Capillary-scale polarimetry for flowing streams

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A micro-polarimeter with a 40 nl probe volume was configured so that it is compatible with capillary-scale flowing stream analysis. The optical configuration consists of two polarizing optics, a capillary, a laser source and a photodetector which is very simple to configure with low cost components. This unique polarimeter is based upon the interaction of a linearly polarized laser beam and a capillary tube, in this case one with an inner diameter of 250 µm. Side illumination of the tube results in a 360° fan of scattered light, which contains a set of interference fringes that change in response to optically active solutes. Solutes that exhibit optical activity are quantifiable and are detected by analyzing the polarization state of the backscattered light. The ability of the instrument to make extremely sensitive optical activity measurements in flowing streams is shown by the determination of (R)-mandelic acid, with a detection limit of \(66 \times 10^{-6} \text{M} (507 \times 10^{-12} \text{g})\), and the non-optimically active control, glycerol. Additionally, the detector was configured to minimize refractive index perturbations.

Introduction

Considering the miniaturization trend in analytical chemistry and the desire to reduce solvent consumption, small sample size requirements and reduced analysis costs for sample evaluation, a polarimeter compatible with capillary-scale analysis schemes is desirable. Even though polarimetry is employed in many identification and purity applications, there has been very little success in developing a sensitive sub-microliter volume polarimeter. Many attempts have been made to incorporate polarimetry detection with small volume analysis. Such attempts include the work of Yeung et al. and Mayster et al. PDR-CHIRAL have made the most recent advance in developing a small volume polarimeter, which appears to be a commercial variation of Yeung’s previous work. This system has been shown to have detection limits at the 10 µM level for most solutes when employed as a detector for high performance liquid chromatography.

Although performance improvements have resulted from these efforts, low signal-to-noise ratios, large-volume flow cells and complex optical arrangements still limit the use of polarimetry. Generally, the flow cells employed to date have remained far too large for capillary-based separation techniques. Yet, in the recent past, Hankins and Bornhop demonstrated that polarimetry can be performed at nanogram levels in nanoliter volumes by modifying the micro-interferometric backscatter detector. Although the capillary polarimetric detector (CPD) could probe nanoliter volumes, it was still limited in that only relative changes in optical activity could be measured; it was incapable of distinguishing between R and S enantiomers, and image processing was needed to extract the polarimetry signal from the fringe pattern, preventing the detector from being used with flowing stream analysis. Here we show that by combining aspects of CPD and conventional polarimetry, capillary-scale flowing stream analysis can be performed for the first time using the micro-polarimeter.

Experimental

Fig. 1 shows a block diagram of the experimental configuration of the micro-polarimeter flow stream analysis and flow injection analysis schemes. As described in detail elsewhere, the micro-polarimeter has an optical train consisting of a 4 mW linearly polarized He–Ne laser (Melles Griot), a Polcor polarizing plate (Corning Optics, Corning, NY, USA) with an extinction ratio of 10 000:1, an unconditioned fused silica capillary (Polymicro Technologies) (id 247 µm, od 348 µm and a polyimide outer coating of 17 µm), a Glan Thompson polarizer and a photodetector. The capillary was mounted on a thermostated aluminum block secured to a micrometer driven translation stage located 60 cm from the laser aperture. Thermal control of the flow cell was achieved with a Peltier cooler (Melcor) and a 4 A, 16 W thermistor-based temperature controller (ILX Lightwave, Bozeman, MT, USA). The Glan Thompson polarizing crystal, acting as the analyzer crystal, was mounted in a micro-calibrated rotation stage (Newport, Irvine, CA, USA) possessing 0.1° resolution. Illumination of the capillary by the laser was done by directing the unfocused laser beam onto the tube. The second fringe left of the centroid of the backscatter interference pattern was analyzed with a photodetector mounted on the same translation stage and directly behind the analyzer crystal.

The active area of the silicon photodetector (44 mm²; PIN 44D, UDT Sensors) was larger than the selected fringe, allowing the entire fringe of interest to be interrogated. The output of the photodetector was amplified (gain = 10) with a low noise pre-amplifier (Standford Research Systems) and then converted to a digital signal using a data acquisition board (PPIO-AIO8, Cyber Research). Temporal display of the signal was done using a PC running a digital strip-chart recorder (Labtech).

