Application of electroosmotically-driven solution displacement for on-chip probing and characterization of protein adsorption

Szu-Wei Tang, Chien-Hsiang Chang, and Hsien-Hung Wei[a)]
Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan

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An alternative strategy for realizing on-chip characterization of protein adsorption is demonstrated using solution displacement with electro-osmotic flow in microchannels. The idea is illustrated by studying adsorption of bovine serum albumin (BSA) on polydimethylsiloxane surfaces. Through tracking the zeta potential change using the current monitoring technique, we quantify how the surface coverage of BSA varies with time and the BSA concentration. The Langmuir kinetic model is employed to capture the transient behavior of the adsorption and to determine both the adsorption and desorption rate constants. The Langmuir isotherm is also established in line to account for the sorption equilibrium. © *2010 American Institute of Physics*. [doi:10.1063/1.3459688]

Electro-osmotic flow (EOF) is the fluid motion over a charged surface under an application of an electric field. As EOF is basically driven by an electric body force within a thin electric double layer of thickness $\lambda = 10-100$ nm next to the surface, in a macroscopic view it looks like a plug flow moving at the Smoluchowski slip velocity:[1]

$$U = -\frac{\varepsilon \zeta E}{\eta}, \tag{1}$$

where ζ is the zeta potential of the surface, E is the applied electric field, ε and η are the permittivity and viscosity of the electrolyte solution, respectively. One of applications using EOF is to displace one solution by another with a small conductivity difference to measure the surface zeta potential of a microchannel.[2] Since the concentration interface between the solutions is essentially flat and progresses at a constant speed according to Eq. (1), this uniform sweeping leads to a steady change in the electric resistance over the channel. Because the conductivity difference is small, the resulting electric current can be taken an approximated form by neglecting contributions from $O((1-\sigma_2/\sigma_1)^2)$ or higher and shown to vary almost linearly with displacement time t_d (Refs. 3 and 4) as follows:

$$\frac{\Delta I}{I_2} \approx \frac{U t_d}{L}\left(1-\frac{\sigma_2}{\sigma_1}\right) \quad \text{for } t_d \leq L/U, \tag{2}$$

where $\Delta I/I_2$ is the relative current change with respect to the initial current I_2, L is the channel length, and σ_1 and σ_2 stand, respectively, for the conductivities of the displacing and displaced solutions. As $\Delta I/I_2$ is nearly proportional to U, ζ can be readily determined by Eq. (1) through the slope of a current-time curve by fitting the data using Eq. (2). According to the Debye–Hückel theory, the surface charge density q_s is directly proportional to ζ via[5]

$$q_s = \varepsilon \zeta / \lambda. \tag{3}$$

Hence, how much charge a surface bears can also be obtained by this current monitoring method. The technique has recently been used to determine the surface zeta potentials of composite microchannels.[4]

In this paper, we explore the use of EOF-driven solution displacement for studying protein adsorption in view of its wide range of involvements in functional assembly, biochemical modification, molecular recognition, and receptor-ligand interactions. Since protein is a polypeptide having many ionizable groups on its backbone, its adsorption behavior can be elicited from its electrokinetic features. Upon protein molecules land onto a substrate, they soon form a monolayer-like charged sheet covering the substrate, modifying the amount of net charges and hence the apparent surface charge density. If a Poisson–Boltzmann equilibrium between the surface charges and the bulk ions is immediately established upon the adsorption, we postulate that the surface charge density change Δq_s is proportional to the amount of the adsorbed proteins Γ, and so is the surface zeta potential change $\Delta\zeta$ according to Eq. (3):

$$\Delta q_s \propto \Delta\zeta \propto \Gamma. \tag{4}$$

By comparing the measured surface zeta potentials before and after the adsorption using the current monitoring method, we can determine the surface coverage by adsorbed proteins. Compared to existing techniques such as quartz crystal microbalance,[6] surface plasmon resonance,[7] and total internal reflection microscopy,[8] the present method is simple and does not require sophisticated mechanical or optical setup. More importantly, it offers a more straightforward way for studying protein adsorption without having to carry out the measurement in different environments.

