Study on surface properties of PDMS microfluidic chips treated with albumin

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Electrokinetic properties and morphology of PDMS microfluidic chips intended for bioassays are studied. The chips are fabricated by a casting method followed by polymerization bonding. Microchannels are coated with 1% solution of bovine serum albumin (BSA) in Tris buffer. Albumin passively adsorbs on the PDMS surface. Electrokinetic characteristics (electro-osmotic velocity, electro-osmotic mobility, and zeta potential) of the coated PDMS channels are experimentally determined as functions of the electric field strength and the characteristic electrolyte concentration. Atomic force microscopy (AFM) analysis of the surface reveals a “peak and ridge” structure of the protein layer and an imperfect substrate coating. On the basis of the AFM observation, several topologies of the BSA-PDMS surface are proposed. A nonslip mathematical model of the electro-osmotic flow is then numerically analyzed. It is found that the electrokinetic characteristics computed for a channel with the homogeneous distribution of a fixed electric charge do not fit the experimental data. Heterogeneous distribution of the fixed electric charge and the surface roughness is thus taken into account. When a flat PDMS surface with electric charge heterogeneities is considered, the numerical results are in very good agreement with our experimental data. An optimization analysis finally allowed the determination of the surface concentration of the electric charge and the degree of the PDMS surface coating. The obtained findings can be important for correct prediction and possibly for robust control of behavior of electrically driven PDMS microfluidic chips. The proposed method of the electro-osmotic flow analysis at surfaces with a heterogeneous distribution of the surface electric charge can also be exploited in the interpretation of experimental studies dealing with protein-solid phase interactions or substrate coatings. © 2009 American Institute of Physics. [doi:[10.1063/1.3243913](https://doi.org/10.1063/1.3243913)]

I. INTRODUCTION

Polydimethylsiloxane (PDMS) is a favorite material for microfluidic chip fabrication due to its properties such as high optical transparency, biocompatibility, or undemanding microfabrication.^{1–3} Electro-osmotic flow (EOF) is often employed in PDMS and other microchips for various biomicrofluidic applications.⁴ The knowledge of electro-osmotic characteristics is then essential for microchip design and process control.

Electro-osmotic characteristics⁵ (electro-osmotic mobility and zeta potential) of PDMS were obtained by many researchers who used various measurement techniques. Current monitoring

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technique is one of the most exploited;^{6–13} however, other techniques such as amperometric detection method,¹⁴ cyclic voltametry,¹⁵ imaging of a caged fluorescent dye,^{16,17} and particle tracking technique¹⁸ were also employed.

Data published in the literature differ significantly. This fact results from both various surface treatments of the PDMS chips (oxygen plasma treatment, possible adsorption from an electrolyte, use of coatings) and various compositions of electrolytes (*pH*, concentration). Electro-osmotic mobilities in a native PDMS were reported to be smaller than those where the PDMS surface was oxidized by plasma treatment.⁹ The surface properties of the oxidized PDMS change in time as well as the values of the electro-osmotic mobilities that significantly decrease when the treated surface is exposed to air.⁹ The electrokinetic characteristics slightly differ if the microchannel PDMS network is sealed against either another PDMS layer or a glass layer.^{9,11}

PDMS was also chemically modified to enhance the EOF intensity. For instance, Luo *et al.*¹⁹ added undecylenic acid to the PDMS prepolymer and the EOF mobility rose up to $7.6 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. When Vickers *et al.*¹⁵ hydrophilized PDMS by an extraction process followed by air plasma treatment, the EOF mobility increased up to $6.8 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. Roman *et al.*²⁰ fabricated microchips with SiO_2 particles homogeneously distributed within the PDMS polymer matrix having the EOF mobility equal to $8.3 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. Choi *et al.*⁷ modified the PDMS surface by cetyltrimethylammonium bromide which even reversed the EOF, i.e., changed the fixed electric charge polarity. Bao *et al.*¹⁴ reported the influence of various cations contained in phosphate buffer solutions on the electro-osmotic velocity in a PDMS channel. They also attributed the EOF emergence to the cation and anion adsorption on PDMS walls.

Nonlinear dependences of the electro-osmotic mobility on ionic strength were experimentally obtained by Spehar *et al.*¹¹ The authors fitted the experimental data with two functions

$$\mu_{EO} = k_1 \log(I) + k_0, \quad (1)$$

$$\mu_{EO} = k_0 + k_1 I^{-0.5}, \quad (2)$$

where μ_{EO} and I are the EOF mobility and the ionic strength, respectively. Equation (1) provided a better fit. The dependence of the electro-osmotic mobility on the ionic strength can be explained by a change of the zeta potential. Kirby *et al.*²¹ derived two approximate relations expressing the zeta-potential dependence on ionic strength. The first relation bears the logarithm form and is valid for high values of zeta potentials. The second one follows the inverse square root dependence and is applicable for low values of zeta potentials (up to 25 mV).

Because PDMS microchips find their use in a variety of bioapplications, a few papers were aimed at the electro-osmotic transport of buffers containing biomacromolecules.^{6,10,22} These molecules often interact with the PDMS surface due to hydrophobic interactions generally resulting in a decrease of EOF intensity. For instance, the presence of bovine serum albumin (BSA) in transported electrolytes decreased the electro-osmotic velocity in microsystems made from twelve different plastic substrates.¹⁰ Zhou *et al.*²² evaluated the EOF mobility in a BSA treated PDMS microchannel to be $1.7 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. We summarized some available data on the electro-osmotic mobilities in various PDMS devices in Table I.

