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Improving the sensitivity of HPLC absorption detection by cavity ring-down spectroscopy in a liquid-only cavity

L. van der Sneppen, A. Wiskerke, F. Ariese *, C. Gooijer, W. Ubachs

Laser Centre, Vrije Universiteit, De Boelelaan 1081-1083, 1081 HV Amsterdam, The Netherlands

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Abstract

A previously described liquid-only cavity flow cell is used to assess the feasibility of improving absorbance detection limits in liquid chromatography (LC) using cavity ring-down spectroscopy (CRDS). In this miniaturized cavity there is an optical path length of only 2 mm between the mirrors, which at the same time form the walls of the flow cell. Typical ring-down times are 65–75 ns for the eluent blank. The performance of the presented flow cell compares favorably to conventional absorbance detection: the baseline noise is determined to be 2.7×10^{-6} A.U. using averaging over 1 s. The concentration detection limits are between 15 and 20 nM (injected concentrations) for compounds with a molar extinction coefficient of 1.0–1.4 $\times 10^4$ M^{-1} cm^{-1} at the laser wavelength of 532 nm. The baseline noise as well as the absolute concentration detection limit is lowered by a factor of 30 as compared to measurements with a typical conventional absorbance detector. With an extra band broadening of only 15%, the flow cell is suitable for LC analysis.

Keywords: Cavity ring-down spectroscopy; CRDS; Liquid-only cavity; Absorption detection; Azo-dyes

1. Introduction

Improving the limits of absorbance detection for non-fluorescent analytes by liquid chromatography (LC) is a main challenge in analytical chemistry method development. Several laser-based techniques have been or currently are explored for this purpose (for a review, see Ref. [1]). Such techniques are attractive especially if miniaturization is aimed at since, contrary to lamp emission, a laser beam can easily be focussed to a very small spot without significant loss of power. It should be noted, however, that conventional absorbance detection is based upon the measurement of a small intensity difference on a large background signal, so that the sensitivity is determined by the accuracy with which $\frac{\Delta I}{I}$ can be measured. Rather than a high-intensity light source, a stable source is required. Other absorbance detection schemes, in which a signal is measured against a zero-background are being explored.

For example, degenerate four-wave mixing is based upon the production of a thermal grating in an absorbing sample using two laser beams. A third laser beam is refracted off this thermal grating, generating a fourth laser beam with an intensity depending on the absorbance of the sample. When applied as a chromatography detector [2,3], turbulence caused by the flow distorts the thermal grating, which puts limitations on this technique.

In thermo-optical absorbance measurements [4,5], absorbance of the pump beam gives rise to a temperature increase in the sample that is proportional to both the absorbance and the pump laser intensity. A second beam, which is scattered off this so-called thermal lens, is utilized to evaluate the magnitude of the absorption. A disadvantage of this technique, which has successfully been applied as a chromatography detector [6,7], is that all possible mechanical vibrations in the set-up should be eliminated.

Recently, a start was made with the implementation of cavity ring-down spectroscopy (CRDS) as an absorbance detector for LC [8,9]. While CRDS is a well-established technique for gas-phase studies [10,11], its application to the liquid phase has only recently gained interest [12–17]. In principle, CRDS offers extremely high sensitivity due to its inherent multi-pass configuration. Furthermore, while the sensitivity of conventional or the aforementioned laser-based absorption techniques is ultimately determined by the stability of the light source, CRDS is based

* Corresponding author. Tel.: +31 20 5987524; fax: +31 20 5987543.
E-mail address: ariese@few.vu.nl (F. Ariese).

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on measuring the decay rate of light that is stored within a stable high-finesse cavity after abruptly terminating the excitation beam, thus higher sensitivities can be obtained. After switching off the excitation beam, the light intensity as measured behind the cavity will decay over time t according to:

$$I(t) = I(0)\exp\left[-[(1-R)+(\alpha+S)L]\frac{ct}{nL}\right] \quad (1)$$

where α denotes the absorption by the analyte, S the absorption and scatter losses introduced by the solvent (both in cm^{-1}), R the reflectivity of the mirrors, c the speed of light, L the path length in the cavity and n is the refractive index of the medium. Of course a more complex equation applies if the absorbing medium does not completely fill the cavity length, as in the design by Refs. [8,9]. Fitting the resulting decay traces to the function $I(t) = I(0)e^{-t/\tau}$ yields values for the ring-down time τ when an absorber is present in the cavity, or τ_0 for an empty cavity (i.e. blank solvent only). Losses due to the mirrors and absorption and scattering by the solvent are comprised in the latter value.

