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Poly(acrylamide-*co*-alkylacrylamides) for Electrophoretic DNA Purification in Microchannels

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We have created a family of water-soluble block copolymers of acrylamide and *N*-alkylacrylamides that are designed to selectively remove proteins from DNA via microchannel electrophoresis. It was hypothesized that the inclusion of hydrophobic subunits in the polymer chain, in sufficient concentration, could lead to protein adsorption due to hydrophobic interactions. A series of *N*-alkylacrylamide comonomers with varying alkyl chain lengths (C4, C6, C8) and also an *N*,*N*-dialkyl group (C6−C6) were synthesized via reactions between acryloyl chloride and the respective alkylamines. Copolymers were synthesized using an aqueous "micellar" polymerization technique, which involves dissolving acrylamide in the aqueous phase while hydrophobic monomers are solubilized in sodium dodecyl sulfate micelles. Copolymers comprising up to 4 mol % of a hydrophobic subunit (as verified by ^{1}H NMR) were synthesized. Polymer molecular weights were determined by tandem gel permeation chromatography−multiangle laser light scattering, and ranged from 1.5×10^6 to 4.3×10^6. Capillary electrophoresis analysis of bovine serum albumin and *β*-lactoglobulin migration in these matrixes revealed that the octylacrylamide and dihexylacrylamide copolymers show the most significant extent of protein adsorption while butylacrylamides show no noteworthy adsorption trend. All copolymer matrixes studied allowed the passage of a dsDNA digest, and displayed some DNA sieving ability at 0.5% (w/w) in TTE (50 mM Tris, 50 mM TAPS, 2 mM EDTA, pH 8.4) buffer. These matrixes are demonstrated in on-chip experiments to adsorb protein, in a step toward meeting the front-end processing goals of *μ*-TAS for genetic analysis applications.

One of the most significant accomplishments of the 20th century was the sequencing of the human genome.[1,2] The availability of the genome sequence will facilitate undertakings in gene therapy, forensic analysis, biological weapon detection, pharmaceutical development, and countless other areas. Over the past several years the development of "lab-on-a-chip" technologies for genetic analysis has been at the forefront of research in the analytical chemistry community and offers the promise of faster, highly parallel, automated, integrated devices. These efforts have been predominantly focused on scaling down current techniques for biomolecule analysis, such as electrophoresis,[3] DNA amplification (polymerase chain reaction (PCR)),[4−6] and on-line fluorescent labeling,[7] and creating ultra-high-throughput devices that can analyze samples in a parallel fashion.[8,9] Although many achievements have been made in scaling down these analytical processes, there remains much work to be done in the development of new materials designed specifically for use in these microfluidic devices.[10,11] Moreover, to date, with a few notable exceptions, published reports have dealt with the analysis of simple and prepurified biological samples, containing only the analytes of interest, and thus have ignored the integration of preprocessing sample treatment steps on bioanalysis chips.

While the extraction and isolation of DNA from cellular material is one of the most commonly used procedures in genetics, molecular biology, and biochemistry, most of the current macroscopic techniques are time-consuming (∼hours) and not amenable to implementation or integration on a microfluidic platform. The major hurdles in this regard are centrifugation and precipitation of DNA. The precipitation and recovery of DNA with ethanol has yet to be accomplished on a chip device. In fact, the associated centrifugation steps required, in both this and other laboratory-scale DNA isolation methods (such as the commonly used Qiagen kit), typically exceed 5000*g*. The scaling laws of centrifugation become unfavorable for small microchip devices, requiring high spin rates, especially as further device footprint miniaturization makes microdevices more portable and affordable. Hence, a simple scaling down of current techniques into the microscale format (centrifugation and precipitation) may not be a promising approach to DNA purification from a complex biological milieu.

For microfluidic devices to be useful "in the field", they must have the capabilities to perform front-end tasks such as the

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processing of raw, complex biological samples to isolate the target analytes of interest, often at low concentrations or of low abundance.[10] Clearly, sample preparation and purification is one limiting step in the creation of an integrated, microscale DNA analysis device, which remains to be fully addressed. Only a few research groups have published demonstrations of on-chip preprocessing and sample purification methods. Among the approaches used are filtration,[12−17] diffusive laminar liquid extraction,[18−21] specific DNA hybridization,[22] and solid-phase extraction (SPE).[23−28]

One of the most promising and generally useful preprocessing techniques demonstrated to date is on-chip SPE with the use of silica-based beads 1.5−5 μm in diameter, which are typically coated with an alkylsilane. Loading these particles into microdevices so that they form a consistent and uniform bead bed can be difficult.[29] To date, most studies of these systems have concentrated on the separation of small molecules (such as fluorescent dyes). The most successful and biologically relevant demonstration of on-chip sample purification using SPE was reported by Landers et al.[30] Their goal was to extract DNA from cells, on a microfluidic chip, for subsequent genetic analysis by electrophoresis. They report packing a reservoir in a microfluidic device with commercially available SPE beads. Subsequently, they showed that if they loaded whole blood lysate onto the chip, and then eluted the adsorbed biomolecules via hydrodynamic flow with changing buffer conditions, some fractions obtained from the chip contained enough DNA to be used for subsequent (off-chip) PCR with successful amplification. In addition to the desired DNA binding, nonspecific adsorption of proteins and lipids can occur on the beads. A wash step could be included to remove some of these proteins; however, it might be difficult to obtain completely pure DNA (free of proteins and other biomolecules) by this SPE approach.