In this paper, we describe techniques for fabrication of multi-channel microchips from PDMS and methods for determination of their electrokinetic and morphological properties. A non-slip mathematical model of the electro-osmotic flow in a representative part of the microchips is then derived. In the next step, we report experimentally obtained dependencies of the EOF characteristics on the electrolyte concentration and the electric field strength. Finally, the obtained experimental results are confronted with the theoretical ones in the view of the surface concentration of the fixed electric charge and the morphology of the PDMS surface treated by BSA.

TABLE I. Electro-osmotic mobilities in PDMS systems.

Source	PDMS	EOF mobility [$\times 10^{-8}$ m ² V ⁻¹ s ⁻¹]	Conditions
Spehar ¹¹	native, reversibly sealed to PDMS	2.3	<i>N</i> -(2-acetamido)-2-aminoethanesulfonic acid, pH=7.8, <i>I</i> =90 mM
Spehar ¹¹	native, reversibly sealed to glass	2.8	<i>N</i> -(2-acetamido)-2-aminoethanesulfonic acid, pH=7.8, <i>I</i> =90 mM
Ren ⁹	native, reversibly sealed to PDMS	1.1	phosphate buffers, <i>I</i> =15 mM, pH=7
Ren ⁹	oxidized, irreversibly sealed to PDMS	3.7	phosphate buffers, <i>I</i> =15 mM, pH=7
Ocvirk ³⁹	native, reversibly sealed to PDMS	4.5	1 mM phosphate and 10 mM KCl, pH=8
Ocvirk ³⁹	native, reversibly sealed to PDMS	6.4	1 mM phosphate, 10 mM KCl, 1000 μ M sodium dodecyl sulfate, pH=9
Luo ¹⁹	addition of 0.5% w undecylenic acid, sealed to PDMS	7.6	19 and 20 mM HEPES buffer, pH=8.5
Vickers ¹⁵	extracted in organic solvents, oxidized, irreversibly sealed to PDMS	6.8	20 mM TES buffer, pH=7.0, (<i>N</i> -tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid)
Vickers ¹⁵	native, sealed to PDMS	4.1	20 mM TES buffer, pH=7.0
Roman ²⁰	addition of SiO ₂ particles	8.3	1 mM sodium phosphate, 10 mM KCl, pH=8.3
Roman ²⁰	native	4.2	1 mM sodium phosphate, 10 mM KCl, pH=8.3
Choi ⁷	surfacial modified by cetyltrimethylammonium bromide (CTAB)	-5.0	100 mM Tris, 20 mM boric acid, pH=9.3, 0.2 mM CTAB
Zhou ²²	native, reversibly sealed to PDMS	1.7	30 mM phosphate buffer, 10 μ g ml ⁻¹ BSA, pH=6.9

II. EXPERIMENTAL

A. PDMS chip fabrication

Four meandering microchannels (75 mm $\times$ 40 μ m $\times$ 25 μ m) with a reservoir at each end were designed on a single microchip, Fig. 1. The chip was fabricated by means of PDMS casting against a SU8 master. The microchannels were enclosed by bonding to a pre-polymerized PDMS layer. This procedure avoided plasma treatment which alters the surface properties of PDMS.

The SU8 master (mold) was fabricated as follows: A phosphor bronze substrate was cleaned in a diluted nitric acid (68% acid and water in the volume ratio 1:2), washed in deionized water and acetone and finally dried in an oven for 30 min at 100 °C. After cooling to the laboratory temperature, 25 μ m high layer of SU8-2025 (MicroChem) was spin coated on the substrate. This layer was pre-baked for 20 min at 100 °C on a hot plate, exposed through a mask, post-baked for

FIG. 1. PDMS microchip.

4 min at 100 °C and developed. PDMS (Sylgard 184, Dow Corning) was prepared by thorough mixing the pre-polymer and the curing agent in the weight ratio 10:1. After degassing, PDMS was poured onto the SU8 master and again degassed. Then the PDMS layer was placed in a drier at 70 °C for 60 min. The cured PDMS was peeled off the master and the holes for electrolyte delivery were punched at required locations. The PDMS surface was finally cleaned with isopropylalcohol and dried with nitrogen. Another PDMS slab was prepared on a glass substrate and pre-polymerized at 90 °C for 9 min. The two PDMS layers were brought in contact and placed in a drier for 30 min to finish the microchip fabrication. A very good irreversible sealing of the two PDMS layers was obtained.

B. Electrokinetic characteristics

The current monitoring technique^{23,24} was used to evaluate the electro-osmotic characteristics of the PDMS substrate coated with BSA. The used Tris-BSA buffer (Sigma T-6789, 50 mM Tris, 138 mM NaCl, 2.7 mM KCl, pH=8, 1% bovine serum albumin) contains several electroneutral molecules, tris(hydroxymethyl)aminomethane cation ($pK_a=8.1$), anion forms of BSA ($pI=4.6$),²⁵ Na^+ , K^+ , Cl^- , H^+ and OH^- . Consideration of all these compounds and their dissociation interactions brings strong difficulties in the analysis of transport processes. Because the NaCl concentration (138 mM) in the Tris-BSA buffer is much higher than the concentrations of the other ions, we assumed that the Tris-BSA buffer approximately behaves like the uni-univalent Na^+Cl^- electrolyte. Hence the expression “electrolyte concentration” used in the following experimental and modeling sections refers to NaCl concentration in the electrolytes.

