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Measurement of electroosmotic flow in plastic imprinted microfluid devices and the effect of protein adsorption on flow rate

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Abstract

Several commercially available plastic materials were used as substrates in the fabrication of microfluid channels for biochemical analysis. Protocols for fabrication using the wire-imprinting method are reported for polystyrene, polymethylmethacrylate and a copolyester material. Channel sealing was accomplished by low-temperature bonding of a substrate of similar material; therefore, each channel was composed of a single material on all sides. The electroosmotic flow in 25-μm imprinted channels was evaluated for each substrate material. The copolyester material exhibited the highest electroosmotic flow mobility of $4.3 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} which is similar to that previously reported for fused-silica capillaries. Polystyrene exhibited the lowest electroosmotic flow mobility of $1.8 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}. Plots of linear velocity versus applied electric field strength were linear from 100 V cm^{-1} to 500 V cm^{-1} indicating that heat dissipation is effective for all substrates in this range. Electroosmotic flow was reevaluated in the plastic channels following incubation in antibody solution to access the non-specific binding characteristics of a common biochemical reagent onto the substrate materials. All materials tested showed a high degree of non-specific adsorption of IgG as indicated by a decrease in the electroosmotic flow mobility in post-incubation testing. Published by Elsevier Science B.V.

Keywords: Electroosmotic flow; Instrumentation; Imprinted polymers; Polymer microfluid devices; Proteins

1. Introduction

The majority of reported applications in the area of microfluidics have utilized silica/glass microfabricated channels. The motivations for using glass are: (1) photolithographic methods for microfabrication in glass are well established, and (2) the electroosmotic and chromatographic properties of glass have been well-characterized. Therefore, applications employing glass devices have yielded rapid, predictable results. Conversely, applications of plastic microfluid devices have been less successful due to the lack of fundamental knowledge of some basic surface properties of plastic substrates. It is difficult to work in a regime where the surface-to-volume ratio is quite high and surface interactions dominate, yet understanding of fundamental surface characteristics, such as electroosmotic flow and analyte adsorption, is limited.

Interest in the development of plastic microfluid devices is increasing with a growing number of

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methods being reported in the literature for fabricating plastic microfluid channels. Micrometer-sized channels have been produced in various plastic substrates by X-ray photolithography [1], ''soft'' lithography [2,3], plastic ultraviolet photolithography [4,5], hot embossing or imprinting [6,7], laser ablation [8], and injection molding [9]. Although considerable progress has been achieved in the design and development of plastic devices in the last two years, their successful implementation in chemical and biochemical separations remains largely undeveloped.

Electrically driven pumping is still the most prominent mechanism for transporting fluids in microfluid channels [10–13]. Electrically driven pumping includes electroosmotic pumping, electrophoretic movement, and electrohydrodyamic pumping [14]. All of these rely on the incorporation of electrodes into the channel design and the manipulation of the electric field across a solution that results in directed pumping through the channels. Many chemical analyses performed in microfluid channels (and also in the related technique of capillary electrophoresis) employ electrokinetic separation which is the combined effect of electroosmotic pumping and electrophoresis.

It is important to note that electroosmotic pumping is a wall-driven phenomenon and the electroosmotic flow rate under the influence of an applied electric field is largely dependent on the wall charge. For glass, the charge on the surface is negative at neutral pH due to the presence of ionizable siloxyl groups. The charge on the surface of many polymers could be predicted from the fundamental knowledge of the polymer itself, and this might be used to predict the electroosmotic flow rate in microchannels prepared using polymers. Many commercially available polymers, however, have additives and surface treatments including phthalate esters, phosphate esters, epoxy plasticizers and glycol derivatives, to improve the casting or molding process or to stabilize the polymer after curing. As a result, the charge on the plastic surface is not readily predicted from the structure of the bulk polymer without knowledge of the proprietary additives. This lack of surface structure knowledge requires the electroosmotic flow to be measured directly. The purpose of this study is to create microfluid channels in several commercially available plastic substrates, and to evaluate electroosmotic flow in these channels. Thus far, there have been few reports in the literature evaluating the electroosmotic flow in plastic materials [8,15–17]. The channels in this study will also be evaluated for the effect of non-specific protein adsorption on electroosmotic flow rate by monitoring a change in flow following incubation in concentrated antibody solution. This type of data can be very useful for optimizing the choice of material for biochemical applications of microfluid systems.

