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Development of a Conductivity-based Photothermal Absorbance Detection Microchip Using Polyelectrolytic Gel Electrodes

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Abstract

The development and application of polyelectrolytic gel electrodes (PGEs) for a microfluidic photothermal absorbance detection system is described. The PGEs are used to measure changes in conductivity based on heat generation by analytes absorbing light and changing the solution viscosity. The PGEs are suitable for direct contact conductivity measurements since they do not degrade with exposure to high electric fields. Both a 2-electrode system with DC voltages and a 3-electrode system with AC voltages were investigated. Experimental factors including excitation voltage, excitation frequency, laser modulation frequency, laser power, and path length were tested. The limits of detection for the 3-electrode and 2-electrode systems are 500 nM and 0.55 nM for DABSYL-tagged glucosamine, respectively. In addition, an electrokinetic separation of a potassium, DABSYL-tagged glucosamine, Rhodamine 6G, and Rhodamine B mixture was demonstrated.

Keywords

Photothermal absorbance detection; label-free detection; polyelectrolytic gel electrode; microfluidics

1. Introduction

Development of sensitive detection methods for lab-on-a-chip devices has proven challenging given the small dimensions of most microfluidic channels.[1–10] UV-VIS absorbance detection used extensively with LC and HPLC does not provide high sensitivity

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for microchips due to short detection path lengths in the microchannels. The detection sensitivity could be improved by incorporating a Z- or U-cell, but the increased path length would result in band broadening. The absorbance detection sensitivity can be enhanced with optofluidic sample modulation methods but the limit of detection still remains in the μM range.[11,12] Laser induced fluorescence (LIF) detection has shown the highest sensitivity on microchips.[13,14] Most analytes, however, lack sufficient native fluorescence for sensitive detection and require labeling with fluorescence tags for determination. Although fluorescence tagging procedures are well established,[15–17] they add to the complexity of an analysis with the introduction of extra off-line sample preparation steps or with microchip design and operational modifications to accommodate on-chip labeling.

An alternative approach to either UV-VIS or fluorescence is photothermal absorbance detection.[18] When an analyte absorbs an excitation probe light, heat released due to its non-radiative relaxation back to the ground state increases the solution temperature. This temperature increase alters the solution refractive index and viscosity. The refractive index gradient is the basis for thermal-lens microscopy (TLM) and photothermal interferometer sensor.[19–22] Although viscosity changes in capillaries or microfluidic channels are difficult to measure, solution conductivity, which is directly related to viscosity, may be monitored more easily. Additionally, this measurement should be path length independent unlike UV absorbance measurements.

Means for measuring the solution conductivity in both capillary and microfluidic platforms have been developed over the years using both contact and contactless conductivity detection systems.[23–30] Johnston et. al. developed a photothermal absorbance detector using contactless conductivity with laser excitation as the light source for a capillary electrophoresis system.[31] In the course of characterizing the detector, they discovered that the sensitivity of the device was considerably lower than theoretically calculated. Simulations indicated the sensing region is much larger than the thermooptically heated volume due to the capacitive nature of contactless conductivity detection.

Dennis *et. al.* showed that microfluidic-based photothermal absorbance detection could be achieved using contact conductivity measurement with platinum electrodes and a high frequency AC excitation signal.[32] A marked increase in the overall sensitivity and agreement with theoretical calculations was shown, however, a limitation to the applied excitation signal due to electrode degradation was observed. While a limit of detection (SNR = 3) of 5 nM for DABSYL-tagged glucosamine was achieved, any misalignment between the detection region and the laser beam could cause a loss in signal intensity and possibly damage the electrodes. Contacting metal electrodes also necessitate the use of high-voltage capacitors to block the direct current (DC) electrokinetic voltage that may cause electrical breakdown of any detection electronics. These limitations make development of alternative electrode systems capable of working at higher voltages desirable.

