Contact Conductivity Detection in Poly(methyl methacrylate)-Based Microfluidic Devices for Analysis of Mono- and Polyanionic Molecules

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An on-column contact conductivity detector was developed for the analysis of various mono- and polyanionic compounds separated by electrophoresis chips fabricated in poly(methyl methacrylate) (PMMA) using hot embossing techniques from Ni electroforms. The detector consisted of a pair of Pt wires (127 µm diameter) with an end-to-end spacing of approximately 20 µm and situated within the fluidic channel. The waveform applied to the electrode pair was a bipolar pulse with a frequency of 5.0 kHz and was used to reduce the charging current from measurement so that the current recorded at the end of one pulse is more representative of the solution conductivity. Using the detector, separations of amino acids, peptides, proteins, and oligonucleotides were demonstrated. For the amino acids and peptides, free-solution zone electrophoresis was performed. A calibration plot for the amino acid alanine was found to be linear from approximately 10 to 100 nM in a carrier electrolyte consisting of 10 mM triethylammonium acetate. The concentration detection limit was found to be 8.0 nM, with the corresponding mass detection limit equal to 3.4 amol (injection volume ~ 425 pL). The protein separations with conductivity detection were performed using MEKC, in which the carrier electrolyte contained the anionic surfactant sodium dodecyl sulfate (SDS) above its cmc. Near baseline resolution was achieved in the PMMA microchip for a solution containing 8 different proteins. In the case of the DNA fragments, capillary electrochromatography was performed. A calibration plot for the amino acids and peptides, free-solution zone electrophoresis was performed. A calibration plot for the amino acid alanine was found to be linear from approximately 10 to 100 nM in a carrier electrolyte consisting of 10 mM triethylammonium acetate. The concentration detection limit was found to be 8.0 nM, with the corresponding mass detection limit equal to 3.4 amol (injection volume ~ 425 pL). The protein separations with conductivity detection were performed using MEKC, in which the carrier electrolyte contained the anionic surfactant sodium dodecyl sulfate (SDS) above its cmc. Near baseline resolution was achieved in the PMMA microchip for a solution containing 8 different proteins. In the case of the DNA fragments, capillary electrochromatography was performed. A calibration plot for the amino acids and peptides, free-solution zone electrophoresis was performed. A calibration plot for the amino acid alanine was found to be linear from approximately 10 to 100 nM in a carrier electrolyte consisting of 10 mM triethylammonium acetate. The concentration detection limit was found to be 8.0 nM, with the corresponding mass detection limit equal to 3.4 amol (injection volume ~ 425 pL). The protein separations with conductivity detection were performed using MEKC, in which the carrier electrolyte contained the anionic surfactant sodium dodecyl sulfate (SDS) above its cmc. Near baseline resolution was achieved in the PMMA microchip for a solution containing 8 different proteins. In the case of the DNA fragments, capillary electrochromatography was performed. A calibration plot for the amino acids and peptides, free-solution zone electrophoresis was performed. A calibration plot for the amino acid alanine was found to be linear from approximately 10 to 100 nM in a carrier electrolyte consisting of 10 mM triethylammonium acetate. The concentration detection limit was found to be 8.0 nM, with the corresponding mass detection limit equal to 3.4 amol (injection volume ~ 425 pL). The protein separations with conductivity detection were performed using MEKC, in which the carrier electrolyte contained the anionic surfactant sodium dodecyl sulfate (SDS) above its cmc. Near baseline resolution was achieved in the PMMA microchip for a solution containing 8 different proteins.

M icrofabrication technology has proven to be a valuable tool for creating miniaturized devices for applications in many chemical and biochemical assays. The attractive features associated with these devices are potential for system integration in which various processing steps of the assay are included onto the fluidic platform, rapid analysis speeds, construction of highly multiplexed systems, the ability to reduce reagent consumption, and the mass production of devices at minimal costs. As this technology continues to evolve, several areas will require further development to expand on existing capabilities such as increasing system integration without sacrificing the benefits of a small footprint, reducing the cost, labor, and time associated with fabricating devices, and further reducing the size of the device. Indeed, significant progress has been made and continues to be made by many research groups in these areas. For example, efforts have focused on developing microfluidic devices in polymers.¹⁻¹⁵ Polymer-based devices offer a variety of fabrication methods that can be used to rapidly create devices inexpensively, and in addition, these fabrication methods can be used to produce high-aspect-ratio microstructures (HARMs), which can assist in system integration and reducing the size of the device.

One of the consequences of reductions in size of the microfluidic device is the resulting constraint placed on the detector required for readout. For example, the sampling volume in many microfluidic devices is <1 nL and, therefore, significant requirements on the limits of detection (LOD) associated with the detector hardware must be realized to analyze even modest concentrations of material in these microfluidic platforms.