The most significant limitation of SPE beds for rigorous on-chip DNA purification is their limited surface area for adsorption and thus relatively low load capacity. A relatively large diameter, nonporous bead has only its outer surface available for molecular interactions, and this may limit the amount of DNA collected. In a different approach to biomolecule purification, the surface area available for extraction has been increased by the introduction of a monomer solution into microchannels, which is then polymerized in situ to create a well-defined porous structure,[31−33] in a system that showed enrichment factors of 1000 for hydrophobic peptides and green fluorescent protein (from a dilute peptide/protein solution[31,32]). These polymer systems have been shown to be reusable, but are not replaceable, and have yet to be demonstrated for purification of DNA from a complex biological matrix.

In work done by the Mathies group, a very promising on-chip sample preprocessing technique has been investigated, based on the use of specific DNA hybridization to selectively capture DNA strands terminating in a known sequence, for subsequent analysis by electrophoretic DNA sequencing.[22] An anchored, complimentary ssDNA sequence (attached to a polyacrylamide matrix) is used to capture ssDNA products of a Sanger cycle-sequencing reaction. When the products of the PCR reaction were electrophoresed through the "capture gel", the DNA amplicons of interest hybridized while other sample components (salt, primers, polymerase) passed through. Electric field and temperature conditions for optimal DNA capture were identified. This method, while elegant and nicely applicable for PCR and cycle-sequencing reaction product purification, may not be able to purify larger, nonamplified dsDNA targets from raw cell lysate.

The strategy investigated in this work is to purify dsDNA from proteins and (eventually, in future formulations) from other cellular debris using a combination of microchip electrophoresis and hydrophobic adsorption of nongenetic material onto hydrophobically modified polyacrylamide (HMPAM) networks. The first technique, microchannel electrophoresis, enables a kinetic separation by discriminating molecules on the basis of their charge-to-hydrodynamic friction ratio, as well as by their polarity of charging (positive vs negative). DNA, a negatively charged molecule, will migrate toward the higher potential, while positively charged molecules will migrate toward the lower potential, and neutral molecules will remain motionless. Thus, this technique should intrinsically separate DNA from all neutral or positively charged biomolecules. Thus, only negatively charged lipids and proteins must be separated by the second modality, hydrophobic interaction. We have designed copolymers based on a polyacrylamide backbone to irreversibly adsorb proteins, with (presumably) ample surface area for adsorption. The inclusion of hydrophobic subunits in a polymer network could lead to substantial and irreversible protein and lipid adsorption via polymer interactions with the protein's hydrophobic amino acids or lipid alkyl groups, respectively, while (we hypothesized) allowing DNA to freely pass during electrophoresis. Thus, two modalities of purification/isolation are

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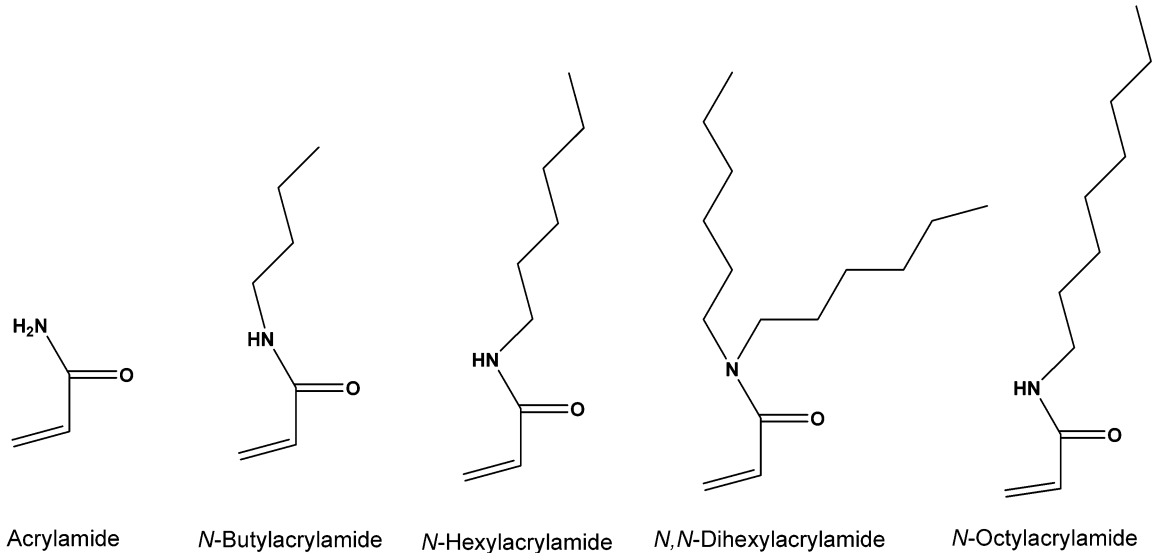

Figure 1. Chemical structures of acrylamide and the hydrophobically modified acrylamides studied.

used to create a microchip-based electrokinetic chromatography "guard column", or protein/lipid sponge. Success in these endeavors could lead to the creation of a technology that will facilitate rapid genetic analyses on integrated microfluidic devices that can accept and process raw cell lysate.