Polyelectrolytic gel electrodes (PGEs) are an attractive alternative to metal electrodes. PGEs are a microfabricated polymer functioning as a salt bridge. They have been applied in cytometry/velocimetry measurements,[33,34] as a micromixer,[35] for electroporation,[36] logic gate,[37] and sample preconcentration.[38–40] One advantage of PGEs compared to

metal electrodes is the ease of microchip fabrication. Whereas the metal electrodes are deposited on the cover slip and require careful alignment over the microchannels, the PGEs can be photopolymerized *in-situ* post-bonding. A second advantage is the PGE can be placed at the same axial position in the separation channel such that the detection region spans across the microchannel. The 2-electrode PGE with this configuration results in no voltage drop between the electrodes induced by the electrophoresis electric potential. In comparison, the 3-metal electrodes are fabricated on the same plane as the channel and have an interdigitated configuration. Thus, the electrophoresis electric field produces a voltage drop between the electrodes, which can interfere with the conductivity measurements. Additionally, the polymer structure results in no oxidation/reduction effects on the electrode surfaces. Therefore, conductivity measurements can be made using direct current, further simplifying the electronics. This paper describes the development and analysis of a 3-electrode PGE microchip based photothermal absorbance detection system, which is compared to a metal electrode based system. In addition, a highly sensitive 2-electrode system using direct current as an excitation signal is also described.

2. Materials and Methods

2.1 Reagents

All materials were used as received without further modification. 2-morpholinoethansulfonic acid (MES), L-histidine, potassium chloride (KCl), Rhodamine 6G, Rhodamine B, diallyldimethylammonium chloride (DADMAC), 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone, N,N'-methylenebisacrylamide, and 3-(trimethoxysilyl)propyl methacrylate (TMSMA) were purchased from Sigma (St. Louis, MO). Glucosamine and 4-(dimethylamino)azobenzene-4'-sulfonyl (DABSYL) chloride were obtained from Supelco (St. Louis, MO). Glacial acetic acid and ethanol were purchased from Fisher Scientific (Fair Lawn, NJ). All solutions were prepared using deionized water filtered through a Barnstead Nanopure Filtration System (Boston, MA). Electrophoresis run solutions were 20 mM MES/20 mM histidine at pH 6.1 unless otherwise stated. A 3 M KCl solution was used as the electrolyte solution in the Ag/AgCl reservoirs.

2.2 Preparation of DABSYL-tagged glucosamine (DABSYL-Glu)

Tagging of glucosamine with DABSYL has been described in detail elsewhere.[31,32] Briefly, 6 mM DABSYL chloride in acetone and 1 mM glucosamine in water were dissolved in 50 mM carbonate buffer at pH 9.0 in a 1:1 ratio. This solution was then heated in a water bath at 75 °C for 12 minutes. After cooling to room temperature, the solution was frozen by immersion in liquid nitrogen and a vacuum oven used to remove any remaining solvent. The residual powder was dissolved in 10 mL deionized water and sonicated for 10 minutes, followed by centrifugation at 15,000 rpm for 15 minutes. This was performed 5 times. The final product was dissolved in deionized water. All experimental studies used 50 µM DABSYL-Glu as the analyte unless otherwise stated.

2.3 Microchip fabrication

The 3-electrode PGE microchips were fabricated using photoresist/chrome coated 0.9 mm thick, 10-cm square B270 glass as the base substrate (Telic, Valencia, CA). The h-line (405

nm) from a maskless SF100 photoexposure system (Intelligent Patterning, LLC, St. Petersburg, FL) was used to expose the desired pattern. After 4.3 s of exposure, the patterned substrate was developed using MF-319 (MicroChem, Corp, Newton, MA) for 1 minute and the chrome layer then etched completely using chrome etchant (Transene, Danvers, MA). The desired pattern was then etched using a 10:1 buffered oxide etchant (Transene) to give channels 46 μm wide at full height and 12 μm deep. The etching progression was monitored using a P-15 profilometer (KLA Tencor, San Jose, CA). Top plates were made from 150 μm thick coverglass (Corning 2940-245, Corning, NY). Access holes of 1-mm were drilled using a MB-1000-1 powder blaster (Comco Inc., Burbank, CA). Substrates were bonded together using a previously published direct bonding technique.[41] Briefly, the surfaces were hydrolyzed in a $\text{NH}_4\text{OH}/\text{H}_2\text{O}_2/\text{H}_2\text{O}$ (2:1:2) solution at 70 °C for 20 minutes. After rinsing with deionized water, the substrates were joined, and annealed at 300 °C for 10 h. A UV curable optical adhesive (Norland, Cranbury, NJ) was used to place cloning cylinders (Fisher Scientific) on top of the access holes to provide fluid reservoirs.