2. Experimental

2.1. Reagents

Plastic sheets were used including the following: acrylic (Lucite CP, ICI Acrylics, Memphis, TN, USA), polystyrene (Corning Costar, Cambridge, MA, USA), copolyester (Vivak, DSM Engineering Plastic Products, Sheffield, MA, USA). Chromel wire (25 μm in diameter) was obtained from Omega (Stamford, CT, USA). Purified goat anti-human IgG and fluorescein isothiocyanate labeled monoclonal mouse anti-human IgG1 (FITC-labeled antibody) were obtained from Sigma (St. Louis, MO, USA). Phosphate buffer, 20 m*M* and 10 m*M* at pH 7.0, were prepared from potassium dihydrogenphosphate and disodium phosphate, also acquired from Sigma.

2.2. Channel fabrication protocol

Channels were fabricated using the wire-imprinting method reported previously [7]. The protocol is shown in Fig. 1 and is briefly described by the following steps. Chromel wire was stretched taut lengthwise over a clean piece of plastic (7.6 cm×2.5 cm). The wire and plastic were then clamped between two clean glass microscope slides and placed into an oven for 7 min at a temperature higher than the softening temperature of the plastic. The assembly was removed from the oven and allowed to cool to room temperature. The clamps and wire were then removed exposing the formed channel.

To cover and seal the channel, a second piece of the same plastic material with similar dimensions was used. Holes (2 mm in diameter) were drilled

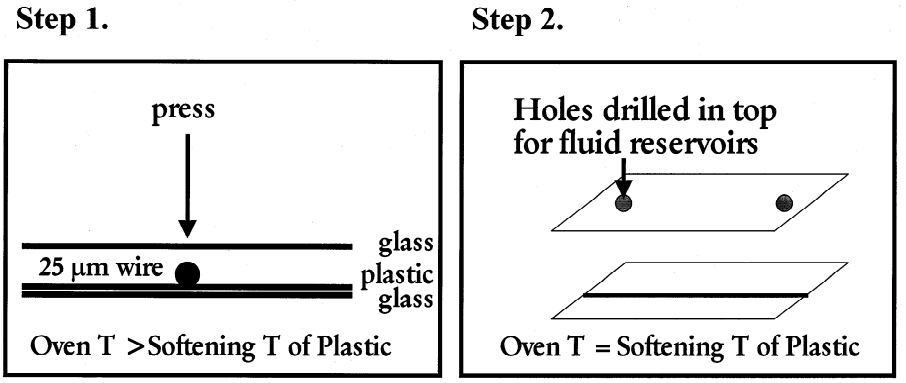

Fig. 1. Preparation of plastic microfluid devices by the wire-imprinting technique.

approximately 5 cm apart through the cover piece prior to sealing. These holes would later serve as wells to contain the buffers. Rough areas around the drilled holes were generally smoothed out by clamping the drilled piece of plastic between microscope slides and heating in the oven for 5 min. The cover piece of plastic was then cleaned with pressurized air to remove all particulates and was placed over the imprinted plastic piece. The two plastic pieces were placed between microscope slides, clamped, and placed into the oven at a temperature just below the softening temperature of the plastic. After 10 min, the assembly was removed from the oven and the completed device was ready for use in experiments. The channels were primed with triply distilled water by placing the water into one well and pulling a vacuum on the other well. The channels remained filled with water until the devices were used.

2.3. Measurement of electroosmotic flow

The electroosmotic flow in the microchannels was determined using the current monitoring method [18]. The method is outlined in Fig. 2. Briefly, the channels were filled by vacuum with a solution of 20 m*M* phosphate, pH 7.0, just prior to use. Electrodes placed into each well were connected to the circuit shown in the figure. The liquid was removed from the wells and equal aliquots of fresh 20 m*M* phosphate buffer were again placed into both wells to reduce error in the measurement caused by evaporation. The high-voltage (HV) power supply was

