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Diode laser-based indirect absorbance detector for capillary electrophoresis

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ABSTRACT

A near-infrared detector has been developed for use in capillary electrophoresis (CE). The detector has a double beam arrangement with signal-to-reference ratioing and operates with a 670-nm diode laser as the light source. The laser beam can be modulated to allow a.c. signal recovery using lock-in amplification,

Near-infrared laser dyes have been investigated as background absorbers, and the singly-charged cationic dye rhodamine 700 found to exhibit suitable characteristics for use in indirect absorbance detection in methanol-water mixtures. Separations of a series of tetraalkylammonium compounds have been performed on both a commercial CE apparatus with indirect UV detection and on a home-made instrument incorporating the diode laser detector. The limit of detection (2 × peak-to-peak noise) for the tetrabutylammonium ion with diode laser-based indirect absorbance is $2 \cdot 10^{-5}$ *M*. Both positive and negative peaks are found for these positively-charged analytes displacing a positively charged background absorber. Transfer ratios for all peaks are quantitated and the results compared with theoretical treatments of displacement in indirect detection. For analytes with mobility less than the indirect absorbing ion, measurement of peak area leads directly to the quantity of analyte present in the sample solution.

INTRODUCTION

The principles of separation in capillary electrophoresis (CE) [1,2] mean that the technique is well suited to analysis of simple inorganic and organic ions. The drawback in separating these types of analytes is often their poor UV-visible absorbance. This problem can be overcome by using indirect detection which does not require the analyte to show a detector response [3]. Indirect UV detection has been used for the analysis of a wide range of organic and inorganic ions [4–6] including quaternary ammonium compounds [7]. The fast run times and high efficiency of these separations offer advantages over alternative methods such as ion chromatography. Laser-induced indirect fluorescence has also been widely studied [8–10]. In these cases intensity stabilisation of gas lasers has ensured high dynamic reserves and hence improved detection limits. In addition lasers are easily focused and are highly monochromatic. Diode lasers are alternatives to argon-ion and helium–cadmium lasers. These devices offer the advantages of small size, low cost through mass production and inherently low noise compared with gas lasers. They have many applications in the area of analytical science [11], including high-performance liquid chromatography [12,13]. Recently a

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diode laser has been used in a fluorescence detector developed for use in CE [14]. Since the output wavelengths of diode lasers are restricted to the red and near-infrared (NIR) region it was necessary to tag amino acids with a suitable fluorophore to perform direct detection. Indirect detection was carried out by adding the dye methylene blue to the background electrolyte (BGE) to serve as the fluorophore. An alkylsilane-derivatised capillary was used to prevent adsorption of methylene blue to the capillary walls.

In the present study a diode laser-based detector has been developed for indirect absorbance. Several NIR dyes have been investigated as potential background absorbers, and rhodamine 700 found to be suitable. Using this technique a series of tetraalkylammonium ions have been analysed, and detector performance compared with lamp-based indirect UV detection. Experimental results are compared with a theoretical treatment of displacement in indirect detection [15–17].

EXPERIMENTAL

Instrumentation

A block diagram of the detector is shown in Fig. 1. The diode laser is a 670-nm device wih a maximum output power of 3 mW (Toshiba T9201) and is controlled by a laser diode power supply (Laser Spectrum TDL-1R). The laser beam is intensity modulated for use with a.c. signal recovery. Output from the laser diode is split by a 60:40 beamsplitter. The transmitted beam is focused by a plano-convex lens ($f = 10$ mm) through the capillary, which is placed behind a 100 x 200 μm aperture, onto the sample detector. The reflected beam is directed onto the reference detector by a mirror. Each detector consists of a silicon photodiode (Centronic OSD50-5T) and an operational amplifier (RS Components OP 27) configured as a current-to-voltage converter with a 2.2 kΩ feedback resistor. The sensitivity of each detector at 670 nm is 920 V W^{-1}. The outputs from the detectors are fed into the differential inputs of a phase-sensitive detector (PSD) (EG&G Instruments 5209). The signal amplitude of one detector is attenuated so that the PSD gives zero output when the capillary is filled with BGE. The output from the PSD is connected to a storage oscilloscope (Nicolet Instruments 3091) which in turn is interfaced to a microcomputer to allow data storage and manipulation.