The readout strategy most commonly used for these devices has relied on laser-induced fluorescence (LIF) due to the fact that it provides exquisite sensitivity with detection limits approaching the single molecule level.¹⁶⁻¹⁸ Unfortunately, most LIF systems do not lend themselves to developing totally miniaturized systems,

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with the detector components oftentimes requiring a much larger footprint compared to the microfluidic device. While several attempts have been made to fabricate miniaturized LIF detectors with on-chip detection, LIF requires analytes that either show intrinsic fluorescence or can be readily associated with (either covalently or noncovalently) labeling chromophores.

Another readout strategy that has shown promise in microfluidic applications is electrochemical detection, such as voltammetric or amperometric detection. The attractive feature of electrochemical detection includes the simple instrumentation required to carry out detection and the favorable sensitivity and limits of detection it offers. However, for amperometric detection, the target material must be intrinsically electroactive, or if it is not, an electroactive species must be appended to the target molecules.

Conductivity detection can be considered an electrochemical technique as well but has the ability to detect any analyte irrespective of whether it contains an electroactive species or not. The only requirement is that the migrating analyte zones possess a conductivity that is different from that of the carrier electrolyte. Another benefit of conductivity is that performance improves with smaller detection volumes, making it an attractive detector for microcolumn separations, such as capillary zone electrophoresis (CZE). To demonstrate the low limits of detection that are obtainable using conductivity detection with CZE, Zare and coworkers described an on-column conductivity detector that possessed a detection volume of 30 pL and could detect approximately $10^{-10}$ lithium ions (1 M).

Recently, reports have appeared describing the integration of contact conductivity into microfluidic devices. In these examples, the detector was fabricated to analyze analyte zones isolated via isotachophoresis (ITP) with the analytes consisting of small inorganic ions or low molecular weight organic ions. However, Guijt et al. described the fabrication of an integrated conductivity detector for the zone electrophoresis separation of several inorganic ions and organic acids. In this device, the Pt electrodes required for conductivity detection were vapor-deposited onto a glass cover slip and consisted of approximately 200 nm thick electrodes spaced by 25 μm and configured orthogonal to the electrophoretic flow direction (contact conductivity detection). Using a sinusoidal voltage waveform applied between the Pt electrodes, the conductivity detection concentration detection limits for several organic anions were determined to be in the range of 5 μM. These same authors constructed a contactless conductivity detector fabricated in a four-electrode geometry. To reduce analyte sorption to the electrode surface causing electrode fouling, a silicon carbide insulating layer was deposited over the electrodes. A concentration detection limit of 20 μM was reported for K+ ions. Also in this report, the authors presented a one-dimensional zone electrophoretic separation of a two-component peptide mixture.

While conductivity detection can be used in microchip formats for the analysis of a variety of species, improvements in its detection limit performance and its utility to detect analytes other than inorganic ions or small organic ions must be realized. For conductivity detection, the analytical response (G, conductivity) is described through the following expression:

$$G = \frac{A_+ + A_-}{1000K}$$

Here $A_+$ and $A_-$ (S cm$^2$ equiv$^{-1}$, S = siemens) are the limiting ionic conductances of cations and anions in solution, respectively, C is the concentration (M), and K is the cell constant (K = L/A, where L is the distance between the electrode pair and A is the area of the electrodes). Clearly, increasing the area of the electrodes and/or reducing the spacing between the electrodes can improve the detection limits of the conductivity measurement. In addition, one must reduce the contribution of Faradaic currents to the measured current. One approach to accomplish this is to use a bipolar pulse waveform. In this format, successive voltage pulses of equal amplitude and duration but opposite polarity are applied to the conductivity electrodes with the current passing between the electrodes measured at the end of the second pulse. If the pulse frequency is appropriately chosen with respect to
to the cell time constant (defined as the time to charge the double layer), the electrical double layer does not have sufficient time to form, which can minimize Faradaic reactions from occurring at the electrodes. In addition, since the bipolar pulses are of equal amplitude and time duration but opposite polarity, the measured current is effectively free from charging currents. Therefore, the measured current primarily results from solution Ohmic resistance. The attractive feature of this format is that the electrodes can be configured directly on-column maintaining column (separation) efficiency.

In this paper, we wish to describe a simple and sensitive bipolar-pulse contact conductivity detector integrated directly to a PMMA-based microfluidic device for the detection of various mono- or polyanionic molecules (amino acids, peptides, proteins, or nucleic acids). The PMMA device was configured to separate the ionic materials using several different electrophoresis formats such as free-solution zone electrophoresis (amino acids and peptides), micelle electrokinetic chromatography (proteins), or capillary electrochromatography (nucleic acids) in a fluidic channel of 3 cm effective length. To perform the electrochromatography separations, the PMMA walls were modified using chemistry recently described in our group to attach a C18 reverse-phase to the PMMA device. By addition of an ion-pairing agent to the carrier electrolyte, reverse-phase separations of DNA fragments could be carried out using conductivity detection. The sensitivity, limits of detection, and stability of the contact conductivity detector will be discussed as well.

**EXPERIMENTAL SECTION**

**Microfabrication of the PMMA Chips.** The fluidic devices were embossed from Ni electroforms fabricated via LIGA techniques, which can be used to make many replicates from this single master. The sections below briefly describe the steps involved in fabricating the electroform (molding die) and the production of the final polymer microparts using hot embossing.