PGEs were fabricated into the microfluidic device, based on previous work.[33] In this study, a coating procedure prior to photopolymerization was added in order to covalently link the PGE to the channel. TMSMA was chosen as the linker.[42] A 200 μL solution of TMSMA was diluted in 40 mL ethanol, and just before use, 1.2 mL of dilute acetic acid (1:10 v/v glacial acetic acid:water) was added. The fabricated glass chip was filled with the dilute TMSMA solution and sealed with parafilm for 3 min. Afterwards, the chip was washed with ethanol and dried. The microchip was then filled with a monomer solution consisting of 65% DADMAC, 2% 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone and 2% N,N'-Methylenebisacrylamide. Polymerization was initiated by exposure to UV light (365 nm) via a J200 UV Exposure System (OAI, Milpitas, CA) 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.

Two-electrode PGE microchips were fabricated using both photoresist/chrome coated 0.9 mm thick, 10-cm square B270 glass and photoresist/chrome coated 2.3-mm thick, 10-cm square quartz as the base substrate (Telic, Valencia, CA). The glass substrate was used to make microchips with 12 μm deep and 55 μm wide (full height) channels. The fabrication procedure for this chip was identical to that of the 3-electrode PGE microchip. Quartz substrates were used to make microchips with 24 μm deep and 107 μm wide (full height) channels. Etching and drilling procedures were identical to those of the 3-electrode PGE microchips. Top plates were made from 150 μm thick quartz coverslips (Structure Probe, Inc., West Chester, PA). Quartz substrates were bonded together by annealing at 1085 °C for 10 h, based on previously published protocols.[41]

2.4 Optical system

The 3-electrode PGE microchip photothermal setup using an alternating current for excitation is shown in Figure 1. Briefly, an adjustable power Innova 300 FReD laser operated at 488 nm (Coherent, Santa Clara, CA) was the light source. The laser light was modulated at 20 Hz (unless otherwise stated) using an SR540 optical chopper (Stanford Research Systems, Sunnyvale, CA). The laser light was spatially filtered before being

focused using a long working distance 40X microscope objective (Olympus, Center Valley, PA) to give a sub-10 μm spot. A PL-A741 machine vision camera (PixeLINK, Ottawa, ON) equipped with an F/2 fused silica singlet lens and two neutral density filters (FNQ057 and FNQ065, Melles Griot, Irvine, CA) were used to visualize placement of the laser spot. Based on the optical components used, only 9.6% of the laser power was delivered to the interrogation region when the chopper was open.

2.5 Electronic components

PGE microchips were connected to the detection circuit using Ag/AgCl electrodes immersed in a 3M KCl solution. Based on calculations using the microchip dimensions and buffer conductivity, more than 99% of the measured impedance between the Ag/AgCl electrodes originates between the PGEs. The electronic components for the 3-electrode PGE microchip were similar to those used previously.[32] Two DS345 digital function generators (Stanford Research Systems) were used to supply the AC excitation signal to the conductivity cell. All measurements were taken under zero background conditions by adjusting the phase and voltage of the function generators to null the background conductivity, using a master-slave configuration. The frequency used for all studies, with the exception of frequency dependent studies, was 100 kHz, based on the lock-in amplifier's frequency input limit. The excitation signal passed through a 3 kV, 100 pF radial disc capacitor (Panasonic-ECG, Secaucus, NJ) prior to application to the Ag/AgCl electrode. Once the signal passed through the detection cell, it went through another 3 kV, 100 pF radial disc capacitor to prevent any DC voltage from reaching the detection electronics.

An OPA602 operational amplifier (Texas Instruments, Dallas, TX) was used in a current-to-voltage configuration with a 1 M Ω feedback resistor (Multicomp, Chicago, IL), giving a gain of 10^6 VA^{-1} . Power was supplied to the operational amplifier using a Model 1301 Power Supply (Global Specialties, New Haven, CT) at $\pm 15 \text{ V}$.