MATERIALS AND METHODS

Monomer Synthesis and Recovery. Hydrophobic *N*-alkyl- and *N*,*N*-dialkylacrylamide monomers were created by the reaction between acryloyl chloride and a corresponding alkylamine. The synthesis procedure used to create these monomers is similar to that reported by McCormick.[34] The amines, including butylamine, hexylamine, octylamine, and dihexylamine, and acryloyl chloride (hazardous) were purchased from Sigma-Aldrich (Milwaukee, WI) and used without further purification. Briefly, the reaction is carried out in a round-bottom flask chilled to 0 °C. An amine, for example, hexylamine, is dissolved to 10 mM in 60 mL of methylene chloride. Triethylamine is included at equal molarity to neutralize the hydrochloric acid byproduct of the reaction. Acryloyl chloride is dissolved in 60 mL of methylene chloride (to a 10 mM concentration) and added to the reaction vessel using an addition funnel under equal pressure. The alkylacrylamide product is then extracted by three consecutive additions of aqueous solution: 100 mM NaOH, 100 mM HCl, and finally water. Following the final extraction, the mixture is placed in a rotary evaporator to remove the methylene chloride. A schematic illustration of the series of *N*-alkylacrylamide comonomers created with varying alkyl chain lengths (C4, C6, C8) as well as an *N*,*N*-dialkylacrylamide (C6−C6) is presented in Figure 1.

Polymer Synthesis and Recovery. The polymers synthesized for this research were created by a technique called "micellar" polymerization,[35−40] which has many similarities to emulsification polymerization. The reaction medium is primarily water, with microdomains of sodium dodecyl sulfate (SDS) above its critical micelle concentration (cmc). The hydrophobic comonomers, which are not water-soluble, are dissolved in the interiors of the SDS micelles. This technique allows the copolymerization of acrylamide with hydrophobic (and otherwise water-insoluble) *N*-alkylacrylamides. The use of SDS microdomains of varying size also allows direct control over polymer properties such as the average concentration (mol %) of hydrophobe incorporation and average hydrophobic block length within the copolymers.[35,38]

All polymerization reactions were conducted in either a 300 or a 500 mL four-neck flask (Kontes, Vineland, NJ), with 100 mL of water as the basis for the reaction volume. To water are added acrylamide (Amresco, Inc., Solon, OH) and SDS (Sigma, St. Louis, MO). Typically, 3 wt % acrylamide and 2.5−3 g of SDS are initially dissolved. The mixture is stirred for 30 min, undisturbed, and then degassed by nitrogen bubbling for an additional 30 min. After sufficient bubbling time has removed oxygen from the reaction medium and allowed for hydrophobic monomers to be dissolved, the initiator 4,4′-azobis(4-cyanovaleric acid) (ACVA) is quickly added, and allowed to mix into the solution at room temperature. The vessel is submersed into a water bath held at 50 °C to begin the polymerization. Free-solution polymerization of acrylamide occurs until the propagating chain encounters a micelle, at which point the hydrophobic moieties contained within the micelle are added to the polymer chain; after this, the acrylamide polymer continues to propagate in free solution.[35] After 4 h of reaction, the vessel is removed from the water bath for copolymer recovery.

The precipitation of HMPAM with acetone, followed by dialysis against water, is used to purify the copolymer products. The polymer solution is first diluted with an additional 100−300 mL of water, precipitated with acetone, and then physically removed from solution. The polymer is further rinsed with acetone and allowed to air-dry. The dried copolymers are redissolved and then purified by dialysis against deionized distilled water using Spectra/Por cellulose ester dialysis membranes (Spectrum, Gardena, CA), having a molecular weight cutoff of 100000, with frequent water

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Table 1. Summary of the Properties of Copolymers Used in These Experiments

polymer	hydrophobe concn (mol %)	M_W ($\times 10^{-6}$)	PDI
LPA	0	1.38	1.8
	0	2.59	1.6
LPA-*co*-butylacrylamide	0.06	2.93	1.5
	0.08	3.13	1.4
	0.14	2.34	1.7
	0.18	2.03	1.6
	0.21	1.96	1.9
	2.84	1.54	1.5
	3.01	1.53	1.8
LPA-*co*-hexylacrylamide	0.21	2.25	1.7
	0.23	2.19	1.6
	0.27	2.17	1.7
	0.42	2.78	1.5
	1.18	1.52	*a*
	4.29	3.83	*a*
LPA-*co*-octylacrylamide	0.12	2.10	*a*
	0.14	3.44	1.4
	0.19	1.41	1.4
	0.26	*a*	*a*
	0.27	6.49	*a*
	0.27	*a*	*a*
	0.31	4.29	*a*
	0.31	5.04	*a*
	0.42	1.58	*a*
	0.45	2.60	*a*
	3.28	2.10	*a*
LPA-*co*-dihexylacrylamide	0.06	3.26	1.5
	0.12	2.07	*a*
	0.13	*a*	*a*
	0.17	*a*	*a*
	0.19	*a*	*a*
	0.19	*a*	*a*
	0.21	*a*	*a*
	0.22	2.78	*a*
	0.23	*a*	*a*
	0.24	*a*	*a*
	0.25	*a*	*a*
	0.27	*a*	*a*
	0.29	*a*	*a*

[a] Could not be accurately determined.

changes over 10 days, and then the polymers are freeze-dried. In total, approximately 50 batches of copolymers with varying molecular weights, hydrophobic moieties, and concentrations (mol %) of hydrophobe were created for these studies. Additionally, linear polyacrylamides (LPAs) were synthesized using essentially the same conditions to serve as control, hydrophilic polymers. Table 1 shows the polymers that were used in the capillary and chip electrophoresis experiments we discuss here.