In experiments in which the root mean square (RMS) baseline noise of the detector was measured a 675-nm diode laser (Phillips CQL80D) was used. Intensity modulation of the laser beam was achieved by two methods. In the first the current to the diode laser was sinusoidally modulated to give a corresponding variation in the laser output intensity. In the second method acoustooptic modulation [18] was employed. The acousto-optic modulator (Isomet 1205C-1) was connected to a 1 W radiofrequency (RF) driver (Isomet 232A-1) and the modulation

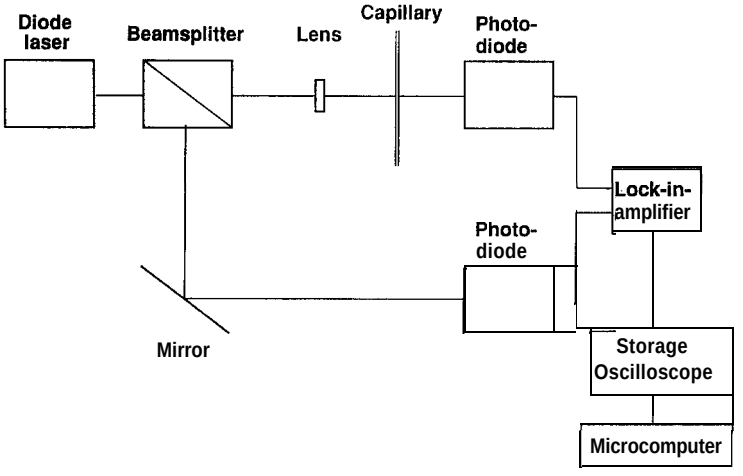

Fig. 1. Detector block diagram.

signal to the RF driver was supplied by a signal generator (Farnell LF1). The modulator was placed before the beamsplitter and the diffracted first-order beam was blocked, allowing the zeroth-order beam to pass. For experiments with no intensity modulation, an 820 nm diode laser (Spindler and Hoyer DC25F) was used. The sensitivity of each detector at this wavelength is 990 V W^{-1}. Outputs from the detectors were connected to a differential amplifier (Tektronix AM502) and the differenced output was fed to a 10-Hz 6-pole Butterworth low-pass filter.

Capillary electrophoresis instrumentation

The diode-laser-based detector was incorporated into a home-made CE apparatus. The system uses a 30-kV high-voltage power supply (Glassman High Voltage PS/MJ30P0400-22) and has the option of either vacuum or electrokinetic sample introduction. A vacuum is created by a water pump connected to a reservoir and the injection accurately timed using a solenoid valve (Lee Company LFAA1200118H) controlled by a timing circuit. A Beckman P/ACE system 2100 with UV detection at 254 nm was used in lamp-based indirect detection studies.

The capillaries used are fused silica 75 μm I.D. (Polymicro), either 70 cm long (35 cm to the detector window) on the home-made instrument or 57 cm long (50 cm to the detector) on the Beckman apparatus.

Reagents

Chemicals used were phosphoric acid, sodium hydroxide, methylene blue and tetrapropyl-, butyl-, pentyl- and hexylammonium bromides (Aldrich), tetramethylammonium hydroxide (Sigma), tetraethylammonium chloride (BDH) and the laser dye rhodamine 700 (Lambda Physik). All samples were used without further purification, dissolved in the BGE and filtered (0.2 μm Millipore) before injection.

RESULTS AND DISCUSSION

Noise analysis

To determine the RMS baseline noise of the detector the capillary was tilled with water and the reference beam was attenuated by an optical flat with a reflection loss of 7%, enabling the detector to be calibrated and its output to be expressed in absorbance units (AU). Using current modulation, a modulation depth of 85% was found to be the maximum obtainable whilst still retaining a good sine wave. With a time constant of 100 ms, an RMS baseline noise level of $2 \cdot 10^{-6}$ AU was achieved. The RMS noise level was found to be slightly higher ($5 \cdot 10^{-6}$ AU) when operating with acousto-optic modulation or in the absence of any modulation at all. With acousto-optic modulation or in d.c. it is possible to operate the diode lasers in power-control mode with optical feedback which stabilises output intensity [19]. By way of comparison, the Beckman P/ACE instrument has an RMS baseline noise of $5 \cdot 10^{-5}$ AU with an equivalent time constant.