The current monitoring technique is based on the measurement of the electric current flowing through a microcapillary under a constant difference of the electric potential. If the electrolyte concentration varies at the microcapillary inlet, the averaged electrolyte conductivity in the microcapillary changes together with the observed electric current.

The experimental procedure, which we used in our study, can be shortly described as follows: (i) the microchannel was filled with the blocking buffer Tris-BSA (10 min) to coat the PDMS surface, (ii) the channel was filled with Tris-BSA buffer of a chosen concentration c_1 , (iii) a constant DC voltage $\Delta\Phi_T$ was imposed on the channel, (iv) when a constant electric current

FIG. 2. Geometries of the computational domains, $H=25 \mu\text{m}$, $L=100 \text{ nm}$: a) flat surface, b) ruffled surface represented by quadratic Bézier curves, A_R is the roughness amplitude, c) step-like surface with the rounding radius 1 nm . L_C and L_U represent the lengths of the charged and uncharged surfaces, respectively. The thick solid line denotes surface areas with a fixed electric charge.

response was obtained, Tris-BSA buffer with concentration of $c_2=2c_1$ was introduced into the inlet (anode) reservoir, (v) the duration of the electric current increase $\Delta\tau$ was measured. We also define the characteristic electrolyte concentration of this experiment $c_0 \equiv (c_1 + c_2)/2$.

The EOF mobility μ_{EO} can be evaluated from

$$v_{EO} = \mu_{EO} E, \quad (3)$$

where v_{EO} and E are the net flow velocity and the electric field strength, respectively. Assuming a linear distribution of the electric potential along the channel (constant E)

$$E \approx -\Delta\Phi_T/L_T, \quad (4)$$

the EOF mobility is given by

$$\mu_{EO} = -L_T^2/(\Delta\tau \Delta\Phi_T), \quad (5)$$

where L_T is the microchannel length. Zeta potential ζ is related to the EOF mobility via the Smoluchowski equation^{5,26}

$$\mu_{EO} = -\zeta\epsilon/\eta \quad (6)$$

where η and ϵ are the dynamic viscosity and the electrolyte permittivity, respectively.

C. AFM surface characterization

PDMS samples were analyzed by the NTEGRA-Prima AFM system (NT-MDT). Diamond-like carbon tips with diameter $1\text{--}3 \text{ nm}$ (NT-MDT, NSG01-DLC) were used. The measurements were carried out in a semi-contact mode in air.

Two types of PDMS samples were characterized: (i) uncoated PDMS and (ii) PDMS coated with BSA. To prepare the coating, the PDMS surface was covered by the blocking Tris-BSA buffer for 10 min . In the next step, the surface was dried and cleaned by a compressed air.

III. MATHEMATICAL MODEL

As the experimental microchips contain long channels with constant surface properties, the electrokinetic transport can be studied only in a short “representative” segment of the channels, Fig. 2. We assume that the width of the channel is large enough in order to describe the problem

TABLE II. Fixed model parameters.

Parameter	Description	Value
ε	electrolyte permittivity	$6.95 \times 10^{-10} \text{ F m}^{-1}$
η	electrolyte viscosity	$8.91 \times 10^{-4} \text{ Pa s}$
F	Faraday's constant	96487 C mol^{-1}
R	molar gas constant	$8.314 \text{ J mol}^{-1} \text{ K}^{-1}$
T	temperature	298 K
D_+	cation diffusivity	$1.5 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$
D_-	anion diffusivity	$2 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$
L_T	total capillary length	$7.5 \times 10^{-2} \text{ m}$
L	length of the capillary segment	$1 \times 10^{-7} \text{ m}$
A_R	roughness amplitude	$8.56 \times 10^{-9} \text{ m}$

as two-dimensional. Three different geometries of the channel surface are considered: Perfectly flat with homogeneous or heterogeneous distribution of the fixed electric charge (a), ruffled with heterogeneous distribution of the fixed electric charge (b), and step-like with heterogeneous distribution of the fixed electric charge (c). The choices of the surface geometries are discussed in the result section.

The mathematical model is based on the approach used in works.^{27,28} The velocity $\mathbf{v}$ and pressure p fields are described by the Stokes equation²⁹ (Reynolds number is close to zero) containing the coulomb force term and by the continuity equation for an incompressible Newtonian fluid

$$\nabla p + q \nabla \Phi = \eta \nabla^2 \mathbf{v}, \quad \nabla \cdot \mathbf{v} = 0, \quad (7)$$

where Φ and q are the electric potential and the volume concentration of the mobile electric charge, respectively. The electric potential field in a system with a constant electrolyte permittivity ε is described by the Poisson equation in the form

$$\nabla^2 \Phi = -q/\varepsilon. \quad (8)$$

Sodium (subscript +) and chloride (subscript −) ions are dominant in the used Tris buffers. If concentrations of the other ions are neglected, the volume concentration of the electric charge is given by

$$q = F(c_+ - c_-), \quad (9)$$

where F and $c_{\pm}$ are the Faraday constant and the molar ion concentrations, respectively. The concentration fields can be evaluated using the steady state molar balances of ions in dilute electrolytes

$$\nabla \cdot \mathbf{J}_{\pm} = \nabla \cdot \left(\mathbf{v} c_{\pm} - D_{\pm} \nabla c_{\pm} \mp \frac{D_{\pm} F}{RT} c_{\pm} \nabla \Phi \right) = 0, \quad (10)$$

where the total ion flux intensities, $\mathbf{J}_+$ and $\mathbf{J}_-$, are given by the sum of the convective, diffusion and electromigration contributions. The symbols $D_{\pm}$, R and T denote the ion diffusivities, the molar gas constant and temperature, respectively. The values of the fixed model parameters are listed in Table II.

