



Single colloid electrophoresis

I. Semenov^{a,*}, O. Otto^{a,b}, G. Stober^a, P. Papadopoulos^a, U.F. Keyser^b, F. Kremer^a

^a Institute for Experimental Physics I (MOP), University of Leipzig, Linnestrasse 5, 04103 Leipzig, Germany

^b Cavendish Laboratory, University of Cambridge, Cambridge, UK

ARTICLE INFO

Article history:

Received 20 March 2009

Accepted 11 May 2009

Available online 18 May 2009

Keywords:

Electrophoresis

Electroosmosis

Electrophoretic mobility

Single colloids

Optical tweezers

ABSTRACT

Optical tweezers enable one to trap a *single* particle without any mechanical contact and to measure its position *and* the forces acting on it with high resolution (± 4 nm, ± 160 fN). Taking advantage of a specially designed microfluidic cell the electrophoretic response of the colloid under study and the electroosmotic effect on the surrounding medium are determined using the *identical* colloid. The former is found to be by more than one order of magnitude larger than the electroosmotic effect. It is shifted in phase with respect to the external field, hence giving rise to a *complex* electrophoretic mobility which can be theoretically described by a strongly damped driven harmonic oscillator model. By exchanging the medium surrounding the colloid it is possible to deduce the (KCl) concentration dependence of the single colloid electrophoretic response. The results are compared with conventional Zetasizer measurements.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

The electrophoretic response of colloidal dispersions and the electroosmotic flow of ionic media are a classical topic of experimental and theoretical colloidal physics [1–3]. Usually experiments are carried out on colloidal dispersions [4–6], but few studies exist where the electrophoretic response of a *single isolated* colloid is determined [7–10]. For that optical tweezers are employed, experimental tools having an extraordinary resolution in measuring the position of a colloid and the forces acting on it [11–15].

In the present study a specially designed microfluidic sample cell is used to measure *separately* both the electrophoretic and the electroosmotic response using the *very same* colloid. Both effects are studied dependent on the strength and frequency of the external electric field and by variation in the ionic concentration of the electrolyte.

2. Materials and methods

2.1. Optical trap

The experimental setup (Fig. 1) consists of an epifluorescence microscope (Axiovert 200, Carl Zeiss, Jena, Germany) with a water-immersion objective (magnification: 60 $\times$, numerical aperture 1.2, Olympus, UPlanApo/IR). For optical trapping a diode pumped YAG:Nd³⁺ laser (1064 nm, 1 W, LCS-DTL 322, Laser 2000, Wessling, Germany) is used. At the chosen wavelength absorption

and heating are negligible [16]. To stabilize the laser a feedback loop is used controlling its power. The beam is expanded, coupled into the back aperture of the microscope objective, and focused into a diffraction-limited spot to form an optical trap within the sample cell. Illumination is provided by a 150 W cold-light source (LQ 1700, Linos, Göttingen, Germany).

Images are taken using a monochrome CMOS camera (Microtron MC1310, Unterschleissheim, Germany) having a 1280 $\times$ 1024 array of square pixels, each with 8-bit resolution in intensity. To enlarge the diffraction pattern of the colloid a magnification lens (1.6 $\times$) is inserted into the optical path in front of the camera. This limits the imaged region of interest down to 100 $\times$ 100 pixels and allows data acquisition frame rates up to 5099 fps to be achieved.

Based on image analysis the center position of the colloid under study is determined with an accuracy $\sim \pm 4$ nm. This means for the electrophoretic mobility a relative error of $\sim 5\%$.

2.2. Measurement cell

The microfluidic cell (Fig. 1) consists of an open-ended channel of rectangular cross section (height, 1 mm; width, 300 μ m) with reservoirs at both ends fitted with platinum electrodes [17,18]. It is made out of a micromachined poly-methyl-met-acrylate (PMMA) spacer enclosed between a microscope slide (thickness $d = 1$ mm) at the top and a coverslip at the bottom (thickness $d = 160$ μ m), which are fixed by UV-sensitive glue. The electrophoretic response is measured at position A (see Fig. 1) whereas the electroosmotic effect of the surrounding medium is studied at B (Fig. 1). The zero value of the external sinusoidal electric AC field between the Pt electrodes is indicated by a LED flash (duration: 0.130 ms) which is recorded by the CMOS camera. The small sized

* Corresponding author.

E-mail address: semenow@rz.uni-leipzig.de (I. Semenov).

