Tuning direct current streaming dielectrophoresis of proteins

Asuka Nakano, Fernanda Camacho-Alanis, Tzu-Chiao Chao,
and Alexandra Ros^{a)}

*Department of Chemistry and Biochemistry, Arizona State University, Tempe,
Arizona 85287, USA*

(Received 13 July 2012; accepted 18 July 2012; published online 2 August 2012)

Dielectrophoresis (DEP) of biomolecules has large potential to serve as a novel selectivity parameter for bioanalytical methods such as (pre)concentration, fractionation, and separation. However, in contrast to well-characterized biological cells and (nano)particles, the mechanism of protein DEP is poorly understood, limiting bioanalytical applications for proteins. Here, we demonstrate a detailed investigation of factors influencing DEP of diagnostically relevant immunoglobulin G (IgG) molecules using insulator-based DEP (iDEP) under DC conditions. We found that the pH range in which concentration of IgG due to streaming iDEP occurs without aggregate formation matches the pH range suitable for immunoreactions. Numerical simulations of the electrokinetic factors pertaining to DEP streaming in this range further suggested that the protein charge and electroosmotic flow significantly influence iDEP streaming. These predictions are in accordance with the experimentally observed pH-dependent iDEP streaming profiles as well as the determined IgG molecular properties. Moreover, we observed a transition in the streaming behavior caused by a change from positive to negative DEP induced through micelle formation for the first time experimentally, which is in excellent qualitative agreement with numerical simulations. Our study thus relates molecular immunoglobulin properties to observed iDEP, which will be useful for the future development of protein (pre)concentration or separation methods based on DEP.
© 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4742695>]

I. INTRODUCTION

The complexity of many biological samples demands for powerful separation techniques. Standard bioanalytical techniques to characterize complex protein samples reach their limits under extreme conditions, i.e., at low analyte concentration as well as in complex biological fluids. Moreover, the concentration of many biomarkers in body fluids is extremely small ($<nM$). Additionally, sample sizes may be limited, especially for diagnostic purposes, where little tissue or body fluid is available. Thus, efficient methods combining low sample consumption with the ability to concentrate analyte in small volumes are required.

Here, we study a novel migration phenomenon for proteins, namely dielectrophoresis (DEP), realized in a tailored microenvironment. In DEP, polarizable objects are manipulated in inhomogeneous electric fields. This is in contrast to electrophoresis (EP), where particles are transported and separated according to their charge and friction relation in homogeneous electric fields. As DEP is a technique relying on electric field gradients, it has the potential to separate biomolecules according to their dielectric properties as well as concentrate them. The technique therefore has high relevance for analytes with low concentration, which need to be

^{a)} Author to whom correspondence should be addressed. Electronic mail: Alexandra.Ros@asu.edu. Telephone: +1-480-965-5323. Fax: +1-480-965-2757.

enriched prior to separation or analysis. Concomitantly, DEP has the potential to provide high molecular specificity.^{1,2}

Two major techniques are commonly used to produce electric field gradients for DEP manipulations. The first approach includes microstructured electrodes,³ often embedded in microfluidic channels. The approach is based on metal patterning techniques. Disadvantages of this method include electrical reactions as well as fouling of electrodes.⁴ Another approach is insulator-based DEP (iDEP), in which electric field gradients are created by integrating non-conductive obstacles in a microfluidic device.^{4–6} In iDEP, the application of electric potentials via electrodes immersed in the microdevice reservoirs allows the decoupling of electrode effects within the large reservoir volume. The technique requires larger applied potentials to achieve high electric fields within the microdevice but establishes homogeneous electric fields throughout the entire depth of the microfluidic channel. Moreover, it requires only one microstructure material, which makes it amenable for soft lithography. Recent advances in DEP realizations include the development of contactless DEP⁷ and 3D carbon electrodes.⁸

DEP is well understood for spherical particles^{9,10} and excellent models exist to describe DEP of biological cells.^{11–13} However, current knowledge on biomolecule DEP is less developed, although applications of DNA DEP have been shown in the past. For example, several studies have demonstrated DNA manipulation and trapping,^{4,14–18} reaching down to several tenths of base pair DNA length.^{15,19,20} Analytical applications of DNA DEP have been early proposed by Washizu *et al.*¹⁴ and recent studies revived this approach exploiting the length dependency of DNA DEP for separation. For example, a field flow fractionation device for DNA was recently demonstrated by Lao *et al.*²¹ Moreover, both the separation of linear DNA by size²² and DNA topoisomers²³ were demonstrated in gel-free environment.

Despite the examples of DNA DEP, the field of protein DEP is in its infancy.^{24,25} Indeed, classical DEP theory for homogeneous particles in solution predicts that extremely high field gradients are necessary to achieve DEP forces significant for manipulation of biomolecules a few nm in size. However, the manipulation of proteins by DEP has recently been demonstrated.^{14,26–31} As anticipated, high field strengths and gradients are required while DEP under DC and AC conditions was reported. Interestingly, proteins with molecular weight smaller than 100 kDa were successfully accumulated on electrodes,^{14,26} carbon nanotubes,²⁷ within droplets,²⁸ and using nanopipettes.¹⁹ Hölzel *et al.* showed the DEP induced trapping of the large multimeric protein R-phycoerythrin at metal electrode gaps;³⁰ whereas Bakewell *et al.* investigated frequency dependent DEP behavior of avidin.³¹ Recently, an iDEP device²⁹ as well as a constriction device^{32,33} was employed to trap proteins.

