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Electrorotation of Colloidal Particles and Cells Depends on Surface Charge

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ABSTRACT The importance of surface conductivity to the frequency-dependent polarizability and the rotation of particles in circular electric fields (electrorotation) is emphasized by various theoretical and experimental investigations. Although surface conductivity seems to be naturally related to the ionic double layer, there is rare experimental evidence of a direct relationship. To highlight the role of surface charges in electrorotation, an apparatus was developed with a symmetrical three-electrode arrangement for field frequencies between 25 Hz and 80 MHz. The three-dimensional electrostatic field distribution between the electrodes was evaluated numerically. With this device, rotating, gradient, and homogeneous electric fields of defined precision and homogeneity could be applied to slightly conducting suspensions. Surface properties of monodisperse latex particles (∅ 9.67 μm), carrying weak acid groups, were characterized by suspension conductometric titration. This procedure determined the amount of carboxyl groups and showed that strong acid groups were missing on the surface of these particles. To obtain the electrophoretic mobility, the spheres were separated by free-flow electrophoresis, and the ζ-potential was calculated from these data. Single-particle rotation experiments on fractions of specified electrophoretic mobility were carried out at frequencies between 25 Hz and 20 MHz. By analyzing the pH dependence of the rotation velocity, it could be shown that the rotation rate is determined by surface charges, both at the peak in rotation rate near the Maxwell-Wagner frequency (MWF) and at low frequencies. The inversion of the rotation direction at the MWF peak for vanishing surface charges was demonstrated. An analytical model for the double layer and dissociation on a charged surface was developed that is valid for low and high ζ-potentials. This model could provide convincing evidence of the linear dependence of the MWF rotation velocity on surface charge.

INTRODUCTION

The idea of evaluating the dielectric properties of matter by measuring the torque on spheres in circular electric fields originated in the last century (Arnò, 1896). In 1921, P. Lertes measured the torque on filled glass spheres in a rotating high-frequency field and determined the dielectric losses of fluids as predicted by Max Born in 1920. The usefulness of this method for the measurement of the permittivity of highly conducting substances was demonstrated at about the same time (Furth, 1924). This technique can be used as well to assess dielectric and electric properties of microscopic objects like cells and colloids (Arnold and Zimmermann, 1982). Measurements can be made by observing the rotation of a single particle, but it is also possible to use electrorotational light scattering (Gimsa et al., 1995; Prüger et al., 1997) to measure bulk properties in suspensions. A technique related to electrorotation is dielectrophoresis (Kaler and Jones, 1990), which makes use of the translational force on particles in AC gradient fields. Together, both methods provide a useful tool for the nondestructive determination of dielectric and electric properties of cells and their suspensions (for a review see Foster et al., 1992).

Direct use can also be made of the mechanical torque, translational, and attractive forces to construct devices for the nondestructive manipulation of cells. Detailed knowledge of the underlying forces in electric fields and their dependencies will improve the construction of microsystems that handle and move single cells (Schnelle et al., 1993; Fuhr et al., 1994) or separate cell suspensions according to their electrical properties (Becker et al., 1995).

When the dielectric permittivity and conductivity of latex particle suspensions were measured, a low-frequency relaxation at 0.5 kHz (∅ 1.17 μm particles) was found (Schwan et al., 1962). This could be explained by a diffusion-controlled relaxation within a two-dimensional surface layer with enhanced surface conductivity (Schwarz, 1962). The importance of this relaxation for electrorotation is emphasized by theoretical approaches (Fuhr, 1985; Sauer and Schlögl, 1985; Arnold et al., 1987) in the low-frequency range (<10 kHz) as well as for higher frequencies around the peak in rotation rate near the Maxwell-Wagner frequency (MWF). Although the surface conductivity seems to be naturally related to the ionic double layer, there is rare experimental evidence of a direct relationship (e.g.. Arnold et al., 1987); there does not exist a clear interrelation between ζ-potential and surface conductivity. Especially at low frequencies and conductivity, the two-dimensional description of the surrounding layer may not be sufficient to explain electrorotation at these frequencies. Volume forces on the counterion layer may contribute to the total force on a particle (Dukhin and Shilov, 1974; Chew and Sen, 1982a; Fixman, 1983).

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The present study addresses how electrorotation spectra depend on surface charge and ζ-potential.