Two SR810 digital lock-in amplifiers (Stanford Research Systems) were used for demodulating the AC excitation frequency and laser modulation frequency in all cases. The external reference setting was used on both lock-in amplifiers. The first lock-in amplifier reference was supplied by a split signal from the master DS345 function generator, and was thus based on the AC excitation frequency, giving a conductivity signal. The second lock-in amplifier reference was supplied by the "f" output of the SR540 optical chopper, and was thus based on the laser light modulation frequency providing the photothermal signal. The first lock-in amplifier was set with a 1 ms time constant and a 24 dB/octave slope, giving a bandwidth of 159 Hz, for all experiments except in studies involving variations in the modulation frequency, in which the time constant was changed to give the narrowest bandwidth possible. The output of the first lock-in amplifier was sent to the second (photothermal) lock-in amplifier. The second lock-in amplifier was set with a 100 ms time constant and a 24 dB/octave slope for all cases, giving a bandwidth of 1.59 Hz. The sensitivity of each lock-in amplifier was chosen such that the maximum signal could be obtained for a given range.

The output of both lock-in amplifiers was digitized using a USB-6229 DAQ card (National Instruments, Austin, TX) and collected on a personal computer using custom software

written in LabVIEW 8.0 (National Instruments). Data analysis was performed in Igor Pro 6 (Wavemetrics, Lake Oswego, OR) and included median filter baseline subtraction prior to analysis.

2.6 Electrophoresis system

Electrophoresis was performed using an in-house built high voltage power supply with six model 10A12-P4 UltraVolt high voltage modules (Ronkonkoma, NY). Voltages were applied to the chip reservoirs using platinum wires, and controlled by a custom program written in LabVIEW using the analog output of a USB-6229 DAQ board. Gated-injection and electrophoresis voltage profiles were determined based on visualization using a TE300 inverted microscope (Nikon, Melville, NY) equipped with a 20X objective, a high pressure mercury lamp, and a NTE/CCD-512-EBFT CCD camera (Roper Scientific, Trenton, NJ). [43]

3. Results and Discussion

3.1 Three-electrode PGE

Electronic and optical parameters, including excitation voltage, AC excitation frequency, laser power, and laser modulation frequency were tested using a 3-electrode PGE microchip and DABSYL-Glu as the analyte of interest. A pair of excitation electrodes (E1 and E2 in Figure 1) were used and set for destructive interference of the excitation signals. Nulling the background signal improves the detection of low signal intensities. A third electrode (E3 in Figure 1), placed axially from the excitation electrodes, served as the detection electrode. A plot of the photothermal signal to noise ratio (SNR) versus excitation voltage showed an expected linear increase (Figure 2a). While the maximum signal intensity continued to increase with excitation voltage, a signal of 5 V_{p-p} was chosen to allow comparison to previously published results using a metal electrode based microchip.[32] With the metal electrode microchip, the photothermal SNR increased with excitation frequency up to the lock-in amplifier's limit (102 kHz), however this was not seen with the PGE microchip (Figure 2b). Although the photothermal signal intensity increased linearly with the excitation frequency, the baseline noise also increased possibly because the background signal nulling through the PGEs has a frequency limit. The maximum SNR was observed at 20 kHz and at higher frequencies the SNR remained constant. Variation of the optical parameters also showed an expected trend with the photothermal SNR increasing linearly with increasing laser power (Figure 2c). The photothermal SNR initially increased and then decreased with increasing laser modulation frequency due to insufficient time for solution heating and cooling at higher modulation frequencies (Figure 2d). The overall photothermal SNR reached a maximum around 20 Hz, as optical chopper instability increased the baseline noise at lower frequencies.