The symmetric boundary conditions were used on the symmetry axis (the top part of the segments). The periodic boundary conditions were applied on the inlet and outlet boundaries (the left and right parts of the segments) except for the electric potential for which

FIG. 3. Mesh of finite elements used for the ruffled geometry: a) one quarter of the computation domain, b) mesh detail above the bottom boundary.

$$\Phi|_{\text{inlet}} = \Phi|_{\text{outlet}} + \Delta\Phi_L, \quad (11)$$

where $\Delta\Phi_L$ is a change of the electric potential per the segment length L . We assume approximately linear distribution of the electric potential (a constant electric field strength along the microchannel), i.e., $E = -\Delta\Phi_L/L$. Thus, if the capillary length L_T , the total potential difference imposed on the capillary $\Delta\Phi_T$, and the segment length L are known, the parameter $\Delta\Phi_L$ can be easily estimated from $\Delta\Phi_L \approx \Delta\Phi_T L / L_T$. The non-slip boundary conditions for the velocity and the normal molar flux intensities equal to zero were considered on the dielectric surface (the bottom part of the segment)

$$\mathbf{v}|_{\text{wall}} = 0, \quad \mathbf{n} \cdot \mathbf{J}_{\pm}|_{\text{wall}} = 0, \quad (12)$$

where $\mathbf{n}$ is the normal vector to the surface. The following boundary conditions were chosen for the electric potential

$$\mathbf{n} \cdot \nabla \Phi|_{\text{charged wall}} = -\sigma/\epsilon, \quad \mathbf{n} \cdot \nabla \Phi|_{\text{uncharged wall}} = 0, \quad (13)$$

where σ is the surface concentration of the fixed electric charge. Zero reference pressure and electric potential were considered at one point of the segment.

This set of model equations was numerically solved using the nonlinear stationary solver of the Comsol Multiphysics software. Because the proposed mathematical model describes transport phenomena in the entire spatial domain including the extremely thin region with a nonzero concentration of the electric charge (so called electric double layer—EDL), a hybrid triangle-rectangle spatial discretization was developed. The rectangle finite elements with various aspect ratios (up to 500) were used in EDL and the triangle elements in the rest of the domain, Fig. 3. Purely triangle meshes with a high element density were exploited for the step-like surface geometry. The main characteristics of all meshes are summarized in Table III. It can be seen that the finite element density across EDL is extremely high in order to capture the concentration and potential gradients.

TABLE III. Mesh parameters.

Geometry	Mesh type	No. elements	Min. step [nm]	Max. step [nm]	Max. aspect ratio (rectangle)
Flat	hybrid	12800	0.002	15	500
Ruffled	hybrid	12800	0.002	15	500
Step-like	triangle	25400	0.08	50	-

Numerical solutions were validated with the use of an analytical solution for the perfectly flat segment.⁵ If one studies the electro-osmotic flow in a cylindrical microchannel, an exact solution can be found in.³⁰ Mesh convergence studies were carried out for the other segments with surface heterogeneities to avoid significant numerical errors.

The mathematical model was used for estimation of the parameter σ . The “optimal” parameter value was identified by minimizing a penalty function defined as the sum of squares of deviations between the experimentally and numerically obtained points on concentration dependencies of the electro-osmotic mobility.

IV. RESULTS AND DISCUSSION

A. AFM characterization

The root mean square (RMS) surface roughness of the uncoated PDMS was 0.85 nm and the average roughness was 0.68 nm.

An example of the AFM image of the coated PDMS surface is plotted in Fig. 4. BSA molecules do not regularly cover the PDMS surface. The protein layer has a “peak and ridge” structure that was observed, for example, when BSA adsorbed on treated³¹ or untreated³² polystyrene (PS) or gold.³³ The maximal vertical height of our protein layer is about 15 nm. BSA molecules have ellipsoid shape with characteristic dimensions 14 nm × 4 nm × 4 nm.³² Thus BSA can adsorb in two limit orientations: (i) the “end-on” with the characteristic height 14 nm or (ii) the “side-on” with the characteristic height 4 nm.^{32,33} It seems that no orientation is preferred on the PDMS substrate because one can observe peaks of various heights, Fig. 4. Width of the peaks attains hundreds of nanometers. Hence, we assume that larger peaks are formed by BSA aggregates. Huang and Gupta³⁴ suggested that the aggregate formation can be caused by protein unfolding upon the BSA adsorption. Then the internal hydrophobic domains are available for a close binding of other BSA molecules.