The device layout (Figure 1A) was designed using AutoCAD (Autodesk Inc., San Rafael, CA), which was read by a GCA M ann 3600 pattern generator (Seattle, WA) to optically write the desired pattern to an optical mask. Microfeatures were printed on a 5 in. x 5 in. plate, which consisted of a chromium-coated quartz plate with a positive photoresist layer. The device possessed a 15 µm wide separation channel of 4 cm total length (effective length = 3 cm). Guide channels for the wires used for conductivity detection were designed with a width of 130 µm. Following development of the exposed resist, the plate was subjected to a Cr etching solution to produce the optical mask.

The X-ray mask was prepared on a Kapton film with the desired device topography transferred to the film using optical proximity printing. A 25 µm-thick Kapton film was stretched and glued to a

Figure 1. (A) Topographical layout of the PMMA-based microchip with integrated conductivity detector: injection channel length = 1.0 cm; separation channel length = 4.0 cm; effective separation channel length = 3.0 cm; channel width = 15 µm; channel height ~85 µm. The solution reservoirs are (1) sample reservoir, (2) buffer reservoir, (3) waste reservoir, and (4) receiving reservoir. (B) SEM of the Ni electroform embossing die taken near the waste reservoir (3). (C) Optical micrograph of PMMA microchip that was assembled with a coverplate and electrodes and then cut down the center of the fluidic channel. (D) Optical micrograph of the conductivity detector (T-cell, electrode gap ~ 20 µm) integrated to the PMMA microfluidic device. In this micrograph, the coverplate was not assembled to the fluidic substrate. The working electrode and reference electrodes possessed a 127 µm diameter and were placed 0.5 cm upstream from the receiving reservoir.

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The Ti ring to which 50 Å of Cr and 300 Å of Au were deposited. SU-8 (negative photoresist, Microchem, Newton, MA) was spin-coated onto the Kapton film at 900 rpm for 20 s (Headway Research Inc., PWM 101 Spinner). After a prebake for 1.5 h at 96 °C, the optical mask was placed over the Kapton film and flood UV exposure of the assembly was carried out for 20 s (Oriel UV exposure System, Stratford, CT). The unexposed SU-8 photoresist was removed by developing following a 20 min postbake at 96 °C. The thickness of the photoresist (30–40 μm) was measured using a surface profiler (Alpha-Step 500, Tencor, San Jose, CA). Following development, plasma cleaning of the Kapton film was performed to remove any remaining photoresist. Au was then electroplated onto the Kapton film at 2 mA/cm² for 2 h.

The molding tool (see Figure 1B) was fabricated by electroplating Ni microstructures onto a stainless steel support, which served as the plating base. A 5 in. diameter, 3 mm thick polished stainless steel plate was activated using a C-12 activator solution (Puma Chemical, Warne, NC) followed by electroplating in a NiCl bath to form a thin layer (<5 μm) of Ni. Then, a 1 μm layer of PMMA (9% PMMA in methyl methacrylate, MMA) was spin-coated onto the plating base and baked at ~180 °C for 1 h. The spin-coated PMMA film was coated with M MA, which was used to bond a 3 mm thick PMMA sheet to this base. The sheet PMMA served as a positive resist during X-ray lithographic patterning. The PMMA was fly cut to slightly higher than the desired structure height to account for final polishing and device assembly. The PMMA/stainless steel plating base assembly was then exposed to an X-ray beam at the Center for Advanced Microstructures and Devices (CAMD) with the X-ray mask positioned in front of the assembly. After exposure, the PMMA was developed in GG developer (60% 2-(2-butoxyethoxy)ethanol, 15% morpholine–2-(2-butoxyethoxy)ethanol, 5% ethanolamine, 20% water). The sample was then rinsed in a GG rinse solution (80% 2-(2-butoxyethoxy)ethanol, 20% water). C-12 activator was again used to remove any oxides that may have formed on the exposed metal surfaces prior to final Ni electroplating.

The desired Ni structures were plated out of a Ni sulfamate bath onto the exposed areas of the stainless steel base. The plating was done at 55 °C under continuous stirring at pH 4.0. Following electroplating, the Ni parts were surface ground to remove excess Ni and to planarize the top of the features. The sample was then lapped to a mirror finish. The raised Ni structures were measured to be 95 μm tall by step profilometry following complete processing. Finally, the unexposed PMMA was removed by dissolving in methylene chloride. An SEM of the finished metal electroform used for embossing parts is shown in Figure 1B.