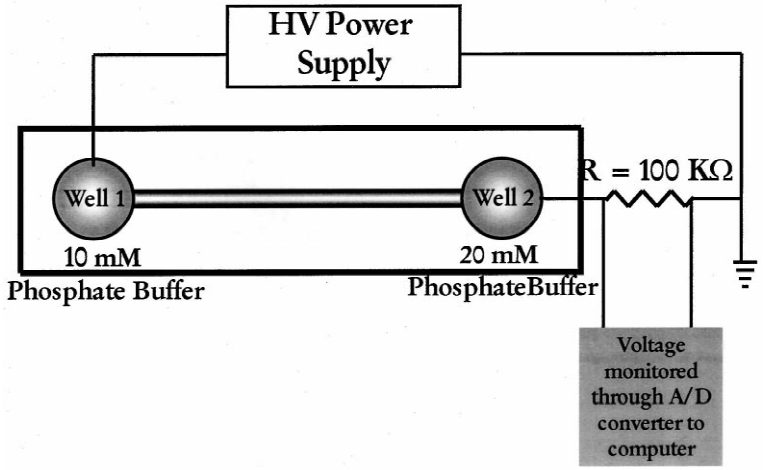

Fig. 2. Measurement of electroosmotic flow rate using the current monitoring method.

immediately switched on and the output voltage across a 100 KΩ resistor in the circuit was measured and recorded on the computer. The voltage was used to determine the steady current in the channels. This measurement provided a baseline current for the condition when 20 m*M* phosphate completely filled the channel. After completing this measurement, the power supply was turned off and the liquid was removed from both wells. An aliquot of 10 m*M* phosphate, pH 7.0, was then placed into well 1 and an aliquot of 20 m*M* phosphate, pH 7.0, was placed into well 2. Both wells were again filled simultaneously. The high voltage power supply was then switched on and the voltage drop across the resistor was monitored. The voltage measurements acquired by the computer were then converted to current by dividing by the 100 kΩ resistance.

In this method, an initial steady current was measured when the channel was filled with a higher conductivity buffer. When a second buffer of lower conductivity was introduced from one well, the current decreased until the channel was completely filled with the second buffer. At this point, a second steady state current was achieved. Typical results of these experiments are shown in Fig. 3 using a copolyester microchannel and measuring the current at an applied electric field strength of 300 V cm^{-1}. The first measurement in Fig. 3 (line A) started at a maximum value which was representative of the current when 20 m*M* phosphate buffer filled the channel. The current immediately began to decrease indicating that the channel was filling with 10 m*M* phosphate buffer. A minimum value for the current was obtained when the channel was completely filled with the lower conductivity buffer (10 m*M* phosphate). The time required to reach the second steady state current, *t*, was the amount of time for the second buffer to completely fill the microchannel. The wells were filled simultaneously with equal volumes in each experiment to minimize error in the measurement caused by flow induced from hydrostatic pressure [19]. This procedure was repeated in each channel six times varying the voltage on the high-voltage power supply so that the electric field

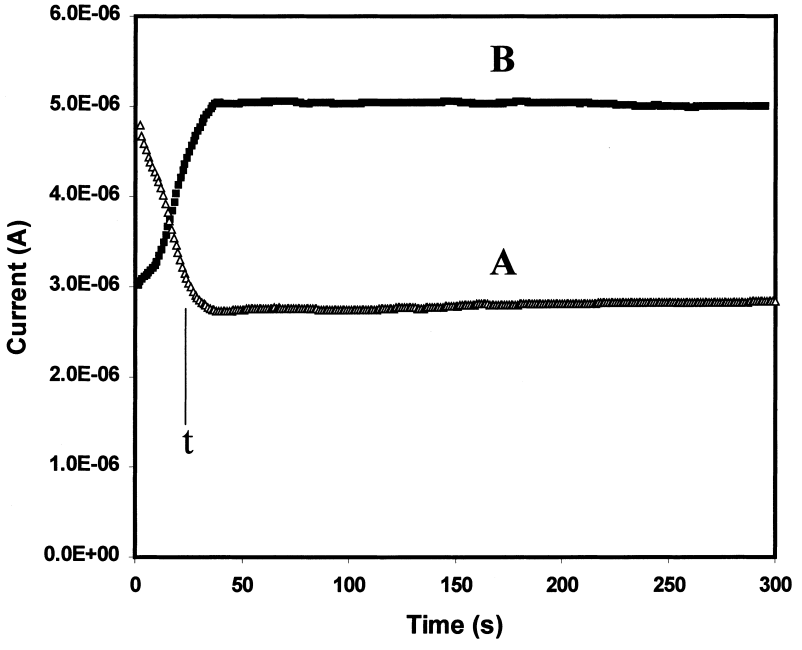

Fig. 3. Data obtained from current monitoring method using copolyester channel with an applied electric field strength of 400 V cm^{-1}. Line A (▲) represents the data obtained when the electric field was applied with 10 m*M* phosphate in well 1 and 20 m*M* phosphate in well 2. Line B (■) represents the data obtained when the electric field was applied with 20 m*M* phosphate in both well 1 and well 2. See Experimental for detailed description.

