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Miniaturized Cavity Ring-Down Detection in a Liquid Flow Cell

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A novel method for applying cavity ring-down spectroscopy in the liquid phase, compatible with LC analyses, is presented. The core of the setup is a home-built cavity ring-down flow cell (cell volume 12 μL) that is constructed using a silicon rubber spacer, which is clamped leak-tight between two high-reflectivity mirrors. The mirrors are in direct contact with the liquid flow, which provides for a small path length and short ring-down times. Inside the cavity there are no windows, reflection losses, or Brewster angles to be considered. Due to the small size of the presented cavity geometry, the setup can be implemented in conventional-size LC apparatuses. With a flow injection setup, a detection limit of 2.5 nM was obtained for Crystal Violet in ethanol, and the linear dynamic range of the system is at least 2 orders of magnitude. The method has the potential to become a powerful alternative for commercial LC UV/visible absorbance detectors.

In liquid chromatography (LC), direct optical absorption is the generic and commonly used method to detect absorbing analytes and their concentration in eluting compounds. Almost any analyte absorbs light in the visible or ultraviolet range, and standard absorption detection shares the advantages of simplicity and versatility. Since direct absorption techniques are based on monitoring attenuation of light intensity, they are fundamentally limited by the intensity fluctuations of the light sources employed, where currently $\Delta I/I$ values of 5×10^{-5} are reached.¹ An option for improving the limit of detection (LOD) in LC lies in the increase of the optical path length without altering the detector cell volume, and therefore, Z-shaped cells of typically 8-mm optical path length and 1-mm diameter are commonly used. With the use of liquid core waveguide flow cells, optical path lengths as long as 30–50 cm can be obtained,^{2,3} but the number of applications is still limited. Considering the broad applicability of absorption detection in LC, it is highly relevant to improve sensitivity and detection limits, but at the same time, the chromatographic restrictions of LC should be complied with. In view of chromatographic band-broadening effects, even in conventional-size LC the detector cell volume should be limited to

typically 20 μL , while for micro- and especially nano-LC, the constraints on detection volumes are obviously much more serious.

The invention of the cavity ring-down (CRD) detection technique⁴ has introduced an elegant manner for improving sensitivity in direct absorption. CRD is based on the injection of a short laser pulse into an optical resonator with high-reflectivity mirrors, followed by the detection of the light leaking away through a mirror. Since its principle is based on measuring the rate of decay of an optical cavity, CRD in effect circumvents the common limitations of absorption measurements: the decay time is independent of the intensity of the light pulse exciting the optical resonator, and hence, pulsed laser sources with pulse-to-pulse intensity fluctuations of $\Delta I/I$ in excess of 10^{-2} can be used without problem. Furthermore, the use of cavities in CRD inherently increases the interaction path length, since most of the light is detected after hundreds or thousands of round trips. Numerous applications in gas-phase spectroscopy and dynamics, using the original pulsed variant of CRD, as well as cavity-enhanced and cavity phase-shift variants, have been reported.⁵ The extension to evanescent-wave absorption outside an optical resonator has shown promising applications for sensitively detecting films of solid^{6,7} or liquid material.⁸

Application of CRD to detection in the liquid phase is still in its infancy, but at the same time, the first reports bear great promise. Xu et al. demonstrated CRD detection for a study of weak overtone absorption in bulk benzene with an extended cell placed at Brewster angle inside a resonator to minimize Fresnel reflection losses.⁹ A breakthrough, bringing CRD in the realm of LC, was established through the work of Snyder and Zare,¹ who designed a cell aligned inside a CRD resonator, having a double Brewster configuration to reduce reflection losses at both the air–glass and the glass–liquid interfaces. Their design involves an optical path length of 300 μm through the liquid flow within a resonator of 1 m, and ring-down transients of several microseconds are measured. Analysis of variations of the typical decay times yielded a LOD of 92 nM (at $\epsilon = 9 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) for LC measurements in a cell volume of 10 μL (of which 0.5 μL illuminated). Hallock