MATERIALS AND METHODS

The actual experiments were carried out in free media far away from surfaces. Measurements were not performed on a microscopic slide glass, as was done in similar experiments (e.g., Arnold and Zimmermann, 1982; Fuhr, 1985). This procedure also has the advantage that the homogeneity of the electric field and the boundary conditions for the steady-state hydrodynamic problem of a rotating sphere are well defined (Kirchhoff, 1883).

All rotation experiments were made in a symmetrical three-electrode arrangement. The measuring chamber consisted of three planar, polished platinum electrodes of 3 mm × 2 mm surface area (Fig. 1 *a*). Each of them was adjusted within a distance of 1.25 mm to the point of symmetry (Fig. 1 *b*). For visual observation, the chamber was closed airtight at its top and bottom with quartz windows. Each electrode was equipped with a phase- and amplitude-measuring device mounted together with the end-stage hybrid amplifiers and the measuring chamber itself on a printed circuit board. The sinusoidal input signals were supplied by three Hewlett-Packard generators (HP-3336C) and were automatically adjusted to the desired AC field in the volume. With this setup either gradient, homogeneous or rotating electric fields of ≥100 V/cm could be generated at frequencies of 25 Hz to 20 MHz.

The board with the measuring chamber was built into a thermostatted housing mounted on a manipulator to allow a three-dimensional positioning of the chamber relative to a fixed microscope. The rotating particles inside the three-electrode chamber were observed through the windows by means of a long-range microscope objective.

An analog computer connected to the three-dimensional positioning device was used to indicate when the actually focused region inside the measuring chamber leaves the internal ellipsoid (Fig. 1 *c*), to ensure that only measurements with a specified error in electric field strength ($\Delta E^2 <$ 5%; Eq. 2) were taken into account.

At low frequencies electrode polarization occurs. Therefore impedances of similar Pt electrodes were investigated in electrolyte solutions covering the relevant concentration range. The electric field strength was corrected by the frequency-dependent electrode polarization impedance, according to the phenomenological equation of Fricke (1932) and Schwan (1967), who found power functions of frequency for R_p and C_p. The factors a, a', b, and b' were determined experimentally with an electrode distance varying method (see Blum et al., 1995):

$$R_p = a \cdot \left(\frac{\omega}{\omega_r}\right)^{b} \qquad \frac{1}{\omega C_p} = a' \cdot \left(\frac{\omega}{\omega_r}\right)^{b'} \tag{1}$$

(ω_r is an appropriate, arbitrarily chosen reference frequency for the low-frequency polarization (50 Hz here)).