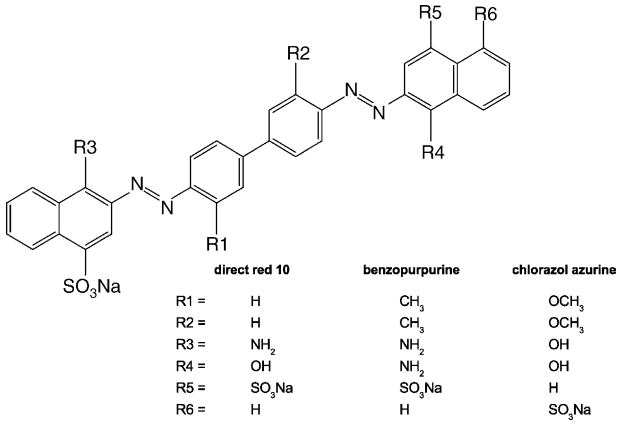
Exploratory studies indicate that CRDS is promising for detection in LC [8,9]. In these studies, a liquid flow cell that was carefully designed to approach the correct Brewster's angle at each of the four interfaces, was placed inside a cavity. Although the effective path length of the cell was only 0.3 mm, the set-up employed a 1 m cavity. This large mirror separation ensured that the decay transients were significantly longer than the laser pulses and the response time of the detection system.

As an alternative, one could develop a set-up in which there is only liquid between the mirrors. This has been explored by Hallock et al. for a cell of large dimensions [13]. In our previous study [18], the feasibility of applying this approach to a μl-sized flow cell was tested using flow injection measurements. This approach has some fundamental advantages: there are no losses due to scattering of additional surfaces in the cavity. Furthermore, since no Brewster's angles have to be considered, a large range of different eluents as well as gradient elution should be compatible with this configuration. Since τ is very short due to the small mirror separation, a repetition rate of several MHz followed by signal averaging could in principle be compatible with the proposed set-up. The disadvantage of the short decay time is that accurate determination of τ is more difficult. Short laser pulses and a fast read-out system are required, and the instrumental response time should be kept as short as possible in comparison to the decay.

This paper shows that the second approach can also be successfully implemented as an LC detector. With our liquid-only cavity which has an optical path length of 2 mm and a volume of 12 μl, we report detection limits that are significantly lower than those achieved with a conventional absorbance detector provided with a U-shaped flow cell.

2. Experimental

The performance of CRDS as a detector for LC separations was tested with a mixture of azo dyes (direct red 10, benzopurpurine, and chlorazol azurine, all obtained from Sigma–Aldrich)



	direct red 10	benzopurpurine	chlorazol azurine
R1 =	H	CH_3	OCH_3
R2 =	H	CH_3	OCH_3
R3 =	NH_2	NH_2	OH
R4 =	OH	NH_2	OH
R5 =	SO_3Na	SO_3Na	H
R6 =	H	H	SO_3Na

Fig. 1. Structures of the dyes in the test mixture: direct red 10 (FW = 697.66 g/mol), benzopurpurine (FW = 724.73 g/mol) and chlorazol azurine (FW = 758.70 g/mol).

of which the structures are shown in Fig. 1 and the absorption spectra in Fig. 2. All three have an extinction coefficient in the range of 1.0–1.4 × 10^4 M^{-1} cm^{-1} at the laser wavelength of 532 nm, no fluorescence was observed for these compounds. The LC separation was carried out isocratically, the eluent was 50% (v/v) 10 mM potassium phosphate buffer (pH 7.4) in HPLC-grade methanol. The flow rate was set to 0.8 ml/min with an Applied Biosystems 400 solvent delivery system; 50 μl of sample was injected using a six-port injection valve. The column was a Chromsep Microspher (Varian) C_{18} 100 mm × 4.6 mm (length × internal diameter) reversed phase column equipped with a guard column. For comparison, the same separation has been performed using a conventional UV–vis absorbance detector (Separations, Applied Biosystems 759a, 8 μl U-shaped flow cell with 8 mm optical path length) that was set to 532 nm. This wavelength, quite appropriate for detection of the dyes concerned (see Fig. 2), was also used in the CRDS measurements. The CRDS set-up was similar to the one described in our previous study focusing on flow-injection [18] and is schematically depicted in Fig. 3. Mirrors ($R \geq 99.996\%$ at 532 nm, 50 mm

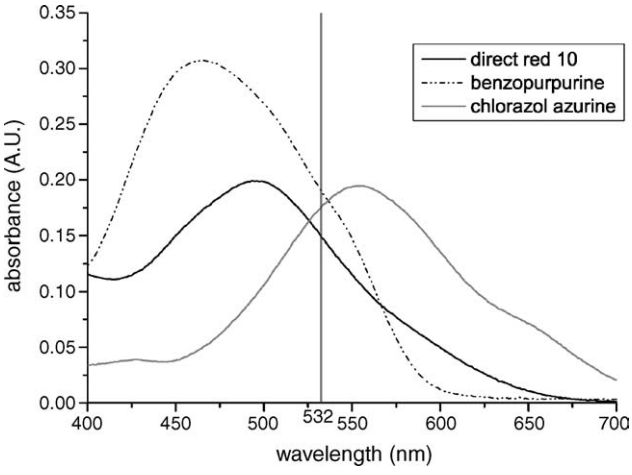


Fig. 2. Absorption spectra of the separate dyes. The concentration of the dyes is 10 ppm in 100 mM aqueous potassium phosphate buffer, pH 7.4.