Copolymer Characterization. To determine the molecular weights and polydispersities of the synthesized polymers, the samples were fractionated by GPC prior to on-line MALLS and refractive index detection, using a Waters 2690 Alliance separations module (Milford, MA) with Shodex (New York, NY) OHpak columns SB-806 HQ, SB-804 HQ, and SB-802.5 HQ connected in series. In this tandem GPC−MALLS mode, the effluent from the GPC system flows directly into a DAWN DSP laser photometer and Optilab DSP interferometric refractometer connected in series (both Wyatt Technology, Santa Barbara, CA). A 100 μL portion of each sample was injected into the tandem GPC−MALLS system at a concentration of ~0.5 mg/mL. The flow rate was 0.3 mL/min, and the mobile phase consisted of 100 mM NaCl, 50 mM NaH_2PO_4, and 200 ppm NaN_3. The tandem GPC−MALLS data were processed using ASTRA software from Wyatt Technology. ASTRA was used to calculate the molecular weights (MWs) and polydispersity indices (PDIs) of the analyzed polymers. All analyses were repeated at least three times. The two processing parameters used to analyze the hydrophobic copolymers were known $dn/dc = 0.111$[35] and the AUX calibration constant. The presence of <5 mol % hydrophobic comonomer does not significantly alter the value of dn/dc.[35,40] Results for the polymers used in this study, which could be determined, are shown in Table 1.

The copolymers were characterized using a Mercury 400 MHz ^{1}H NMR spectrometer (Varian, Palo Alto, CA) to determine the amount of hydrophobic monomer incorporated into the copolymer (i.e., the concentration (mol %) of hydrophobe). An assigned NMR spectrum for a poly(acrylamide-*co*-hexylacrylamide) previously existed in the literature[40] and served as a guide for our NMR assignments and hence the alkylacrylamide incorporation determinations. Specifically, hydrogens found in the polymer backbone have representative peaks at 1.7 and 2.2 ppm,[40] and the area under these peaks was compared with peak areas for the terminal alkyl methyl hydrogens found at 0.8 ppm, to estimate the amount of hydrophobic subunit incorporated. Table 1 shows the results.

Capillary Electrophoresis. Performance testing of HMPAM matrixes, at a polymer concentration of 0.5 wt %, was done by capillary electrophoresis (CE) on a Bio-Rad Biofocus 3000 (Bio-Rad, Hercules, CA). Capillaries were dynamically coated to reduce electroosmotic flow and protein adsorption by first rinsing with 1 M HCl, followed by a 0.5 wt % solution of hydrophilic polyDuramide, or poly(*N*-hydroxyethylacrylamide).[41,42] Polymer solutions used in biomolecule separations were loaded into the capillary by the application of high pressure (100 psi) from a nitrogen source for 45−60 s. This loading time was more than adequate to pump several column volumes of these polymer solutions at this concentration (0.5 wt %) through the capillary (75 μm inner diameter, 25 cm length, and 20 cm to the detection window). For each experiment, fresh polymer matrix was loaded. UV absorption was used for simultaneous biomolecule detection at 214 and 260 nm wavelengths. Unless otherwise noted, all protein samples for CE were dissolved in water at 1 mg/mL, from stock solutions of 10 mg/mL, and prepared freshly before the experiment. Proteins studied were purchased from Fisher Scientific (Pittsburgh, PA) and included bovine serum albumin (BSA), pepsin, α-lactalbumin, β-lactoglobulins A and B, trypsin inhibitor, and acylase. DNA samples (a 50 bp ladder, and a ϕX174-Hae III dsDNA digest) were purchased from Invitrogen (Carlsbad, CA). Sample injection was performed electrophoretically, by the application of 20 kV (800 V/cm) for 3 s. The running buffer was 1× TTE (50 mM Tris, 50 mM TAPS, 2 mM EDTA, pH 8.4), and electrophoretic separations were performed at 400 V/cm with a ~18 μA current.

Microchip Electrophoresis. The electrophoresis of DNA and proteins in microfluidic chips was conducted using a system custom-built for our laboratory by engineers at ACLARA BioSciences (Mountain View, CA) and designed to allow sensitive, multi-color laser-induced fluorescence (LIF) detection. The instrument consists of an electrical subsystem, which supplies voltage

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to the microfluidic device, and an optical subsystem, which allows the detection of fluorescent moieties as they migrate past a determined position in the channel. Both subsystems are operated using a single program (from ACLARA BioSciences) written in LabView software.

The power subsystem consists of a high-voltage power supply with independent control of four electrodes. Each electrode can be set to a desired voltage between 0 and 4.5 kV or, alternatively, may be floated (disconnected) from the circuit. The software allows the user to set and hold the voltage state of all four electrodes for an arbitrary duration. Multiple steps may be programmed for novel separation schemes or complex chip functions. Additionally, four controllable, low-voltage outputs allow for the digital control of external devices, e.g., a heater. The optical subsystem is a confocal, epifluorescence system in which fluorophores are excited by a JDS Uniphase Series 2214-30sl single-line, 488 nm argon ion laser (San Jose, CA). Mirrors direct the laser beam into a TE200 inverted, epifluorescence microscope. The beam is then passed through a band-pass filter, reflected off a dichroic mirror, and focused through a Nikon 10×/0.45 microscope objective into the center of the microfluidic channel, producing a laser spot size of ~10 μm. Emitted fluorescence is collected through the same objective and is passed through the dichroic mirror, followed by a second, wide-band-pass filter (Chroma Technology, Brattleboro, VT). The spectrum of the filtered light is measured by directing it through a transmission grating and focusing it onto a high-quantum-efficiency, 532 × 64 pixel charge-coupled device (CCD) cooled to −15 °C (Hamamatsu Corp., Bridgewater, NJ). Pixel binning is applied to the data from the CCD camera to quantify the intensity of emission over a particular range of wavelengths, calibrated by Raman scattering lines of solvents. Data collection can be accomplished at rates up to 50 Hz. The CCD output is collected, binned, low-pass filtered, and stored using a program written in LabView.