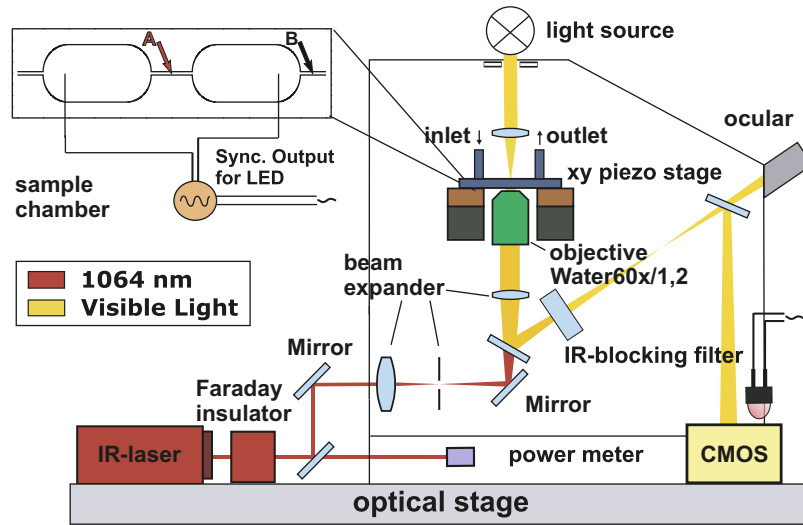


Fig. 1. Scheme of the experimental setup. Particles are imaged using an epifluorescence microscope accomplished with a high-resolution CMOS camera. Scheme of the sample cell used to measure the electrophoretic (colloid at position A) or the electroosmotic (colloid at position B) response. For the phase measurement of the electrophoretic response an LED flash indicates the zero value of the external electric field.

cross section of the channel between the reservoirs leads to a high resistance, exceeding all other possible resistors in the system (e.g., potential drop at electrical double layer of electrodes), and ensures a uniform electric field distribution at position A (Fig. 2).

Single spherical polystyrene (PS) colloids of diameter $d = 2.23 \mu\text{m}$ are used. All measurements are carried out at a height of $50 \mu\text{m}$ above the cell's ground in potassium chloride (KCl) solutions of varying concentrations to which as a buffer 10^{-6} M Tris is added.

2.3. Numerical calculation

The numerical calculation of the voltage profile U along the channel is carried out by integrating the following equation using a 4th order Runge–Kutta algorithm

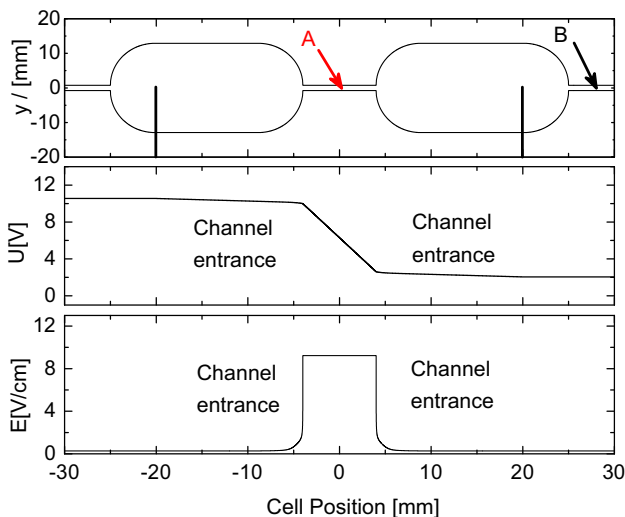


Fig. 2. The fluidic cell consists of an open-ended narrow channel (width, $300 \mu\text{m}$; height, 1 mm) in which two reservoirs with Pt electrodes (diameter = 0.2 mm , the tip is along the axis) are incorporated. For electrophoretic measurements the colloid under study is placed in position A and for electroosmotic studies in position B. Potential drop (middle) and electric field distribution (bottom) within a channel having a length l of 8 mm as calculated for a potential difference of 1 V at the electrodes.

$$R = \rho \int \frac{dx}{A(x)}, \quad (1)$$

where R is the total channel resistance, ρ the specific electric resistance of the ionic solution, $A(x)$ the cross section at given channel position, and dx the step size for integration. The entrance and exit resistance of the channel is computed using the approximation for a disk-like pore from [19–21]. By solving the integral the electrical field E follows directly from the Poisson equation and computing the numerical derivation of the potential profile

$$\begin{aligned} \text{div} \mathbf{E} &= \frac{\rho}{\epsilon}, \\ \mathbf{E} &= -\text{div} \mathbf{U} \end{aligned} \quad (2)$$

The electrode resistance is found to be much smaller than the channel resistance (Fig. 2) in accord with [22,23].