strength across the channel was 100, 200, 300, 400, 500 or 600 V cm^{-1}. Between each measurement of electroosmotic mobility, the channel was refilled with the higher conductivity buffer (20 m*M* phosphate). This was accomplished by placing 20 m*M* phosphate buffer into both wells and reapplying the electric field. The current was monitored during this step also (Fig. 3, line B) to ensure that the channel was completely filled with the higher conductivity buffer prior to the next experiment. Calculations of the electroosmotic flow rate were made at each field strength.

2.4. Calculations of electroosmotic flow

The electroosmotic flow rate was measured in three different plastics using the current monitoring method. The time required for the second buffer to completely fill the microchannel, *t*, was then used to calculate electroosmotic flow rate using the following equation: $v_{eof}=Lt^{-1}$ where *L* is the length of the channel and *t* is the time it takes for the second buffer to completely fill the channel. An average electroosmotic flow rate was calculated from the measurement taken from four to six different applied field strengths. The electroosmotic mobility is described as the electroosmotic flow rate normalized for the field strength, or $v_{eof}E^{-1}$, where *E* is the electric field strength.

2.5. Determination of antibody adsorption

Following the determination of electroosmotic flow in the native plastic channel, the buffers were removed from the wells and a 25-μl aliquot of $1.6\cdot10^{-5}$ mol l^{-1} purified antibody was placed into well 1. Vacuum was applied to well 2 for 5 min to move the antibody solution into the channel. The device was then covered to prevent evaporation, and placed into the refrigerator overnight. The antibody solution was then replaced with 20 m*M* phosphate buffer solution and pulled into the channel using a vacuum. Measurements of electroosmotic flow were then repeated. A very high current was generally an indication that the antibody solution had not been completely removed from the channel since the original antibody solution was prepared in phosphate buffered saline with a high concentration of salt (150 m*M*). If a high current was encountered, the channel was rinsed again and the measurement was repeated.

2.6. Injections of fluorescently labeled antibody

Antibody adsorption to the microchannel walls was also tested by injecting aliquots of FITC-labeled antibody into the channels and monitoring the fluorescence signal measured as the individual samples moved through the channels. Fluorescence was monitored near the end of the microchannel using a photomultiplier tube detector (Hamamatsu, Bridgewater, NJ, USA) mounted to the top port of a fluorescence microscope equipped with a mercury lamp for excitation. Emission was detected through a 515 nm long-pass filter. Discreet injections of the FITC-labeled antibody solution were achieved using a variable volume sample fill method described previously [20]. For the purposes of these experiments, devices were fabricated with two perpendicular cross channels to perform discreet injections.

3. Results and discussion

3.1. Imprinting plastic substrates

Creating a reproducible impression in the plastic substrates using the wire-imprinting technique required heating the substrate to a temperature higher than the softening temperature of the plastic. There was some flexibility in the imprinting temperature; however, if the temperature was too high, the plastic melted causing it to adhere to the glass support. Conversely, the sealing temperature was quite critical since as little as one degree above the optimal temperature caused the channels to soften and deform, and a single degree below optimal temperature often prevented the channel from completely sealing. The advantage of the wire-imprinting procedure is its simplicity; devices can be made in approximately 15 min in almost any analytical laboratory with minimal instrumentation. Optimal results were achieved using an oven with good temperature control and with circulating air (such as the oven of an analytical gas chromatograph) preventing ''hot spots'' that caused channel deformation. Conditions were optimized for fabricating and sealing the acrylic, polystyrene and

Table 1
Optimized conditions for sealing and fabrication of the plastics studied

Plastic	Imprinting time and temperature (plastic piece No. 1)	Smoothing time and temperature (plastic piece No. 2)	Sealing time and temperature (both pieces)
Acrylic	110°C, 7 min		108°C, 10 min
Polystyrene	112°C, 7 min	112°C, 5 min	105°C, 10 min
Copolyester	80°C, 7 min	80°C, 5 min	75°C, 10 min

copolyester sheets listed previously. These conditions are summarized in Table 1 for each of the plastics tested. For the acrylic material, the area around the drilled holes was easily smoothed using a drill bit to remove rough shards of the plastic. For softer plastics, the rough areas could not be effectively removed in this manner, therefore, the device was smoothed by heating in the oven for a short time prior to sealing.