It was noted that exact cancellation of the sample and reference photodetector signals was not possible, which indicates differences in their frequency responses. Detector noise was found to increase when modulation was employed. This would imply that noise in the modulated signal is amplified by these differences in sample and reference detectors. To eliminate this problem it may be possible to obtain improved performance using a.c. signal recovery with acousto-optic modulation and one detector, such that the signal and reference beams are canceled optically rather than electronically [12]. Mechanical vibrations of detector components were also found to contribute to the noise. Movement of the laser beam focal point in the capillary detection volume can lead to fluctuations in the intensity of light reaching the sample photodiode and so to noise on the output of the detector. This noise is not canceled by differencing, since the same intensity fluctuations are not observed at the reference photodiode, where positioning of the laser beam is not critical.

Detector output noise showed no significant dependence on the frequency of modulation over the range 30 Hz to 100 kHz, indicating that the detector is not flicker-noise limited. The contribution to noise from detection electronics was also found to be small.

Indirect detection

Electropherograms of two NIR-absorbing dyes were obtained in order to assess their suitability as indirect background absorbers. Methylene blue run in a pH 2.5 buffer showed peak tailing characteristic

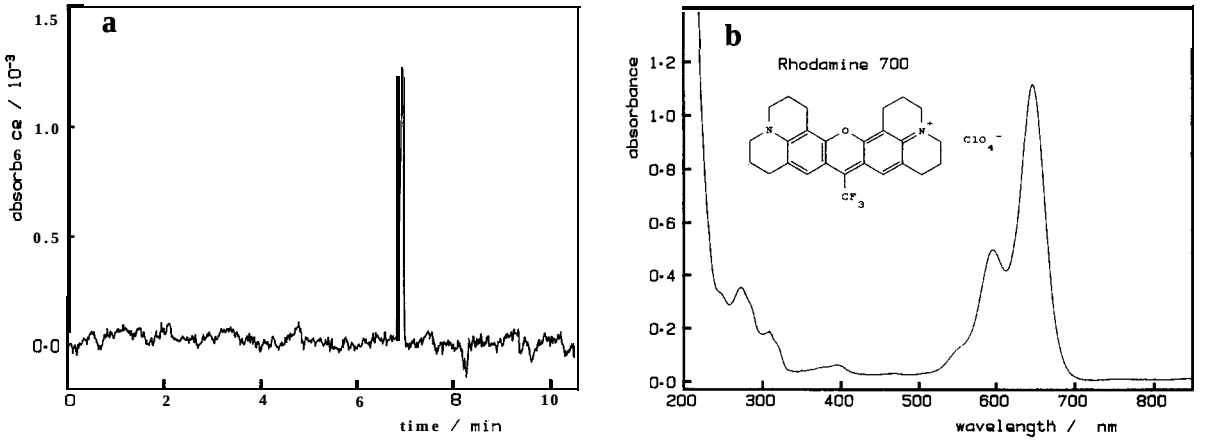

Fig. 2. Electropherogram, structure and spectrum of rhodamine 700. (a) Electrophoresis conditions: buffer, 20 m*M* aqueous sodium phosphate pH 3.5–methanol (70:30, v/v); capillary, 70 cm × 75 μm (35 cm to the detector); applied voltage, 20 kV; temperature, ambient; absorbance detection at 670 nm; injection. 2 sat ΔP = 2900 Pa: concentration, 1 m*M*. (b) Concentration 1.25 . 10^{-5} Min water-methanol (70:30, v/v).

of adsorption of the positively-charged dye to the capillary surface, as noted by Higashijima *et al.*[14]. In contrast, the laser dye rhodamine 700 gives a much narrower peak with no signs of wall binding (Fig. 2a). In order to dissolve rhodamine 700 at a concentration of ca. 1 m*M* suitable for indirect detection, the addition of organic cosolvent is necessary at levels up to 50% (v/v). The structure and spectrum of the dye are shown in Fig. 2b. The absorbance at 670 nm in water-methanol is only one sixth of that at 642 nm (λ_{max}) but has the advantage of being similar to the absorbance at 254 nm, the wavelength chosen for indirect UV detection.