Experiments were carried out with T3550 glass microchips (Micronit, Enschede, The Netherlands) dynamically coated to reduce electroosmotic flow and protein adsorption by first rinsing with 1 M HCl followed by a 0.5 wt % solution of polyDuramide, or poly(*N*-hydroxyethylacrylamide) synthesized in our laboratory.[41,42] The chips have a standard, 100 μm "offset T" injector,[43−45] a 3.5 cm separation distance, and 50 μm wide by 20 μm tall channels. Samples were injected by grounding the sample well and applying 300 V on the sample waste for 20 s while floating the remaining electrodes. Pullback during the separation step was at 350 V on both sample and sample waste wells, with 1400 V on the analysis waste, providing a separation field strength of 350 V/cm.

Proteins for chip analysis were labeled with FITC, a reagent purchased from Fisher Scientific (Pittsburgh, PA), by following a standard labeling protocol, and purified via elution from a packed Sephadex G-25 column. The final protein sample concentration was estimated to be ~0.5 mg/mL.

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RESULTS AND DISCUSSION

Monomer and Polymer Synthesis and Purification. The physical appearance of each alkylacrylamide differed, and the products varied from liquids (butylacrylamide and dihexylacrylamide) to solids (hexylacrylamide and octylacrylamide). As the hydrophobicity of the monomer increased, its water solubility decreased, and this made the extraction process easier. Reaction yields were typically greater than 90%, and the products were determined to be pure by ^{1}H NMR (data not shown). Copolymer synthesis was relatively easy to accomplish in good yield, according to the methods described. The results of the copolymer characterization according to molecular weight and the concentration (mol %) of hydrophobe are shown in Table 1. The obtained molecular weights were relatively large, ranging from 1.4×10^6 to 6.5×10^6, while PDIs averaged 1.6 and ranged from 1.4 to 1.9. Copolymers including modified acrylamides with long alkyl chain lengths (C8, C6−C6) and/or a relatively large concentration (mol %) hydrophobe (≥0.5 mol %) yielded weak light-scattering and refractive index signals on the GPC−MALLS system, most likely because they were not fully recovered from the columns due to copolymer interactions with the GPC packing material or because they had molecular weights greater than the fractionation potential of the column. In these cases, the molecular weight and polydispersity could not be accurately determined. It is assumed that the molecular weights of these copolymers are similar to those of the other copolymers, given the identical synthesis conditions. We are currently investigating methods that will allow the accurate analysis of these polymers by tandem GPC−MALLS, e.g., the use of a different solvent system. However, as discussed below, the molecular weight of the polymers did not seem to be an important variable for the intended application.

Capillary and Chip Electrophoresis using HMPAM and LPA Networks. Two sets of control experiments were performed by CE and chip electrophoresis using HMPAM and LPA networks. The first experiments confirmed that both DNA and proteins could be co-injected from a single sample and separated electrophoretically in a linear polyacrylamide matrix (0.5 wt %) without any apparent detrimental effects such as aggregation, adsorption, or peak broadening. These were done by CE with UV detection simultaneously at 214 and 260 nm (to see protein and DNA molecules, respectively). Since both DNA and proteins are routinely separated by polyacrylamide gel electrophoresis and by CE in LPA solutions, these results were not unexpected. However, to our knowledge there has been no report of these two very different types of analytes being co-injected and separated by CE in a single run. DNA peaks elute first, followed by proteins (data not shown). Some size-based separation of DNA occurs, and the native proteins are separated by their intrinsic differences in electrophoretic mobility. Protein peak efficiencies and resolution were similar to those obtained by free-solution electrophoresis in a polyDuramide-coated capillary, i.e., without the LPA separation matrix. The second control experiment involved the separation of a mixture of dsDNA molecules of different sizes through HMPAM solutions by both CE and chip electrophoresis. As shown in the microchip electropherogram provided in Figure 2, a dsDNA sizing ladder is nicely resolved in an HMPAM matrix in less than 125 s; peak efficiencies for this separation averaged 5 million plates/m. Additionally, we believe that the hydrophobic moieties

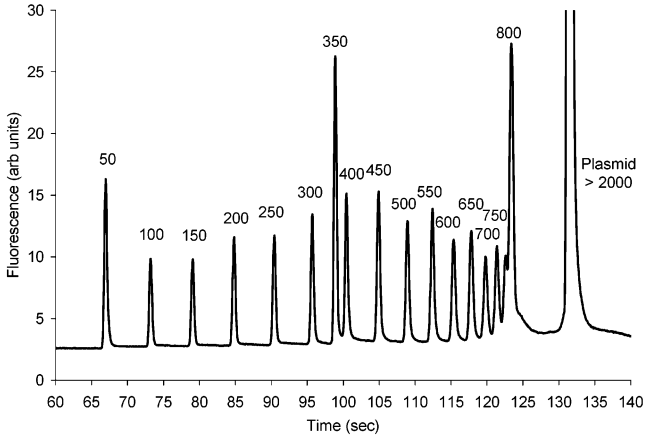

Figure 2. A control experiment: microchip electropherogram of a 50 bp dsDNA ladder, freely migrating through a 2 wt % HMPAM copolymer solution (MW = 4.3 × 10^6 with 0.31 mol % octylacrylamide). The separation is both rapid (<125 s) and highly efficient (average of 5.5 million plates/meter). The field strength was 240 V/cm with a 3.5 cm separation distance and a 2.5 μA current.