3. Theoretical background

The electrophoretic mobility μ_e of a particle or molecule is defined as the factor of proportionality between its velocity v_e with respect to the surrounding medium and the local electric field E : $v_e = \mu_e E$. The same holds for the electroosmotic flow velocity v_{eo} of a liquid a relation in terms of an electroosmotic mobility μ_{eo} as $v_{eo} = \mu_{eo} E$ [10].

A colloidal particle in a focused laser beam encounters a dominating gradient force that enables one to trap the particle close to the geometrical focus. In good approximation, the colloid can be described as a particle in a three-dimensional harmonic potential that in its turn is characterized by two spring constants, one in the axial and one in the radial direction. For simplicity, only the one-dimensional motion along the radial coordinate x is analyzed, where the corresponding force constant is k_{trap} .

The particle's motion can be described by the Langevin equation

$$m\ddot{x}(t) + 6\pi\eta r v_e(t) = F^{\text{electric}} - k_{\text{trap}} x(t), \quad (3)$$

where η is the viscosity of the surrounding medium and r the radius of the particle. The frame of the optical trap is used as reference for determining the absolute velocity $\dot{x}(t)$ of the particle, where $v_e(t) = \dot{x}(t) - v_{eo}(t)$.

A colloidal particle loses momentum rapidly as a result of viscous drag. The characteristic time for this decay is about 10^3 times

shorter than the temporal resolution of the experiment (0.2 ms); consequently the inertial term can be safely neglected.

There are three forces acting (directly or indirectly) on the particle: forces caused by the external electric field, photonic forces of the optical trap, and Stokes frictional forces. Both the particle and the liquid are affected by the imposed electric field, and a proper estimate of the particle displacement can be obtained only when the fluid motion is taken into account. The total frictional force on the particle in the stationary case depends on its relative velocity with respect to the surrounding fluid:

$$F^{\text{electric}} = 6\pi\eta r\mu_e E(t). \quad (4)$$

Hence Eq. (3) turns into

$$6\pi\eta r(\dot{x}(t) - v_{eo}(t)) = 6\pi\eta r\mu_e E(t) - k_{\text{trap}}x(t). \quad (5)$$

As shown experimentally below (Fig. 3) the electroosmotic effect is by more than one order of magnitude smaller than the electrophoretic response. Hence $v_{eo}(t) \ll \dot{x}(t)$ and $\mu_{eo} \ll \mu_e$.

Since Eq. (5) is linear, the expression for the electric field $E(t)$ and the displacement $x(t)$ can be written in complex form

$$\begin{aligned} E(t) &= E_0 e^{i(\omega t)}, \\ x(t) &= a e^{i(\omega t - \phi)} \end{aligned} \quad (6)$$

where E_0 is the maximum amplitude of the external electric field, ω its frequency, a the maximum amplitude of the displacement, and ϕ the phase shift ($a, \phi \in \mathbb{R}$ (real numbers)). Substituting into Eq. (5) delivers the characteristic equation

$$i\omega a e^{i\phi} = \mu_e E_0 - \omega_c a e^{i\phi}, \quad (7)$$

where ω_c is the corner frequency of the optical trap

$$\omega_c = \frac{k_{\text{trap}}}{6\pi\eta r}. \quad (8)$$

Separating real and imaginary parts in Eq. (7) yields as solution for the amplitude

$$a = \frac{\mu_e E}{\omega_c \sqrt{1 + (\omega/\omega_c)^2}}, \quad (9)$$

which results in the formula for a driven oscillator.

