (*i.e.* analysis at a wavelength where the fluorescent molecules do not absorb),[3] shifted subtractive Raman spectroscopy[4] and the use of the Kerr gate effect in time resolved Raman spectroscopy.[5] In addition SERRS [a combination of SERS (surface enhanced Raman spectroscopy) and RRS] can also be employed, both to overcome fluorescence and the analytically (relative) low-sensitivity *generally* associated with Raman scattering of molecules in solution. Interestingly different kinds of media/substrates (*e.g.* silver and gold colloids,[6] nanoshell colloids[7] and "gelcolls" which employ a hydrophilic swelling polymer (such as polyacrylic acid) in combination with a metal colloid[8]) have been and are being developed for obtaining SERRS signals of analytes. Although the theory of SERRS is still being developed, the observed SERRS signal enhancement over RRS is currently hypothesised to be caused by a combination of effects: the "classical electromagnetic field (EM) enhancement"[9] and the "chemical effect".[10] For maximum sensitivity, SERRS requires controlled aggregation of the colloidal sol used.[11] In fact surface enhancement of the Raman signals is dependent on the size of the colloidal particles as well as the exciting wavelength employed. This is because the surface plasmon absorption bands of metals such as silver and gold show wavelength dependent shifts for metal particles with sizes $> \lambda/20$ (λ = wavelength of the incident light) and surface enhancement is achieved by choosing the Raman excitation wavelength to coincide with the plasmon band.[12]

Vat dyes such as indigo (or 2,2′-bis-indole; (**1**)), besides being of significant historical interest in *e.g.* Coptic textiles[13] and icons,[14] hold a large part of the current dyestuff market (~3% of the total production of dyes)[15] for the coloration of cellulosic fibres. Its major industrial application is the dyeing of clothes (blue jeans) and other blue denim products. Indeed new, contemporary methods for their synthesis[16] or those of their analogues are of industrial, economic and academic importance. The dye indigo carmine (**2**) is used extensively as *e.g.* a food colorant (E.C. number E132) and as a diagnostic tool in a number of medical applications.[17] In an attempt to develop novel applications of SERRS the technique has been used to monitor and analyse the synthesis of the dye indigo carmine from indigo (see Fig. 1) using a silver sol as the SERRS substrate/medium.

A silver sol, for use as a SERRS substrate, was prepared *via* a modified Lee–Meisel procedure.[12,18] The physico-chemical characteristics of the silver sol obtained were determined by UV–VIS spectroscopy,[19] transmission electron spectroscopy as well as photon correlation spectroscopy[20] and were in accord with those reported in the scientific literature. For SERRS analysis of indigo the silver sol was aggregated using 35 μL of 1 mol dm^{-3} HCl. However, in order to analyse both synthesized and reference samples of indigo carmine, aggregation of the silver sol was achieved by addition of 150 μL of a 0.01% aqueous solution of poly(l-lysine). Typically the foregoing were added to 2 mL of silver colloid, which was prepared by diluting 1 mL of the stock silver sol with 1 mL of deionized water. SERRS reference spectra of indigo and indigo carmine, solubilized in methanol, were obtained at concentrations of ~10^{-6} mol dm^{-3}. The synthesis of indigo carmine from indigo[21] was conducted by adding 7 mg of indigo to 5 mL of concentrated sulfuric acid at room temperature. The reaction mixture was left to stand for 1 h and the pH of the solution adjusted to 2.3. 150 μL of this solution was then diluted five-fold with methanol and a 150 μL aliquot added to the pre-prepared aggregated silver sol and the SERRS spectrum collected. The SERRS spectra were collected at 5–10 min intervals during the conversion of indigo to indigo carmine. SERRS spectra were obtained using a Labram Raman spectrometer (Instruments S. A., Ltd.) equipped with a 1800 grooves/mm holographic grating, a holographic super-notch filter (Kaiser), an Olympus BX40 microscope, and a Peltier-cooled CCD (MPP1 chip) detector. A helium–neon laser provided exciting radiation of 632.8 nm. The laser beam was attenuated by a 10% neutral density filter, resulting in a laser power of 0.8 mW at the static sol and solution samples in all experiments. All SERRS spectra were collected by using a 180° back-scattering geometry. An Olympus microscope objective, having a magnification of ×10 and a numerical aperture of 0.25, was used both to focus the incident laser light and to collect the back-scattered Raman light.

Fig. 2 shows the SERRS spectra of indigo, indigo carmine synthesized from indigo and indigo carmine. Spectral assignments for selected vibrational bands, in the 1800–200 cm^{-1} region, of the SERRS spectra of a reference sample of indigo carmine and indigo carmine synthesized from indigo are given in Table 1. It is immediately obvious, both from the data in Table 1 and the SERRS spectra shown in Fig. 2, that the synthetic and reference samples of indigo carmine are identical. This conclusion was reinforced by mass spectroscopy/NMR data (not shown) of the two compounds.

The synthesis of indigo carmine from indigo is, under the experimental conditions employed, complete within ~30 min (Fig. 3).

In Fig. 3 the ratio $A_1 : A_2$ is plotted *versus* time, where A_1 represents the area (calculated using the Labram software) of the vibrational bands between 522 and 626 cm^{-1} for indigo carmine and A_2 represents the area between the same wavenumber limits for indigo.