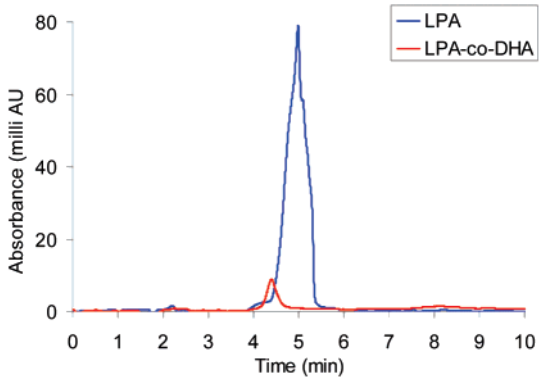

Figure 3. Capillary electropherograms comparing BSA peak elution in polyacrylamide (blue) and poly(acrylamide-*co*-dihexylacrylamide) (red). Injection of protein was at 800 V/cm for 3 s. Significant adsorption of BSA onto the HMPAM occurs (26000−1600 mAU min). The copolymer molecular weight is 1.5 million, and the copolymer contains 0.122 mol % dihexylacrylamide. The polyacrylamide molecular weight is 1.4 million. The field strength was 400 V/cm, with typically an 18 μA current.

in the copolymer may interact with each other to help stabilize the copolymer network, thus enhancing DNA separation. Separation of the same DNA sample in an LPA network, roughly matched in molecular weight and used at the same concentration, was substantially poorer (data not shown). Further, comparative studies of DNA separations in HMPAM vs LPA matrixes are ongoing.

Adsorption of Proteins onto HMPAM Networks. In the next set of CE experiments, a relatively large amount of protein sample was injected into the capillary (i.e., under nonanalytical conditions) to allow accurate determinations of the extent of protein adsorption onto or load capacities of the various copolymer networks. The extent of protein adsorption onto the copolymer networks was determined by comparing the peak height and peak area of the eluted proteins relative to benzoic acid, which acted as an internal standard. We found that the extent of protein adsorption onto the HMPAMs was governed by both the amount of hydrophobic subunit incorporated and the type of hydrophobic moiety. Typical CE electropherograms obtained after BSA migration through both an LPA matrix (with no hydrophobic content) and a hydrophobically modified polyacrylamide copolymer (with 0.122 mol % *co*-dihexylacrylamide), under identical CE injection conditions, are compared in Figure 3. As seen in the figure, both the peak height and the peak area for BSA are dramatically reduced in the HMPAM trace, compared to the results obtained with the acrylamide homopolymer (peak areas of 1600 vs 26000 mAU min, respectively).

Capillary electrophoresis experiments using BSA and β-lactoglobulins A and B were conducted to determine how the type and amount of hydrophobic moiety in the copolymers affect protein adsorption. Data obtained from six electropherograms were averaged for each copolymer matrix of varying concentration (mol %) of hydrophobe and type of hydrophobic moiety. The averaged protein peak areas were then normalized to the benzoic acid peak area to correct for slight variations in the amount injected, which could occur even though identical sample concentrations and injection conditions were used. The protein peak area was plotted against the concentration (mol %) of hydrophobe in the copolymer (Figure 4). The effects of alkyl chain length and the overall concentration (mol %) of hydrophobe in the copolymer on protein adsorption are shown in Figure 4A for BSA and Figure 4B for β-lactoglobulins A and B. These microchannel electrophoresis experiments reveal that *N*,*N*-dihexyl- and *N*-octylacrylamide copolymers enable the most significant protein adsorption, as might be expected given that these are the most hydrophobic monomers studied. *N*-Hexylacrylamide copolymers showed moderate adsorption, while butylacrylamides exhibited essentially none. Because both BSA and the lactoglobulins adsorb to the copolymer matrixes in the same manner (compare parts A and B of Figure 4), there appears to be a generalized trend that increasing the hydrophobicity of the copolymer increases the extent of protein adsorption. Differences in the molecular weights of the copolymers were found to not significantly alter the migration time of the proteins nor the amount of adsorption observed, using solutions of 0.5 wt % dissolved polymer (data not shown).

A sample containing a mixture of proteins and dsDNA was injected under analytical conditions, with the application of 10 kV (400 V/cm) for 8 s, and analyzed by capillary electrophoresis. In particular, the sample consisted of a dsDNA digest at 100 μg/mL and BSA at 1 mg/mL in water. A comparison of the results obtained for the CE separation of this mixed DNA/protein sample through a linear polyacrylamide network and a hydrophobically modified polyacrylamide network (*co*-dihexylacrylamide) is shown in Figure 5. Dual detection at 214 and 260 nm was used to distinguish and detect both DNA and proteins during the same experiment. In the protein-sensitive electropherograms, Figure 5A, protein peaks appear large relative to those of the DNA, while in the DNA-sensitive electropherograms, Figure 5B, both DNA and protein peaks have moderate heights (due to protein absorption of UV light resulting from the presence of aromatic amino acid side chains). DNA, having a high electrophoretic mobility, elutes first, followed by the protein. We found that complete adsorption of the BSA is accomplished using this dihexylacrylamide-containing copolymer matrix, as determined by the absence of a peak for the injected BSA. In the protein-sensitive electro-