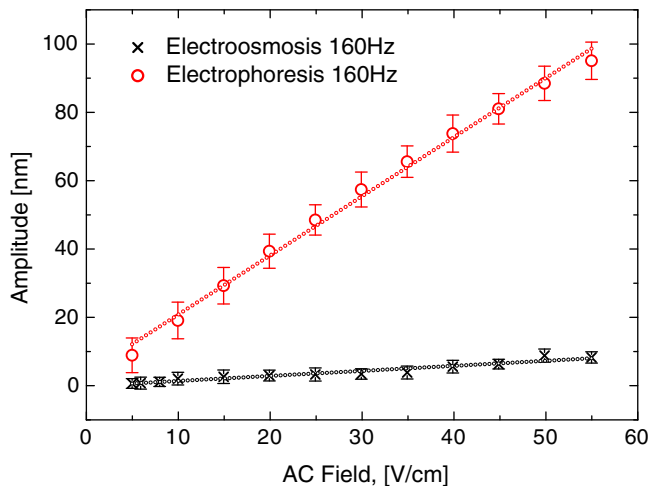


Fig. 3. Amplitude vs. strength of the external electric AC (160 Hz) field for a negatively charged PS colloid (diameter: 2.23 μm) in an aqueous solution of 10^{-5} KCl molar concentration. The electrophoretic (circles) and the electroosmotic (crosses) responses are measured for the identical colloid. The dashed lines represent a fit of experimental data.

The particle motion lags behind the driving field by a frequency-dependent phase

$$\varphi = \arctan \frac{\omega}{\omega_c}. \quad (10)$$

In the limit $\omega \ll \omega_c$ the amplitude becomes frequency independent, while the phase is zero. For $\omega \gg \omega_c$ the response decreases inversely with frequency and the phase shift approaches $\pi/2$. For our system at a laser power of 0.2 W the typical value of the corner frequency ω_c is $1880 \pm 60 \text{ s}^{-1}$.

4. Results and discussion

To compare and to separate the electrophoretic and the electroosmotic forces effects the identical colloid is placed either in position A resp. B of the same microfluidic cell (Figs. 1 and 2). For both—within the limits of experimental accuracy—a linear dependence on the external electric field is found. The electroosmotic effect turns out to be by more than one order of magnitude smaller than the electrophoretic response of the colloid at sufficiently high frequencies (Fig. 3).

The measurement of the phase of the electrophoretic response is much less accurate than that of the amplitude. This is caused by the frame rate of the CMOS camera of maximum 5099 fps which corresponds—at a frequency of the external electric field of 160 Hz—to a maximal uncertainty in the determination of the phase up to $\pm 11^\circ$. Therefore further analysis will focus on the amplitude. For the electroosmotic effect it is—with the present experimental resolution—not possible to determine the phase with respect to the external electric field. Hence the sign of $v_{eo}(t)$ in Eq. (5) is not defined and thus the electrophoretic response can be measured only with an accuracy of $\sim 10\%$ due to the electroosmotic effect.

In order to prove the homogeneity of the local electric field in the microfluidic cells the electrophoretic response of single colloids is measured dependent on the channel length (Fig. 4). Coinciding electrophoretic mobilities are obtained. Excellent reproducibility is achieved in subsequent runs in which—for the identical col-

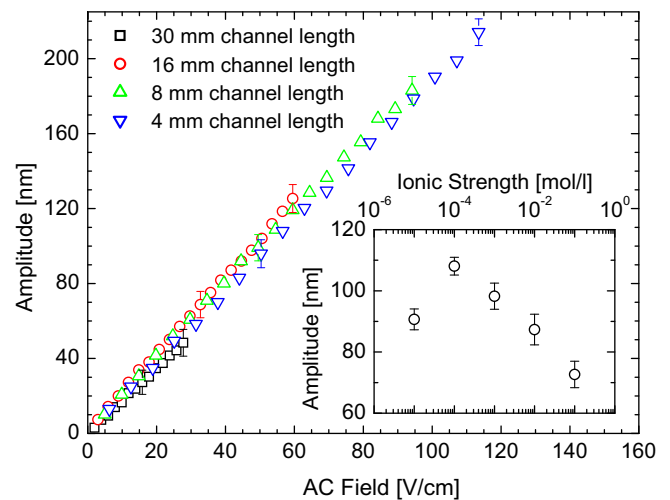


Fig. 4. Amplitude vs. strength of the external electric AC (160 Hz) field for the electrophoretic response of a single negatively charged PS colloids (diameter: 2.23 μm) in a buffered solution (10^{-5} M KCl and 10^{-6} M Tris) for varying channel lengths l as indicated. Inset: test of reproducibility of the single colloid electrophoretic response as proven by six subsequent runs per point in which—for the identical colloid—the KCl concentration is varied in the entire range between 10^{-5} and 10^{-1} mol/L within the channel length l of 8 mm. The circles indicate the mean value; the error bars the standard deviation. Laser power 0.2 W.

loid—the KCl concentration for the surrounding medium is varied between 10^{-5} and 10^{-1} mol/L (Fig. 4, inset).