3.2. Electroosmotic flow in plastics

The electroosmotic flow rate was measured in 25-μm wire-imprinted channels prepared in the acrylic, polystyrene and copolyester sheets. The electroosmotic flow rate is known to be a function of several parameters with one of the most important parameters being the surface charge on the channel walls. Determination of the electroosmotic flow rate in the various plastics is therefore indicative of the charge density on the plastic surface. The direction of the flow indicates whether the charge is positive or negative. Measurements of electroosmotic flow rate were made using the current monitoring method described in the previous section. Diffusion of the second buffer into the channel was found to be a problem when the initial current value, upon introduction of the lower conductivity buffer, was significantly different from first steady state current measurement. If significant effects of diffusion were noted, the measurement was repeated.

A single run or determination of the electroosmotic mobility was made by measuring the electroosmotic mobility at various electric field strengths on a single channel on one day and then averaging these values to obtain a single data point. The within-run (single run, one day) measurements of electroosmotic mobility gave reproducible values with average relative standard deviations of 9.4%, 11.9% and 8.4% for the acrylic, copolyester and polystyrene materials, respectively. Values of electroosmotic mobility obtained at the lowest electric field strength (100 V cm^{-1}) were the least reliable in this measurement. It was difficult to obtain an accurate determination of t at low field strengths because the slope of the line in the plot of current versus time was very low. The average run-to-run relative standard deviations calculated from separate runs made on the same microfluid channel on different days were 11.9%, 7.3% and 5.8% for acrylic, copolyester and polystyrene channels, respectively. Between runs, the channels were stored filled with 20 m*M* phosphate buffer solution. The device-to-device relative standard deviations for electroosmotic mobility were 16.1% for four different channels prepared in the copolyester sheet and 28.2% for five different channels prepared in the acrylic sheet. All of the copolyester devices were prepared from a single sheet of material. Devices prepared in acrylic were made from sheets acquired from two vendors which may account for the higher variability in the measured electroosmotic mobility. It is important also to note that the channels were rinsed only with aqueous solutions prior to use, therefore only water-soluble contaminants could have been removed from the device prior to measurement of the electroosmotic flow.

The effect of electric field strength on electroosmotic flow rate is shown in Fig. 4 for the three plastic substrates. The flow rate was linearly dependent on field strength for each of the plastics implying that heat dissipation in these substrates is effective with fields of 600 V cm^{-1} or less. The electroosmotic flow rates and the electroosmotic mobilities for all plastics are compared in Table 2. Also included in this table are data for fused-silica capillaries [21], and laser-ablated polystyrene [8] obtained from the literature. It can be seen that the