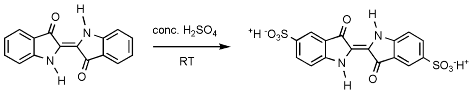

Fig. 1 Chemical structures of indigo (**1**) and indigo carmine (**2**). The reaction conditions for conversion of (**I**) to (**2**) are indicated.

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Very similar kinetic profiles to the one shown in Fig. 3 were obtained by analysing other indigo carmine vibrational features. Although SERRS has been extensively used for the analysis of dyes (see *e.g.* reference 22) and many compounds have been synthesized as model analytes for SERRS (see *e.g.* reference 23) to our knowledge this is the first report whereby SERRS has been used to monitor the synthesis of a dye molecule.

This study shows that SERRS can be used to monitor synthetic reactions at the micromolar level, as exemplified by the indigo carmine concentration of *ca.* 5×10^{-6} mol dm^{-3} in the final silver sol. Thus the ultrasensitivity of the SERRS technique together with the ease and rapidity of analytical measurements makes this a powerful tool for monitoring the progress of synthetic reactions (especially reactant(s) and/or product(s) which are fluorescent) conducted at the microscale level. In addition SERRS affords high structural specificity, in terms of vibrational band frequencies, for identifying reaction products (in relation to reactant(s)) when compared to other techniques such as NMR (a relatively poor limit of detection), fluorescence and UV–VIS absorption spectroscopy (the λ_{max} of indigo and indigo carmine are virtually identical).

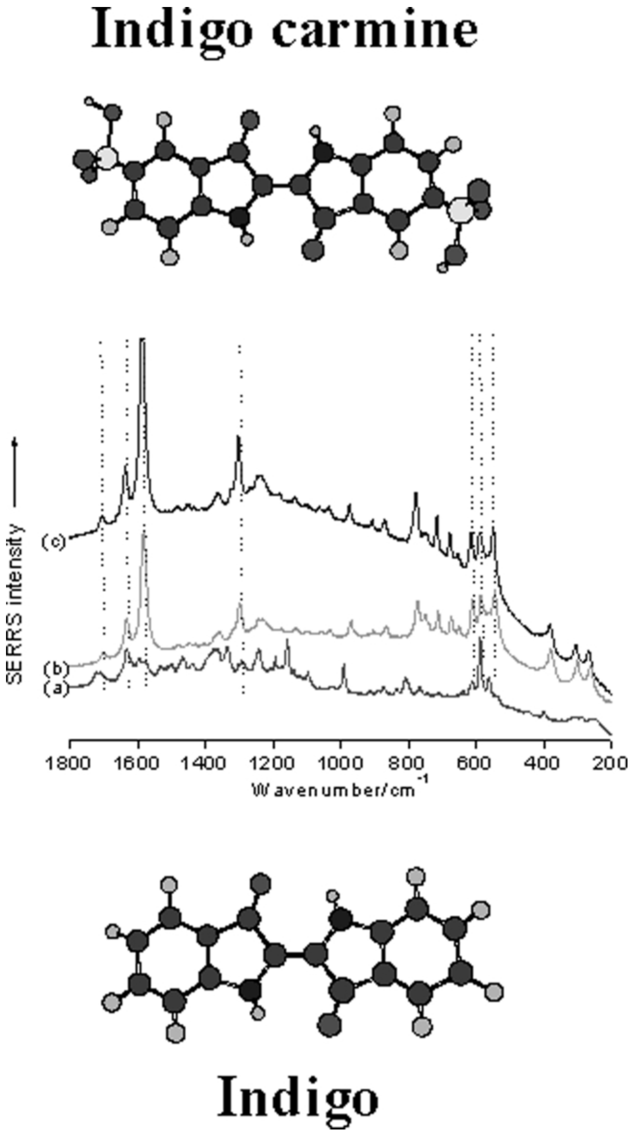

Fig. 2 SERRS spectra of (a) indigo, (b) indigo carmine synthesized from indigo and (c) a "reference" sample of indigo carmine.

Table 1 Assignments of selected Raman vibrational bands for reference/synthesized indigo carmine

Vibrational frequency/cm^{-1}	Band assignment
1701	C=O str.
1632	C—C str., C—H bend
1582	C=C str.
1298	$-SO_3^-$ sym. str.
611	$-SO_3^-$ bend
586, 546	C=C—CO—C bend

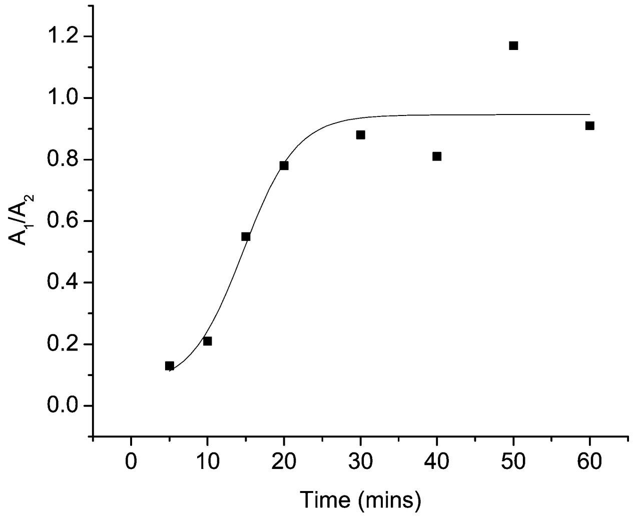

Fig. 3 Plot showing the rate of formation of indigo carmine from indigo.

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