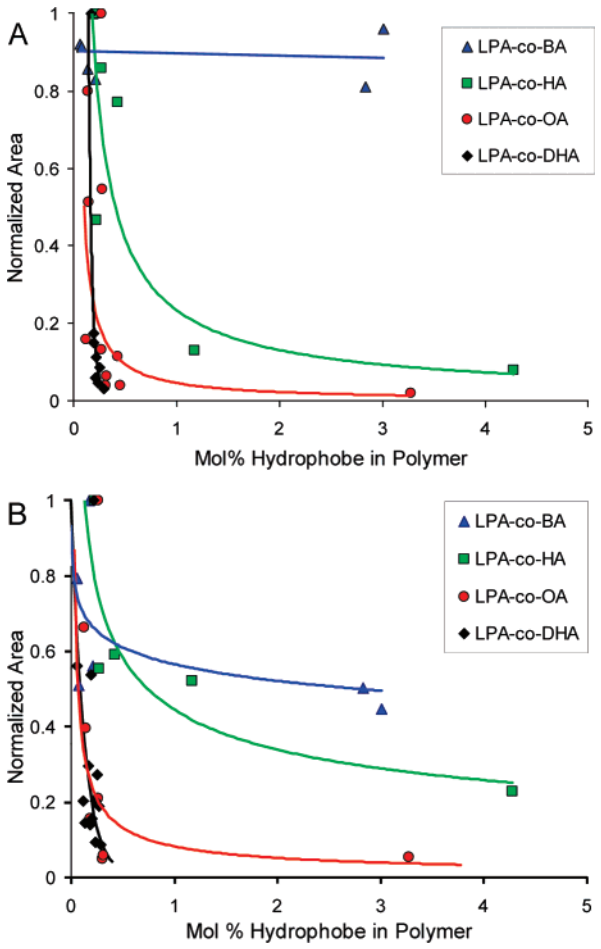

Figure 4. Analysis of BSA adsorption onto copolymers containing different alkylacrylamide comonomer units, with peak areas normalized to the benzoic acid peak area at each concentration (mol %) of hydrophobe (A). Adsorption trends for β-lactoglobulins A and B are very similar to those for BSA migration through hydrophobically modified copolymers (B), thus indicating a potential general trend. Lines are drawn to aid the eye.

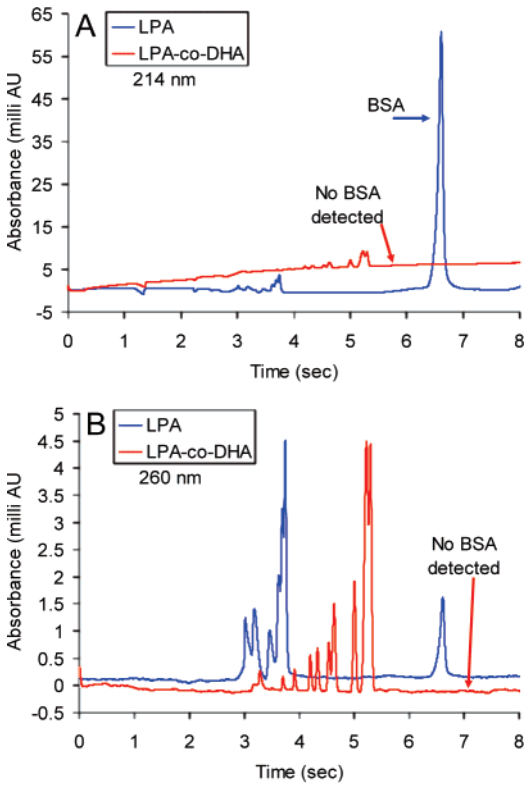

Figure 5. Capillary electropherograms of a protein−DNA sample mixture, showing the essentially complete adsorption of protein (BSA) on a hydrophobically modified polyacrylamide matrix. Protein-sensitive UV detection is done at 214 nm (A), while DNA-sensitive detection is at 260 nm (B). Polymer molecular weights (LPA and HMPAM) are ~2 million, with 0.25 mol % dihexylacrylamide in the HMPAM, a 20 cm detection length, and a field of 400 V/cm. Extending the displayed separation time does not reveal the presence of a BSA peak in the HMPAM electropherogram. The size-based separation of DNA in HMPAM is better than that in a homopolyacrylamide matrix of similar molecular weight at the same concentration. Injection of the DNA−protein mixture was done at 400 V/cm for 8 s.

pherograms with detection at 214 nm (Figure 5A), a peak for BSA is clearly visible for electrophoresis through polyacrylamide, while it is completely absent in the HMPAM trace. In the DNA-sensitive channel, 260 nm (Figure 5B), both the DNA and protein can be detected after electrophoresis through the polyacrylamide, while only DNA elution is detected in the HMPAM. Additionally the dihexylacrylamide-containing copolymer is seen to provide significantly better size-based separation of a dsDNA digest, possibly due to the earlier onset of a physically cross-linked polymer network with hydrophobic interactions between alkyl matrixes.[37,40,46] Thus, essentially complete adsorption of a control protein, serum albumin (the main constituent in blood plasma), with no adverse affect on DNA separation, has been accomplished using a novel HMPAM network.