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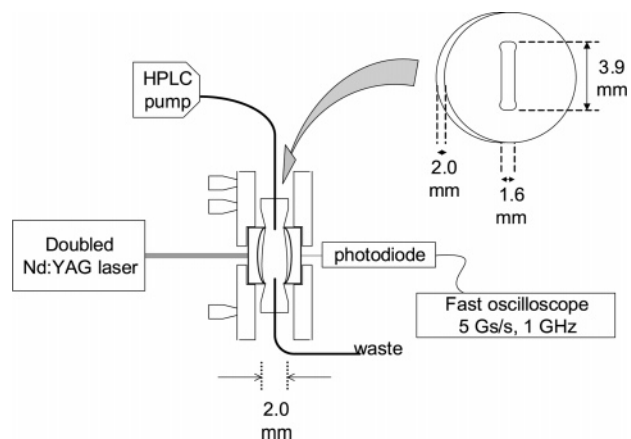


Figure 1. Schematic diagram of the flow cell (not to scale) and the setup. The outer boundaries of the cavity ring-down flow cell are formed by the concave mirrors, pressed leak-tight to the sides of a silicon rubber spacer. Flow is introduced via capillary tubing (shown as thick black lines) inserted in the spacer, shown in detail at the upper right.

et al. demonstrated that the use of refractive index-dependent Brewster-faced cells can be circumvented, since in some cases the liquid can be brought in contact with dielectrically coated mirrors.¹⁰ In their study on bulk liquids, a single-pass path length of 20 cm was used and typical ring-down times of 200–400 ns were recorded.

In the present study, we report on a miniaturization of the thin flow cell setup of Snyder and Zare¹ by forming a miniresonator with two mirrors at a typical separation of 2 mm in contact with the fluid as in ref 10. Whereas the flow cell as suggested by Hallock et al.¹⁰ is not suitable for HPLC detection purposes, the presented flow cell with a volume of 12 μL meets the chromatographic demands on the detector cell volume. For the analysis of small variations in the resulting optical transients, special care has to be taken to minimize the response time of the optical detection system. Our current limit of detection is estimated to be comparable to that of ref 1 in an experiment with flow injection, using Crystal Violet (CV) dissolved in ethanol. Several options to further increase the sensitivity of the miniature setup are envisioned and will be discussed.

EXPERIMENTAL SECTION

The design of our miniature cavity ring-down flow cell, built from two highly reflective mirrors separated by 2 mm, is schematically depicted in Figure 1. The mirrors, which are in direct contact with the liquid, are sealed in 10-cm-diameter plate holders equipped with micrometer screws for cavity alignment. A 2-mm-thick silicon rubber spacer determines the single-pass cavity length, while a hole in the spacer forms the flow cell (volume 12 μL). The shape of the hole is as elliptic as possible to sustain laminar flow inside the cell in order to minimize unwanted LC band broadening. The laser beam of maximum 1.6-mm diameter is directed through the flow cell and illuminates a volume of $\sim 4 \mu\text{L}$. Fused-silica capillary tubing (external diameter 0.32 mm, internal 0.25 mm) is inserted in the spacer in order to introduce the liquid flow into the cell.