We demonstrate the idea by studying adsorption of bovine serum albumin (BSA, Mw=66430 Da) on a polydimethylsiloxane (PDMS) surface. Both kinetic and equilibrium studies are carried out. Various BSA solutions are prepared by adding BSA of 1–100 ppm (in weight) to 18mM sodium phosphate buffer at pH=7. Because the buffer's pH is higher than the isoelectric point of BSA $\approx$ 4.7, BSA in this buffer is negatively charged with the zeta potential of -5 mV (measured by using Zetasizer 3000 HS, Malverm). The featured device consists of a PDMS microchannel and two reservoirs at the two ends of the channel. The channel is made by standard photolithograph techniques and has the dimensions

[a)]Author to whom correspondence should be addressed. Electronic mail: hhwei@mail.ncku.edu.tw.

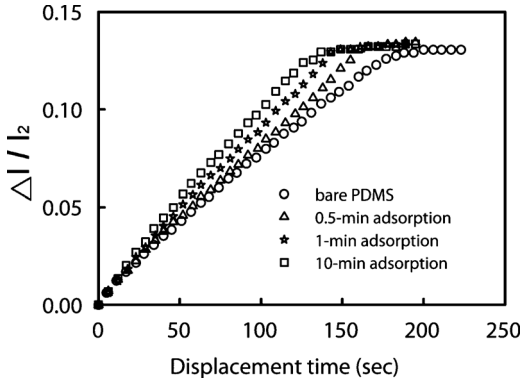

FIG. 1. Plot of the measured current change $\Delta I/I_2$ vs displacement time for adsorption of 10 ppm BSA with different adsorption periods.

of 300 μm in width, 90 μm in height, and 4 cm in length. Prior to the experiment, the whole system is treated by O_2 plasma at 0.3 torr for 5 min.

In the kinetic study, 18 mM buffer with the desired BSA concentration is filled into the microchannel. Different incubation periods of 30 s–120 min are used to examine how the amount of adsorbed BSA varies with time and to identify the time required to reach sorption equilibrium. Right after incubation, 5 mL of 18 mM BSA-free sodium phosphate buffer solution is injected into the channel to wash out non-adsorbed BSA molecules on the surface. This step is to keep the state of the surface as close as that at the moment we stop the incubation. To measure the surface zeta potential of a PDMS microchannel with/without BSA, 20mM sodium phosphate buffer (of conductivity $\sigma_1=2.73$ mS/cm) is used to displace 18mM one (of conductivity $\sigma_2=2.41$ mS/cm) without added BSA under an electric field of 100 V/cm across the channel. The estimated double layer thickness is 6 nm. The measured zeta potential of the channel in the absence of BSA is -33.5 ± 1.0 mV, in good agreement with -37.3 mV estimated by an empirical formula reported previously.[9]

As for the equilibrium study, the incubation is kept for 2 h using 18 mM sodium phosphate buffer with added BSA at a given concentration. The same buffer without added BSA is used to wash out non-adsorbed BSA after the incubation. The zeta potential measurement is again carried out by displacing 18 mM buffer with 20 mM buffer. But we add BSA to the 20 mM buffer at the same BSA concentration as the one used in the incubation. This procedure ensures that the as-established equilibrium between the surface and the bulk during the incubation does not change significantly in the zeta potential measurement.

Figure 1 shows typical transient current responses obtained from the kinetic study, clearly revealing that there are indeed changes in the zeta potential due to BSA adsorption.[10] According to Eqs. (1) and (2), since the slope of a current-time curve is nearly proportional to ζ, the observed slope increase by prolonging adsorption time (i.e., incubation time) suggests that ζ increases in amplitude during the adsorption. Because a bare PDMS surface is negatively charged, this amplitude increase in ζ implies that the surface becomes more negatively charged due to landing of negatively charged BSA onto the surface. As there is electrostatic repulsion between the surface and adsorbed BSAs, the adsorption is perhaps driven by much stronger van der Waals attractions