Separations of six tetraalkylammonium salts using rhodamine 700 as a background absorber are shown in Fig. 3. Fig. 3a gives results from an automated instrument with UV detection at 254 nm and Fig. 3b from the home-made instrument with detec-

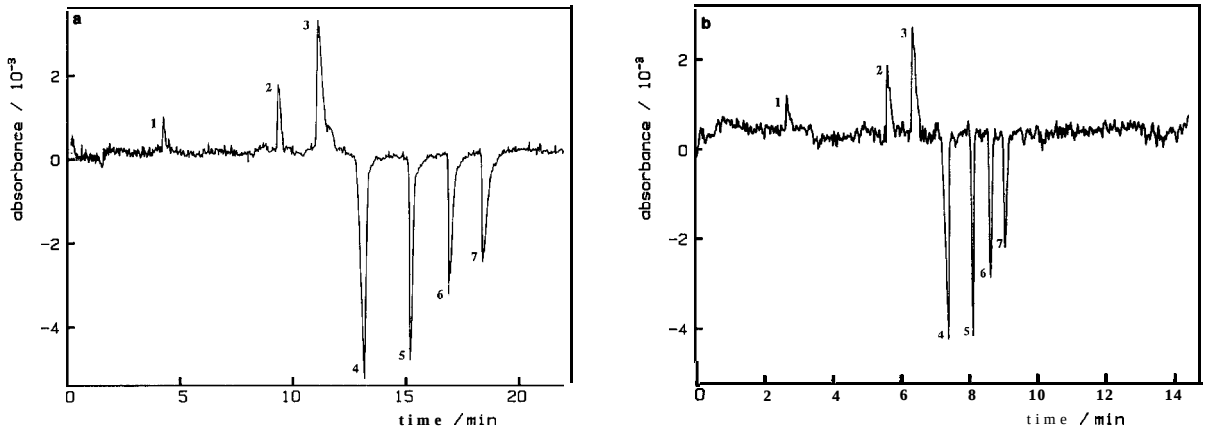

Fig. 3. Electropherogram of 6 tetraalkylammonium ions. Peaks: 1 = $(CH_3)_4N^+$; 2 = $(C_2H_5)_4N^+$; 3 = $(C_3H_7)_4N^+$; 4 = system peak; 5 = $(C_4H_9)_4N^+$; 6 = $(C_5H_{11})_4N^+$; 7 = $(C_6H_{13})_4N^+$. Conditions: buffer, 1 m*M* aqueous sodium phosphate pH 3.6–methanol (50:50, v/v); applied voltage, 20 kV; injection, 10 s at 5 kV; sample concentration 0.2 m*M*. (a) Capillary, 57 cm x 75 μm (50 cm to the detector); temperature, 30°C; UV absorbance detection at 254 nm. (b) Capillary, 70 cm × 75 μm (35 cm to the detector); temperature, ambient; absorbance detection at 670 nm.

tion at 670 nm. Positive peaks are observed for the tetramethyl-, ethyl- and propylammonium ions whilst tetrabutyl-, pentyl- and hexylammonium ions produce negative peaks. Positive peaks indicate an increase in concentration of background absorber present at the detector, whilst negative peaks indicate a decrease. The migration time of the system peak corresponds to the mobility of the rhodamine cation.