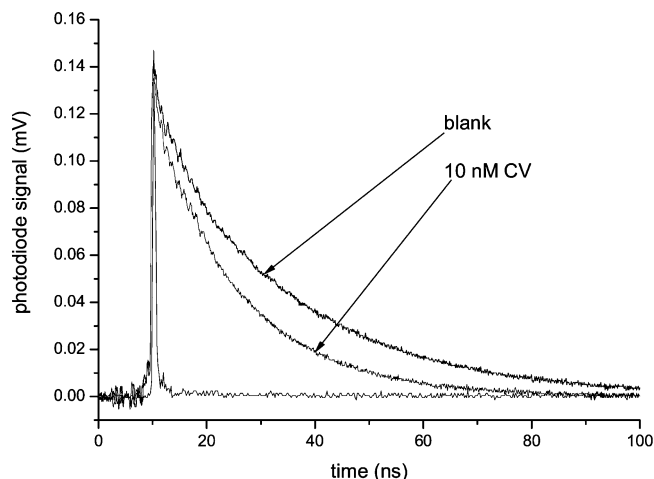


Figure 2. Cavity ring-down signal as measured on the oscilloscope for pure ethanol in flow (blank; upper curve) and after injection of 100 μL of a 10 nM CV–ethanol solution (lower curve). Also displayed is the temporal response curve of the detection system.

In view of the short single-pass optical path and the resulting short ring-down times, the instrument response function must be kept as short as possible. For this purpose, we use laser pulses of 100-ps duration from a Q-switched, mode-locked, and frequency-doubled (532 nm) Nd:YAG laser (Quante), a fast sampling oscilloscope of 1-GHz analog bandwidth (Tektronix 5104 5 Gs/s), and a fast 4-GHz bandwidth photodiode (PHD400, Becker & Hickl). In combination, they yield a response time of ~ 600 ps fwhm, negligible with respect to typical decay times observed of 20–30 ns. Laser pulses of 0.15 mJ incident pulse energy are coupled into the cavity at a repetition rate of 10 Hz, limited by the laser. With highly reflective coated mirrors (REO Inc., Boulder, CO) having radius of curvatures (roc) of 50 mm the miniature cavity is easily aligned, in contrast to mirrors with roc = 20 or 50 cm.

All measurements are performed in flow on the test compound CV (color index 42555, 98% Aldrich Chemical, Milwaukee, WI) dissolved in ethanol (ethanol absolute $\geq 99.5\%$, Baker). The solvent is degassed by sonication prior to use; the extinction coefficient for CV in ethanol at 532 nm is determined at 54 500 $\text{M}^{-1} \text{cm}^{-1}$. Flow (flow rate 1 mL/min) is introduced with an LC pump (Gynkotek Separations 300) combined with a six-port valve equipped with a 100- μL injection loop. Dilute solutions of CV in ethanol are injected in the continuous flow of pure ethanol for the CRD detection measurements.

RESULTS AND DISCUSSION

Figure 2 displays some typical decay transients as recorded in pure ethanol and in a 10 nM CV–ethanol solution after injection of a 100- μL plug; the displayed transients are taken at the time window when the analyte plug has reached the flow cell. No signal averaging is applied. The fast transient corresponds to the photodiode signal of a laser pulse directly measured and represents the instrument response function. All transients are registered by the oscilloscope at a maximum sampling rate of 5 Gs/s, so that 500 data points are available on a 100-ns decay transient (typically corresponding to 3τ). $1/e$ Decay times τ are extracted

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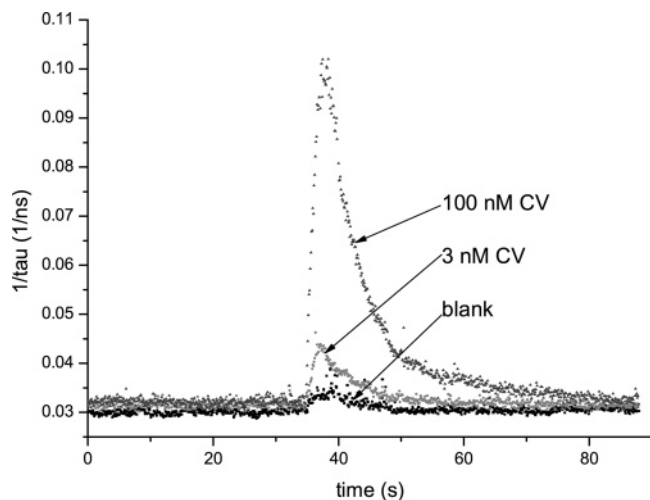