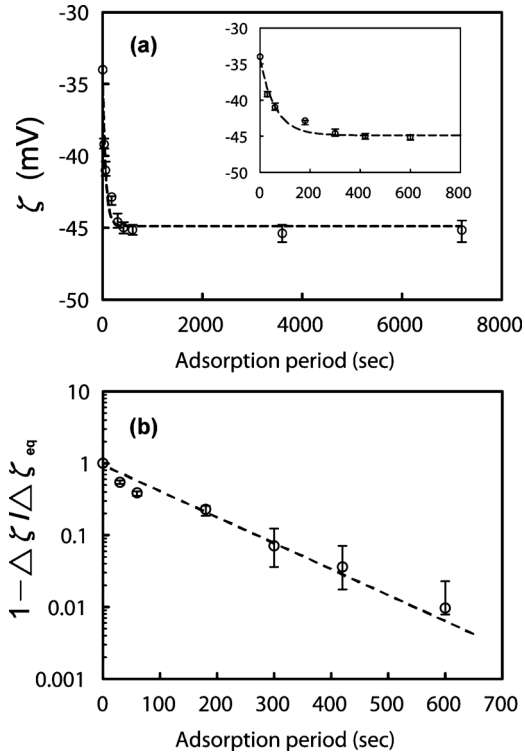

FIG. 2. (a) Plot of the measured zeta potential against adsorption period. The fitted curve is ζ (in mV)$=-33.5+\Delta\zeta_{eq}[1-\exp(-kt)]$ with $\Delta\zeta_{eq}=-11.4$ mV and $k=7.3\times10^{-3}$ s^{-1}. (b) Semilog plot of $(1-\Delta\zeta/\Delta\zeta_{eq})$ vs adsorption period for indicating the exponential decay of the zeta potential change at the rate of $0.434k=3.17\times10^{-3}$ s^{-1} through the slope of the plot.

or hydrophobic affinity[11] that can overcome the repulsion to keep BSAs on the surface. Plotting the measured zeta potential against adsorption time in Fig. 2(a), we find that the zeta potential drops from -33.5 to -44.5 mV within 1000 s and shows no significant change afterwards. So the adsorption is quite fast and takes merely a few minutes to reach equilibrium.

We also employ the Langmuir kinetic model[12] to explain the zeta potential behavior seen in Fig. 2(a) as follows:

$$\frac{d\Gamma}{dt}=\alpha C(\Gamma_\infty-\Gamma)-\beta\Gamma, \tag{5}$$

where Γ_∞ is the maximum surface packing density of BSA, α the adsorption rate constant, β the desorption rate constant, and C the bulk concentration of BSA. Together with Eq. (4), the solution of this model gives how Γ varies with time through the measured zeta potential change as follows:

$$\frac{\Gamma}{\Gamma_{eq}}=\frac{\Delta\zeta}{\Delta\zeta_{eq}}=1-\exp(-kt), \tag{6}$$

where $\Delta\zeta_{eq}$ is the zeta potential change corresponding to the equilibrium surface concentration $\Gamma_{eq}=\alpha C\Gamma_\infty/k$ and $k=\alpha C+\beta$ is the rate constant whose inverse gives the characteristic time scale for establishing a sorption equilibrium. Therefore, at a given C we can determine the values of $\Delta\zeta_{eq}$ and k by fitting the data of Fig. 2(a) using Eq. (6). At $C=10$ ppm, we find $\Delta\zeta_{eq}=-11.4$ mV and $k=7.3\times10^{-3}$ s^{-1}. The latter is comparable to 2.38×10^{-3} s^{-1} reported previously for BSA adsorption on similar surfaces using UV-spectrophotometery.[13] Fig. 2(b) plots $(1-\Delta\zeta/\Delta\zeta_{eq})$ against adsorption time t to reflect how fast

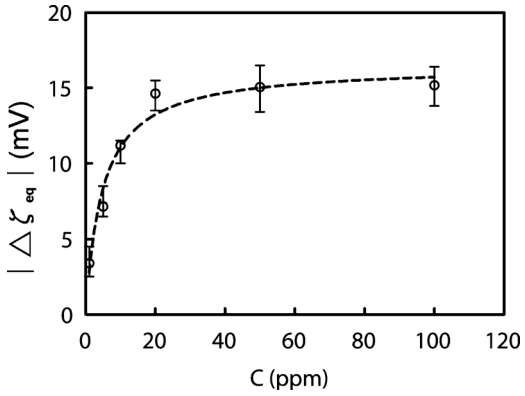

FIG. 3. The measured equilibrium zeta potential change as a function of the BSA concentration C. The dashed curve is the fitted profile according to the Langmuir isotherm Eq. (7).

available adsorption sites diminish as approaching the equilibrium. It clearly shows the trend predicted by Eq. (6) and reveals that it takes about 600 s to reach 99% of the equilibrium surface coverage.