These findings are impressive and they revealed that DEP can indeed generate forces high enough to encage proteins at specific locations. It is worthy to point out that DEP *trapping*, i.e., the accumulation of particles at a specific location, is not always required for analytical applications. Field-flow fractionation based on DEP^{34–37} or sorting at restrictions³⁸ are examples, in which DEP *trapping* is not necessary. Indeed, slight differences in DEP behavior are sufficient in order to take advantage of biomolecule DEP for separation.

A major problem in understanding biomolecule DEP and hence the development of analytical applications remains the fact that experimental conditions differ in studies on protein DEP up to date.^{19,26–32} This is not only due to the particular special arrangements at the microscale used to generate high electric field gradients, but also a consequence of the solvent conditions and intrinsic protein properties. Moreover, a generic theoretical model predicting protein polarizability and accounting for the large protein variety has not been developed yet. DEP based transport phenomena have been studied following Maxwell Wagner O’Konski theory³⁹ while assuming a globular protein shape⁴⁰ and on the basis of experimentally reported protein conductivity.²⁵ For nanocolloids, corrections to the classical Maxwell Wagner O’Konski approach were suggested due to the apparent size dependent crossover frequencies.^{41,42} These refinements take into account charge movement in the Stern layer and the diffuse layer.^{43,44} A standard model employing Poisson–Nernst–Planck equations was also suggested accounting for ionic migration, convection, diffusion, and electrophoretic migration.^{45,46} The latter has shown to predict the polarizability of short rod-like DNA adequately.⁴⁷ These refined theories may also be

applied to reveal insight into protein DEP but were not yet substantiated with experimental data. Additionally, molecular dynamics simulations allow the prediction of dielectric properties of proteins which will be useful to estimate DEP forces and the frequency dependence of protein DEP⁴⁸ but are limited to a few selected proteins.^{48–51}

Moreover, other factors not solely resultant from the polarization of proteins affect DEP applications. For example, if aggregate formation is occurring the observed DEP forces may be much higher at a given potential, and thus do not reflect the DEP properties of protein monomers. Aggregation can be influenced to a great extent by pH and temperature changes, which therefore also have to be controlled carefully in DEP applications. Indeed, we could recently show that the pH in a typical iDEP experiment can change throughout the entire channel within 10–30 min⁵² depending on the buffer and applied electric field. To improve solubility and avoid aggregate formation, surfactants are often employed in bioanalytical applications⁵³ and the consequence on protein DEP has not been studied. It is likely, however, that surfactants interacting with the protein change their polarizability. Furthermore, temperature effects are likely to occur due to the high electric fields employed promoting aggregation in DEP experiments.³² Thus, various factors may significantly alter DEP behavior of proteins in analytical microfluidic devices. To the best of our knowledge, a thorough investigation of these effects influencing protein DEP has not been conducted previously and is important for future applications involving immunoglobulins.

In our previous work, we were able to demonstrate protein DEP streaming with insulator-based DEP.²⁵ Our data demonstrated that DEP is suitable to concentrate proteins in tailored microfluidic devices. In addition, we developed a convection-diffusion model that qualitatively captured this protein DEP streaming behavior. Here, with both the experimental microfluidic tool and the theoretical model at hand we extend our previous studies to investigate factors influencing protein DEP. In particular, we study the influence of pH, surfactant concentration, and electrokinesis resulting both from electrophoretic and electroosmotic components as well as aggregation on DEP. Moreover, we were able to demonstrate the tuning of the dielectrophoretic protein behavior, i.e., positive to negative DEP, by adequate choice of surfactants under DC conditions.

II. MATERIALS AND METHODS

A. Chemicals

Si wafers (4 in.) were obtained from University Wafer. The negative photoresist SU-8 2007 and developer were purchased from Microchem (Newton, MA, USA). (Tridecafluoro-1,1,2,2-tetrahydrooctyl)dimethylchlorosilane (TDTS) for wafer silanization was purchased from Gelest (Morrisville, PA, USA). Sylgard184 composed of the silicon elastomer base and the curing agent for poly(dimethylsiloxane) (PDMS) was obtained from Dow Corning Corporation (Midland, MI, USA). Potassium phosphate monobasic, sodium phosphate dibasic, poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (F108), and 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Alexa-Fluor 488 chicken anti-rabbit immunoglobulin G (IgG) conjugates were obtained from Invitrogen (Carlsbad, CA, USA). Deionized (DI) water was supplied from a Synergy purification system (Millipore, USA). Glass slides of 0.15 mm thickness were purchased from Fisher Scientific, and Pt wire from Alfa Aesar (Ward Hill, MA, USA).

B. Microchip fabrication

A standard photolithographic technique⁵⁴ was employed to fabricate the inverted microstructure used as a master wafer for softlithography. In detail, a Si wafer was spincoated with SU-8 at a proper rpm to obtain a desired thickness, exposed under UV light through a chrome on soda-lime photomask, and developed in SU-8 developing solution. After silanization of the wafer, the softlithography step was performed by casting PDMS on the master wafer, followed by curing in an oven for 4 h at 85 °C. Subsequently, the cured PDMS was peeled off from the

wafer, and the 2 mm diameter reservoir holes were manually punched through the PDMS piece at both ends of a 1 cm long channel. The resultant PDMS piece and 0.15 mm thick glass slide were sonicated in isopropanol and DI water baths and blow-dried with nitrogen. In order to tightly seal the PDMS with the glass slide, both surfaces were activated by treatment with oxygen plasma (PDC-001 Harrick Plasma, Harrick, USA) for 1 min at the highest RF setting. A 5 mm thick PDMS slab with 5 mm diameter reservoir holes above the microchip reservoirs was pressed on the top of the device to enlarge the reservoirs and to hold the Pt wire electrode in position.