Figure 3. Recording of a time trace representing continuously monitored ring-down events after injection of an absorber in a flow of ethanol. From bottom up: blank; 3 nM CV; 100 nM CV.

from the transients by fitting to a single-exponential decay. The ring-down signal is represented by the following function:

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

where α_{anal} denotes absorption by the analyte and α_{solv} absorption and scattering by the solvent (both in cm^{-1}). R is the reflectivity, c is the speed of light, t is the time, L is the path length in the cavity, and n is the refractive index of the medium. Fitting yields values for τ , the decay time with the analyte sample present, and for τ_0 , the decay time of the background, which includes effects of the limited mirror reflectivity and all absorption and scattering effects of the solvent. The absorbance due to the analyte then follows from

$$\alpha_{\text{anal}} = 2.303\epsilon C = (n/c)\left[\frac{1}{\tau} - \frac{1}{\tau_0}\right] \quad (2)$$

where ϵ is the molar extinction coefficient (in $\text{M}^{-1}\text{cm}^{-1}$) and C the concentration (in M). We note that a $1/e$ decay time of 25 ns, as typically observed, corresponds to an effective absorption path length of 5.5 m. This explains the gain in sensitivity by the CRD method; in a $1/e$ time interval, the light pulses traverse the cavity 2750 times.

The fitting procedure is performed on-line (at 10 Hz) without loss of events, such that absorption in the flow cell can be monitored constantly. This allows for an on-line registration of time traces, during times when an injected plug is passing the CRD flow cell. Typical results of the absorption (proportional to $1/\tau$) in such time traces are presented in Figure 3 comparing injected plugs of a blank, a 3 nM, and a 100 nM CV–ethanol sample. The figure shows that at higher concentrations only minor band broadening and limited tailing is observed, even less than usually dealt with in conventional-size LC.

A calibration curve was measured over a concentration range of 1–100 nM CV in ethanol. The integrated signal on the $1/\tau$ traces is plotted as a function of concentration in Figure 4. The

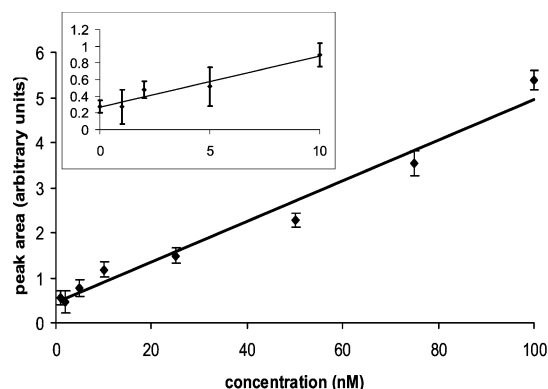


Figure 4. Calibration curve for CRD detection of CV in ethanol. In each case, a 100- μL plug is injected at a flow rate of 1 mL/min; the area under the peaks in the $1/\tau$ traces is plotted versus concentration. Each data point represents an average over three measurements, with the error bar showing the standard deviation. The inset shows the measurements of the low CV concentrations in detail. For this measurement series, each data point is the average of at least six measurements, the error bar again shows the standard deviation.

R^2 value for this calibration curve was 0.973 (for 8 points, each measured in 3-fold), which indicates a satisfactory linear dynamic range over at least 2 orders of magnitude. For a 100 nM solution of CV, the absorbance over a distance of 5.5 m (corresponding to $1/\tau$) is 0.3. Since deviations from the Lambert–Beer law are normally not seen for an absorbance lower than 1, no saturation effects are expected to take place.