Implementation on a Microfluidic Device. Fluorescently labeled trypsin inhibitor and BSA were first analyzed, each individually, in an LPA matrix to allow for unambiguous peak identification. Trypsin inhibitor, with seven lysine residues (MW ≈ 20000), shows one predominant product of the FITC labeling reaction, and hence exhibits one tall, sharp peak, while the much larger BSA, having many more lysine residues (MW ≈ 66000), has multiple FITC labeling reaction products and consequently appears as a relatively broad peak. Overlays of electropherograms conducted with copolymers of varying hydrophobic content are shown in Figure 6. The concentration of octylacrylamide in the copolymers ranged from 0 mol % (LPA) to 0.31 mol % . In these electropherograms, the fluorescence intensities and migration times of both protein peaks have been normalized to those of trypsin inhibitor (which was found, in earlier experiments, to be nonadsorbing during CE through HMPAM networks, as further discussed below). We see that copolymers with increasing octylacrylamide content incrementally adsorb more BSA. In particular, the peak height and area for BSA are reduced in octylacrylamide matrixes when compared to those for LPA, and the migration time of BSA becomes longer as more hydrophobic copolymers are used. The removal of BSA from the sample, although incomplete in this case, demonstrates the potential of this purification technique for the selective removal of this highly abundant blood plasma protein by HMPAMs in a microfluidic device. Since, as shown in Figure 5, DNA peaks typically elute well before protein peaks, a simple "slowing" of protein peaks by HMPAM networks also could be enough to effect the purification

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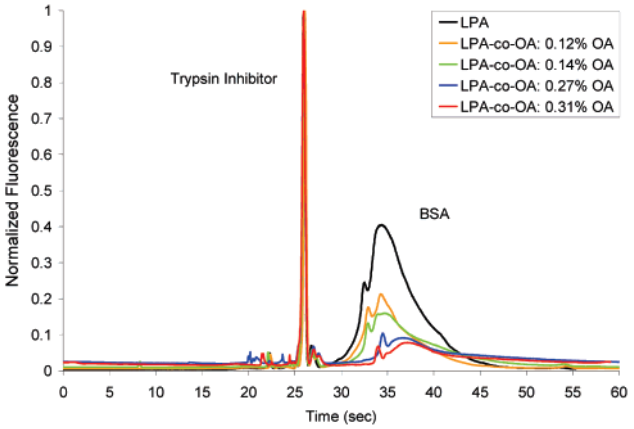

Figure 6. Electropherogram overlays showing the results of BSA migration through 0.5 wt % octylacrylamide (OA) containing copolymer matrixes. The separation field strength was 350 V/cm, with a ~3 μA current, 1× TTE buffer, and a detection length of 3.5 cm. Note that the separation is completed within 50 s. Fluorescence is normalized to the peak area for fluorescently labeled trypsin inhibitor, which acts as an internal standard. The extent of BSA adsorption is seen to increase with increasing content of the hydrophobic OA monomer in the copolymers.

of DNA away from BSA and other proteins (one could simply turn off the field after DNA peaks elute).

As described above in the discussion of Figure 4, similar trends for the adsorption of BSA and β-lactoglobulins A and B onto hydrophobically modified polyacrylamides indicate that retention on HMPAM networks would be observed for many different proteins. However, two of the proteins we investigated (trypsin inhibitor and α-lactalbumin) were found not to adsorb strongly to HMPAM matrixes at 20 °C, the standard run condition we used in these studies. These results, though still anecdotal since we have only studied the migration and adsorption of a few proteins, indicate that it may be more difficult to remove low-molecular-weight and/or hydrophilic proteins by adsorptive electrophoresis. This is a logical result since native (folded) proteins have mostly non-surface-exposed hydrophobic residues which may remain unavailable for adsorption; however, even hydrophilic proteins typically have numerous hydrophobic monomers buried inside their folded structures. The use of higher temperature as an agent to denature proteins should thus expose hydrophobic amino acid residues, making them available for adsorption onto HMPAM networks. Higher temperature also typically strengthens the hydrophobic effect, which is entropically driven. We will explore this phenomenon further using HMPAMs with a more complex mixture of proteins, on temperature-controlled microfluidic devices. The polymer formulations and electrophoresis conditions we present here, however, may be of great interest to the proteomics community since this is a method for the removal of serum albumins, which may facilitate analyses aimed at less abundant proteins.

CONCLUSIONS

The inclusion of an upstream biosample processing technology such as on-line DNA purification has so far been mostly overlooked by the μ-TAS community, with a few notable exceptions. In this work, novel copolymers have been created that adsorb proteins via hydrophobic interactions during electrokinetic chromatography in both capillaries and microchips, with apparently no dsDNA adsorption, and no adverse effects on dsDNA separation. These copolymers are synthesized by micellar polymerization, a technique that allows otherwise water-insoluble monomers to be incorporated into a water-soluble polymer. The concentration (mol %) of hydrophobe in the copolymer can be analyzed by ^{1}H NMR. The amount and nature of the hydrophobic moiety play important roles in separation of DNA from proteins. By increasing both the length of the alkyl group and the total amount of alkylacrylamide (or dialkylacrylamide), a greater extent of protein adsorption occurs during both capillary and microchip electrophoresis. Our results show that dihexylacrylamide and octylacrylamide have the greatest potential to adsorb proteins away from a complex biological mixture, and that conditions of electrophoresis in HMPAMs can be manipulated to freely allow dsDNA migration with complete adsorption of serum albumins, the most abundant proteins in blood plasma. Success in this work could aid in the miniaturization and automation of time-consuming tasks that presently cannot easily be implemented on a portable, rapid-analysis microfluidic device. Clearly, the elimination of these steps has useful implications for DNA-based detection of biological pathogens and other medical diagnostics in a traditional clinical setting, as well as in nontraditional medical environments.

ABBREVIATIONS

ACVA, 4,4′-azobis(4-cyanovaleric acid); BA, butylacrylamide; BSA, bovine serum albumin; DHA, dihexylacrylamide; GPC, gel permeation chromatography; HA, hexylacrylamide; HMPAM, hydrophobically modified polyacrylamide; LPA, linear polyacrylamide; MALLS, multiangle laser light scattering; OA, octylacrylamide; PDI, polydispersity index; SDS, sodium dodecyl sulfate; SPE, solid-phase extraction.

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