Limit of detection

The limit of detection (LOD) using the laser-based detector for the tetrabutylammonium ion was determined from the electropherogram shown in Fig. 4. The LOD was taken with the ratio of signal to peak-to-peak noise level equal to 2: 1 and found to be $2 \cdot 10^{-5} M$. This may be compared with a value for the LOD calculated knowing the transfer ratio and the dynamic reserve [3]. The transfer ratio, *TR*, is defined as the number of indirect absorbing ions displaced by one analyte ion and the dynamic reserve, *DR*, is the ratio of the signal produced by the BGE to the noise on top of the signal. For the electropherogram shown in Fig. 4, *DR* measured using the output RMS noise is 1300. The concentration limit of detection, c_{lim}, is given by [3]

$$c_{lim} = \frac{c_m}{TR \cdot DR} \quad (1)$$

where c_m is the concentration of the background absorber. With the value of *TR* equal to 1.1, determined experimentally for the tetrabutylammonium ion as discussed in the next section, c_{lim} is calculated to be $7 \cdot 10^{-7} M$. The corresponding c_{lim} with the signal to peak-to-peak noise (6 x RMS noise [20]) ratio equal to 2:1 is $8 \cdot 10^{-6} M$. The dilution factor of the analyte ion from injection to peak maximum at the detector was measured to be 2.3, giving a calculated LOD of $2 \cdot 10^{-5} M$ in agreement with the experimental value.

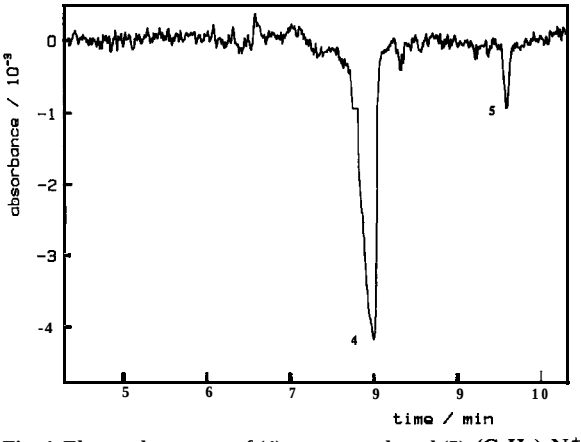

Fig. 4. Electropherogram of (4) system peak and (5) $(C_4H_9)_4N^+$ used to determine the LOD. Conditions as in Fig. 3b except sample concentration 0.03 m*M*.

Transfer ratios

Transfer ratios were measured for all the tetraalkylammonium ions in the electropherograms of Fig. 3a and b. Negative peaks in indirect detection (positive displacement) have positive *TR* values [3], and therefore *TR* values for positive peaks (negative displacement) must carry the opposite sign. Peak areas represent the amount of background absorber displaced by each analyte. To obtain the peak areas in units of moles, the areas from the electropherograms (AU · s) were normalised by multiplying by the observed volume flow-rate of the analyte ($dm^3 s^{-1}$) and dividing by the signal response of the rhodamine 700 (AU $dm^3 mol^{-1}$). The quantity Q_i of an ion *i* injected electrokinetically is given by [21]

$$Q_i = (\mu_{eff,i} + \mu_{eo}) E \pi r^2 c_i t \quad (2)$$

where $\mu_{eff,i}$ is its effective electrophoretic mobility [16], c_i its concentration, μ_{eo} the electroosmotic mobility, *E* the electric field strength, *r* the capillary internal radius and *t* the injection time. The transfer ratio can be calculated by dividing the amount of rhodamine displaced by the amount of analyte injected.

Results based on these calculations are shown in Table I. The *TR* values obtained experimentally at 254 nm and 670 nm for analyte ions eluting after the system peak are close to unity and in good agreement with results predicted by simple theory [3], which assumes a 1: 1 charge displacement of the monitored background ion by the analyte ion. In cases where the transfer ratio can be predicted from the analyte mobilities, these results show that measurement of peak area leads directly to the quantity of sample present in the sample solution. Since this does not require construction of any calibration plots with standard solutions, such a treatment could be of particular value in quantification of unknown species.