The detection limit for a signal-to-noise ratio of 2 (2 times the standard deviation of the blank, $n = 5$) is determined at 2.5 nM for CV–ethanol solutions with $\epsilon = 54\,500 \text{ M}^{-1} \text{ cm}^{-1}$ at 532 nm. It should be noted that this LOD is primarily a result of the rather large blank signals obtained; injection plugs of pure ethanol produce time traces deviating from background as shown in Figure 3. This relatively high blank signal cannot be attributed to carryover of CV remains, since the replacement of all possible contamination sources did not result in a lower blank. The LOD obtained with the setup of Snyder and Zare¹ is comparable to ours; their LOD value of 92 nM at $\epsilon = 9000 \text{ M}^{-1} \text{ cm}^{-1}$ may be scaled to CV–ethanol solutions at 532 nm ($\epsilon = 54\,500$) resulting in a LOD of 15 nM. The factor of 6 difference can be explained by their cell path length of 300 μm , which is a factor of 7 shorter. The setup of ref 1 (with a flow cell of 10 μL of which 0.5 μL illuminated) has proven to be suitable as an LC detector. Similarly, the present demonstration using injected plugs of dissolved analytes into a real flow cell closely mimics LC conditions.

In principle, there is no difference in terms of sensitivity between a CRD cavity of length $L = 1 \text{ m}$ (as in the case of ref 1) with a short flow cell (of $d \text{ mm}$) inserted, or a geometry where $L = d$ (in the present case) as long as the absorption solely occurs in the analyte. Indeed similar sensitivities are found. A practical difference lies in the time of duration of the decay transients, which in the present case is as short as 100 ns for 3τ analyzed transients, much shorter than in ref 1. This has the advantage, not yet benefited from with the 10-Hz laser repetition rate, of scaling the experiment to a multiple MHz repetition rate, with the prospect of increasing the sensitivity via signal averaging.

The use of a miniature flow cell formed by mirror surfaces, bringing the eluent directly in contact with the dielectric coating,

warrants some discussion on its behavior under operating conditions. Ring-down times of 25–30 ns, obtained in air, represent mirror reflectivities of $R \approx 99.98\%$. The ring-down times are found to be slightly shorter (typically 20%) for the mirrors in contact with liquid ethanol for the same cavity geometry. Considering the refractive index ($n = 1.36$) for ethanol, however, a larger decay time is expected (see eq 1).

Such a decrease of the ring-down times could be explained by several effects. Higher overtones of the $-OH$ vibrations in ethanol (fundamental $\sim 3350\text{ cm}^{-1}$) are expected to cause absorption in the visible range. For example, the attenuation coefficient for water at 532 nm is $0.67 \times 10^{-3}\text{ cm}^{-1}$, including also Rayleigh scattering.¹¹ A possible additional effect is caused by lowering of the mirror reflectivity when liquid is brought into contact with the surface layer of the dielectric multilayer coating. We note that the mirrors did not degrade significantly after extended use of the CRD mirrors in contact with ethanol.

CONCLUSION

In this study, a new approach to implementing cavity ring-down absorption measurements in the liquid phase is presented. In this approach, the liquid is in direct contact with the cavity mirrors, but in comparison with ref 10, the cell volume has been reduced by more than 3 orders of magnitude, making the setup fully compatible with LC. Despite the inherently short decays (ns instead of μs range), the sensitivity of the method is satisfactory, and our measurements in a liquid flow are as sensitive as

previously reported measurements.¹ A considerable advantage of the system is that there are no surfaces in the cavity itself. Scattering losses are therefore minimal, and there are no Brewster's angles to be considered. Consequently, a large range of different eluents (with different refractive indices) can be successfully used. Furthermore, the short decay time ensures that the system can be improved, using higher repetition rate lasers (up to several MHz) and subsequent averaging.

A 3 nM injection plug of CV in a continuous flow of ethanol could clearly be distinguished. When the peak area under the $1/\tau$ traces is measured as a function of concentration, a linear relationship is observed; the linear dynamic range is at least 2 orders of magnitude. Considering its linear response and small size, the presented cavity geometry can be implemented in conventional-size LC. The band broadening that is observed when measuring in a flow is negligible.

A major challenge lies in the extension of sensitive CRD detection techniques to the ultraviolet range, where most analytes have strong(er) absorptions.

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