![Fig. 1 Block diagram of the micro-polarimeter. PP, polarcor polarizer; FC, temperature controlled flow cell; SP, syringe pump; GT, Glan Thompson polarizer; PD, photodetector; PA, low noise pre-amplifier; DAC, data acquisition board; PC, personal computer.](image)
Solutes, both optically active and non-optically active [(R)-mandelic acid and glycerol in the concentration ranges 0–5 and 0–7.67 mM, respectively], were evaluated under both constant flowing and flow injection analysis (FIA) conditions. The constant flowing system consisted of a syringe pump (Model 22, Harvard Apparatus), a stream of water at a flow rate of 150 μL min⁻¹ and a capillary 45 cm in length. For the FIA experiment, a sub-microliter (0.5 μL) injection valve (Valco) was inserted between the syringe pump and the flow cell. The capillaries connecting the syringe pump to the injection valve and the capillary running from the injection valve to the flow cell were 24 and 28 cm, respectively. A flow rate of 50 μL min⁻¹ was used for the FIA experiments. In all experiments, the flow cell was thermostatted at 25 °C.

Results and discussion

The micro-polarimeter’s optical set-up,⁹ was derived from Hankins and Bornhop;⁹ it differs in that the changes in total fringe intensity facilitate quantitative optical activity measurements. In this configuration, capillary-scale, flowing stream analysis can be performed using the micro-polarimeter, for the first time in polarimetry. However, here the signal is obtained using a slightly different method than originally described.⁹ For flowing stream investigations, it is convenient to fix the transmission axis of the analyzer plate at 45° with respect to the polarization state of the interrogated fringe. In this orientation, when an optically active solute is introduced into the probe volume, a change in intensity (voltage) should result as the plane-polarized light is rotated in either the left or right direction. The polarimetry signal in this configuration is based on changes in fringe intensity, hence the active area of the photodetector must be larger than the size of the fringe being analyzed. Since changes in intensity indicate the presence of optically active solutes, no change in output voltage should be observed for non-optically active solutes as long as the fringe remains on the surface area of the detector.

The response of the micro-polarimeter to optically active solutes under dynamic conditions was first determined by flowing a continuous stream of either the blank or a solution of (R)-mandelic acid through the capillary maintained at 25 °C. The following procedure was adopted: first, using a syringe pump, a stream of flowing water at a flow rate of 150 μL min⁻¹ was induced into the capillary (45 cm in length). The pressure and temperature of the system were allowed to stabilize, which on average took approximately 1.5 min. Detector alignment was then achieved by translating the X–Y translation stage with the analyzer crystal and the photodetector until the second fringe was centered on the active surface area of the detector. Next, the analyzer crystal was rotated about its central axis until the transmission axis of the polarizer was orientated at an angle of 45° with respect to the polarization vector of the backscatter fringe and secured in place for the duration of the experiment. Once alignment was achieved, the output voltage of the micro-polarimeter gathered water for 30 s. Next, one at a time, a series of (R)-mandelic acid solutions (0–5 mM), each prepared in distilled water from the same source as the blank, was continuously flowed through the detector and a signal (relative change in voltage) was obtained for the sample. This concentration range was selected to prevent large-scale refractive index perturbations from shifting the fringe of interest off the active surface area of the photodetector. Therefore, the resultant signal could be attributed to only a change in polarization. Between each sample solution, the capillary was rinsed well with distilled water to eliminate sample contamination. Note that for the analysis of the (R)-mandelic samples, the samples were simply injected and the output from the detector was recorded.

The result of this experiment is shown in Fig. 2. Using the average voltage difference between the blank run and each solution, a calibration curve was generated (Fig. 2) in triplicate, where relative optical rotation signal is plotted versus concentration. As expected, the relative optical rotation signal increased in a linear fashion with increasing solute concentration.

Even though the experimental configuration is relatively simple, this new detector shows good reproducibility and excellent sensitivity. The limit of detection at the 99.9% confidence level was calculated to be 66 × 10⁻⁶ M for (R)-mandelic acid. This is impressive considering that it was obtained using a 250 μm id capillary, an inexpensive laser and photodetector and a probe volume of 40 nL. At the current detection limit, only 507 pg of (R)-mandelic acid is present within the probe volume. For the analysis of a constant stream of sample, the concentration sensitivity of the micro-polarimeter compares well with that of the best, low volume commercially available polarimeters,⁵ yet is achieved in a probe volume three decades smaller.