Figure 4 shows that there is a large uncovered part of the PDMS surface among BSA molecules/aggregates. This part forms approximately one half of the whole surface. According to an image analysis of the obtained 3D data, we determined the percentage of the uncoated part to be equal to 57%. This is surprising with respect to the fact that the BSA concentration in the Tris-BSA buffer is high (1%). A similar observation was published by Butler *et al.*³⁵ They used immunoglobulins and other proteins. The authors suggested that adsorption of proteins on PDMS can be locally difficult because small air bubbles can be bound on the polymer surface.

AFM images reveal important facts. The EDL formation is prevented at the PDMS surface covered by the BSA molecules because the fixed electric charge is shielded by this protein. Further, the RMS roughness increased from 0.85 nm to 5.34 nm and the average roughness from 0.68 nm to 4.28 nm on the coated PDMS surface. It means that the immobilized BSA molecules also increase the hydrodynamic resistance at the PDMS surface.

Hence, we suggest three possible arrangements characterizing a representative part of the coated PDMS surface, Fig. 2. In our concept, the covered part of the PDMS surface does not contribute to the formation of the mobile electric charge in the electrolyte, i.e., the concentration of the fixed electric charge in the covered parts is considered to be zero.

FIG. 4. AFM images of the surface coated with BSA: a) 3D image of a representative sample of the surface, b) height profile of the sample that corresponds to white line in a).

B. Electrokinetic properties

An example of a typical experiment used for the electro-osmotic velocity evaluation is shown in Fig. 5. The used electric current monitoring technique enables to clearly determine the duration of the electrolyte transport along the entire length of the microchannel. The electric current increase is not linear. In all experiments, we observed that the current response is a convex function of time. The origin of this finding was theoretically revealed and discussed in work.²⁴

1. Electric field strength characteristics

Experiments aimed at the evaluation of the electro-osmotic mobility and zeta potential were performed for two different characteristic electrolyte concentrations. The obtained dependencies are linear in the considered interval of the electric field strength, Fig. 6. Each experimental point is represented by the arithmetic mean of the net velocity obtained from five independent measurements. 90% confidence intervals are also plotted. With the use of Eq. (5), the electro-osmotic mobility was evaluated to be $(1.08 \pm 0.02) \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $(2.35 \pm 0.06) \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ for the higher ($c_0 = 0.104 \text{ M}$) and the lower ($c_0 = 0.010 \text{ M}$) characteristic concentration, respectively. From Eq. (6), the corresponding zeta-potentials are $-(13.8 \pm 0.3) \text{ mV}$ and $-(30.1 \pm 0.8) \text{ mV}$. The obtained values are in agreement with the data reported by Zhou *et al.*²² for a PDMS substrate treated by BSA.

FIG. 5. Measurement of the electro-osmotic velocity: Electric current time course. Duration of the electric current increase is equal to $\Delta\tau$, $E=13.7 \text{ kV m}^{-1}$, $c_0=0.013 \text{ M}$.

2. Concentration dependencies

In the next step, dependencies of the net velocity on the electrolyte concentration were measured for two imposed electric field strengths, Fig. 7. Each point again represents the arithmetic mean of five independent measurements. The net velocity nonlinearly decreases with increasing concentration for the both electric field strengths. The zeta-potential and electro-osmotic mobility vary with increasing concentration from -38 mV to -13 mV and from $3.0 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ to $1.0 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, respectively.

The experimental results were fitted with two functions, Eq. (1) and (2), as suggested by Spehar *et al.*¹¹ Our results are in agreement with data published in their work. The concurrence is both qualitative (the shape of the dependence, confidence of the two fits) and quantitative (the range of concentrations and the corresponding electro-osmotic mobilities).

FIG. 6. Dependencies of the electro-osmotic flow velocity on the electric field strength. Circles: $c_0=0.104 \text{ M}$, squares: $c_0=0.010 \text{ M}$. The solid lines denote linear fits of the experimental data.

FIG. 7. Dependencies of the electro-osmotic flow velocity on the characteristic electrolyte concentration. Circles: $E = 13.7 \text{ kV m}^{-1}$, squares: $E = 11.0 \text{ kV m}^{-1}$. The solid and the dotted lines denote nonlinear fits of the experimental data according to the relations, Eq. (1) and Eq. (2), respectively.

It can be seen that the logarithm dependence, Eq. (1), fits the experimental points significantly better than the inverse square root function, Eq. (2), for the both electric field strengths. This fact is consistent with the finding that the logarithmic dependence is mostly applicable for low electrolyte concentrations, e.g., $c_0 < 100 \text{ mM}$ on silica.²¹ On the other hand, the obtained value of the zeta-potential is suspiciously low (close to 25 mV). According to the theory, the experimental data should be fitted better by the inverse square root function. Hence, we decided to find possible origins of this discrepancy.

C. Effect of electric charge heterogeneities

The entire surface of the PDMS substrate cannot contribute to the electro-osmotic transport of the electrolyte due to the immobilized BSA molecules. Considering the homogeneous distribution of the electric charge along the PDMS surface [$L_C = 100 \text{ nm}$, $L_U = 0 \text{ nm}$, Fig. 2(a)], the dependence of the net velocity on the characteristic concentration c_0 can be evaluated with the use of an exact expression.⁵ It gives the relation between the zeta potential and the electric charge surface concentration⁵

$$\zeta = \frac{2RT}{F} \operatorname{arcsinh} \left(\frac{\sigma \lambda F}{2RT\epsilon} \right), \quad (14)$$

which can be substituted into Eq. (6). The symbol λ denotes the Debye length that is proportional to the inverse square root of the characteristic electrolyte concentration

$$\lambda = \left(\frac{RT\epsilon}{2c_0 F^2} \right)^{0.5}. \quad (15)$$

We further consider that the surface concentration of the fixed electric charge on PDMS is approximately constant on the studied interval of the electrolyte concentrations. The optimal σ value can be then found by the optimization procedure (see the section Mathematical model). It can be observed that Eq. (14) is not able to satisfactorily fit the experimental data, see the dotted line in Fig. 8.