C. Chip preparation

Prior to the experiment, the channels were treated with 500 μ M F108 in phosphate buffer in the case of static F108 coating condition. After overnight incubation, F108 solution exchanged with the buffers to be used for the subsequent DEP experiments. Channels employed under dynamic coating conditions were filled throughout the experiments with pH 8 phosphate buffer containing 3 mM F108 while no buffer exchange was required prior to experiments. The phosphate buffers were prepared with various pHs from 6.5 to 8. Acetate-acetic acid and tris-HCl were used to prepare pH 4 and pH 9 buffers, respectively. All buffer pHs were assessed with a pH meter (SB70P symphony, VWR, USA). For the DEP experiments, the reservoirs were filled with 50 μ l phosphate buffer containing 1.7 mM CHAPS. The inlet reservoir was filled with 3.3 nM fluorescently labeled IgG solution. Pt electrodes attached to both reservoirs were connected to the high voltage power supply (HVS448 6000 V, LabSmith, Livermore, CA, USA) to apply DC voltages.

D. Detection and data analysis

For fluorescence microscopy imaging, an inverted microscope (IX 71, Olympus, Center Valley, PA, USA), with a 60 $\times$ objective (LUCPlan FL N, Olympus, Center Valley, PA, USA), a mercury burner (U-RFL-T, Olympus, Center Valley, PA, USA), and fluorescent filter set (exciter ET470/40, dichroic T495LP, emitter ET525/50, Olympus, USA) was used. Images were acquired at 5 images per second using a CCD camera (Quantum 512 SC, Photometrics, Tucson, AZ, USA) and Micro-Manager software (University of California, San Francisco, CA, USA) and analyzed with IMAGE J software (version 1.43).

A concentration factor, R , was calculated as: $R = (I_{SC} - I_{NS}) / (I_B - I_{PDMS}) \times 100$. Here, I_{SC} denotes the fluorescence intensity at concentrated streaming regions when voltage was applied for DEP streaming to occur, while I_{NS} is the intensity at the non-streaming regions. I_B is the average fluorescence intensity with 140 V/cm applied voltage while no DEP streaming is observed. I_{PDMS} is the intensity of PDMS background aside from the channel. The depletion factor was calculated similarly except I_{SC} was substituted with I_{SD} , which denotes the fluorescence intensity at depleted streaming regions when negative DEP is observed. For both, concentration and depletion factor calculations, the intensities were calculated as the average of a defined number of pixels in each area and averaged over a total of 10 frames.

III. NUMERICAL SIMULATION

To understand DEP behavior of proteins, estimating the DEP force is of importance. As motivated in the Introduction, a generic model describing protein polarization is still lacking, so that we relate our theoretical considerations on well-established classical theories for solid particles. Although this approach surely does not take into account all subtleties of protein polarization, it allowed others⁴⁰ and us²⁵ to study transport phenomena based on DEP in microfluidic environments. Because this approach is the basis for our numerical simulations, it is briefly reviewed here. We estimate the DEP force of proteins with an ellipsoid model¹⁹ assuming an ellipsoid shape of IgG in solution.⁵⁵ The DEP force acting on a prolate ellipsoid particle under DC condition is then given by^{19,56}

$$F_{dep} = \frac{4}{3} \pi abc \epsilon_m \left(\frac{\sigma_p - \sigma_m}{Z\sigma_p + (1-Z)\sigma_m} \right) \nabla E^2, \quad (1)$$

where a , b , and c ($a > b = c$) are the radii of an ellipsoid, ϵ_m the permittivity of the medium, $\mathbf{E}$ the electric field and Z the polarization relaxation factor. The modified Clausius-Mosotti factor, f_{MCM} , is defined by:^{10,25}

$$f_{MCM} = \frac{\sigma_p - \sigma_m}{Z\sigma_p + (1-Z)\sigma_m}. \quad (2)$$

When F_{dep} is balanced with the drag force,²⁵ we can determine the DEP mobility (μ_{dep}) as

$$\mu_{dep} = \frac{2abc\epsilon_m f_{MCM}}{9\eta\bar{R}}, \quad (3)$$

where η is the medium viscosity and $\bar{R}$ is the mean translational coefficient.²⁵

First, we computed the electric field and ∇E^2 in the iDEP device at an applied electric field of 4200 V/cm to match our experimental conditions. The simulation results show the maximum electric field strength of 1×10^6 V/m and the strongest ∇E^2 value of 8×10^{17} V²/m³ at the tip where the vertices face each other. Based on the calculated field strength and ∇E^2 , we simulated the concentration profiles for both DEP modes. Previously, we used Eqs. (1)–(3) to determine the DEP mobility, μ_{dep} in case of positive and negative DEP. Using the reported value for protein conductivity of 24.6 S/cm, a μ_{dep} of 8.6×10^{-24} m⁴/V²s was obtained in the case of positive DEP.²⁵ By assigning a protein conductivity of zero, the negative DEP case can be modeled, while μ_{dep} results as -7.14×10^{-24} m⁴/V²s.²⁵ Utilizing these parameters and our maximum limits of potential application over a cm long channel, the values obtained for the DEP force indicate that electrokinetic forces cannot be overcome by DEP. Hence, streaming DEP is observed. This situation has been previously reported for proteins by us²⁵ and for nanoparticles by others.^{5,57}

Previously, we could show that a convection-diffusion model adequately represents the protein migration behavior in the presence of DEP, electrokinesis, and diffusion.²⁵ Here, the total flux is given as

$$\mathbf{J} = -D\nabla c + c[\mathbf{u}_{ek} + \mathbf{u}_{dep}], \quad (4)$$

Where D is the diffusion coefficient, c the concentration of the particles, $\mathbf{u}_{dep}$ the dielectrophoretic velocity, and $\mathbf{u}_{ek}$ the overall electrokinetic velocity, resulting from electrophoresis and electroosmosis. Upon solving the model with COMSOL MULTIPHYSICS 4.2 at steady state conditions, satisfying qualitative agreement between simulation and experiments was found.²⁵ Initially, we employed a diffusion coefficient of 3.89×10^{-11} m²/s (Ref. 25) and an electrokinetic mobility, μ_{ek} , of 1.5×10^{-8} m²/Vs. We will use this model to study the dependence of streaming iDEP of proteins in this work with a particular focus on the influence of the protein concentration with respect to pH, protein aggregation, electrophoretic and electroosmotic contributions.