The embossing system consisted of a PH1 Precision Press model TS-21H-C(4A)-5 (City of Industry, CA). A vacuum chamber was installed into this press to remove air (pressure ~0.1 bar) so complete filling of the die could take place. The wafers inserted into the press were 133 mm in diameter, and the maximum area that could be patterned was 100 mm. Before molding, all residual water present in the polymer had to be removed. At room temperature, water adsorption to PMMA is ~0.4% and this amount must be reduced to 0.1% for proper embossing. Therefore, the PMMA wafers (Goodfellow, Berwyn, PA) were baked in an oven at 80 °C for 8 h. The die was coated with a release agent, MoldWiz (Axel, Woodside, NY), to improve demolding. During embossing, the molding die was heated to 150 °C and pressed into the PMMA wafer with a force of 1000 lbs for 4 min. During this process, the die was heated to 160 °C. After 4 min, the press was opened and the polymer part removed and cooled. The PMMA wafer was maintained at 85 °C throughout the demolding process.

**Assembly of Microchip with Integrated Conductivity Detector.** The conductivity detector (see Figure 1C,D for optical micrographs) was constructed from platinum wires (Scientific Instrument Services, Ringoes, NJ) with diameters of 127 μm. Two pieces of platinum wire were cut to ~2.5 cm in length, and the end of each wire was polished with a diamond polishing compound starting with 600 μm grit paper then moving down to 25, 15, 3, and finally 1 μm grit paper (Buehler, Lake Bluff, IL). Between individual polishings, the wires were washed with 18 M Ω-cm deionized water (ddH₂O) and sonicated for 2 min. After polishing, the ends of the wires were inspected under a 20× stereomicroscope to ensure a blunt end had been formed and inserted into guide channels (130 μm width) embossed into the PMMA microchip. Through inspection of the wires under the stereomicroscope, they were situated with an end-to-end spacing of ~20 μm terminating at the separation microchannel. Once situated properly, the Pt wires were tacked down using a small amount of epoxy and the entire fluidic channels and wires covered with a PMMA coverplate by thermal annealing at 107 °C for 12 min in a GC oven with the PMMA pieces clamped between two glass plates.

**Apparatus and Conductivity Detector.** The operating platform for the electrophoretic separations consisted of a high-voltage power supply (CZE1000R, Spellman, Plainview, NY). The leads were connected to an in-house built high-voltage relay (switching driven by a 5 V signal) with four outputs distributed to each of the solution reservoirs configured on the microchip (see Figure 1A). The switching signal for the relay was generated using a universal data acquisition board (PCI-1200, National Instruments, Austin, TX). In addition, the applied voltage for the electrophoresis was controlled by one of the DAC outputs of this board.

The bipolar pulse waveform for the conductivity detector was generated by an in-house fabricated circuit, which was controlled by a National Instruments controller board. The pulse frequency was typically 5.0 kHz, and the pulse width was 100 μs. The pulse width was selected to be short compared to the cell time constant, which was defined by the time to charge the double layer (for a 127 μm Pt wire, the cell time constant was ~250 μs). The potential of one electrode was maintained at virtual ground while the potential at the other electrode was controlled by the generated bipolar waveform. The current between the electrode pair was measured 5 μs prior to the rising edge of every bipolar pulse (±0.5 V) and averaged over the electrophoresis sampling time (1 s) to improve S/N. The data acquisition and controlling software was written in Labview (National Instruments).

**Microelectrophoresis Separations.** All electrophoretic separations were carried out at ambient temperature and run in reverse mode (injection end cathodic, detection end anodic). Injection was initiated by applying a positive high voltage to the sample waste reservoir (point 3; see Figure 1A) and grounding the sample reservoir (point 1) for the appropriate amount of time to completely fill the cross channel. Points 2 and 4 were also grounded during injection. The cross channel was designed (offset “T”) to
have a load volume of 425 pL (length 250 μm). Calculation of the injection plug variance ($\sigma_{inj}^2$) from $l_{inj}^2/12$, assuming a rectangular injection plug, yielded a value of $5.2 \times 10^{-5}$ cm$^2$ for $\sigma_{inj}^2$. For typical electrophoretic separations using this chip design (see Figures 2–5), $\sigma_{tot}^2 \sim 3.0 \times 10^{-3}$ cm$^2$, and therefore, $\sigma_{inj}^2$ represents less than 2% of $\sigma_{tot}^2$. Following injection, a positive high voltage was switched to point 4 and point 2 was grounded. The sample and waste reservoirs were set to 10% of the high voltage applied to reservoir 4 and acted as pullback voltages to prevent sample leakage from these channels during the separation. (Caution! The electrophoresis uses high voltages and special care should be taken when handling the electrophoresis electrodes.)

**Free-Solution Zone Electrophoresis.** Free-solution electrophoresis was carried out on the amino acids (Sigma Chemical, St. Louis, MO) using a triethylammonium acetate electrolyte (10 mM, pH 7.0), or a solution of peptides (Sigma Chemical) using a phosphate carrier electrolyte (100 μM, pH 5.0, Sigma Chemical). The appropriate concentration of amino acids or peptides were made from stock solutions diluted in the carrier electrolyte and electrokinetically injected into the cross channel using the voltage pattern described above. Electrophoresis for the amino acids and peptides was performed using a field strength of 150 V/cm with a bipolar pulse amplitude of 0.5 V.