Hence, we consider that the PDMS surface can be divided into two parts, Fig. 2(a). One part represents the surface where no BSA molecules are immobilized (L_C), i.e., the surface electric charge concentration should be similar as on the non-treated PDMS substrate. The other part

FIG. 8. Dependencies of the electro-osmotic flow velocity on the characteristic electrolyte concentration for the flat geometry Fig. 2(a). a) $E = 13.7 \text{ kV m}^{-1}$, circles: Experiments, solid line: $L_C = 62 \text{ nm}$, $\sigma = -1.80 \times 10^{-2} \text{ C m}^{-2}$, dotted line: $L_C = 100 \text{ nm}$, $\sigma = -0.884 \times 10^{-2} \text{ C m}^{-2}$, b) $E = 11.0 \text{ kV m}^{-1}$, circles: Experiments, solid line: $L_C = 57 \text{ nm}$, $\sigma = -1.83 \times 10^{-2} \text{ C m}^{-2}$, dotted line: $L_C = 100 \text{ nm}$, $\sigma = -0.805 \times 10^{-2} \text{ C m}^{-2}$.

represents the surface where BSA molecules occupy the PDMS surface (L_U). In this region, we assume that $\sigma \rightarrow 0 \text{ C m}^{-2}$. A two parametric optimization procedure was then carried out to identify the proper L_C and σ values.

As shown in Fig. 8, the dependence of the net velocity obtained by the numerical analysis of the model with the electric charge heterogeneity can perfectly fit the experimental data. The fixed electric charge is effectively located on about 60% of the PDMS surface (57%–62%). This finding is also in good agreement with the AFM observation (approximately 57% of uncoated surface). The local electric charge concentration on the charged part of PDMS (the L_C domain without BSA) is then about $-1.8 \times 10^{-2} \text{ C m}^{-2}$. The charge concentration averaged over the entire PDMS surface (computed as the weighted arithmetic mean) is about $-1.1 \times 10^{-2} \text{ C m}^{-2}$.

D. Effects of the surface roughness

The AFM analysis revealed that the surface roughness of the PDMS with the adsorbed BSA molecules is several times higher than that of the native PDMS. To study the effect of the surface roughness on the electro-osmotic flow numerically, we approximated the real surface profile with: (i) ruffled surface represented by Bezier curves of the second order, Fig. 2(b), and (ii) step-like surface with rounded corners, Fig. 2(c). In both cases the height of the peaks corresponded to the double of the average surface roughness measured by AFM. We again considered that the BSA

FIG. 9. Dependencies of the electro-osmotic flow velocity on the characteristic electrolyte concentration for the ruffled surface (solid line) and the step-like surface (dotted line). $E=13.7 \text{ kV m}^{-1}$, circles: Experiments; solid line $L_C=74 \text{ nm}$, $\sigma=-2.5 \times 10^{-2} \text{ C m}^{-2}$; dotted line: $L_C=89 \text{ nm}$, $\sigma=-3.1 \times 10^{-2} \text{ C m}^{-2}$.

molecules cover a certain part of the PDMS surface which then cannot contribute to the EOF flow. Therefore, the same parametric optimization procedures as the above were performed to find out the values of the fixed electric charge σ and the length of the charged surface L_C . The length L_U corresponds to the half width of the peaks (Fig. 2).

The results of the optimization procedure are plotted in Fig. 9. It can be found that the modeled curve for the ruffled geometry fits the experimental data better than the curve for the step-like geometry. The local surface concentration of the electric charge σ and the length of the charged surface L_C for the ruffled surface were $-2.5 \times 10^{-2} \text{ C m}^{-2}$ and 74 nm , respectively, for the step-like geometry $-3.1 \times 10^{-2} \text{ C m}^{-2}$ and 89 nm , respectively. These results predict the fraction of the surface covered by BSA to be 26% or 11%, respectively.

The values of the optimized parameters (L_C , $|\sigma|$) are much higher for the ruffled and step-like geometry than those for the flat geometry. The results obtained for the flat geometry are also in a good agreement with the AFM observation. One of the possible explanations of this fact relies on a limited potential of the spatially two-dimensional models. The AFM picture of the PDMS layer coated with BSA shows that there are “valleys” among the immobilized protein molecules. As the surface electric charge is located dominantly in these valleys, the charged electrolyte (in EDL) is forced to flow among the protein molecules but not over them. The typical Debye layer thickness for the used electrolytes is about 1 nm (it depends on the used electrolyte concentration) but the vertical dimensions of the immobilized protein can exceed 10 nm . Thus the ruffled and step-like geometries cannot describe the real electrolyte behavior at the coated surface. The classical electro-osmosis probably emerges only in the valleys whereas zero flow is induced at the protein surface. In our opinion, the flat geometry with heterogeneous distribution of the electric charge can predict such behavior because this geometry linearly combines the charged and uncharged parts on the flat surface but no vertical flow over the uncharged proteins is forced. For a better understanding of the above described problem, spatially three-dimensional models reflecting the real geometry of the coated PDMS surface should be developed. In such models, the entire flow pattern can be probably obtained and analyzed.

