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Thermal Lens Spectrometry

R. D. Snook

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The use of redox mediator modified glucose oxidase in amperometric enzyme electrodes

By P. N. Bartlett and V. Q. Bradford
Department of Chemistry, University of Warwick, Coventry CV4 7AL, U.K.

Glucose oxidase, modified by the covalent attachment of ferrocenyl groups, has been shown to undergo direct oxidation at clean metal electrodes (Degani & Heller 1987, 1988; Bartlett *et al.* 1987). Since modified enzymes of this type do not require a freely diffusing mediator and can be oxidized at modest overpotentials they are attractive for application in biosensors and in bioelectronics.

The use of ferrocene monocarboxylic acid and ferrocene acetic acid modified glucose oxidase has been studied in membrane enzyme electrodes for glucose. It was found that the lifetime of these devices is limited by the stability of the oxidized form of the ferrocenyl substituent attached to the enzyme. In buffered aqueous solution this is of the order of minutes for the ferrocene monocarboxylic acid modified enzyme and of the order of hours for the ferrocene acetic acid modified material.

We have also studied the modification of glucose oxidase using tetrathiafulvalene (TTF) and tetracyanoquinodimethane (TCNQ). It is known that electrodes made from TTF.TCNQ carry out the oxidation of unmodified flavoproteins (Albery *et al.* 1987*a*); however, the mechanism remains controversial (Albery *et al.* 1987*b*; Kulys 1986). We reasoned that planar hydrophobic molecules, such as TTF and TCNQ, might be incorporated into the hydrophobic regions of the protein. Our results suggest that this is indeed the case, and we have shown that glucose oxidase, modified with TTF or TCNQ, undergoes oxidation at metallic electrodes.

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Thermal lens spectrometry

By R. D. Snook
DIAS, UMIST, PO Box 88, Manchester M60 1QD, U.K.

Thermal lens spectrometry is a laser-based technique that can be used for extremely sensitive spectrophotometric analysis in nanolitre volumes of solutions.

In thermal lens spectrometry (Jun Shen & Snook 1989*a*) a laser is used to excite chromophores in solution. Non-radiative decay routes of the excited chromophore

leads to local heating of the solvent which in turn leads to a refractive index change in the beam/sample interaction volume. For most solvents the change in refractive index with temperature is negative ($-\mathrm{d}n/\mathrm{d}T$) which causes the solution to behave as a diverging lens. For a gaussian beam profile this causes a reduction in beam intensity at the beam centre which can be monitored in the far field of the thermal lens using a pinhole aperture and photomultiplier detector. The thermal lens signal, θ is related to the absorbance of the chromophore through the following equation

$$\theta = -2.303 P(\mathrm{d}n/\mathrm{d}T) A/\lambda k.$$

The sensitivity of the technique is therefore power dependent, P being the power of the pump beam, λ the laser wavelength, k the thermal conductivity of the solution and A the absorbance of the solution. The technique can be used to gain the same information as conventional UV/visible spectrophotometry but limits of detection are improved by as much as three orders of magnitude because of the power enhancement factor. The determination of U^{VI} and Cu^{II} in solution was described using a novel mode-mismatched two beam thermal lens spectrometer. Absorption coefficient detection limits of 10^{-4} and 10^{-7} cm^{-1} for these species were found in nanolitre volumes of solution.

The thermal lens technique also allows the thermal power absorbed to be measured. The quantum efficiency Q_f of fluorescent molecular species in solution can therefore be measured using the thermal lens technique (Jun Shen & Snook 1989*b*) as the total absorbed power (excitation and thermal power) can be quantified. This method was outlined, and results for the quantum efficiency (Q_f) of sodium fluorescein in ethanol and 0.1 M NaOH were presented ($Q_f = 0.92-0.97$).

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Enantiometric purity determination to 1% level using a laser-based polarimetric HPLC detector

By David M. Goodall and Zecai Wu
Department of Chemistry, University of York, Heslington, York YO1 5DD, U.K.

The development of laser-based polarimetric detectors for high-performance liquid chromatography (HPLC) (Yeung *et al.* 1980; Bobbitt & Yeung 1986) with noise levels in the range of 0.1–10 μ° has provided a significant advance in the quantitation of chiral molecules.

We have designed an instrument based on an 820 nm diode laser which has the advantages of low source flicker noise and compact design (Lloyd *et al.* 1989). Detection limits were found to be in the range 0.1–2 μg, dependent on the specific rotation of the chiral molecule and the chromatographic peak width (Goodall *et al.* 1990).

Enantiomeric purities of resolved enantiomers and enantiomer mixtures have been determined using reversed-phase achiral chromatography with polarimetric and