The photothermal signal intensity for the 3-electrode PGE microchip was comparable to that of the metal electrode microchip; however, the noise level for the PGE microchips was higher. The limit of detection ($3 \times \text{SNR}$) achieved with the 3-electrode PGE microchip using parameters providing a good comparison to the metal electrode microchip (400 mW laser modulated at 20 Hz; excitation of 5 V_{p-p} at a frequency of 100 kHz) was 500 nM for

DABSYL-Glu. Using identical conditions, the metal electrode based microchip limit of detection was 37 nM for the same analyte. This difference in sensitivity can be explained by electrode configuration. In the metal electrode design, the sensing electrode was placed between two excitation electrodes in the same plane, with the electrodes orthogonal to the microchannel. In this design, the two excitation electrodes effectively work to null the baseline signal. However, in the 3-PGE setup, the electrodes are in a triangular orientation at the microchannel wall as shown in Figure 1. In this configuration, the two excitation electrodes (E1 and E2) not only interact with the detection electrode (E3), but also directly with each other, subsequently decreasing the signal intensity (See Supporting Information). This configuration does not take advantage of PGEs, namely, having a vertical electrode that can be used with DC excitation. Incorporating a DC measurement circuit requires removal of the AC coupling capacitors between the detection circuit and the Ag/AgCl electrodes. This will lead to an electrical breakdown of the measurement circuit from the high voltage power supply used for flow control. To mitigate this problem, we have investigated the use of an alternative microchip utilizing a 2-electrode PGE design.

3.2 Two-electrode PGE microchip

The 2-electrode PGE microchip photothermal setup using a direct current for excitation is shown in Figure 3. To prevent any potential electrical breakdown in the measurement circuit, a redesigned system was developed (Figure 4). All resistors were low current-noise RR series high-precision metal film chip resistors (Susumu, Kyoto, Japan). A 400 mV bias voltage was applied between the two PGEs through a pair of Ag/AgCl electrodes. The nonpolarizable Ag/AgCl electrode was chosen to be compatible with the DC measurement as well as to minimize noise. The Ag/AgCl electrode pair maintained a stable current with the 400 mV bias voltage for longer than 30 min. The current flowed through the detection PGE and its corresponding Ag/AgCl electrode to the operational amplifier (U1). Because the measurement circuit is electrically floating and the current direction caused by the 400 mV bias voltage is orthogonal to the main microchannel, the measurement does not interfere with the electrophoretic separation. This amplifier was an OPA602 (Texas Instruments) and was operated as a current to voltage converter. The OPA602 was chosen because of its low input current noise. R1 was 50 k Ω and C1 was 10 nF giving a current to voltage gain of 5×10^4 VA⁻¹ and a lowpass cutoff frequency of 300 Hz. The signal was then highpass filtered with a cutoff frequency of 0.5 Hz to suppress the baseline signal and amplified again using an ultra-low noise instrumentation amplifier (INA217, Texas Instruments), U2. This highpass filter and instrumentation amplifier combination is the key feature of the measurement circuit to achieve a high SNR, as follows. The cutoff frequency is given by $1/(2\pi RC)$ where R and C are the resistance and capacitance of a passive RC lowpass or highpass filter. To get the cutoff frequency as low as 0.5 Hz, a high resistance and/or high capacitance is required. High resistance should generally be avoided as the inherent thermal noise of a resistor increases with $\sqrt{R}$. Therefore, a high capacitance (C2), 220 μ F (tantalum capacitor, Nichicon, Kyoto, Japan), with a low resistance (R2), 1.4 k Ω , was chosen. Most capacitors with this value do not have good response at high frequencies. Therefore, it is undesirable to incorporate this capacitor in the direct highpass filter topology, where a signal passes through the capacitor. For this reason, the highpass filter was created by combining a lowpass filter (R2 and C2) with subtraction, where the instrumentation amplifier, U2, served

to differentially amplify the output of U1 and the lowpass filtered output of U1. The highpass filtered signal is amplified at U2 with a resistor (R3) of 20 Ω , giving a gain of 500. The total effective current to voltage gain is $25 \times 10^6 \text{ VA}^{-1}$. The amplified signal then passed through an isolation amplifier, U3 (ISO122, Texas Instruments), and finally sent to a lock-in amplifier to demodulate the laser modulation frequency. The lock-in amplifier was set with a 300 ms time constant and a 24 dB/octave slope for all cases, giving a bandwidth of 0.53 Hz.