**Figure 2.** (A) Calibration plot for alanine constructed by integrating the area under each peak and averaging over five runs (vertical error bars) for a concentration range of ~15–100 nM. Alanine was electrophoresed using TEAA as the background carrier electrolyte (10 mM, pH 7.0). (B) Free-solution zone electrophoretic separation of 100 μM amino acid mixture consisting of (1) alanine, (2) valine, (3) glutamine, and (4) tryptophan in an unmodified PMMA microchip using indirect, contact conductivity detection. Electrophoretic conditions: 3 s electrokinetic injection time; $E = 150$ V/cm for the electrophoresis; detector operated at 5.0 kHz with a bipolar pulse amplitude of ±0.5 V.

**Figure 3.** Free-solution zone electrophoretic separation of a peptide mixture (~0.23 μM total peptide concentration) consisting of (1) bradykinin, (2) bradykinin fragment 1–5, (3) substance P, (4) [Arg$^9$]-vasopressin, (5) luteinizing hormone, (6) bombesin, (7) leucine enkephalin, (8) methionine enkephalin, and (9) oxytocin in an unmodified PMMA microchip. The solid line represents the 3rd electrophoretic run on this chip, and the dotted line is the 35th electrophoretic run on the same chip. Detection was accomplished using indirect, contact conductivity. Separation conditions: carrier electrolyte 100 μM phosphate (pH 5.0); 3 s electrokinetic injection time; $E = 150$ V/cm for the electrophoresis; detector operated at 5.0 kHz with a bipolar pulse amplitude of ±0.5 V.

**Figure 4.** MEKC separation of a protein mixture (1.7 μM total protein concentration with all proteins at similar concentrations within the mixture) in an unmodified PMMA microchip consisting of (2) lysozyme, (3) trypsin inhibitor, (4) carbonic anhydrase, (5) ovalbumin, (6) serum albumin, (7) phosphorilase B, (8) β-galactosidase, and (9) myosin detected using indirect, contact conductivity detection. Benzoic acid (1) was added to the mixture as an internal standard. Electrophoresis conditions: carrier electrolyte 100 μM TRIS HCl with 1% SDS (pH 9.2); 3 s injection time; $E = 250$ V/cm for the electrophoresis; detector operated at 5.0 kHz using a bipolar pulse amplitude of ±0.5 V.

**Micellar Electrokinetic Chromatography (MEKC).** MEKC of the protein mixture (BioRad, Laboratories, Hercules, CA) used a TRIS HCl electrolyte (100 μM, pH 9.2, BioRad Laboratories)
laboratories for conductivity detection. Briefly, the carrier ion-pair reverse phase chromatography method developed in our laboratories was carried out using an electrolyte and electrokinetically injected into the cross channel with 1% sodium dodecyl sulfate, SDS, above its cmc. The appropriate concentrations of proteins were diluted in the carrier electrolyte and electrokinetically injected into the cross channel as described above. Electrophoresis for the proteins was performed using a field strength of 250 V/cm with a bipolar pulse amplitude of ±0.5 V. The field strength used for the separation was 100 V/cm. The conductivity detector was operated at a frequency of 5.0 kHz and a pulse amplitude of ±0.5 V.

CEC was performed in open channels of the PMMA microfluidic devices, whose surfaces had been modified using chemistry developed in our laboratories. Briefly, the modification chemistry consisted of converting the methyl ester groups of the polymer backbone into an amine-terminating surface by adding N-lithioetylene-diamine to the microchip channels. After the reaction, the PMMA microchannels were washed with 2-propanol and water. The amine-modified chips were then purged with nitrogen and neat n-octadecane 1-isocyanate (Sigma-Aldrich Chemical) added. Following this reaction, the PMMA chips were washed with copious amounts of hexanes, toluene, and acetone and subsequently dried.

**Measurement of Electroosmotic Flows (EOF).** The EOF was measured by using the method described by Zare and co-workers. The procedure involved filling the entire chip with a buffer of low ionic strength and the appropriate pH. After filling the chip, one reservoir was emptied and filled with the same buffer but of lower ionic strength. An electric field was then applied to the reservoirs containing the low and high ionic strength buffer, and the current was monitored continuously. After the current had plateaued, the time to reach this plateau was secured from the plot from which the linear velocity could be calculated. Dividing this linear velocity by the electric field strength produced the EOF (cm²/(V s)).