E. Effective electric charge of BSA

In the previous analysis, we assumed that the immobilized BSA molecules do not contribute to the electro-osmotic flow, i.e., the effective surface electric charge of the BSA molecule is close to zero. Because the experiments were carried out at $pH=8$, which is quite above the isoelectric point of BSA ($pI \sim 4.6$), the BSA molecules have to be negatively charged. Böhme and Scheler showed that the effective electric charge number of BSA under $pH=8$ is about -12 .³⁶ The corresponding surface charge density on a BSA molecule is then approximately $-5 \times 10^{-2} \text{ C m}^{-2}$ (the characteristic BSA diameter equal to 6 nm was considered). Under such conditions, the BSA immobilization should accelerate the electro-osmotic flow at the PDMS surface.

This is in a clear contradiction with our findings. We carried out control experiments in order to compare the electro-osmotic mobility in the PDMS chip with and without the BSA coating under the same conditions (Tris buffer, $pH=8$, $c_0=0.104 \text{ M}$, $E=13.7 \text{ kV m}^{-1}$). The obtained electro-osmotic mobilities were equal to $(1.08 \pm 0.02) \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ for the BSA coated PDMS and to $(2.56 \pm 0.03) \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ for the native PDMS. The decrease of the electro-osmotic mobility after the protein treatment indicates that the average surface charge density significantly reduced. It seems that the mobility decrease after a protein treatment is a common feature of many plastics.^{10,22,37} The generally accepted explanation of this phenomenon is given in monograph.³⁸ In a stronger electrolyte, the counter-ions from the electrolyte firmly bind (the counter-ions are thus immobile) by the electrostatic interaction to the ionized groups of proteins that then behave as electroneutral molecules. We should note that the Böhme and Scheler experiments³⁶ were carried out in deionized water.

The previous discussion implies that our assumption on the zero effective electric charge of the immobilized BSA is correct.

V. CONCLUSIONS

The electrokinetic and surface properties of the PDMS chips treated with BSA molecules were investigated. The electro-osmotic mobilities and zeta potentials were evaluated from the electro-osmotic velocity vs. electric field strength dependencies giving the values $(1.08 \pm 0.02) \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $-(13.8 \pm 0.3) \text{ mV}$ for the electrolyte concentration of 0.104 M and $(2.35 \pm 0.06) \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $-(30.1 \pm 0.8) \text{ mV}$ for the electrolyte concentration of 0.010 M. The observed effect of electrolyte concentration on the electro-osmotic velocity was further studied in detail. The experimental dependence of the electro-osmotic velocity on the electrolyte concentration was nonlinear and well fitted with a logarithmic function.

The AFM analysis of the native and the BSA treated PDMS chips showed an increase in the average surface roughness from 0.68 nm to 4.28 nm and also significant heterogeneities of the protein layer. This observation implied that (i) the increased surface roughness offers a higher resistance to the electro-osmotic flow, and (ii) the BSA molecules cover only a part of the PDMS surface and partially shield the fixed electric charge. These implications were supported by the fact that the theoretical dependence of the EOF velocity on the electrolyte concentration derived for a flat surface with a homogeneous distribution of the surface electric charge did not satisfactorily fit the experimental data.

Assumption of the existence of the heterogeneously distributed surface electric charge gave a better fit of the experimental data for all the studied geometries. However, different values of the electric charge surface density and the fraction of the surface covered by the BSA molecules were predicted for the suggested geometries. When compared to the AFM analysis, the flat surface geometry with the heterogeneous distribution of the electric charge seems to provide the most realistic results giving the value of the local surface charge density of -0.018 C m^{-2} and the fraction of the surface coated with BSA of 40%.

The obtained results give us a deeper insight into the processes in the vicinity of the PDMS surface coated by BSA. One can expect that a similar behavior will be typical for other proteins on various substrates. The precise electro-osmotic dosing of samples and washing buffers is one of

the crucial steps of many lab-on-a-chip bioassay applications (heterogeneous immunoassays etc.). We showed that the developed mathematical model can help to precisely predict and possibly control the sample dosing under various electrolyte concentrations.

The use of knowledge about the microchannel surface properties and the advanced mathematical model for the explanation of the concentration dependencies of the electro-osmotic characteristics is the main novelty of this paper.

