DEVELOPMENT AND ANALYSIS OF A MICROFLUIDIC PHOTOTHERMAL ABSORBANCE DETECTOR USING POLYELECTROLYTIC GEL ELECTRODES

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ABSTRACT

We report on a sensitive photothermal absorbance detection method for use with a microfluidic chip using polyelectrolytic gel electrodes (PGEs). PGEs are microfabricated into glass substrates by photopolymerization, enabling contact measurement of conductivity without suffering redox effects from the application of a high electric field. Photothermal techniques involve heating of the solution through the radiationless relaxation of optically excited states, which subsequently results in a change in the solution viscosity. The microfluidic based photothermal system monitors the solution viscosity by measuring changes in conductivity. Experimental factors including laser modulation frequency, and laser power were studied. In addition, an electrokinetic separation of potassium, DABSYL-tagged glucosamine (DABSYL-Glu) has been demonstrated.

KEYWORDS: Photothermal Absorbance Detection, Polyelectrolytic Gel Electrode, Conductometry

INTRODUCTION

Laser induced fluorescence detection provides unparalleled sensitivity [1]; however, most analytes such as proteins, peptides, and DNA do not have appropriate fluorescence properties required to achieve high detection sensitivity, therefore, tagging is required. Although fluorescent tagging is routinely performed, tagging can often interfere with further analysis techniques, such as mass spectrometry, due to changes in the mass-to-charge ratio of the analyte [2]. Therefore, an alternative detection technique for measuring native molecules would be preferable. UV absorbance is one such intrinsic property that is widely used, but its path length dependence limits its application to longer optical path lengths devices, which are difficult to achieve with most microfluidic devices. However, a photothermal induced conductivity change relies on the temperature induced viscosity change of the sample, which is path length independent [3].

PGEs can conveniently be placed laterally in microchannels, which allows the sensing region to be interrogated orthogonally to the flow without interfering with the probing laser source. The PGEs can be used with DC voltages for measuring the conductivity without redox effects on the electrode surfaces, further simplifying the measurement electronics. This paper describes the development and analysis of a 2-electrode PGE microchip based photothermal absorbance detection system using direct current conductivity measurements.
EXPERIMENTAL

Microchips were fabricated using soda lime glass as the base substrate [4]. Microchannels were 12-μm deep and 55-μm wide. PGEs were fabricated into the microfluidic device, as described previously [5]. Briefly, the microchip was filled with a monomer solution consisting of 65% DADMAC, 2% 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropionophenone and 2% N,N′-Methylenebisacrylamide. Polymerization was initiated by exposure to UV light (365 nm) at 4.8 mW cm"2 for 20 s using an electrode mask to define the PGE dimensions. A 1 M KCl solution was then used to flush the microchip of any remaining monomer. Solution conductivity was measured using a pair of PGEs that were electrically connected to a conductometer via Ag/AgCl electrodes. A 488-nm argon-ion laser was modulated by an optical chopper, and then focused between the PGEs. The laser modulation frequency was referenced to a lock-in amplifier to demodulate the photothermal signal from the conductance signal (Figure 1).

RESULTS AND DISCUSSION

Figure 2 shows an example of the conductance signal and its corresponding photothermal signal. 50-μM DABSYL-Glu/5 mM potassium was used as a sample in a 20 mM 2-morpholinoethansulfonic acid/20 mM histidine buffer solution at pH 6.1. DABSYL-Glu absorbs 488-nm light while potassium does not. After a gated injection of sample, a potassium peak was observed in the conductance signal and the modulated DABSYL-Glu signal followed. In the photothermal signal trace, only the DABSYL-Glu peak was found. This shows an additional advantage of a combined conductivity/photothermal detection technique as analytes that do not absorb light can be differentiated from those that do absorb. The photothermal signal intensity decreased with increasing laser modulation frequency due to insufficient time for solution heating and cooling. However, the overall photothermal signal-to-noise ratio

Figure 1. 2-electrode PGE microchip system for photothermal absorbance measurements. The DC conductometry circuit was connected through Ag/AgCl electrodes. The lock-in amplifier was referenced to the laser modulation frequency to recover the photothermal signal.

Figure 2. A separation of potassium and DABSYL-Glu. The conductivity plot shows the output of the time-dependent conductometry signal and the photothermal plot shows the output of the lock-in amplifier.
(SNR) reached a maximum near 50 Hz, as optical chopper instability increases the baseline noise at lower frequencies (Figure 3). The photothermal signal also showed the expected trend of increasing linearly with increasing laser power (Figure 4).

![Figure 3. Signal-to-noise ratio versus laser modulation frequency.](image1)

![Figure 4. Photothermal signal versus laser power.](image2)

CONCLUSIONS

In this study, a SNR over 18,000 was achieved with 50 μM DABSYL-Glu sample and 366 mW of laser power, giving a limit-of-detection 8.3 nM. This detection limit corresponds to less than $10^{-6}$ optical absorbance units which is equivalent or better than conventional optical absorbance measurements performed with 1-cm pathlengths.

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REFERENCES