In the equilibrium study, Fig. 3 shows the measured equilibrium zeta potential changes for various values of the BSA concentration C. It indicates that the amount of adsorbed BSA rises sharply as C is increased for low $C(<20$ ppm) and becomes saturated at high $C(>20$ ppm). To capture the observed trend, Eq. (6) in the long-time limit gives the well-known Langmuir isotherm:

$$\frac{\Gamma_{eq}}{\Gamma_{\infty}} = \frac{\Delta\zeta_{eq}}{\Delta\zeta_{eq,max}} = \frac{KC}{1+KC}, \tag{7}$$

where $\Delta\zeta_{eq,max}$ is the maximum zeta potential change corresponding to Γ_{∞} and $K=\alpha/\beta$ is the isotherm constant. Fitting the data in Fig. 3 using Eq. (7), we find $\Delta\zeta_{eq,max}=-15.8$ mV and $K=195$ mL/mg$(=0.195$ ppm$^{-1})$. The latter is not far from 24 mL/mg found in the previous study on BSA adsorption on polypropylene surfaces using quartz crystal microbalance.[6] Moreover, combining $K=\alpha/\beta$ in the equilibrium study and $k=\alpha C+\beta$ in the kinetic study (at $C=10$ ppm), we can determine the two rate constants as follows: $\alpha=4.83\times10^{-4}$ ppm^{-1} s^{-1} and $\beta=2.47\times10^{-3}$ s^{-1}.[14] The fact that $\alpha C/\beta(=KC)\approx2$ at $C=10$ ppm indicates that at this particular BSA concentration the strength of adsorption is about twice stronger than desorption. The equilibrium surface coverage is then $\Gamma_{eq}/\Gamma_{\infty}\approx0.66$ according to Eq. (7). Using Eqs. (3) and (4), the estimated surface concentration is about 10 BSAs per 10^3 nm^3, which is the same order of magnitude as that when the surface is nearly covered by BSA of approximately 10 nm in size. Together with the fact that the measured parameters k and K are within typical ranges, we conclude that the present approach does capture some essences of the system. This also implies that Eq. (4) is an adequate conjecture to elicit the natures of the actual adsorption process.

We have demonstrated the use of solution displacement in studying the adsorption of BSA protein on PDMS surfaces. By postulating that the surface charge density change is proportional to the amount of adsorbed BSA, we reveal how the surface coverage varies with time and the BSA concentration by measuring zeta potential changes using the current monitoring method. With the aid of the Langmuir kinetic model, we are able to quantify both the transient and equilibrium behaviors of the adsorption. More importantly, we can determine the kinetic rate constants and establish the Langmuir isotherm for characterizing the adsorption. The present microfluidic scheme not only offers a renewed strategy for realizing on-chip characterization of protein adsorption without using additional markers but also has potential applications in molecular detection and sensing at microscales.

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[9] B. J. Kirby and E. F. Hasselbrink, Jr., Electrophoresis **25**, 203 (2004); The reported formula is ζ (in mV)$=6.75-29.75$pC for $6.5<$pH<7. With pC≈1.48 for the 20 mM buffer, the estimated ζ is -37.3 mV.
[10] The actual current changes are not strictly linear in time, which is attributed to the $O((1-\sigma_2/\sigma_1)^2)$ terms neglected in Eq. (2). We also use the unsimplified equation to determine the zeta potential. The result merely has a few percent of difference compared to that using Eq. (2) because of this small nonlinear effect.
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[14] In principle, both α and β can be determined solely by the kinetic study, as they can be obtained by plotting k against C if sufficient data of k are available in a wide range of C. At high $C(>10$ ppm), however, an accurate kinetic study would hardly be attainable, since the adsorption rate would become so fast that the system would have already reached an equilibrium by the time we measure the zeta potential. In other words, because the surface concentration now changes very rapidly, it is impossible in practice to rinse the channel and to load the solutions in time while still keeping the surface not changed significantly prior to the zeta-potential measurement. At the other extreme, too low C such as 1 ppm will not give enough data showing discernable zeta potential changes $(<3$ mV) for determining k.