For the 2-electrode PGE microchips, a DC excitation voltage of 400 mV was chosen since a higher excitation voltage can generate a local electroosmotic flow (EOF) between the PGEs and cause band broadening. Thus, only optical parameters were studied for the 2-electrode PGE microchip. Unless otherwise stated, all experiments conducted using the 2-electrode PGE microchip were completed using 20 mM MES/20 mM His/20 mM KCl at pH 6.1 as the running buffer. All other optical, electrophoresis, and data acquisition components were identical to those of the 3-electrode PGE microchip system.

The effect of laser modulation frequency on the SNR showed a strong dependence on environmental noise sources (Figure 5b). This experiment was conducted using an excitation laser source at 400 mW. If the laser modulation frequency was increased, the signal strength decreased, as was observed with the 3-electrode PGE microchip (Figure 5a). While the photothermal signal decreased with increasing laser modulation frequency, the noise remained low ($\sim 3 \mu\text{V}$) until a multiple of 60 Hz was reached, where the noise increased to values around 30 μV due to power-line noise. Signal collection at these frequencies decreased the SNR below 10,000. The maximum SNR was obtained at 50 Hz (420,000 and 230,000 for the 24 μm and 12 μm deep channels, respectively) as shown in Figure 5b. The noise level showed no significant difference between the 24 μm (quartz) and 12 μm (B270 glass) deep channels.

Based on the 3-electrode PGE microchip experimental results, a linear increase in the photothermal signal was expected with increased laser power. For this experiment, four different microchips were used; two had 12 μm deep channels (55 μm maximum width), while the other two had microchannels that were 24 μm deep (107 μm maximum width). These results showed a channel depth dependence, as well as slight chip-to-chip variability (Figure 6a). However, as shown, using a microchip with 12 μm deep channels, it was still possible to obtain a SNR of about 100,000 using a 500 mW laser source modulated at 150 Hz. When the channel depth was increased to 24 μm , the maximum SNR reached over 220,000 using a 500 mW laser source modulated at 150 Hz. Using the same laser output with a metal electrode based microchip, a SNR of only 10,000 was observed for a 30 μm deep channel using an excitation of 5V_{p-p} at 100 kHz and optical modulation at 20 Hz.

Using the 2-electrode PGE microchip, a concentration study was performed in the range from 80 nM to 50 μM DABSYL-Glu using a 400 mV excitation voltage and a laser power of 400 mW modulated at 150 Hz. This study was performed with both 24 μm and 12 μm deep microchips (Figure 6b). The results showed a linear increase with concentration. Based on these results, the limit of detections for the 12 and 24 μm deep 2-electrode PGE microchip were 1.39 and 0.55 nM, respectively, giving far better sensitivity than its 3-electrode

counterpart. Although theoretically, the photothermal process is path length independent, the photothermal signal was attenuated in the 12 μm deep channel microchip compared to the 24 μm deep channel microchip (Figures 5 and 6). Heat dissipation through the glass or quartz substrates is believed to cause this path length dependence. As channel dimensions get smaller and smaller, the surface to volume ratio increases, and therefore, heat dissipation to the substrate also increases reducing the overall signal intensity.

A separation of potassium, Rhodamine 6G, Rhodamine B, and DABSYL-Glu was performed on the 2-electrode PGE microchip using a laser power of 400 mW modulated at 150 Hz. Using a separation field of 285 V cm^{-1} and a 1 second gated injection, the entire separation was fully base line resolved in under 50 seconds, as seen in Figure 7. The noise peaks at time 0 generated by the voltage changes during the gated injection shown in the conductivity trace did not appear in the corresponding photothermal data. This is because they did not contain the frequency necessary to be picked up by the photothermal lock-in amplifier (i.e., the modulation frequency). Also, the potassium peak in the conductivity data did not appear in the corresponding photothermal data because this molecule did not absorb 488 nm light. Therefore, only Rhodamine 6G, Rhodamine B, and DABSYL-Glu were separated and detected in the photothermal signal. This shows an added advantage of a combined conductivity/photothermal detection technique as analytes that do not absorb light can be detected separately from those that do based on the data collection frequency.