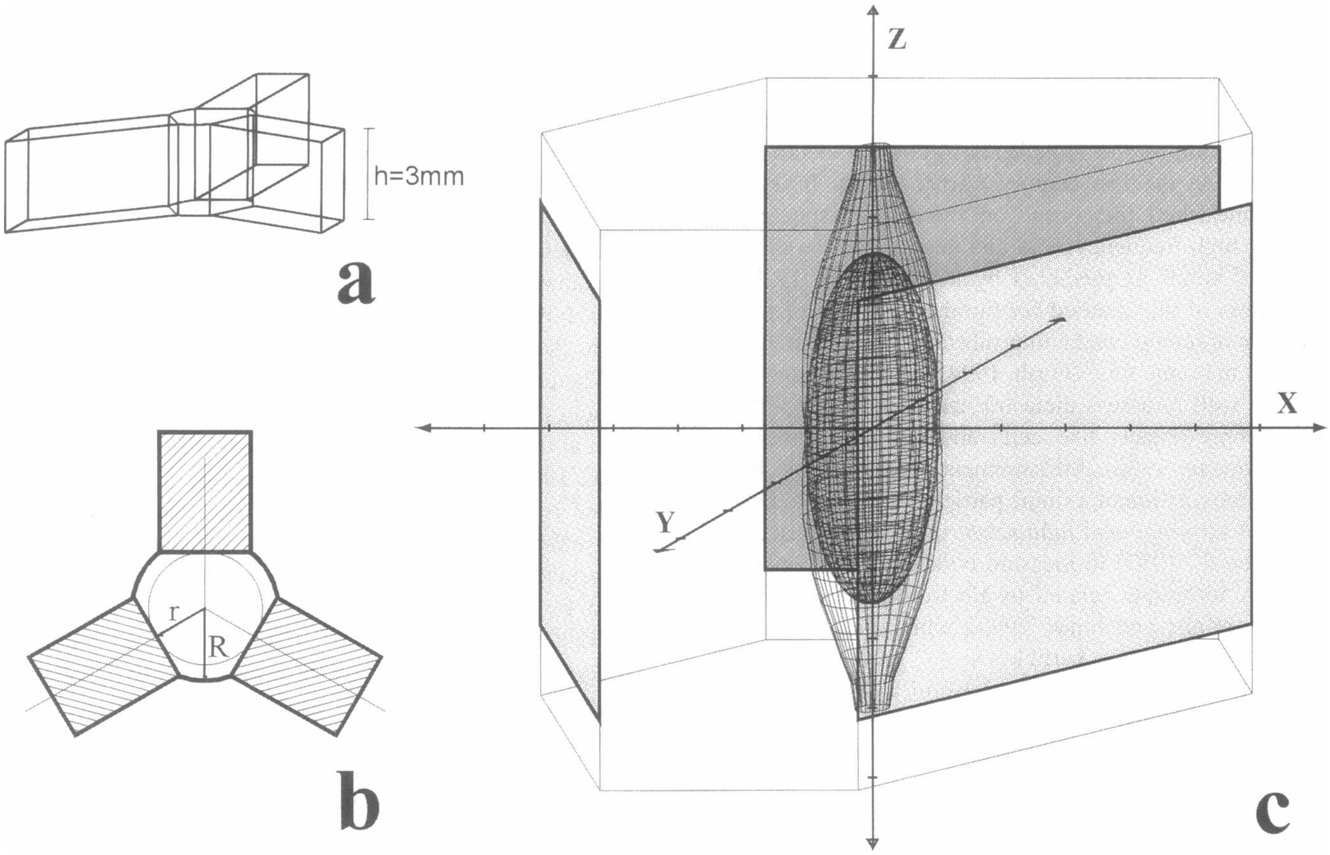

FIGURE 1 (*a*) Scheme of the rotating field chamber (h = 3.0 mm). Electrodes were made of massive platinum blocks with planar 2.0 × 3.0 mm surfaces exposed to the electrolyte solution. Microscopic observation was performed through two quartz windows sealed with silicone rubber to the surfaces above and under the electrodes. (*b*) Top view of the electrode arrangement along the central axis. The electrode surfaces have r = 1.25 mm distance to the vertical axis of symmetry, and the volume has a R = 1.6 mm radius. (*c*) Time-invariant volume between the electrodes, where relative inhomogeneities ΔE^2 are smaller than 5% (see Eq. 2). The enclosed ellipsoid shows the volume in which all measurements took place. The electrostatic potential distributions were calculated numerically for possible potential states of the electrodes during one cycle, assuming that the front windows (*top* and *bottom*) were at a distance of 0.5 mm to the electrodes, which is the worst case possible.

To determine the actual field strength distribution and the region of homogeneity of the electric field in the chamber, a finite-element calculus was performed. To achieve the three-dimensional potential distribution, the geometry was covered with a 55 × 55 × 40 discretization net while symmetry relationships were taken into account (symmetry along the *x-y* plane and the *z-y* plane, Fig. 1 *c*). Electrostatic potential distributions for phases from 0° to 120° were calculated in steps of 5°. With these results the quadratic error relative to the central electric field vector was determined according to the following equation:

$$\Delta E^2 = \frac{|\vec{E}(x, y, z) - \vec{E}_0(x, y, z = 0)|^2}{|\vec{E}_0(x, y, z = 0)|^2} \tag{2}$$

Fig. 1 *c* shows the inner volume in which the relative error of the quadratic field strength does not exceed 5%, and the enclosed ellipsoid shows the analog-computer controlled volume in which all measurements were made.

For the measurements a sphere in this 5% region was focused, then the field was switched on and the rotating sphere was tracked until it reached the boundary of the 5% region. The whole procedure took between 10 s and some minutes for a particle. The individual rotation velocities were determined off-line by analyzing the video sequences. The time between different orientations was stopped several times, from which the individual standard deviation of the rotation rate was calculated. Rotation of the particles could easily be detected by eye, because nearly all polystyrene beads supplied by Polyscience Ltd. showed small inhomogeneities. Diameters of the spheres were checked by their video image; those differing by more than 5% from the mean diameter were rejected.

