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Cavity ring-down spectroscopy for detection in liquid chromatography at UV wavelengths using standard cuvettes in a normal incidence geometry☆

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Abstract

Liquid chromatography (LC) with cavity ring-down spectroscopy (CRDS) detection, using flow cuvettes (put under normal incidence inside the ring-down cavity), is demonstrated. Fresnel reflections are maintained within the capture range of a stable cavity of 4 cm length. This method circumvents the need for specific Brewster's angles and possible mirror degradation is avoided. The flow cuvettes are commercially available at low cost. At 355 nm (the frequency-tripled output of a Nd:YAG laser), the system surpasses the performance of conventional absorbance detectors; the baseline noise was 1.3×10^{-5} AU and detection limits (injected concentrations) were between 40 and 80 nM for nitro-polyaromatic hydrocarbons with an extinction coefficient ε of $7.3–10.2 \times 10^3$ M^{-1} cm^{-1}. The system was also tested at 273 nm, but in the deep UV the reflectivity of the currently best available mirrors ($R \geq 99.91\%$) is still too low to show a significant improvement as compared to conventional UV–vis detection.

Keywords: Cavity ring-down spectroscopy; Liquid chromatography; Detection method; Flow cuvettes; Nitro-polyaromatic hydrocarbons

1. Introduction

Liquid chromatography (LC) is used in many different research areas, often with UV absorbance detection. Especially for low concentrations or poorly absorbing analytes, the improvement of absorbance detection limits remains a relevant task.

Cavity ring-down spectroscopy (CRDS) is a laser-based absorption technique which owes its high sensitivity (i.e., low minimum detectable absorbance) to the multi-pass effect and the independence towards light source intensity fluctuations [1,2]. A stable optical cavity is built with high-reflectivity mirrors, in which an injected laser pulse will typically make thousands of round-trips. The effective path length through the sample can be as large as meters or even kilometers. Furthermore, instead of light intensity differences, the rate of decay of the light is measured separately after each pulse. Thus, light source fluctuations play an insignificant role. In recent years, CRDS has become an accepted method for trace measurements in the gas phase, but substantial progress has also been made in applying CRDS to the liquid phase [3–9] and developing it as an LC absorption detector [10–14].

Xu et al. measured a weak overtone of liquid benzene between 588 and 613 nm with one or two cuvettes placed inside a resonator; Fresnel reflection losses were minimized by putting the cuvettes under Brewster's angle [3]. Alexander studied reaction kinetics of nitrate radicals with terpenes using the same geometry [7]. Hallock et al. studied reaction kinetics of Methylene Blue using a different approach: in this study, the mirrors were brought in contact with the liquid, forming the outer boundaries of a large-volume flow cell [4,6]. In the group of Loock, fiber-loop ring-down spectroscopy has been applied to the liquid phase [5,8]. Whereas the sensitivity of this approach is still limited, these fiber-loop ring-down devices may nevertheless become promising tools in remote sensing of liquids. Fiedler et al. built a broadband (about 200 nm) cavity enhanced absorption spectrometer, employing cuvettes in which the earlier mentioned weak overtone in liquid benzene was remeasured [9].

In developing CRDS as a detection method for LC, of course the flow cell volume and design is of crucial importance. Furthermore, emphasis should be on features like ease-of-operation and robustness of the system as a whole. Different geometries have been described in the literature [10–14]. Reflection losses can be

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minimized by putting a suitable flow cell under Brewster's angle inside a ring-down cavity, and the group of Zare successfully used this approach to enhance the performance of absorption detection in LC, both for pulsed [10] and continuous-wave [11] CRDS. Their flow cell, with a path length of 300 μm through the liquid, was designed to have the correct Brewster's angle at both the air–glass and the glass–liquid interfaces. For LC measurements with continuous-wave CRDS detection at 488 nm and using a cavity length of no less than 1 m, they typically obtained ring-down times of several μs and a baseline peak-to-peak noise of 2×10^{-7} absorbance units (AU = εCl as in the Lambert–Beer law) [11].

Another approach is to use the full cavity length for measurements by designing a flow cell in which the outer boundaries are formed by the cavity mirrors [4,6,12–14]. No surfaces or Brewster's angles inside the cavity need to be considered, and a large range of eluents with different refractive indices can be used. This is advantageous from an ease-of-operation and system robustness point of view. In such a methodology, the cavity length has to be made small (in order to have a μl-sized flow cell, compatible with LC analysis) and therefore the ring-down times are much shorter. Such short ring-down times do not allow for very accurate determination of the decay rate and hence limit the minimum detectable absorbance. However, although not practiced yet, the repetition rate of the laser and detection system can in principle be increased up to the MHz-range for extra signal averaging.

