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THERMAL LENS CALORIMETRY

APPLICATION TO CHROMATOGRAPHIC DETECTION

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SUMMARY

The laser-induced thermal lens effect has been applied to the calorimetric detection of absorbing samples having negligible fluorescence quantum yields. A simple but fast method for numerically fitting the thermal lens transient data, during the 0.25-sec period while the sample cools, allows the instrument to serve as a real-time absorbance monitor. Preliminary results, using 190 mW laser power, indicate detection limits of $A_{min} \approx 1.5 \cdot 10^{-5}$ cm^{-1} for a 5-sec response time.

INTRODUCTION

The incorporation of laser sources into spectroscopic detectors for liquid chromatography[1–3] has produced improvements in limits of detection. While the most significant advances have been made in fluorescence detection due to the large excitation intensity of the laser, a similar advantage should exist for detecting samples having insignificant fluorescence quantum yields by a calorimetric absorbance measurement using a laser source. This concept was first proposed by Kreuzer[4] for thermocouple calorimetry and first demonstrated by Oda and Sawada[5] for laser-induced photoacoustic spectroscopy. In this work, we report the suitability of a related technique, thermal lens calorimetry[6], for detection of liquid chromatographic eluents.

The thermal lens effect, first reported by Gordon *et al.*[7], produces a time dependent divergence of a chopped laser beam due to the additional heat deposited in an absorbing sample at the center of a Gaussian laser beam profile, where the intensity is greatest. When the sample is located one confocal length beyond a waist in the beam, the strength of the thermal lens can be determined by the loss of intensity from the center of the beam measured by a detector having its field of view restricted by a pinhole. For a motionless sample, the intensity change is governed by thermal diffusion[7,8]

$$I_{bc}(t) = I_{bc}(o)\left[1 + \frac{\theta}{1 + t_c/2t} + \frac{1}{2}\left(\frac{\theta}{1 + t_c/2t}\right)^2\right]^{-1} \quad (1)$$

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where θ is proportional to the absorbance of the sample, A

$$\theta = -2.303P(\mathrm{d}n/\mathrm{d}T)\,A/\lambda k \qquad (2)$$

$$= 2.303\,EA$$

where P is the laser power, $(\mathrm{d}n/\mathrm{d}T)$ is the change in refractive index with temperature, λ is the laser wavelength and k is the thermal conductivity. It is convenient to group the terms which effect sensitivity into a constant, E, which is the enhancement of the linear response portion compared to Beer's law[6]. The time constant, t_c, depends on the radius of the laser beam in the sample, ω

$$t_c = \omega^2\,\varrho\,C_p/4k \qquad (3)$$

where ϱ is the density and C_p is the heat capacity of the sample.

The modification of this theory, to account for the additional heat transport which occurs when the sample is flowing, has been recently studied[9]. For sufficiently slow flow-rates, the effect of mixing within the path of the laser beam can be modeled as a perturbation increase to the effective thermal conductivity. This allows the data to be fit to the thermal diffusion response, which is extremely beneficial in reducing the uncertainty of the measurement[8], while E and t_c are somewhat smaller due to the increase in the effective value of k.

EXPERIMENTAL

The argon ion laser based, thermal lens calorimeter is shown in Fig. 1. The major construction details for the instrument have been previously published[10]. The laser beam, $\lambda = 458$ nm, $P = 190$ mW, is focused by a 33 cm focal length lens, L, through an electronic shutter, S, and an 18-μl, 1 cm pathlength flow cell, C, obtained from Helma Cells, Inc. The beam propagates along a 5 m folded optical path to a Silicon Detector Corp., photovoltaic detector, D, which is constrained to view the center of the beam profile by a pinhole, P, having an aperture of 2.5 mm radius.