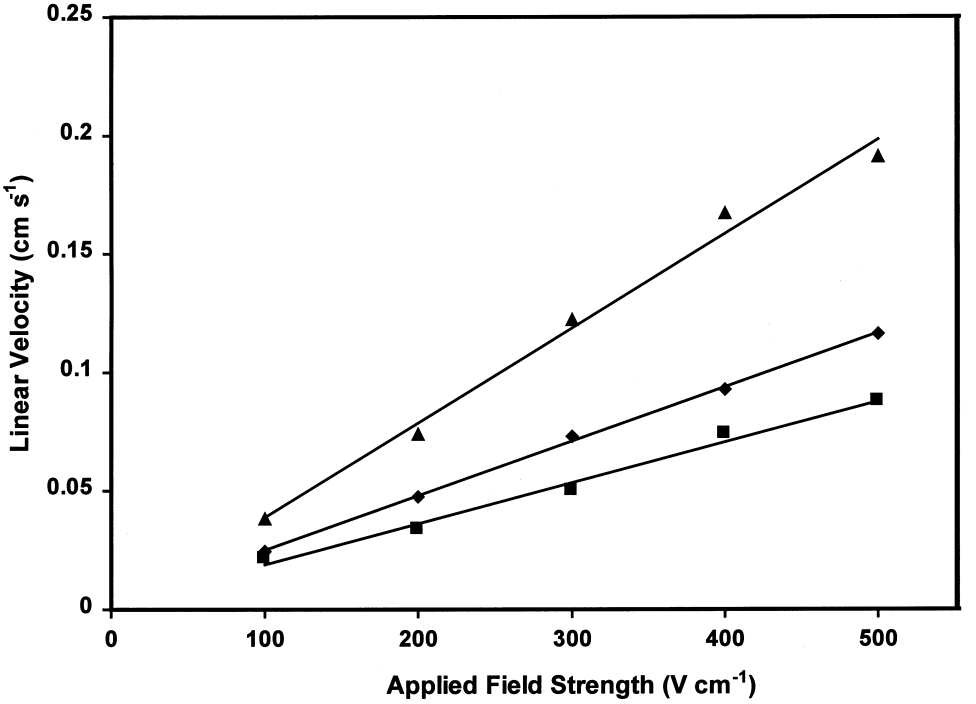

Fig. 4. Linear velocity versus applied electric field for three types of plastic: ♦ acrylic, r^2=0.9896; ▲ copolyester, r^2=0.9896; ■ polystyrene, r^2=0.9881.

flow rates for the plastics examined in this study cover a wide range; the lowest flow rate associated with the polystyrene imprinted sheet and the highest associated with the copolyester sheet. The general direction of flow in each plastic was toward the negative electrode as is the case for glass. The copolyester sheet exhibited an electroosmotic mobility similar to the fused-silica capillary. One advantage of using plastics for chemical separations is the possibility of obtaining a broad spectrum of electroosmotic flow rates. Substrates may be chosen based on the type of electroosmotic flow needed for a particular application. In the separation and analysis of chemical species that have the same type of charge, such as DNA, it is desirable to eliminate electroosmotic flow or maintain electroosmotic flow in the opposite direction from the migration of analytes to improve the separation resolution. In this case, polystyrene may be the most useful substrate. However, when the chemical species are oppositely charged it may be advantageous to have some significant electroosmotic flow to move all species in the same direction past the detection window. In this case, plastics exhibiting a higher electroosmotic flow

Table 2
Electroosmotic flow rates and mobilities for the plastics studied

Substrate	Linear velocity at 300 V cm^{-1} (cm s^{-1})	Electroosmotic flow mobility (cm^2 V^{-1} s^{-1})	*n* (number of measurements)
Fused silica [18]	$1.5 \cdot 10^{-1}$	$5.0 \cdot 10^{-4}$	
Acrylic imprinted	$7.5 \cdot 10^{-2}$	$2.5 \cdot 10^{-4}$	71
Copolyester imprinted	$1.3 \cdot 10^{-1}$	$4.3 \cdot 10^{-4}$	50
Polystyrene imprinted	$5.4 \cdot 10^{-2}$	$1.8 \cdot 10^{-4}$	20
Polystyrene laser ablated [8]	$9.7 \cdot 10^{-2}$	$4.5 \cdot 10^{-4}$	

rate, such as the copolyester, may be the most applicable to this separation.

The value of electroosmotic flow for the polystyrene-imprinted channel was much lower than that value previously reported for laser-ablated polystyrene channels. Although some variation might be expected between plastics obtained from different vendors, differences may also be induced by the fabrication procedure itself. It has been reported that the process of laser ablation in air chemically alters the surface by introducing charges from incorporated oxygen reactive species. This increase in surface charge should, thereby, increase the electroosmotic flow rates in ablated channels. The value that was obtained for the imprinted channels is presumably from the native plastic with little to no chemical alteration as a result of the channel fabrication procedure. This may account for the lower flow rates measured for imprinted channels when compared to laser-ablated polymer channels.

The plastics tested were substrates that could be effectively sealed to the same material using a low-temperature bonding procedure. For this reason, polycarbonate was not included in this study, although it is reported to exhibit good properties for biochemical measurements. Many literature reports of plastic devices utilize different materials for bonding thereby creating devices that are essentially hybrids of two materials. It is probable that the electroosmotic flow rates will be altered by the effect of using dissimilar materials. This is the first report of electroosmotic mobility measured in plastic devices with a single substrate material forming all sides of the channel.