IV. RESULTS

In this section, we report on our experimental study of protein DC iDEP streaming dependent on pH, electrophoretic and electroosmotic mobilities as well as surfactant interaction and discuss the experimental data with the aid of numerical simulations.

A. Dependence on pH

First, we investigated the influence of pH on the DEP behavior of IgG within a pH range of 4–9 in the presence of the zwitterionic additive CHAPS. CHAPS is known to improve protein solubility⁵³ in bioanalytical applications and was previously reported to prevent protein aggregation in iDEP systems.²⁵ We started with pH 8 in low conductivity buffer and as a result,

high fluorescence intensity was observed in streamlines along the posts (Figure 1(a)) resembling DC iDEP streaming.²⁵ Note that this streaming pattern occurs for proteins exhibiting positive DEP while this pattern changes for negative DEP (as discussed below).

We then proceeded with DEP experiments with other pH values, while keeping the buffer medium conductivity at 0.01 S/m. As a result, two main effects were found. First, protein aggregates started to form below pH 6.5 and above 8 regardless of the presence of CHAPS (Figures 1(e) and 1(f)). Immediately after applying a voltage, large amounts of aggregates started to flow within the channel and hindered further experiments by clogging the channel and adsorption on the PDMS surface. Interestingly, the DEP behavior has changed from

FIG. 1. (a)–(f) Fluorescence microscopy images of IgG streaming iDEP in an array of triangular posts. The scale bar indicates 20 μm and the flow direction is from top to bottom. Below each image in (a)–(d) is a profile intensity plot at the position marked in (a) (black line). (a)–(d) Streamlines are observed due to positive DEP at an applied electric field of 4200 V/cm with pH buffer media in the range of pH 6.5–8: (a) pH 8, (b) pH 7.5, (c) pH 7, (d) pH 6.5. Positive DEP streaming is decreased from (a) to (d) while decreasing the medium pH results as an decrease of the concentration. (e) and (f) Protein aggregates are formed regardless of the presence of CHAPS at (e) pH 4 and (f) pH 9.

streaming to trapping—a strong indicator for larger particles resulting from aggregate formation. We conclude that at pH below 6.5 and above 8, aggregation is favored even in the presence of CHAPS. We attribute this to a pronounced degradation and conformational changes within the protein.

In contrast, in the pH range between 6.5 and 8, aggregate formation was not apparent and iDEP streaming according to positive DEP was observed. Figures 1(a)–1(d) show streamlines resulting for pH 8 to 6.5, whereas the intensity profile at the midpoint line between the two rows of posts is plotted under each representative image. As clearly shown in Figures 1(a)–1(d), the protein concentration is enhanced with increasing buffer pH. This phenomenon was investigated more quantitatively by calculating the concentration factor. Figure 2 shows protein concentration factors with varying pH between 6.5 and 8. Both for electric fields of 2800 and 4200 V/cm, we observed that the protein concentration is enhanced towards higher pH. The largest concentration factor of 71% is obtained at pH 8 at 4200 V/cm.

The variation of the pH clearly demonstrates a working range for IgG DEP streaming. Both below pH 6.5 and above pH 8, aggregation hampers protein streaming. This is in contrary to recently published results of the Lapizco-Encinas group on DC iDEP of bovine serum albumin.²⁹ While this group reports on protein particle trapping in a DC iDEP device at similar solution conductivities, we remark that electric fields and gradients thereof are much larger in our device. Comparing their data²⁹ with ours²⁵ we conclude that even at an order of magnitude higher electric fields and ~ 2 – 3 order of magnitude larger ∇E^2 DC iDEP streaming is apparent, both theoretically and experimentally (this paper). This is remarkable, as we even employed a larger protein (150 kDa IgG compared to 60 kDa bovine serum albumin²⁹) from which we would expect a larger DEP force according to classical theory. We thus suspect that protein aggregation—barely resolvable in fluorescence microscopy studies—can enhance DEP forces leading to trapping at even lower electric fields. We furthermore find from calculations of the trapping criterion as developed previously^{58–60} that the trapping condition is not satisfied in either iDEP device (see supplementary material⁶¹).

It is interesting to note that the working pH range is relevant for diagnostic applications, in which circumneutral conditions are essential for affinity reactions of immunoglobulins with antigens. Although our experiments are carried out at low buffer conductivity, our work complements protein DEP studies under physiological conditions in nm-sized constriction devices, in which trapping was observed.^{32,33} Thus, the resulting optimum pH range for iDEP as observed here is well suited for applications combining DEP manipulation with biological

FIG. 2. Concentration factor observed experimentally for an applied electric field of 2800 and 4200 V/cm for changes in medium pH ranging from pH 6.5 to 8.

immunoassays. The concentration increase in the range of pH 6.5 to 8 additionally demonstrates that IgG streaming behavior is indeed influenced by pH. In the following, we thus investigated potential pH-dependent factors influencing iDEP migration such as electroosmosis and electrophoresis.