**RESULTS AND DISCUSSION**

**Fabrication of Conductivity Detector.** The detector was fabricated by inserting (using a stereomicroscope) polished Pt wires into guide channels embossed in the fluidic substrate to accommodate the electrodes and allowed reproducible lateral placement of the electrodes within the fluidic device. These guide channels were 130 μm wide and 95 μm in deep. The Pt wires were 127 μm in diameter. Therefore, the wires were taller than the height of the microstructures. Attempts to make the microstructures of the appropriate height to accommodate the circular wires completely, which would have allowed insertion of the Pt wires following assembly of the device, were found to be difficult due to the various grinding and lapping processes and the slight compression of the embossed microstructures during device assembly (structure height ~50 μm following assembly). For this reason, we decided to place the wires in an unassembled device and then heat anneal the coverplate to the substrate once the wires had been properly positioned within the fluidic device. For this to work properly, sealing of the coverplate around the electrodes was critical. It was found that the wires during assembly formed impressions into the coverplate, similar to the wire imprinting described above. Electroosmotic flow (EOF) is measured by using the method described by Zare and co-workers. Two reservoirs containing the low and high ionic strength buffer, of low and high ionic strength and the appropriate pH. After filling the reservoirs containing the low and high ionic strength buffer, the current was monitored continuously. After the current had plateaued, the time to reach this plateau was secured from the plot from which the linear velocity could be calculated. Dividing this linear velocity by the electric field strength produced the EOF (cm²/(V s)).

![Figure 5](image-url) **Figure 5.** CEC separation of a double-stranded DNA ladder (400 ng/mL) in an unmodified (A) and C18-modified (B) PMMA device. The ladder consisted of 100, 200, 400, 800, 1200, and 2000 bp fragments. The PMMA microchannel was modified by chemically attaching a C18 phase to its surface. The mobile phase used for this separation was 25% acetonitrile and 75% aqueous phase containing 50 mM TEAA (ion-pairing agent, pH 7.4). Detection was accomplished using indirect, contact conductivity detection. The conductivity cell was operated at 5.0 kHz and a pulse amplitude of ±0.5 V. The field strength used for the separation was 100 V/cm. Peak assignments were based on work reported in ref 44 for this same sizing ladder and using ion-paired reverse-phase chromatography.

**Capillary Electrophromatography (CEC).** CEC separation of the double-stranded oligonucleotides was carried out using an ion-pair reverse phase chromatography method developed in our laboratories for conductivity detection. Briefly, the carrier electrolyte consisted of 25% acetonitrile and 75% water containing 50 mM triethylammonium acetate (TEAA) serving as the ion-pairing agent. The oligonucleotides analyzed were a low mass backbone into an amine-terminated surface by adding N-lithioetylene-diamine to the microchip channels. After the reaction, the PMMA microchannels were washed with 2-propanol and water. The amine-modified chips were then purged with nitrogen and neat n-octadecane 1-isocyanate (Sigma-Aldrich Chemical) added. Following this reaction, the PMMA chips were washed with copious amounts of hexanes, toluene, and acetone and subsequently dried.

**Measurement of Electroosmotic Flows (EOF).** The EOF was measured by using the method described by Zare and co-workers. The procedure involved filling the entire chip with a buffer of low ionic strength and the appropriate pH. After filling the chip, one reservoir was emptied and filled with the same buffer but of lower ionic strength. An electric field was then applied to the reservoirs containing the low and high ionic strength buffer, and the current was monitored continuously. After the current had plateaued, the time to reach this plateau was secured from the plot from which the linear velocity could be calculated. Dividing this linear velocity by the electric field strength produced the EOF (cm²/(V s)).
more convenient temperatures. Also seen from the micrograph of Figure 1D is that the width of the channel was somewhat wider than that of the wire potentially leaving unswept volumes near the detector, which could give rise to memory effects. However, as can be seen from Figure 1C, the walls of the fluidic channels were slightly compressed around the wires due to assembly, and from inspection of our data, no memory effects were observed in the detector response.

**Figures of Merit for Conductivity Detector.** Recently, we reported on the fabrication and characterization of a contact conductivity detector used in micro-liquid chromatography. The detector, similar in design to that used here, was found to produce a mass detection limit for KCl using direct conductivity detection of 3.46 ng when operated in a bipolar pulse format. The conductivity detector analytical figures of merit were evaluated here by analyzing signals generated from the free-solution electrophoresis of four, nonlabeled amino acids using a 10 mM TEAA carrier electrolyte (pH 7.0). A calibration plot covering the concentration range of 15–80 nM for alanine is shown in Figure 2A along with an electropherogram for the amino acid mixture (see Figure 2B). The correlation coefficient for this plot was determined to be 0.994. The concentration limit of detection (LOD) was determined by measuring the S/N ratio for a series of electropherograms with the amino acid concentration adjusted near the anticipated LOD as determined from the regression plot. Inspection of these data indicated a concentration LOD of 8.0 nM at a S/N ratio = 3/1.

The free-solution separation of the amino acids was carried out in a reverse mode, in which the injection end was cathodic and the detection end anodic. While PMMA does show an electroosmotic flow (EOF) that runs from anode to cathode, it is smaller than that observed for fused silica at this same pH. The EOF of an unmodified PMMA microchip measured using this carrier electrolyte was found to be $(2.17 \pm 0.03) \times 10^{-4} \text{cm}^2/\text{Vs}$ and changed minimally with pH over the range of 3–10, consistent with our previous data. At the pH used for these separations, the amino acids exist in their anionic form and, therefore, migrate toward the anodic reservoir only if their electrophoretic mobility is greater than the EOF, which is the case here because all amino acids were observed by finish line format conductivity detection.