All chemicals used were of analytical grade. Water was distilled twice before it was degassed in vacuum and was kept under nitrogen atmosphere until use. Latex particles (Polybeads carboxylated™, mean diameter 9.67 μm, SD = 0.49 μm) were supplied by Polyscience (St. Goar); these had a surface charge density characterized by the manufacturer as 0.12 meq COOH/g. Although the beads were supplied as detergent-free, it was nevertheless advisable to wash them extensively to remove traces of chemicals that might be carried over from the manufacturing process. The procedure was as follows. A particle suspension containing ~1% spheres was distributed among several rinsed Eppendorf cuvettes, spun down, and resuspended by vortexing a couple of times. Finally, the suspension was dialyzed against water for 2 days to remove traces of free ions diffusing from deeper regions of the spheres. After this treatment conductivity in the surrounding media remained constant.

Monodisperse latices normally possess additional sulfate groups that originate from chemicals used in the polymerization process, e.g., initiators (e.g., persulfate) and copolymers (Fischer and Nölken, 1988). The nominal surface charge density given by the manufacturer (0.12 meq COOH/g) gives neither information about the solution-accessible part of COOH groups in the surface layer, nor the quantity of sulfate groups contributing to the surface charge. Furthermore, changes of the apparent surface charge density by aging or by oxidation of hydroxyl groups (Shirahama and Suzawa, 1984) must be taken into account. For this reason, a detailed analysis of the chemical composition and structure of the surface by conductometric titration according to the method of Labib and Robertson (1980) was necessary.

This procedure required all of the particles (~0.5 g) equilibrated in a 5 mmol HCl solution for 24 h. After washing as described above, particles were immersed in 40 ml water. This suspension was subject to a conductometric titration with HCl and NaOH to determine the absolute amount of carboxyl and residual sulfate groups at the surface. A syringe in combination with a perfusor (no. 1831; Braun, Melsungen, Germany) was used to add HCl or NaOH (10 mmol both) at a constant rate to a steered, thermostatted vessel under a nitrogen atmosphere. Conductivity was measured with a conductivity-measuring probe (WTW, Weilheim, Germany) and an impedance analyzer (Hewlett Packard 4192A) at 1.592 kHz. The pH was monitored with a measuring chain connected to a pH meter (Knick, Berlin, Germany). Measured values were digitized and sampled on a personal computer. Raw conductivity data had to be corrected for the constant efflux of electrolyte through the reference electrode diaphragm of the pH-measuring chain. Further correction was made for the volume increases caused by the addition of NaOH and HCl solutions. The remaining salt-contaminated suspension was concentrated by centrifugation, dialyzed against water, and washed again in the above-described procedure.

Preliminary electrorotation experiments yielded a broad range of rotation rates at all field frequencies, although all other parameters were kept constant. To carry out the electrorotation experiments on fractions with well-defined ζ-potentials (with reduced variation), it was thought necessary to separate particles according to their surface charge on a free-flow electrophoresis apparatus (ElphorVaP-5; Bender and Hobein, Munich, Germany). The electrophoresis buffer (Hannig and Heidrich, 1990) contained 2.2 g triethanolamine, 0.4 g potassium acetate, 18.8 g glycine, and 2.0 g glucose per liter of solution, adjusted to pH 7.2 with acetic acid. Obtained fractions were washed as described above. Suspensions for all rotation experiments were made of these specified fractions diluted in 0.4 mol/liter sucrose solution for density compensation. Particle concentrations were chosen between 3 mg and 10 mg in 20 ml, except for the pH variation experiment (Fig. 5), where smaller quantities were used. Conductivity and pH of the different solutions used in the described experiments were adjusted with HCl, NaOH, and KCl solutions containing 0.4 mol/liter sucrose. Conductivity and pH measurements throughout the electrorotation experiments were done according to the following protocol: 1) Samples of the suspension were drawn at the inlet of the measuring chamber before and after each experiment. After the particles were removed from the bulk solution by filtering, negligible quantities of indicator *para*-nitro-phenol (PNP) (10^{-6} mol/l) or methyl red (5.0×10^{-7} mol/liter) was added and the pH was checked photometrically. 2) The volume in the system (~0.25 ml) was replaced every 5–10 min, and the conductivity was checked with a conductivity measuring cell placed in the outlet tube near the rotation chamber. 3) If a sufficient quantity of solution was collected in a syringe at the outlet, the pH was checked as described before.

RESULTS

Conductometric titration

The total number of charged groups as determined by the conductometric titration was 6.9 μeq/g polymer. This is much lower than the nominal charge density of 0.12meq/g, showing that the particle core is not accessible by the medium. The quantity of dissociable groups leads to a total surface charge density of 1.17 neq/cm^2. An upper boundary of the number of sulfate groups could be estimated to be ~0.097 neq/cm^2. Taking this very conservative value, the ratio of sulfate group density with respect to the COOH surface density of 1.07 neq/cm^2 is, at maximum, 10% or even less.