3.3. Effect of antibody adsorption on electroosmotic flow in plastics

Microchip immunoassays with electrokinetic separation of components have several advantages over other immunoassay approaches since they are homogeneous, require small sample and reagent volumes, and may be successfully carried out in low electric fields. Few immunoassay separations have been reported in the literature using plastic microchannel devices [7,19,21] which may be related to the problem that some common plastics exhibit a high degree of non-specific protein adsorption. Glass/silica capillaries also demonstrate a characteristically high degree of protein adsorption; therefore, many protein separations are conducted in capillaries coated with a biocompatible polymer. With the wide range of chemical properties available from commercial plastics, it should be possible to find a native material that exhibits a low degree of non-specific adsorption so that channel modification would be unnecessary.

To evaluate the non-specific adsorption of a test protein to the imprinted plastic channels, monoclonal antibody was incubated in the channels overnight. The electroosmotic mobility was measured in each channel before and after incubation to provide an indication of any changes in the surface caused by the adsorbed protein. The results are shown in Fig. 5. From these data, it can be seen that all plastics tested for the purposes of this study exhibited a high degree of adsorption as indicated by the significant decrease in electroosmotic mobility in the channels. Although the flow was decreased in all cases, there was no evidence of flow reversal in the devices following protein adsorption. The ratio of electroosmotic mobility before and after antibody incubation is 0.26, 0.14 and 0.26 for acrylic, polystyrene and copolyester, respectively. The reduction of electroosmotic mobility (and electroosmotic flow) is mainly a result of blocking the surface charge on the plastic with adsorbed protein. This high degree of non-specific adsorption makes plastic devices suitable for use in heterogeneous adsorption immunoassays where adsorbed antibody reacts with soluble antigens. The change in flow that results from non-specific adsorption might prove problematic for homogeneous solution-phase immunoassays unless the surfaces were pre-treated with the adsorbing antibody. Such pretreatment with the antibody prior to the assay would minimize the change in flow that occurs upon adsorption during the analysis.

Antibody adsorption in the microchannels was also evaluated by sequentially injecting equal aliquots of fluorescently labeled antibody into the channels and monitoring the resulting peak height. As shown in Fig. 6, the first three injections of antibody solution resulted in low, but increasing, peak heights indicating that much of the injected protein adsorbed to the wall in these first injections. After the fourth injection, the maximum peak height