The extended time required for migration of the amino acids to the detector indicates that their apparent mobility is small. The apparent mobility for alanine was measured to be $3.34 \times 10^{-6} \text{cm}^2/(\text{V s})$, giving a value of $2.50 \times 10^{-3} \text{cm}^2/(\text{V s})$ for its electrophoretic mobility. For comparison, the electrophoretic mobility of alanine was calculated from published data using a capillary column and indirect fluorescence detection and found to be $3.05 \times 10^{-4} \text{cm}^2/(\text{V s})$ ($\mu_{\text{app}} = 8.7 \times 10^{-4} \text{cm}^2/(\text{V s})$, taken from ref 49), similar to the value calculated here.50

**Separation of Peptides Using Free-Solution Electrophoresis.** To demonstrate the ability to detect the presence of other polyanionic species, several peptides were separated using free-solution zone electrophoresis (carrier electrolyte was 100 $\mu$M phosphinate, pH 5.0). In Figure 3 is shown the resulting free-solution electropherogram of this peptide mixture. As can be seen, baseline separation of all 9 peptide fragments was achieved in this 3 cm length channel (effective length) with the separation requiring $<250$ s at this field strength. The conductivity response was again negative, indicating that the analyte zones possessed a conductivity below that of the carrier electrolyte. It was found that after performing 35 separations on this device (see Figure 3), the detector response degraded by $\sim50\%$. We also note that the amino acid and protein assays produced similar results in terms of degradation of detector response. However, for the oligonucleotides assays (see below), no degradation in the response of the detector was observed over the lifetime of the chip. Fortunately, the ease of fabrication (hot embossing) and assembly of the device with its detector electrodes makes it simple to replace failed devices.

**Separation of Proteins Using MEKC.** Protein separations and detection represent a unique analytical challenge in light of recent efforts devoted to proteome research and the diverse nature of proteins. When proteins are separated by capillary or micro-electrophoresis techniques, they can be detected via direct UV absorption (only if the protein contains a chromophoric amino acid residue) or laser-induced fluorescence following either covalent or noncovalent labeling with fluorescence being the preferred mode for microchips. In this series of experiments, we were interested in determining if contact conductivity detection could be used in PMMA based microchips for the analysis of proteins separated by MEKC.

In Figure 4 is shown an electropherogram of a protein mixture that was analyzed on an unmodified PMMA microchip using conductivity detection. In this example, the carrier electrolyte contained SDS (anionic micelle) as the pseudostationary phase. Clearly, even with the addition of SDS to the carrier electrolyte, we were able to detect signals generated from the migrating

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protein bands. As can be seen from the electropherogram, most of the protein components were sufficiently resolved, except for ovalbumin and serum albumin ($R_s = 0.41$) as well as phosphorlyase B and $\beta$-galactosidase ($R_s = 0.57$). The calculated plate number for myosin was found to be $2.54 \times 10^4$ (calculated using $N = 5.54t_{mig} W_{j}^{1/2}$), which yielded a value of $1.17 \times 10^{-4}$ cm for the height equivalent to a theoretical plate (H). Calculation of H assuming that the major contribution to band broadening is longitudinal diffusion produced a value of $1.33 \times 10^{-4}$ cm, close to the observed plate height for this separation. Therefore, diffusion is the major zone broadening mechanism for our protein separations and not extracolumn effects (finite injection plug length or detector length) or solute–wall interactions.

In this example (see Figure 4), the total protein concentration was $1.7 \mu M$, which resulted in signals above the background. The attractive feature of conductivity detection in this case is that the proteins did not require labeling with a fluorophore, which makes them readily accessible for downstream processing, such as proteolytic digestion followed by mass spectral fingerprinting for identification purposes. In addition, detection is nondestructive maintaining all material in an analyzable form.

**CEC Analysis of Oligonucleotides.** We have developed a micro-column reverse-phase separation of double-stranded DNA molecules using conductivity detection as an efficient method for the purification of PCR products. In this separation, a packed microcolumn consisting of C18-coated particles was used for the separation of a low mass ladder and isolation of a PCR product. The mobile phase contained acetonitrile as the organic modifier and an ion-pairing agent, TEAA. We were interested in adapting this separation for a PMMA-based microchip with conductivity detection. Recently, a CEC separation of neutral organic dye molecules in a glass-based open channel chip has been described. In this work, the coating (stationary phase) consisted of octadecylsilanes covalently attached to the glass surface using siloxane-based chemistry. Shallow channels were found to give smaller plate heights as predicted from theory for open-channel chromatography. While our channels were not shallow, they were designed to be narrow ($15 \mu m$) to yield acceptable plate heights for this separation.

In Figure 5A is shown the CEC analysis of double-stranded DNA fragments using an unmodified PMMA microchip. As can be seen, only one band appeared for the DNA fragments and, in addition, the background signal from the conductivity detector was very unstable. The lack of separation in this case is due to the relatively poor hydrophobic nature of the PMMA surface, allowing minimal partitioning (i.e., low capacity factor) by the ion-paired DNA complexes to the PMMA “stationary phase”. In addition, the instability of the conductivity response was due to dissolution of the PMMA surface by the acetonitrile organic modifier. This observation was supported through inspection of blank PMMA sheets subjected to this mobile phase, which significantly altered the appearance of the polymer.