4. Conclusion

Previously published results show that while a metal electrode based photothermal absorbance detector gives good sensitivity, the fabrication of such microchips is often difficult. In addition, the alignment of such electrodes is a delicate process that has profound effects on the overall signal intensity. An alternative to metal electrodes has been presented that shows superior sensitivity when a DC excitation signal is used with a 2-electrode PGE microchip. Comparable sensitivity can be obtained using an AC excitation signal with a 3-electrode PGE microchip. It should be noted that the PGEs do not suffer the effects of a voltage drop caused by the high separation field as is the case for the metal-based electrodes due to the orthogonal direction of the applied field and are thus amenable for use with a DC voltage. In addition, PGEs offer the advantage of applicability to very small interrogation regions, as the spatial fabrication of these electrodes is precise and external to the separation channel. These experimental results demonstrate superior sensitivity in comparison to conventional UV-VIS spectrophotometers. Our current work is geared towards demonstrating applicability to a wider range of analytes with detection in the UV region.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Polyelectrolytic gel electrodes (PGEs) for a microfluidic conductivity-based photothermal absorbance detection system is developed.
- Both a 3-electrode system with AC voltages and a 2-electrode system with DC voltages were investigated.
- Comparable and superior sensitivity compared with a metal electrode chip can be obtained using a 3-electrode and a 2-electrode PGE microchip, respectively.
- Low detection limit of sub-nM is achieved with clear sample separation.

Figure 1.

Schematic of 3-electrode PGE microchip system for photothermal absorbance measurements. All detection circuits were connected to the chip through capacitors and Ag/AgCl electrodes. Two function generators in a master-slave configuration were set out-of-phase to null the background signal. The first lock-in amplifier was referenced to the excitation frequency, f_e , to get the conductivity signal, while the second lock-in amplifier was referenced to the laser modulation (chopping) frequency, f_c , to get the photothermal signal. The image in the upper-right corner shows the actual PGEs, as well as the focused laser spot. S, B, SW, and A stand for sample, buffer, sample waste, and analysis, respectively. E1 and E2 are the excitation electrodes and E3 is the detection electrode.

Figure 2.

Signal-to-noise ratio (SNR) of DABSYL-Glu on the 3-electrode PGE microchip as a function of excitation voltage (a), excitation frequency (b), laser power (c), and laser modulation frequency (d).

Figure 3.

2-electrode PGE microchip system for photothermal absorbance measurements. The DC conductometry circuit was connected through Ag/AgCl electrodes. The circuits in the dashed line were electrically floated. The lock-in amplifier was referenced to the laser modulation (chopping) frequency, f_c , to get the photothermal signal. The image in the upper-right corner shows the actual PGEs and the focused laser spot.

Figure 4.

Circuit design for DC conductivity measurements used to prevent electrical breakdown. The measurement circuit was electrically isolated and powered by a floating battery. The signal passed through an isolation amplifier before being sent to the lock-in amplifier.

Figure 5. The photothermal intensity (a) and corresponding SNR (b) with respect to laser modulation frequency using the 2-electrode PGE microchip. ■ and ● correspond to the 24 and 12 μm deep channels, respectively.

Figure 6. Response (SNR) of DABSYL-Glu on the 2-electrode PGE microchip as a function of laser power (a) and analyte concentration (b). $\square$ and $\blacksquare$ correspond to 24 μm deep channel microchips, and $\circ$ and $\bullet$ correspond to 12 μm deep channel microchips.

Figure 7.

Separation of potassium, Rhodamine 6G, Rhodamine B, and DABSYL-Glu. The conductivity trace shows the output of the conductometry circuit and the photothermal SNR trace shows the output of the lock-in amplifier. 1, 2, 3, 4, and 5 correspond to gated injection peaks, 2 mM K^+ , 150 μ M Rhodamine 6G, 150 μ M Rhodamine B, and 50 μ M DABSYL-Glu, respectively. The inset shows a magnified view of a 150 Hz modulated conductivity signal. 3', 4', and 5' are photothermal responses of Rhodamine 6G, Rhodamine B, and DABSYL-Glu, respectively.