Electrophoretic mobility distribution

When the 9.67-μm Polybead COOH particles were separated by free-flow electrophoresis, the electrophoretic mobility showed a very broad distribution around a mean value of 4.81×10^{-4} cm^2/Vs with a standard deviation (SD) of 0.22×10^{-4} cm^2/Vs (Fig. 2 *a*). The great variability may have its origin in inhomogeneities of the manufacturing process, but it is also possible that aging, especially with pretreatment, plays a role, as mentioned by other authors (Bangs, 1984). As expected, the effect on the observed rotation speed was pronounced and decreased the standard deviation of the rotation rate to nearly one-third for a given fraction in comparison to the initial composition. The ob-

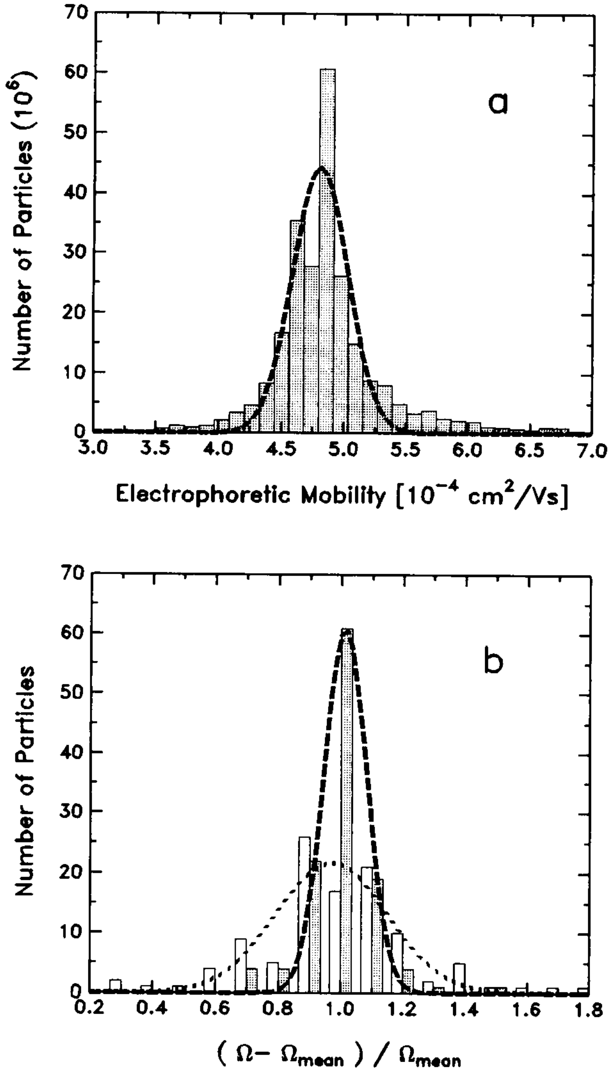

FIGURE 2 (*a*) Distribution of electrophoretic mobility of Polybeads-COOH (Ø9.67 μm) determined by free-flow electrophoresis. The mean value of the distribution is 4.81×10^{-4} cm^2/Vs, with a standard deviation of 0.22×10^{-4} cm^2/Vs (pH = 7.2, 40 V/cm, 19°C). (*b*) Distribution of angular velocity of rotation $\Omega(\nu)$ relative to their mean values $\Omega_{mean}(\nu)$ of all data points at the same electric field frequency ν. Data shown are for electric field frequencies $\nu > 20$ kHz of a preliminary experiment and the experiment shown in Fig. 4 *a*. Compared to the preliminary experiment with unseparated spheres (SD = 0.179), the variation of the rotation velocity was drastically reduced (SD = 0.069) if particles of a single electrophoretic mobility fraction were used in the electrorotation experiment.

tained variability should be even smaller than that shown in Fig. 2 *b*, because the relative mean values of the angular rotation velocities $\Omega(\nu)/\Omega_{mean}(\nu)$ were calculated on the basis of only a few data points per frequency (ν, frequency of the external rotating electric field). This behavior emphasizes once more the importance of surface charge and ζ-potential for electrorotation experiments.