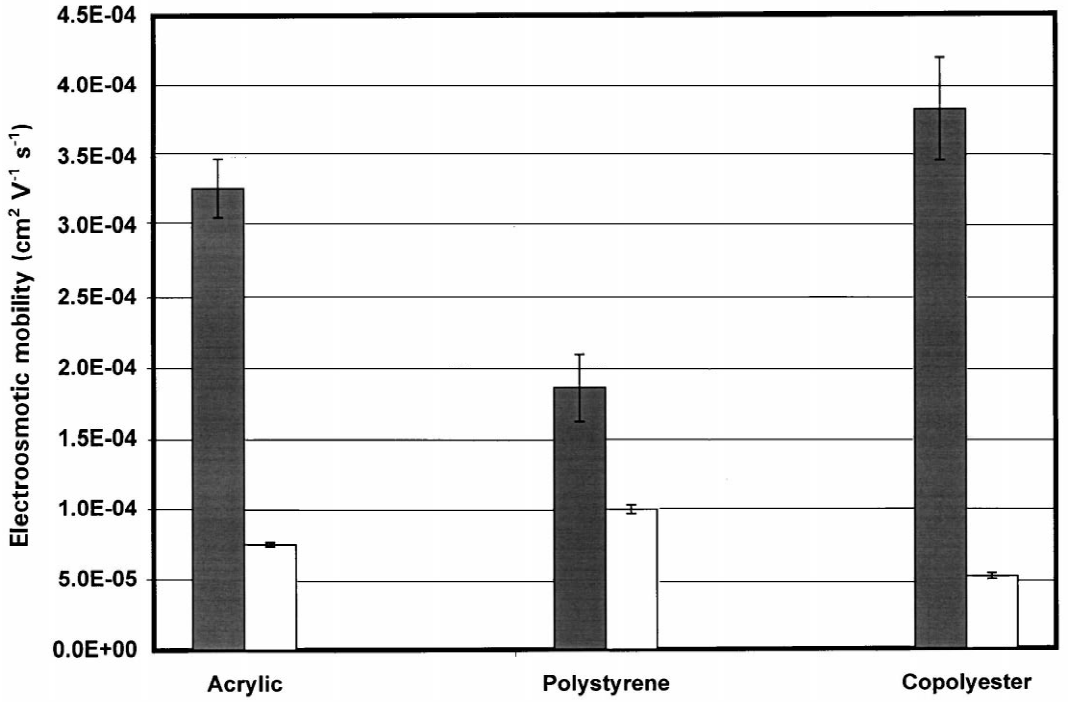

Fig. 5. Comparison of electroosmotic mobility in the native plastic (shaded) versus the antibody-adsorbed (clear) plastic.

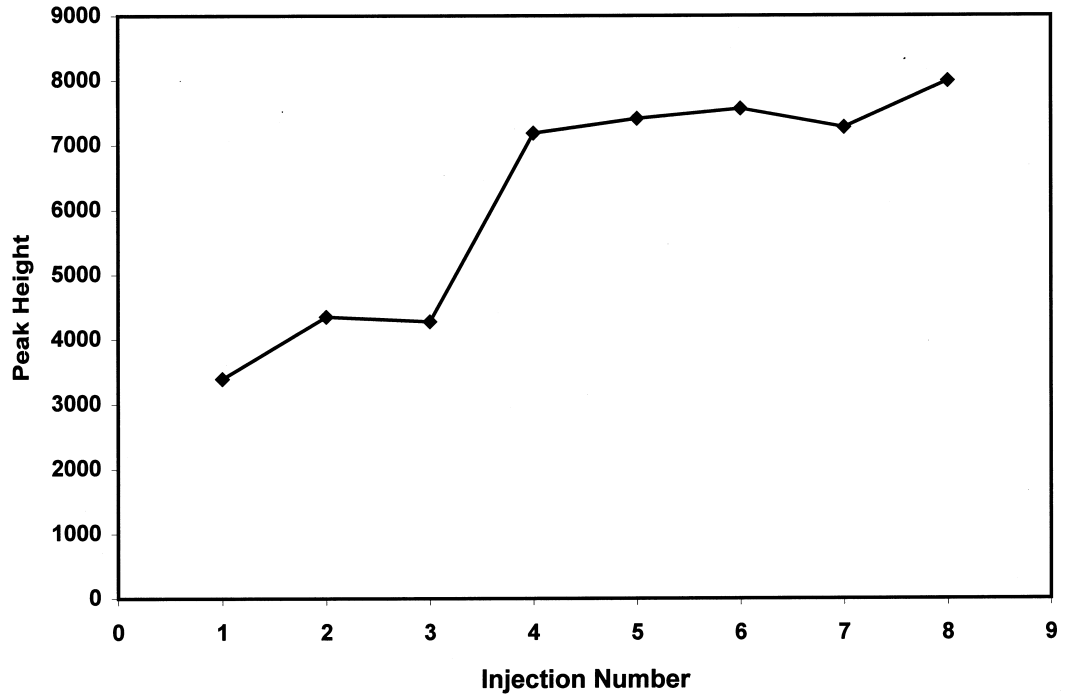

Fig. 6. Measured peak height versus injection number for eight sequential injections of fluorescein-labeled antibody.

was obtained and subsequent injections showed very reproducible results. These results indicated that the protein remained bound to the plastic between injections and that priming the system with antibody led to a stable system suitable for subsequent homogeneous immunoanalysis.

4. Conclusions

We have demonstrated the ability to fabricate microchannel devices rapidly and easily in several common commercially available plastics. This study is also the first characterization of electroosmotic flow in plastic microchannel devices made from a single material. Channels fabricated in the three plastics chosen for this study all exhibited electroosmotic flow in the same direction as glass indicating that the net surface charge in all microchannels was negative. These plastics demonstrated a broad range of electroosmotic mobilities with polystyrene devices having the lowest electroosmotic mobility, and copolyester devices showing the highest electroosmotic mobility. Presumably, the charge is a function of additives and not surface contamination or surface alteration caused by the fabrication technique. To validate these assumptions, we intend to further characterize these and other plastics to determine the chemical groups responsible for introducing surface charge.

5. Disclaimer

Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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