B. Influence of electroosmosis

In order to understand the influence of the electroosmotic transport on DEP streaming, we investigated variations of μ_{ek} (based on EP and electroosmotic flow (EOF)). We consider this an important factor in protein streaming iDEP since strong electrokinetic transport counteracts DEP concentration. A reduction of the competing electrokinetic forces is therefore expected to result in a higher protein concentration effect. Therefore, we studied the influence of the magnitude of EOF on IgG streaming DEP by only considering an electroosmotic mobility, μ_{eo} , and its variations in our numerical model. Thus, all variations in μ_{eo} correspond to changes in the overall μ_{ek} . Figures 3(a)–3(c) depict the concentration profiles for IgG (with positive DEP) in dependence of changes of μ_{eo} . We started with a typical μ_{eo} of $1.5 \times 10^{-8} \text{ m}^2/\text{Vs}$ for oxygen plasma treated PDMS with F108 coating according to a static coating procedure⁶² and varied the mobility values by up to an order of magnitude.

Clearly, we observe from Figures 3(a)–3(c) that protein iDEP streaming is enhanced by reducing the electrokinetic component. The concentration factor can be significantly improved up to 11% for the lowest employed μ_{eo} . Furthermore, this change in the overall μ_{ek} contributes to the characteristic peak formation of the line profiles. Figure 3(d) shows the concentration intensity plots with μ_{eo} ranging from $1.5 \times 10^{-8} \text{ m}^2/\text{Vs}$ to $0.1 \times 10^{-8} \text{ m}^2/\text{Vs}$. Note that the regions of the highest streaming concentration shift inwards with increasing μ_{eo} . Further, these streamlines are broadened towards lower μ_{eo} values.

FIG. 3. DEP streaming simulation for IgG molecules within a triangular post array at 4200 V/cm. (a)–(c) Streaming concentration at steady-state according to positive DEP with varying μ_{ek} of (a) $1.5 \times 10^{-8} \text{ m}^2/\text{Vs}$, (b) $0.5 \times 10^{-8} \text{ m}^2/\text{Vs}$, and (c) $0.1 \times 10^{-8} \text{ m}^2/\text{Vs}$ to reflect changes in EOF. (d) Profile plots of (a)–(c) at the midpoint line of the two rows of posts. μ_{ek} is varied (reflecting changes in EOF) as indicated in the inset while other parameters remain constant with $\mu_{dep} = 8.6 \times 10^{-24} \text{ m}^4/(\text{V}^2\text{s})$ and $D = 3.89 \times 10^{-11} \text{ m}^2/\text{s}$.

We now discuss whether under our experimental conditions changes in the EOF could be responsible for the observed pH-dependence of the streaming iDEP. If μ_{eo} is decreased with increasing pH, improved streaming DEP should be expected, as our simulation results in Figure 3(d) suggest. It is well known that EOF on PDMS and glass stems from deprotonated silanol groups with a pKa of ~ 4.7 .⁶³ It is thus not likely that pH changes in the range of pH 6–9 would considerably contribute to EOF changes. Additionally, the channel walls are coated with the tri-block copolymer F108. A pH influence is thus also not likely to occur under these conditions. To experimentally verify this assumption, we measured μ_{eo} in the range of pH 6–8 under static coating conditions employing F108 as the coating agent. As expected, we could not observe significant changes in the magnitude of EOF (data not shown).

C. Influence of electrophoresis

Next, we discuss the influence of the protein's electrophoretic mobility, μ_{ep} , attributing to the overall electrokinetic transport. Under our experimental conditions, we observed cathodic EOF (Ref. 64). Assuming that μ_{eo} found previously for PDMS/glass channels⁶² is in the same order of magnitude (10^{-8} m²/Vs) as μ_{ep} for proteins,⁶⁵ two scenarios are possible. First, the protein has a positive charge; as a consequence EOF and electrophoresis act in the same direction. The second case arises when EOF and electrophoresis counteract due to negatively charged proteins. In the latter case, the overall electrokinetic transport should be lower in magnitude than in the first case, as a result of the reduced overall μ_{ek} . If strong enough, the overall transport direction changes. As the protein charge is critically influenced by the solution conditions, changes in the electrokinetic transport are expected for different pH.

In order to understand the influence of the protein charge on DEP streaming, we discuss our simulations for variations of μ_{ek} in relation to our experimental data. First, we note that we determined a pI ≤ 5 for the polyclonal IgG used in this experiment (see supplementary material⁶¹). Thus, the negative charge of the protein should rise with an increase in pH. As electrophoresis counteracts EOF in pH > 5 for the employed IgG, a reduced μ_{ek} would be expected. Thus, we would predict an overall stronger DEP streaming concentration with pH values above the pI due to the decreased μ_{ek} . This is underlined by Figure 3(d), indicating increased streaming concentration for lower μ_{eo} . Indeed, we found experimentally that the streaming DEP concentration is enhanced towards higher pH (see Figures 1 and 2).

D. Influence of DEP mobility

With the change in a protein's charge, such as induced through pH changes, the DEP behavior could also be influenced. Assuming that a larger amount of charges relates to a larger polarization of a protein, the DEP behavior would increase for pH conditions far from the pI of a protein. Our numerical simulations allow us to examine this scenario as well. Figure 4 shows the changes in the concentration profile arising from an increase in the protein μ_{dep} . As expected, higher μ_{dep} , arising from stronger DEP forces increases the streaming concentration. Using similar arguments as above for the protein charge state, we conclude that our experiments are also in agreement with an increase in μ_{dep} possibly arising from an increased polarization of the double layer. While our experimental results do not allow us to distinguish between the two cases (increase of DEP or reduction of overall μ_{ek}) we can nonetheless conclude from the simulations that the more pronounced concentration towards higher pH (up to pH 8) is reasonably well explained by our model.