The electrophoretic response of a colloid is a complex quantity as outlined above. The amplitude (Fig. 5) dependent on the strength of the external electric field in aqueous solutions of varying (KCl) concentration has a linear slope. Using Eq. (9) it is readily possible to determine the electrophoretic mobility μ_e . For the phase no dependence on the strength of the external electric field and on the salt (KCl) concentration of the surrounding medium is observed (inset in Fig. 5) in full accord with Eq. (10). The frequency dependence of the amplitude of the electrophoretic response (Fig. 6) can be—within the limits of experimental accuracy—as well described by Eq. (9). This offers an additional possibility for determining the electrophoretic mobility μ_e .

In Fig. 7 results of the single colloid electrophoretic studies are compared with conventional measurements of the electrophoretic mobility (using a Malvern Zetasizer) dependent on the concentration of (KCl) solutions. The electrophoretic mobility is negative due to the negative surface charge of the colloids under study. Excellent agreement is found between the single colloid electrophoretic mobilities as determined from the field strength (Fig. 5) and from the frequency dependence (Fig. 6). The conventional measurements have a much larger experimental uncertainty but confirm a maximum value of the electrophoretic mobility at a concentration of $\sim 10^{-3}$ mol/L. These values are in agreement with previous experimental results for similar monovalent solutions [7].

The concentration dependence of the mobility can be qualitatively explained by the “standard electrokinetic model” [2,25], where for large particle diameters (Smoluchowski limit $\kappa a \gg 1$)

$$\mu_{ep} = \frac{\varepsilon \varepsilon_0 \zeta}{\eta} \quad (11)$$

with ε the dielectric constant, η the surrounding medium viscosity, and ζ the zeta potential. The effective charge of the particle is [1,2,24]

$$Z_{eff} = \left(\frac{e \zeta}{k_B T} \right) \frac{a}{\lambda_B} (1 + \kappa a), \quad (12)$$

where a is the particle radius, e is elementary charge, and

$$\lambda_B = \frac{e^2}{4\pi \varepsilon \varepsilon_0 k_B T} \quad (13)$$

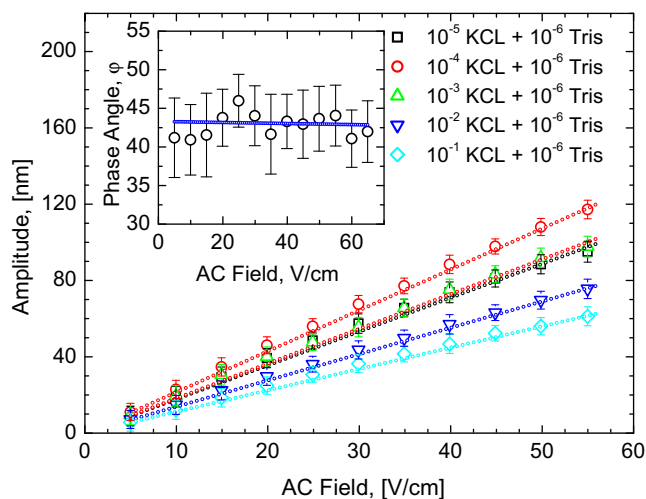


Fig. 5. Amplitude and phase (inset) vs. strength of the external electric AC (160 Hz) field for the identical negatively charged PS colloid (diameter: 2.23 μ m) in aqueous solutions of varying molar (KCl) concentration, as indicated, within the channel length l of 8 mm. Laser power 0.2 W. Dashed lines represent linear fits of Eq. (9) to the experimental data.