Since the micro-polarimeter is a derivative of the micro-interferometric backscatter detector (MIBD)⁷,⁸ and the refractive index (RI) and optical activity are inherently coupled, it was necessary to evaluate the response of the detector to changes in refractive index for molecules which are not optically active. In order to confirm that the signal is produced only by changes in optical activity, this test was done by measuring the polarimetric response of the system for the non-optically active solute glycerol. Using a series of six glycerol solutions ranging in concentration from 0 to 7.6 mM, a calibration curve was generated in triplicate (Fig. 3). This particular concentration range was selected given that it was equal to the RI range of the (R)-mandelic acid solutions. Note that the glycerol calibration curve is relatively flat and that the optical activity signal does not increase or decrease appreciably (±0.03 ± 0.01 V) with changes in concentration. This lack of response confirms that the detector is unaffected by moderate

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**Fig. 2** The three trial average calibration curve of polarization signal versus (R)-mandelic acid concentration using flowing stream conditions.

**Fig. 3** The three trial average calibration curve of polarization signal versus glycerol concentration using flowing stream conditions.
RI changes ($\Delta n = 5 \times 10^{-5}$) and only senses change in the polarimetric signal. Hence the flat glycerol calibration curve verifies that the micro-polarimeter can be configured to respond only to changes in optical activity over a limited but analytically useful concentration range.

While monitoring solute detection with flowing streams at a constant concentration, this shows that the micro-polarimeter can be used for the real-time analysis of flowing streams in capillaries (250 $\mu$m id); it is of value to determine the response of the detector for transient events. In an experiment analogous to capillary-scale FIA conditions, one by one a series of ($R$)-mandelic acid solutions ranging in concentration from 0 to 5 mM were studied. A 0.5 $\mu$L plug of each ($R$)-mandelic acid solution was injected into a 50 $\mu$L min$^{-1}$ flowing stream of water while recording the voltage from the photodetector. As with the flowing stream study, the flow cell was thermostated at 25 °C for this experiment. Two traces for such an experiment were taken, one for 5 and the other for 4 mM ($R$)-mandelic acid (Fig. 4). Each trace shows the response of the micro-polarimeter with two injections performed approximately 50 s apart. Note that for both the 4 and 5 mM traces, Gaussian shaped peaks are obtained with the micro-polarimeter, demonstrating that the detector is compatible with capillary-scale FIA schemes. The response of the detector is related only to changes in polarization and not RI since both the samples are within the concentration range where RI signals were effectively eliminated. Further, a three trial average calibration curve was generated for the micro-polarimeter under FIA conditions. The response in peak height versus concentration was highly reproducible and linear ($r^2 = 0.996$; $3.48 \times 10^{-3}X - 2.28$) for the concentration range studied. The limit of detection at the 99.9% confidence level was calculated to be $1.7 \times 10^{-3}$ M for ($R$)-mandelic acid. At the current detection limit, 13 ng of ($R$)-mandelic acid are present within the probe volume. The concentration sensitivity of the micro-polarimeter under FIA conditions is a factor of 19.6 times less than that for the continuous solute introduction mode. This difference in sensitivity is attributed to mixing and solute peak broadening due to the dead volume at the injection valve.

While our preliminary results indicate the applicability of the detector with capillary-scale FIA schemes, further improvements are feasible. First, the incorporation of an electronic filter would increase the signal-to-noise ratio (S/N) of the measurement. Currently, no electronic filters have been used to condition the output signal of the photodetector. Upon close investigation of the baseline, one observes a noise component that would be effectively attenuated with standard high-pass filtering, reducing the low frequency noise in the output and allowing a lower detection limit to be achieved. Second, optimization of the FIA system dead volume to eliminate band broadening and mixing would result in sharper peaks and higher S/N, improving the performance of the detector under FIA conditions. Ultimately, as mentioned before, by slightly modifying the optical configuration of the micro-polarimeter, the dynamic range of the detector could be increased. The implementation of a larger area photodetector or an array detector would help eliminate larger refractive index perturbations and thus increase the dynamic range. Also, it should be possible to enhance the performance of the system as a polarimetry sensor with the incorporation of a refractive index detection arm for real-time RI compensation.

Conclusion

It has been shown that optically active solutes can be quantified in flowing streams within nanoliter volumes using a simple micro-polarimeter. With the current system, the concentration limit of detection for flowing streams is $6.6 \times 10^{-6}$ M for ($R$)-mandelic acid using a detection volume of 40 nL. At this detection limit and using an unmodified capillary of 250 $\mu$m id, the resolution performance of the detector is $10^{-6}$. Further, preliminary results indicated that the micro-polarimeter is compatible with capillary-scale FIA and with a few modifications the performance of the device can be enhanced.

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References


Fig. 4 Flow injection analysis results for 5 and 4 mM ($R$)-mandelic acid. Each trace shows the results of two 0.5 $\mu$L volume injections into a 150 $\mu$L min$^{-1}$ flowing stream of water.