E. Influence on protein DEP due to surfactants

Our preceding experimental and simulation data demonstrated that highest improvement in DEP concentration is expected around a pH of 8, which can be tuned by decreasing the electrokinetic component and thus favoring DEP vs. streaming. In this study, we employed adsorptive treatment with a tri-block-copolymer (F108) to prevent non-specific protein adsorption and control EOF.⁶² Recently, we could also demonstrate that dynamic coating, in which the coating

FIG. 4. Profile plots for varying μ_{dep} at the midpoint line of the two rows of posts while the other parameters stay constant; $\mu_{ek} = 1.5 \times 10^{-8} \text{ m}^2/(\text{Vs})$ and $D = 3.89 \times 10^{-11} \text{ m}^2/\text{s}$.

agent is apparent in the buffer throughout the entire experiment is able to reduce EOF even further.⁶⁴ Hence, we employed this dynamic coating procedure with 3 mM F108 as final concentration in the buffer and studied its effect on streaming DEP of IgG.

As shown in Figure 5(a), the streaming behavior changed drastically. The regions under which we previously observed concentration (Figures 1(a) and 3) are now depleted. In order to further understand this astonishing DEP streaming behavior, we compared our results to numerical simulations. Interestingly, simulations of negative DEP closely reflect our experimental observations (Figure 5(b)). Therefore, the dynamic coating procedure changed the streaming

FIG. 5. (a) Fluorescence microscopy image of streaming iDEP of fluorescently labeled IgG molecules in an array of triangular posts for 3 mM F108. Concentration depletion along the streamlines is observed due to negative DEP at an applied electric field of 4200 V/cm at pH 8. (b) Normalized concentration as obtained from the simulation within a triangular post array at 4200 V/cm in the case of negative DEP ($\mu_{dep} = -1.28 \times 10^{-23} \text{ m}^4/\text{V}^2\text{s}$). The scale bar indicates 20 μm and the flow direction is from top to bottom. The color bar indicates concentration in arbitrary units.

behavior from positive DEP concentration to negative DEP streaming depletion. To the best of our knowledge, our data are the first to demonstrate negative DC iDEP streaming of a protein caused by a surfactant.

It is noteworthy that these experiments critically depend on the concentration of the dynamic coating agent F108. It has been reported that the tri-block copolymer F108 forms micelles in solution and that this micelle formation is dependent on temperature. Alexandridis *et al.*⁶⁶ found a critical micelle concentration (CMC) of F108 at 25 °C at 3 mM. Our experiments demonstrating negative DEP were performed with 3 mM F108. We therefore propose that micelle formation and integration of IgG therein is responsible for the change in protein DEP behavior. This is likely to occur due to a reduced surface conductivity of the protein micelle so that the Clausius-Mossotti factor changes sign. Note that Eq. (2) indicates that the Clausius-Mossotti factor is negative when the particle conductivity is lower than that of the medium.

In order to investigate whether micelle formation is responsible for the change to negative DEP, we conducted dynamic light scattering (DLS) measurements of the solutions employed for the iDEP experiments (see supplementary material⁶¹). We studied the temperature dependent micellation of F108 alone and with CHAPS and/or IgG added. Our data clearly show that micellation occurred at 3 mM F108 above 30 °C regardless whether CHAPS is added and independent of IgG addition. We thus conclude that the observed iDEP streaming depletion is due to F108 micelle formation.

This negative DEP behavior was further investigated quantitatively by calculating the concentration depletion factor. We note that the depletion effect was enhanced with increasing the applied electric field from 1400 to 4200 V/cm as demonstrated in Figure 6. The largest depletion of 54% was observed with the highest applied voltage of 4200 V/cm in accordance with a similar trend in concentration increase for positive DEP streaming with applied potential. As previously observed in our numerical simulations,²⁵ DEP experiments demonstrated larger depletion than obtained in the simulation (only 15%, see Figure 5(b)). The simulation was conducted using a μ_{dep} value of $-1.28 \times 10^{-23} \text{ m}^4/\text{V}^2\text{s}$ calculated based on the size of the spherical micelle-IgG complex measured by DLS, as well as μ_{eo} of $0.14 \times 10^{-8} \text{ m}^2/\text{Vs}$ for F108 dynamic coated PDMS channels.⁶⁴ We note that the here employed numerical model for DC conditions does not capture the contribution of the double-layer to the protein polarizability in detail.^{44,46} We can, however, speculate that the contribution of double-layer polarization and a decrease in the zeta potential due to the polyethylene glycol chains of F108 forming the micelles results in

FIG. 6. Depletion factor observed experimentally for an applied electric field of 1400, 2800, and 4200 V/cm under 3 mM F108 dynamic coating procedure.

a lower protein conductivity and consequently negative Clausius-Mosotti factor. The influence of dynamic polarization phenomena on nanocolloid polarization was recently reported^{42,44} and may also hold for proteins. In addition to the ionic transport in the double-layer, the electrophoretic motion of proteins may also be responsible for the here observed transition to negative DEP.⁴⁶ The theoretical framework to fully understand protein DEP (including DC iDEP) needs yet to be fully developed and a further study of DC and AC protein DEP may provide more insight into protein polarization and DEP.

V. CONCLUSION

In this study, we conducted a thorough investigation of the factors affecting protein iDEP. We revealed a suitable pH range of pH 6–8, under which IgG iDEP concentration can be performed without aggregation. Moreover, we studied the influence of electrokinesis by numerical simulations in detail. We postulate that our model will be useful in the future, when more quantitative data obtained from independent experimental techniques and theoretical models on biomolecule polarization become available. This will allow the prediction of migration differences for proteins and eventually reveal suitable separation and fractionation conditions for biomolecules based on DEP. Our work is thus an important step towards employing DEP as a new analytical tool for biomolecules.