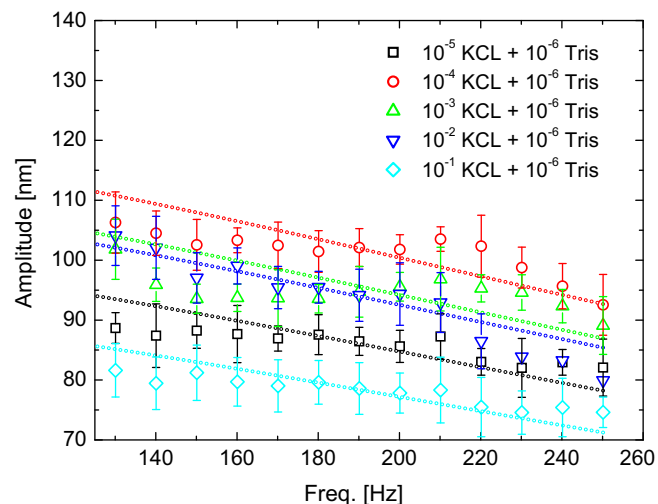


Fig. 6. Amplitude of the electrophoretic response vs. frequency of the external electric AC field (field strength: 50 V/cm) as measured with the identical PS colloid (diameter: 2.23 μ m) in aqueous solutions of varying (KCl) concentration as indicated. The channel length l is 8 mm. Laser power 0.2 W. The dashed lines represent linear fits of Eq. (9) to the data.

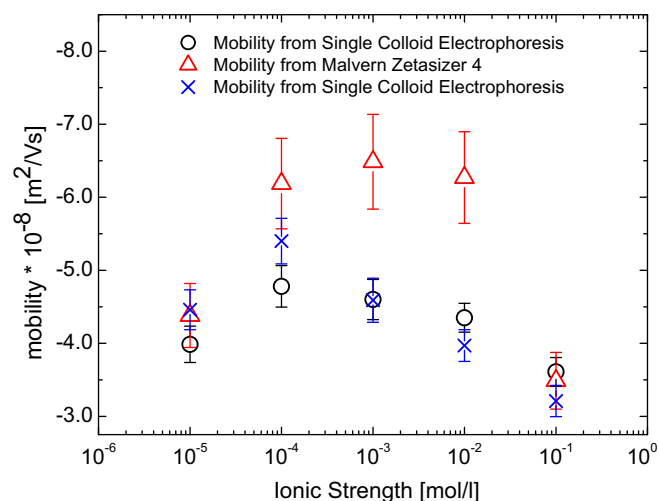


Fig. 7. Comparison between single colloid electrophoretic and conventional Zeta-sizer measurements as outlined for the electrophoretic mobility vs. (KCl) concentration. The black symbols correspond to the results obtained from the field strength dependence (Fig. 5), the blue symbols for the frequency dependence (Fig. 6). Channel length, 8 mm; pH, 8.4; temperature, 23 $^{\circ}$ C; Laser power, 0.2 W. The circles indicate the mean value; the error bars the standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

is the Bjerrum length. The electrostatic interactions are screened on the scale of the Debye length [25]

$$\kappa^{-1} = \sqrt{\frac{\varepsilon \varepsilon_0 k_B T}{2 N_A e^2 I_s}} \quad (14)$$

Here N_A is Avogadro's number and I_s the ionic strength. Evaluating Eqs. (11)–(14) one would expect a monotonic decrease of electrophoretic mobility with salt concentration, $\mu_{ep} \propto I_s^{-1/2}$, due to increased screening from the ionic cloud (in principle, electrical double layer) around the colloid.

The mobility at low concentrations does not have the same trend. This case is outside of the Smoluchowski limit, because $\kappa a = 0.4 < 1$. For low salt concentrations the standard electrokinetic

model predicts that Eq. (11) should be replaced by $\mu_{ep} = \frac{2}{3} \frac{\epsilon \epsilon_0}{\nu} \zeta$ (Hückel limit $ka \ll 1$), explaining the decreasing trend [25]. The “hairy-layer” model [26,27] which was applied to polystyrene beads by Delgado and co-workers [7,28,29] offers another possible explanation. The negatively charged groups are repelled from the colloid surface into the solution. Due to their mutual repulsion, they are oriented approximately perpendicular to the surface, increasing the diameter [28,30] and changing conductivity properties [31] of the hydrodynamic stagnant layer.

5. Conclusions

Based on an optical tweezers setup a method is described which enables one to measure both the electrophoretic response and the electroosmotic mobility of the surrounding ionic medium using a *single identical* colloid. The electrophoretic response is found to be by more than one order of magnitude larger than the electroosmotic effect. It is shifted in time with respect to the applied external electric field and must be hence described by a *complex* electrophoretic mobility which can be quantitatively described by the model of a damped driven oscillator. The data obtained by the single colloid techniques compare—within the limits of experimental accuracy—well with conventional measurements of the electrophoretic mobility using a Zetasizer.

Acknowledgment

Financial support was from the German Science Foundation (Forschergruppe DFG).

References

- [1] R.J. Hunter, *Zeta Potential in Colloid Science*, Academic Press, London, 1981.