Previously, a liquid-only cavity flow cell in which the mirrors are clamped leak-tight around a 2-mm thick silicon rubber spacer has been tested extensively [12–14]. The spacer, with an elliptical hole of 3.9 mm length and 1.6 mm width, acts as a 14-μl flow cell, and flow is introduced via capillary tubings inserted through the sides of the spacer. Using a tunable pulsed laser source set at 457 nm and 100 Hz, a baseline peak-to-peak noise of 2.7×10^{-6} AU was obtained. Since the path length in this system was larger than in refs [10,11] (2 mm, i.e., the full cavity length, compared to 300 μm) absolute concentration detection limits were better than those reported by Bechtel et al. [11].

When solid surfaces (e.g., cuvettes or flow cuvettes) are placed inside a ring-down cavity, care is usually taken to minimize any possible reflection losses using the Brewster's configuration. However, when the cavity length is sufficiently short in order to preserve any reflections off intra-cavity surfaces, a normal incidence geometry can also be used [9,15–19]. As illustrated by Fiedler et al. [9], multi-mode excitation of a cavity gives rise to a certain angular distribution of the light beam, and a Brewster's angle cannot accurately be defined. Actual losses can therefore be larger than the calculated Fresnel reflection losses (which should be negligible at Brewster's angle). Instead of minimizing reflection losses, it can be advantageous to capture the surface reflections on the normal of both sides of the cuvette inside a small, stable cavity. Reflection losses on the inside of the cuvette (where the liquid touches the cuvette surface) are much smaller due to the smaller difference in refractive indices.

In the present paper, we explore whether – using an isocratic LC separation of a test mixture of nitro-poly-aromatic hydrocarbons (PAHs) as a model system – standard flow cuvettes can be used in LC-CRDS in a straight-forward normal incidence configuration, and whether they allow application in the ultraviolet region; for this purpose laser lines at 355 and 273 nm are involved. A major advantage of the zero degree geometry is that there is no need for a carefully designed, custom-made flow cell. Since there are no Brewster's angles to be considered and the liquid is not in direct contact with the mirrors, a large range of eluents with different refractive indices, including more aggressive liquids such as acids, could be used.

2. Experimental

The separation of the nitro-PAHs was carried out isocratically, the eluent was 80% methanol, 20% Milli-Q water. Benzopurpurine and the nitro-PAHs (2-nitrofluorene, 1-nitropyrene and 6-nitrochrysene, see Table 1) were obtained from Sigma–Aldrich (Munich, Germany). The flow rate was set to 0.8 ml/min with an Applied Biosystems 400 solvent delivery system; 50 μl of sample (dissolved in methanol) was injected using a six-port injection valve. The column was a Chromsep Microspher (Varian, Middelburg, The Netherlands) C18 100 mm × 4.6 mm (length × internal diameter) reversed phase column equipped with a guard column. For comparison measurements, we used a Kratos spectroflow 757 UV–visible absorbance detector with an 8-mm path length and 12-μl volume (Applied Biosystems, Foster City, CA, USA), set at the laser wavelength of the CRDS measurements. Steady-state measurements were performed by adding 5-μl aliquots of a 1-ppm aqueous benzopurpurine solution to a standard quartz, 10 mm × 10 mm cuvette filled with 2 ml Milli-Q water.

Quartz cuvettes and flow cuvettes, with a surface roughness of less than 0.2 μm and a parallelism of the windows better than 0.01 mm were obtained from Hellma, Müllheim, Germany. The cuvettes were mounted on a mirror mount permitting separate alignment in the previously aligned cavity. The flow cuvette used in the LC measurements had a volume of 80 μl, a path length of 10 mm and an aperture diameter of 3 mm. Using chromatograms measured with the UV–visible absorbance detector with and without the CRDS flow cuvette on-line, the extra band-broadening introduced by the CRDS flow cuvette was determined to be negligible (around 5% for 1-nitropyrene).

For the CRDS measurements (see Fig. 1), no mode-matching was performed, ensuring multi-mode excitation of our cavities (that are not temperature controlled or locked to a specific laser mode). Transients were recorded using a photomultiplier tube (Hamamatsu, Shimokanzo, Japan) and a fast sampling oscilloscope of 1 GHz analog bandwidth (Tektronix 5104 5 GS/s).

Table 1
Formula weights (F.W.) of the various test compounds and their extinction coefficients at the applied laser wavelengths

Compound	F.W. (g/mol)	ε_{273} (M^{-1} cm^{-1})	ε_{355} (M^{-1} cm^{-1})
Benzopurpurine	724.73	32×10^3	15×10^3
2-Nitrofluorene	211.22	2.7×10^3	10×10^3
1-Nitropyrene	247.26	11×10^3	8.7×10^3
6-Nitrochrysene	273.29	28×10^3	7.3×10^3