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- ¹S. K. Sia and G. M. Whitesides, *Electrophoresis* **24**, 3563 (2003).
- ²J. M. K. Ng, I. Gitlin, A. D. Stroock, and G. M. Whitesides, *Electrophoresis* **23**, 3461 (2002).
- ³T. Fujii, *Microelectron. Eng.* **61–62**, 907 (2002).
- ⁴J. M. Edwards, M. N. Hamblin, H. V. Fuentes, B. A. Peeni, M. L. Lee, A. T. Woolley, and A. R. Hawkins, *Biomicrofluidics* **1**, 014101 (2007).
- ⁵H. Ohshima and K. Furusawa (Marcel Dekker, New York, 1998).
- ⁶Z. A. Almutairi, T. Glawdel, C. L. Ren, and D. A. Johnson, International Mechanical Engineering Congress and Exposition 2007, Vol 11 Pt a and Pt B: Micro and Nano Systems 893 (2008).
- ⁷H. Choi, K. B. Lee, K. R. Yoon, I. S. Choi, S. I. Woo, K. Choi, S. C. Lee, and Y. Kim, *Bull. Korean Chem. Soc.* **25**, 560 (2004).
- ⁸S. Dasgupta, A. A. S. Bhagat, M. Horner, I. Papautsky, and R. K. Banerjee, *Microfluidics and Nanofluidics* **5**, 185 (2008).
- ⁹X. Q. Ren, M. Bachman, C. Sims, G. P. Li, and N. Allbritton, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **762**, 117 (2001).
- ¹⁰H. Shadpour, H. Musyimi, J. F. Chen, and S. A. Soper, *J. Chromatogr. A* **1111**, 238 (2006).
- ¹¹A. M. Spehar, S. Koster, V. Linder, S. Kulmala, N. F. de Rooij, E. Verpoorte, H. Sigrist, and W. Thormann, *Electrophoresis* **24**, 3674 (2003).
- ¹²A. Sze, D. Erickson, L. Q. Ren, and D. Q. Li, *J. Colloid Interface Sci.* **261**, 402 (2003).
- ¹³R. Venditti, X. C. Xuan, and D. Q. Li, *Microfluidics and Nanofluidics* **2**, 493 (2006).
- ¹⁴N. Bao, J. J. Xu, Q. Zhang, J. L. Hang, and H. Y. Chen, *J. Chromatogr. A* **1099**, 203 (2005).
- ¹⁵J. A. Vickers, M. M. Caulum, and C. S. Henry, *Anal. Chem.* **78**, 7446 (2006).
- ¹⁶D. Ross, T. J. Johnson, and L. E. Locascio, *Anal. Chem.* **73**, 2509 (2001).
- ¹⁷D. Ross and L. E. Locascio, *Anal. Chem.* **75**, 1218 (2003).
- ¹⁸S. Devasenathipathy, J. G. Santiago, and K. Takehara, *Anal. Chem.* **74**, 3704 (2002).
- ¹⁹Y. Q. Luo, B. Huang, H. Wu, and R. N. Zare, *Anal. Chem.* **78**, 4588 (2006).
- ²⁰G. T. Roman, T. Hlaus, K. J. Bass, T. G. Seelhammer, and C. T. Culbertson, *Anal. Chem.* **77**, 1414 (2005).
- ²¹B. J. Kirby and E. F. Hasselbrink, *Electrophoresis* **25**, 187 (2004).
- ²²F. Zhou, W. Wang, W. Y. Wu, J. R. Zhang, and J. J. Zhu, *J. Chromatogr. A* **1194**, 221 (2008).
- ²³X. H. Huang, M. J. Gordon, and R. N. Zare, *Anal. Chem.* **60**, 1837 (1988).
- ²⁴W. Schrott, M. Pribyl, J. Stepanek, and D. Snita, *Microelectron. Eng.* **85**, 1100 (2008).
- ²⁵G. Sagvolden, I. Giaever, and J. Feder, *Langmuir* **14**, 5984 (1998).
- ²⁶R. F. Probstein, *Physicochemical hydrodynamics: An Introduction*, 2nd ed. (Wiley and Sons, New York, 1994).
- ²⁷T. Postler, Z. Slouka, M. Svoboda, M. Pribyl, and D. Snita, *J. Colloid Interface Sci.* **320**, 321 (2008).
- ²⁸Z. Slouka, M. Pribyl, D. Snita, and T. Postler, *Phys. Chem. Chem. Phys.* **9**, 5374 (2007).
- ²⁹W. M. Deen, *Analysis of Transport Phenomena* (Oxford University Press, New York, 1998).
- ³⁰S. H. Chang, *Biomicrofluidics* **3**, 012802 (2009).
- ³¹M. M. Browne, G. V. Lubarsky, M. R. Davidson, and R. H. Bradley, *Surf. Sci.* **553**, 155 (2004).
- ³²J. E. Puskas, Y. Dahman, and A. Margaritis, *Biomacromolecules* **5**, 1412 (2004).
- ³³M. Tencer, R. Charbonneau, N. Lahoud, and P. Berini, *Appl. Surf. Sci.* **253**, 9209 (2007).
- ³⁴Y. W. Huang and V. K. Gupta, *J. Chem. Phys.* **121**, 2264 (2004).
- ³⁵J. E. Butler, E. P. Lu, P. Navarro, and B. Christiansen, *J. Mol. Recognit.* **10**, 36 (1997).
- ³⁶U. Bohme and U. Scheler, *Chem. Phys. Lett.* **435**, 342 (2007).
- ³⁷Y. L. Gao, G. Q. Hu, F. Y. H. Lin, P. M. Sherman, and D. Q. Li, *Biomed. Microdevices* **7**, 301 (2005).
- ³⁸W. B. Blanch and D. S. Clark, *Biochemical Engineering* (Marcel Dekker, New York, 1997).
- ³⁹G. Ocivirk, M. Munroe, T. Tang, R. Oleschuk, K. Westra, and D. J. Harrison, *Electrophoresis* **21**, 107 (2000).