TABLE I

PEAK AREAS AND TRANSFER RATIOS IN INDIRECT DETECTION OF TETRAALKYLAMMONIUM IONS WITH RHODAMINE 700

Analyte ion	Peak area (AU)		Transfer ratio		
	Fig. 3a	Fig. 3b	Experimental[a]	Experimental[b]	Theoretical[c]
Tetramethylammonium	0.0024	0.0053	-0.1	-0.3	-0.05
Tetraethylammonium	0.012	0.012	-0.7	-0.7	1.0
Tetrapropylammonium	0.045	0.016	-1.6	-1.1	0.9
Tetrabutylammonium	-0.036	-0.015	1.5	1.1	1.1
Tetrapentylammonium	-0.031	-0.013	1.3	1.0	1.2
Tetralhexylammonium	-0.031	-0.012	1.3	1.0	1.3

[a] Detection at 254 nm (Fig. 3a).
[b] Detection at 670 nm (Fig. 3b).
[c] From computer analysis of transport equations [16].

The positive peaks (negative *TR* values) found for analyte ions with mobility greater than the system peak are not accounted for by simple theory. A more complete description considers the two important conditions that must be satisfied in indirect detection. Firstly, constancy of the Kohlrausch function, *K*, with time and secondly, the condition of electroneutrality. The Kohlrausch function is [22]

$$K = \sum \frac{c_{x,i} z_i}{\mu_{\mathrm{eff},i}} \qquad (3)$$

where z_i is the charge on the ith ion, $c_{x,i}$ its distance-dependent concentration and $\mu_{\mathrm{eff},i}$ has already been defined. By inspection, the requirement that the function is constant with time allows for the concentration of a sample within a zone to increase or decrease (positive or negative peaks). Theory which assumes a 1: 1 charge displacement represents the solution of the Kohlrausch function when the charge and the mobility of the analyte and monitored background ion are matched. However, for a system with a more complex BGE the constancy of *K* does not provide a unique solution. Here it is necessary to consider the transport equations which have the general form

$$-\frac{\partial J_i}{\partial x} = \frac{\partial}{\partial x}\left(\frac{I}{\kappa}\,\mu_{\mathrm{eff},i}\,c_{x,i} + D_i\,\frac{\partial c_{x,i}}{\partial x}\right) \qquad (4)$$

where x is the longitudinal distance, Z the current density, κ the conductivity, J_i is the flux of the ith ion, D_i its diffusion coefficient, and $\mu_{\mathrm{eff},i}$ and $c_{x,i}$ have already been defined. It is not possible to solve the problem using the transport equations with no simplification, but the problem can be simplified by assuming that the interaction between an ion and all other ions in the system are linearly additive [15–17]. The resulting set of linear equations can be solved using eigenvectors as part of a computer program.

This program has been used to calculate *TR* values for the six tetraalkylammonium ions using rhodamine 700 as a background absorber, giving the results shown in Table I. Agreement with experimental findings is good for tetramethyl-, butyl-, pentyl- and hexylammonium ions, although predicted transfer ratios are wrong both in sign and magnitude for the tetraethyl- and propylammonium ions. A possible explanation for this discrepancy is neglect of effects of non-ideality in transport and equilibrium behaviour in the current theoretical treatment.

CONCLUSIONS

A laser-based indirect absorbance detector has been developed using an inexpensive, low-noise diode laser. With the laser dye rhodamine 700 added to the buffer as a background absorber, six tetraalkylammonium salts were separated on an automated instrument with UV detection and on a home-made CE apparatus incorporating the detector. The limit of detection using the laser-based detector for the tetrabutylammonium ion was found

to be $2 \cdot 10^{-5} M$ (2 x peak-to-peak noise). The separation produces both positive, negative and system peaks. Tetraalkylammonium ions with mobilities lower than the monitored rhodamine 700 ion gave negative peaks with transfer ratios close to unity and in good agreement with theory. The three ions with mobilities higher than rhodamine 700 were found to give positive peaks, and current theoretical treatments of indirect detection based on the transport equations are unable to account for all observations. Our results show that the method may be used for quantification of molar amounts of an ion injected, including unknown species, but only for ions with mobility less than that of the monitored background electrolyte ion.

Future work will investigate possible improvements to detector sensitivity including the use of different absorbing buffer additives and will consider the effects on indirect detection of non-ideality in ionic equilibria and transport.

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