Moreover, this work demonstrated a clear distinction between positive and negative iDEP streaming of proteins. For the first time, we were able to demonstrate both positive and negative DEP with the same protein species only by tuning the surrounding environment with a surfactant. We could demonstrate that micelle formation is responsible for the observed negative DEP. In summary, our study provides a basis for understanding protein iDEP under DC conditions. Although concentration factors need to be further improved for analytical applications, this study provides a sound basis to optimize iDEP devices for proteins.

ACKNOWLEDGMENTS

This project was supported by grants from the National Center for Research Resources (5R21RR025826) and the National Institute of General Medical Sciences (8R21GM103522) from the National Institutes of Health. We thank Professor Rebecca Wachter and Dr. J. Nathan Henderson in the Chemistry and Biochemistry Department at Arizona State University for assistance with the dynamic light scattering experiments reported in the supplementary information.

- ¹K. P. Chen, J. R. Pacheco, M. A. Hayes, and S. J. R. Staton, *Electrophoresis* **30**, 1441 (2009).
- ²Z. R. Gagnon, *Electrophoresis* **32**, 2466 (2011).
- ³M. P. Hughes, *Electrophoresis* **23**, 2569 (2002).
- ⁴C.-F. Chou, J. O. Tegenfeldt, O. Bakajin, S. S. Chan, E. C. Cox, N. Darnton, T. Duke, and R. H. Austin, *Biophys. J.* **83**, 2170 (2002).
- ⁵E. B. Cummings and A. K. Singh, *Anal. Chem.* **75**, 4724 (2003).
- ⁶B. H. Lapizco-Encinas, B. A. Simmons, E. B. Cummings, and Y. Fintschenko, *Anal. Chem.* **76**, 1571 (2004).
- ⁷H. Shafiee, M. B. Sano, E. A. Henslee, J. L. Caldwell, and R. V. Davalos, *Lab Chip* **10**, 438 (2010).
- ⁸R. Martinez-Duarte, R. A. G. Iii, K. Abi-Samra, and M. J. Madou, *Lab Chip* **10**, 1030 (2010).
- ⁹T. B. Jones, *IEEE Eng. Med. Biol. Mag.* **22**, 33 (2003).
- ¹⁰T. B. Jones, *Electromechanics of Particles* (Cambridge University Press, New York, USA, 1995).
- ¹¹J. Voldman, *Annu. Rev. Biomed. Eng.* **8**, 425 (2006).
- ¹²P. R. C. Gascoyne and J. Vykoukal, *Electrophoresis* **23**, 1973 (2002).
- ¹³F. F. Becker, X. B. Wang, Y. Huang, R. Pethig, J. Vykoukal, and P. R. Gascoyne, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 860 (1995).
- ¹⁴M. Washizu, S. Suzuki, O. Kurosawa, T. Nishizaka, and T. Shinohara, *IEEE Trans. Ind. Appl.* **30**, 835 (1994).
- ¹⁵S. Tuukkanen, A. Kuzyk, J. J. Toppari, H. Häkkinen, V. P. Hytönen, E. Niskanen, M. Rinkio, and P. Törmä, *Nanotechnology* **18**, 295204 (2007).
- ¹⁶A. Kuzyk, B. Yurke, J. J. Toppari, V. Linko, and P. Törmä, *Small* **4**, 447 (2008).
- ¹⁷M. Kumemura, D. Collard, C. Yamahata, N. Sakaki, G. Hashiguchi, and H. Fujita, *ChemPhysChem* **8**, 1875 (2007).
- ¹⁸H. Dalir, Y. Yanagida, and T. Hatsuzawa, *Sens. Actuators B* **136**, 472 (2009).
- ¹⁹R. W. Clarke, J. D. Piper, L. Ying, and D. Klenerman, *Phys. Rev. Lett.* **98**, 198102 (2007).
- ²⁰L. Ying, S. S. White, A. Bruckbauer, L. Meadows, Y. E. Korchev, and D. Klenerman, *Biophys. J.* **86**, 1018 (2004).
- ²¹A. I. K. Lao and I.-M. Hsing, *Lab Chip* **5**, 687 (2005).
- ²²J. Regtmeier, T. T. Duong, R. Eichhorn, D. Anselmetti, and A. Ros, *Anal. Chem.* **79**, 3925 (2007).
- ²³J. Regtmeier, R. Eichhorn, L. Bogunovic, A. Ros, and D. Anselmetti, *Anal. Chem.* **82**, 7141 (2010).