To carry out this CEC separation, we next modified the PMMA surface by attaching (covalent) a C18 phase to the PMMA surface.

When we measured the sessile water contact angle of this modified PMMA surface, it was found to be $103^\circ$ compared to $63^\circ$ for native PMMA indication of increased hydrophobicity of the C18-modified surface. In Figure 5B is shown a separation of the DNA ladder using the C18-modified PMMA walls with conductivity detection. As can be seen from this electropherogram, the individual DNA fragments were well resolved and the stability of the conductivity signal evident, indicating no dissolution of the PMMA surface upon exposure to this mobile phase. Apparently, the C18 layer protects the underlying PMMA from acetonitrile attack. Also, the peaks observed for the oligonucleotides were negative due to the lower conductivity of the DNA bands compared to the background carrier electrolyte, consistent with our previous data for this type of separation with conductivity detection.

For most CEC separations, the driving force is electrokinetic, which is the result of EOF from the fused silica capillary and the silica microparticles. For native PMMA, it does display an EOF that runs from anode to cathode and possesses a value that is similar to the free solution electroforetic mobility of DNA. Because the electrophoresis was run in reverse mode, the electroforetic mobility of the DNA would run counter to the PMMA’s EOF giving a relatively long migration time. However, for the C18-modified PMMA surface, the EOF was measured to be $-1.9 \times 10^{-4}$ cm$^2$/V s$^{-1}$ (negative sign indicates cathode to anode movement). The reversal of the EOF for this modified chip is due to the diamine foundation layer used to attach the C18 phase to the PMMA substrate. Unreacted surface amine groups result in an excess surface charge that is slightly positive, producing the reversed EOF compared to native PMMA. This results in migration of the DNA fragments toward the anode, because the EOF and electrophoretic mobility of the DNA run in the same direction. It should be noted as well that the presence of these positive charges on the wall of the PMMA could result in anion exchange at the surface in addition to hydrophilic/hydrophobic phase partitioning, giving rise to mixed retention mechanisms. Under these conditions, one typically observes lower plate numbers compared to the absence of these mixed retention mechanisms. Calculation of the plate number for the 800 bp fragment ($t_{mig} = 6 s$, $t_{099} = 89 s$) was found to be $4.1 \times 10^4 m^{-1}$. In our previous work using a packed column and an ion-paired reverse phase separation (no mixed retention mechanism), we were able to achieve plate numbers of $5.1 \times 10^4 m^{-1}$. However, the eddy effects produced by the packed column used in our previous work should significantly reduce the plate numbers compared to the open channel format as used herein. Therefore, the mixed retention mechanism could be giving rise to reduced chromatographic efficiency. Elimination of these mixed retention mechanisms could be accomplished by end capping the residual amine groups, which would result in improved separation efficiency.

**CONCLUSIONS**

An integrated conductivity detector consisting of a pair of Pt electrodes was developed for a PMMA-based microfluidic device and used for the detection of various anionic materials separated by free-solution electrophoresis, M EKC or CEC. Attomole detection limits for alanine was demonstrated, comparable to those obtained using indirect laser fluorescence. The favorable detection
limits resulted from bipolar pulse operation, signal averaging, and small spacing of the electrode pair. Direct analysis of several proteins was presented with no requirement for labeling. In our examples, contact conductivity detection was used, and as such, slight electrode fouling was observed due to adsorption of analytes or other buffer components to the electrode surface. For obtaining quantitative information on a sample, it may thus be necessary to include an internal standard (such as benzoic acid added to the peptide mixture; see Figure 4) into the sample or consider using contactless conductivity detection. However, if only qualitative information is required, then slight degradation in the detector response is not an issue. In addition, due to the ease of fabrication of the polymer fluidic device and integration of the detector electrodes, replacement of the device is relatively simple, quick, and not prohibitively expensive when significant degradation of detector response results in device failure. We also were able to demonstrate that PMMA surfaces could be prepared with modified hydrophobic character, thus allowing the surface to behave as a viable stationary phase for reverse-phase separations. The C18-coated PMMA surface was found to be stable to organic modifiers used in the mobile phase (acetonitrile). Using conductivity detection, an ion-pair reverse phase separation of a low mass double-stranded DNA ladder was shown. The ability to use reverse-phase microchromatography with conductivity detection will serve as a viable platform for isolating certain DNAs required for subsequent analysis with high efficiency.4 From the results presented herein, it is clear that conductivity detection can be used to detect various polyanionic species separated by a variety of electrophoretic formats. While only anionic species were analyzed, the conductivity detector should work for mono- and/or polycationic species as well. And finally, due to the versatility and sensitivity of conductivity detection as well as its ease of implementation, it could potentially be used as a flow sensor in chips possessing a complex network of fluidic channels.

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