The laser calorimeter flow cell is connected in series directly to the outlet of a Beckman-Altex Model 153 UV–VIS detector equipped with a 470 $\pm$ 5 nm wavelength interference filter, which also monitors the eluent from the liquid chromatograph, Beckman-Altex Model 330. The column is ODS on 5-μm silica support, 25 cm

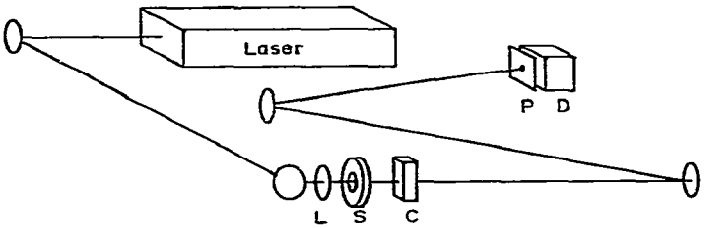

Fig. 1. Thermal lens calorimeter. L = 33 cm focal length lens; S = electronic shutter; C = flow cell; P = pinhole; D = photovoltaic detector.

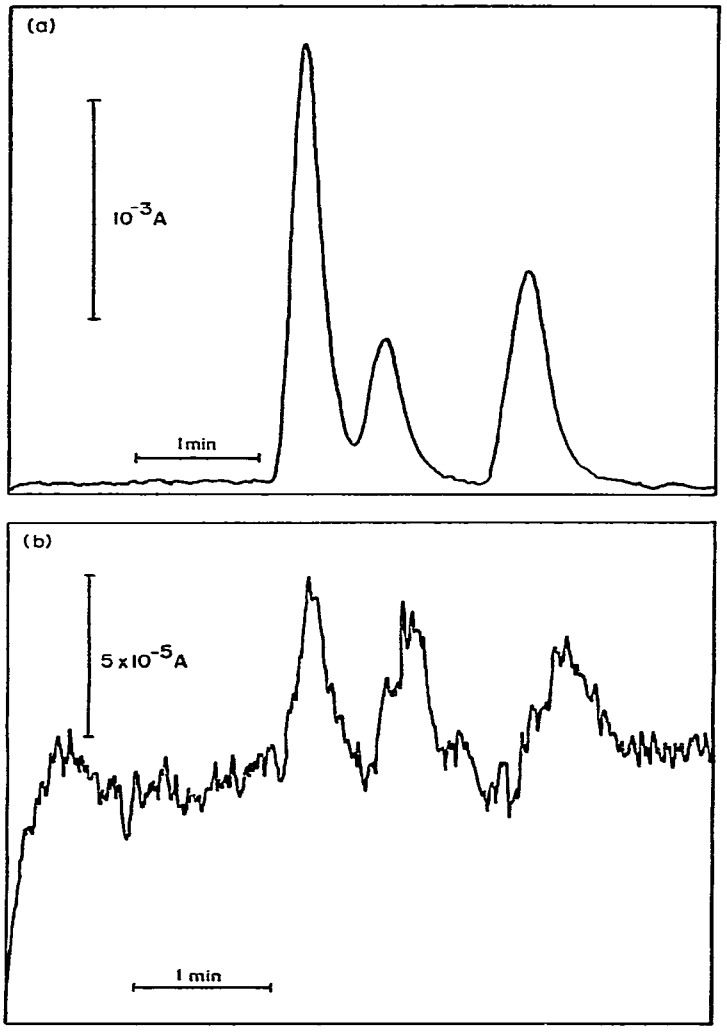

Fig. 2. Nitroaniline isomers detected by thermal lens calorimetry. Order of elution: *para*, *meta* and *ortho*. Amounts injected are: (a), 330, 210, 26 ng; (b), 6.6, 4.2, 0.53 ng. The initial rise on the left (b) is due to the moving average settling from an initial value of $\bar{\theta} = 0$.

× 4.6 mm, held at a temperature of 60°C; the solvent is methanol–water (50:50), pumped at a rate of 1 ml/min. Standard solutions of the positional isomers of nitroaniline were prepared in methanol–water (50:50), and their absorbances at 458 nm are checked spectrophotometrically. Quantitative dilutions of these solutions were combined into mixtures to assess the performance of the instrument.