- ²⁴B. H. Lapizco-Encinas and M. Rito-Palomares, *Electrophoresis* **28**, 4521 (2007).
- ²⁵A. Nakano, T. Chao, F. Camacho-Alanis, and A. Ros, *Electrophoresis* **32**, 2314 (2011).
- ²⁶L. Zheng, J. P. Brody, and P. J. Burke, *Biosens. Bioelectron.* **20**, 606 (2004).
- ²⁷H. Maruyama and Y. Nakayama, *Appl. Phys. Express* **1**, 124001 (2008).
- ²⁸S. Agastin, M. R. King, and T. B. Jones, *Lab Chip* **9**, 2319 (2009).
- ²⁹B. H. Lapizco-Encinas, S. Ozuna-Chacón, and M. Rito-Palomares, *J. Chromatogr., A* **1206**, 45 (2008).
- ³⁰R. Hölzel, N. Calander, Z. Chiragwandi, M. Willander, and F. F. Bier, *Phys. Rev. Lett.* **95**, 128102 (2005).
- ³¹D. J. G. Bakewell, M. P. Hughes, J. J. Milner, and H. Morgan, in *Proceedings of Annual International Conference of the IEEE/EMBS* (1998), Vol. 2, p. 1079.
- ³²V. Chaurey, C. Polanco, C.-F. Chou, and N. S. Swami, *Biomicrofluidics* **6**, 012806 (2012).
- ³³K.-T. Liao and C.-F. Chou, *J. Am. Chem. Soc.* **134**, 8742 (2012).
- ³⁴X.-B. Wang, J. Yang, Y. Huang, J. Vykoukal, F. F. Becker, and P. R. C. Gascoyne, *Anal. Chem.* **72**, 832 (2000).
- ³⁵J. Yang, Y. Huang, X. Wang, X.-B. Wang, F. F. Becker, and P. R. C. Gascoyne, *Biophys. J.* **76**, 3307 (1999).
- ³⁶J. Yang, Y. Huang, X.-B. Wang, F. F. Becker, and P. R. C. Gascoyne, *Biophys. J.* **78**, 2680 (2000).
- ³⁷Y. Huang, X. B. Wang, F. F. Becker, and P. R. Gascoyne, *Biophys. J.* **73**, 1118 (1997).
- ³⁸S. K. Srivastava, J. L. Baylon-Cardiel, B. H. Lapizco-Encinas, and A. R. Minerick, *J. Chromatogr., A* **1218**, 1780 (2011).
- ³⁹C. T. O'Konski, *J. Phys. Chem.* **64**, 605 (1960).
- ⁴⁰N. S. K. Gunda and S. K. Mitra, *Biomicrofluidics* **4**, 014105 (2010).
- ⁴¹N. G. Green and H. Morgan, *J. Phys. Chem. B* **103**, 41 (1998).
- ⁴²I. Ermolina and H. Morgan, *J. Colloid Interface Sci.* **285**, 419 (2005).
- ⁴³S. Basuray and H.-C. Chang, *Phys. Rev. E* **75**, 060501 (2007).
- ⁴⁴S. Basuray and H.-C. Chang, *Biomicrofluidics* **4**, 013205 (2010).
- ⁴⁵H. Zhao and H. H. Bau, *J. Colloid Interface Sci.* **333**, 663 (2009).
- ⁴⁶H. Zhao, *Electrophoresis* **32**, 2232–2244 (2011).
- ⁴⁷H. Zhao and H. H. Bau, *Langmuir* **26**, 5412 (2009).
- ⁴⁸D. V. Matyushov, *J. Chem. Phys.* **136**, 085102 (2012).
- ⁴⁹P. E. Smith, R. M. Brunne, A. E. Mark, and W. F. van Gunsteren, *J. Phys. Chem.* **97**, 2009 (1993).
- ⁵⁰S. Boresch, P. Höchtel, and O. Steinhauser, *J. Phys. Chem. B* **104**, 8743 (2000).
- ⁵¹G. Löffler, H. Schreiber, and O. Steinhauser, *J. Mol. Biol.* **270**, 520 (1997).
- ⁵²A. Gencoglu, F. Camacho-Alanis, V. T. Nguyen, A. Nakano, A. Ros, and A. R. Minerick, *Electrophoresis* **32**, 2436 (2011).
- ⁵³L. M. Hjelmeland, D. W. Nebert, and J. C. Osborne, Jr., *Anal. Biochem.* **130**, 72 (1983).
- ⁵⁴T. T. Duong, G. Kim, R. Ros, M. Streek, F. Schmid, J. Brugger, D. Anselmetti, and A. Ros, *Microelectron. Eng.* **67–68**, 905 (2003).
- ⁵⁵S. Sandin, L.-G. Öfverstedt, A.-C. Wikström, Ö. Wrange, and U. Skoglund, *Structure* **12**, 409 (2004).
- ⁵⁶H. Morgan and N. G. Green, *J. Electrostat.* **42**, 279 (1997).
- ⁵⁷E. B. Cummings, *IEEE Eng. Med. Biol. Mag.* **22**, 75 (2003).
- ⁵⁸R. Davalos, G. McGraw, T. Wallow, A. Morales, K. Krafcik, Y. Fintschenko, E. Cummings, and B. Simmons, *Anal. Bioanal. Chem.* **390**, 847 (2008).
- ⁵⁹J. L. Baylon-Cardiel, B. H. Lapizco-Encinas, C. Reyes-Betanzo, A. V. Chávez-Santoscoy, and S. O. Martínez-Chapa, *Lab Chip* **9**, 2896 (2009).
- ⁶⁰J.-S. Kwon, J.-S. Maeng, M.-S. Chun, and S. Song, *Microfluid. Nanofluid.* **5**, 23 (2008).
- ⁶¹See supplementary material at <http://dx.doi.org/10.1063/1.4742695> for isoelectric focusing, dynamic light scattering, and the trapping condition.
- ⁶²W. Hellmich, J. Regtmeier, T. T. Duong, R. Ros, D. Anselmetti, and A. Ros, *Langmuir* **21**, 7551 (2005).
- ⁶³V. Tandon, S. K. Bhagavatula, W. C. Nelson, and B. J. Kirby, *Electrophoresis* **29**, 1092 (2008).
- ⁶⁴M. Viefhues, S. Manchanda, T.-C. Chao, D. Anselmetti, J. Regtmeier, and A. Ros, *Anal. Bioanal. Chem.* **401**, 2113 (2011).
- ⁶⁵B. Jachimska, M. Wasilewska, and Z. Adamczyk, *Langmuir* **24**, 6866 (2008).
- ⁶⁶P. Alexandridis, J. F. Holzwarth, and T. A. Hatton, *Macromolecules* **27**, 2414 (1994).