- [2] R.J. Hunter, A.E. Alexander, *J. Colloid Sci.* 17 (8) (1962) 781.
- [3] J. Lyklema, *Fundamentals of Interfaces and Colloid Science*, vol. II, Academic Press, New York, 1995.
- [4] M. Medebach, T. Palberg, *Colloids Surf. A* 222 (1–3) (2003) 175.
- [5] T. Palberg, M. Medebach, N. Garbow, M. Evers, A.B. Fontecha, H. Reiber, E. Bartsch, *J. Phys. Condens. Matter* 16 (38) (2004) S4039.
- [6] A.V. Delgado, S. Ahualli, F.J. Arroyo, F. Carrique, *Colloids Surf. A* 267 (1–3) (2005) 95.
- [7] A. Delgado, F. Gonzalez-Caballero, M.A. Cabrerizo, I. Alados, *Acta Polym.* 38 (1) (1987) 66.
- [8] N. Garbow, M. Evers, T. Palberg, *Colloids Surf. A* 195 (1–3) (2001) 227.
- [9] G. Seth Roberts, Tiffany A. Wood, William J. Frith, P. Bartlett, *J. Chem. Phys.* 126 (19) (2007) 194503.
- [10] M.H. Oddy, J.G. Santiago, *J. Colloid Interface Sci.* 269 (1) (2004) 192.
- [11] A. Ashkin, *Proc. Natl. Acad. Sci. USA* 94 (10) (1997) 4853.
- [12] C. Gutsche, U.F. Keyser, K. Kegler, F. Kremer, P. Linse, *Phys. Rev. E* 76 (3) (2007) 031403.
- [13] K. Kegler, M. Konieczny, G. Dominguez-Espinosa, C. Gutsche, M. Salomo, F. Kremer, C.N. Likos, *Phys. Rev. Lett.* 100 (11) (2008) 118302.
- [14] M. Salomo, K. Kegler, C. Gutsche, M. Struhalla, J. Reinmuth, W. Skokow, U. Hahn, F. Kremer, *Colloid Polym. Sci.* 284 (11) (2006) 1325.
- [15] O. Otto, C. Gutsche, F. Kremer, U.F. Keyser, *Rev. Sci. Instrum.* 79 (2) (2008) 023710.
- [16] George M. Hale, Marvin R. Querry, *Appl. Opt.* 12 (3) (1973) 555.
- [17] A. Norlin, J. Pan, C. Leygraf, *Biomol. Eng.* 19 (2–6) (2002) 67.
- [18] E.E. Uzgiris, *Rev. Sci. Instrum.* 44 (1) (1974) 74.
- [19] J.E. Hall, *J. Gen. Physiol.* 66 (4) (1975) 531.
- [20] B. Hille, *Ion Channels of Excitable Membranes*, third ed., Sinauer, 2001.
- [21] G. Stober, L.J. Steinbock, U.F. Keyser, *J. Appl. Phys.* 105 (1) (2009), doi:10.1063/1.3095761.
- [22] O.A. Saleh, L.L. Sohn, *Rev. Sci. Instrum.* 72 (12) (2001) 4449.
- [23] L.J. Steinbock, G. Stober, U.F. Keyser, *Biosens. Bioelectron.* 24 (8) (2009) 2423.
- [24] John C. Crocker, David G. Grier, *Phys. Rev. Lett.* 77 (9) (1996) 1897.
- [25] B. Daunweg, V. Lobaskin, K. Seethalakshmy-Hariharan, C. Holm, *J. Phys. Condens. Matter* 20 (40) (2008) 404214.
- [26] A.G. Van Der Put, B.H. Bijsterbosch, *J. Colloid Interface Sci.* 75 (2) (1980) 512.
- [27] A.G. Van Der Put, B.H. Bijsterbosch, *J. Colloid Interface Sci.* 92 (2) (1983) 499.
- [28] A.V. Delgado, F. Gonzalez-Caballero, R.J. Hunter, L.K. Koopal, J. Lyklema, *J. Colloid Interface Sci.* 309 (2) (2007) 194.
- [29] A. Delgado, F. Gonzalez-Caballero, J. Salcedo, M.A. Cabrerizo, *Mater. Chem. Phys.* 19 (4) (1988) 327.
- [30] J. Lyklema, S. Rovillard, J. de Coninck, *Langmuir* 14 (1998) 5659.
- [31] J. Lyklema, M. Minor, *Colloids Surf. A* 140 (1998) 33.