RESULTS AND DISCUSSION

A major challenge in application of thermal lens calorimetry to detection of transient samples is to have adequately short response time while maintaining the

many advantages[8] of fitting the data to the kinetic response of eqn. 1. Although the time constant can be measured in advance for a particular beam size, solvent composition and flow-rate, there are still two independent parameters, $I_{bc}(o)$ and θ, which describe a given thermal lens transient. An optimum fitting procedure allows both parameters to be adjusted but requires, for a 100 point transient, about 1 min to converge running an efficient FORTRAN algorithm on a DEC LSI-11 microcomputer.

As a compromise between precision and speed, we choose to assign $I_{bc}(o)$ the value of the first data point in the thermal lens transient. This reduces the fit to a single parameter, θ, but adds a component of noise associated with the short-term fluctuations effecting the value of $I_{bc}(o)$. For an N point transient, each point, $I_{bc}(t > o)$, contributes to the average value of θ after applying a correction term which accounts for the time dependence, obtained by rearranging eqn. 1:

$$\theta = \frac{1}{N-1} \cdot \sum (1 + t_c/2t) \{[2I_{bc}(o)/I_{bc}(t) - 1]^{\frac{1}{2}} - 1\} \quad (4)$$

For a 100 point transient, this weighted average can be determined in about 250 msec using the same microcomputer hardware, as above. This speed allows real-time monitoring of the sample absorbance on a sufficiently fast scale for LC detection.

To trade-off unnecessary speed for precision, a digital filter or moving average may be applied to the result of each transient, θ_i. For this study ten experiments were correlated to form an average, $\bar{\theta}_i$, by allowing an individual result, θ_i, only a 10% influence in changing the old value of the average, $\bar{\theta}_{i-1}$:

$$\bar{\theta}_i = 0.9\ \bar{\theta}_{i-1} + 0.1\ \theta_i \quad (5)$$

At the repetition rate of the experiment, 2.0 Hz, this provides a 5.0 sec response time (10–90%).

In order to determine the time constant, required to implement eqn. 4, and the enhancement, needed for interpreting absorbance information from θ, a solution diluted from stock of known absorbance is pumped through the flow cell using a syringe pump operated at rates which closely bracket the flow-rate for chromatography. For each flow-rate, 100 transient are averaged together and fit[8] to the three parameters, $I_{bc}(o)$, θ and t_c. For the chromatographic conditions, methanol–water (50:50), 1.0 ml/min, the enhancement, $E = 120$, and time constant, $t_c = 28$ msec, are found by linear interpolation[9].

These values are then used in a computer routine which gathers 100 point transients of 50 msec duration (2 kHz clock rate), fits the data according to eqns. 4 and 5, and plots $\bar{\theta}_i$, which requires ten transients to settle from the initial value of $\bar{\theta} = 0$. Preliminary results are obtained for the detection of substitutional isomers of nitroaniline, shown in Fig. 2. The peak absorbance values thus measured agree, within the error of measurement, with the values calculated from the known absorbance of the injected sample and the concentration profile of the peak. For the more concentrated runs, agreement was also observed with the commercial optical detector, corrected for the differences in molar absorptivities at the two different wavelengths. The limit of detection, which depends on particular solvent composition and

flow-rate as well as laser power, is determined from the fluctuations in the baseline to be $A_{min} \approx 1.5 \cdot 10^{-5}$ cm^{-1}, in this case.

These preliminary results appear promising for future applications of the thermal lens effect to chromatographic detection. Further work to reduce the laser intensity fluctuations, the primary noise source, must be carried out in order to improve the detection capabilities. Since discrete wavelength, continuous wave, gas lasers (*e.g.*, He–Ne, Ar^+, Kr^+, CO_2, CO), which have the continuous power and spatial coherence required for thermal lens measurements, are uniquely simple to operate and are relatively inexpensive, their use in routine detection applications is plausible. Although their lack of continuous tunability precludes the generation of spectra, they should be quite suitable for single wavelength detection which could allow, for example, functional group monitoring in the infrared. In addition, detection of post-column reaction products in the near-UV and visible would transfer the specificity of the reaction to a detector which is very sensitive, but otherwise only semi-selective.

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