Electrorotation spectra

In the following experiments the influence of surface charge on the rotation spectra (rotation rate versus frequency of the external rotating electric field) was investigated. Two different solutions were prepared with 7-mg spheres of fraction 28 (electrophoretic mobility (EM) = $(4.86 \pm 0.12) \times 10^{-4}$ cm^2/Vs) suspended in 20 ml sucrose solution adjusted to 15 μS/cm (one with NaOH, the other with HCl). Furthermore, a second set of experiments was done with 5-mg particles of fraction 30 (EM = $(4.62 \pm 0.12) \times 10^{-4}$ cm^2/Vs), but in sucrose solutions adjusted to ~22 μS/cm in the same way. Data for the four suspensions are listed in Table 1. Although the particles had been stored in distilled water, the addition to the solutions led to a distinct shift in conductivity. This has its origin in the fact that the number of surface-bound groups is on the same order of magnitude as the total concentration of ions in the solutions. Particularly at low pH, the shift is quite pronounced, indicating that the surface groups in the stored fraction were mainly dissociated. After the measuring chamber was rinsed with adequate solutions, particle rotation was observed at various field frequencies in the range between 50 Hz and 20 MHz. Electrorotation spectra for the four solutions are shown in Figs. 3 and 4, using the dimensionless quantity

$$N_r = \frac{2\eta\Omega(\nu)}{\varepsilon_0 \varepsilon_l E^2} \tag{3}$$

where η is the viscosity of the medium, and $\Omega(\nu)$ is the angular rotation velocity in an external rotating electric field of electric field strength E (amplitude) and frequency ν. The Maxwell-Wagner frequency (ν_0) is defined by

$$2\pi\nu_0 = \omega_0 = \frac{\sigma_l}{\varepsilon_0 \varepsilon_l} \tag{4}$$

σ_l, ϵ_l denote, respectively, the conductivity and the relative permittivity of the surrounding medium l (liquid) or with index s (solid) of the spheres. ϵ_0 denotes the permittivity of the free space.

The relative dielectric constant of the 0.4 mol/liter sucrose solution is $\epsilon_l = 74.5 \pm 1$ at 27°C (Landolt and Börnstein, 1959), which was the normal cell temperature during the experiments.

Whereas the rotation spectra at high pH shows a behavior as described earlier by Arnold et al. (1987) for comparable particles, the spectra for low pH and vanishing surface charge shows a qualitatively different behavior. In the case of low pH, the peak in rotation rate near the MWF inverts, with a clearly pronounced minimum at this point (Fig. 4 *b*). To our knowledge this experiment is the first observation of a rotation in the direction opposite that of the exciting electric field for vanishing surface charge.

Surface charge dependency of the rotation velocity at the MWF

The results from the preceding experiment made it necessary to examine the effects of surface charge and pH in detail. For this purpose it is essential to restrict pH and conductivity to a defined range. Attempts to use various

TABLE 1 Electrophoretic mobility and pH of the suspensions (0.4 mol/liter sucrose) determined during the electrorotation experiment shown in Figs. 3 and 4

No.	Fraction no.	u [10^{-4}] (cm^2/Vs)	σ_{mean} (μS/cm)	MWF (kHz)	Temp. (°C)	pH
1	28	4.86 ± 0.12	11.78 ± 0.12	284 ± 7	27.0 ± 0.1	5.38 ± 0.15
2	28	4.86 ± 0.12	11.70 ± 0.57	274 ± 17	26.8 ± 0.1	7.47 ± 0.1
3	30	4.62 ± 0.12	14.94 ± 2.47	364 ± 64	27.0 ± 0.1	4.13 ± 0.30
4	30	4.62 ± 0.12	22.07 ± 0.36	536 ± 11	27.0 ± 0.1	8.30 ± 0.12

buffers were unsuccessful. We therefore used mixtures of NaOH and acetic acid without any stabilization of the pH, but with a rigorous control of pH and conductivity. The conductivity was chosen to be ~22 μS/cm (MWF $\nu_0 \approx 500$ kHz), and 10 mixtures in the pH range accessible under these conditions were prepared (see Table 2). Quantities of less than 1 mg of particles of fraction 24 (EM = (5.33 ± 0.12) × 10^{-4} cm^2/Vs) were added to 5 ml of each solution. The negligible amounts of spheres gave rise to only a minor alteration of pH when compared with the photometrically obtained values at the outlet of the measuring chamber (before and after the experiment). Volume and tubings were rinsed with ~200 ml of the identical solutions before the system was filled with the suspension. Results of the observed rotation velocities at the MWF are plotted in Fig. 5.

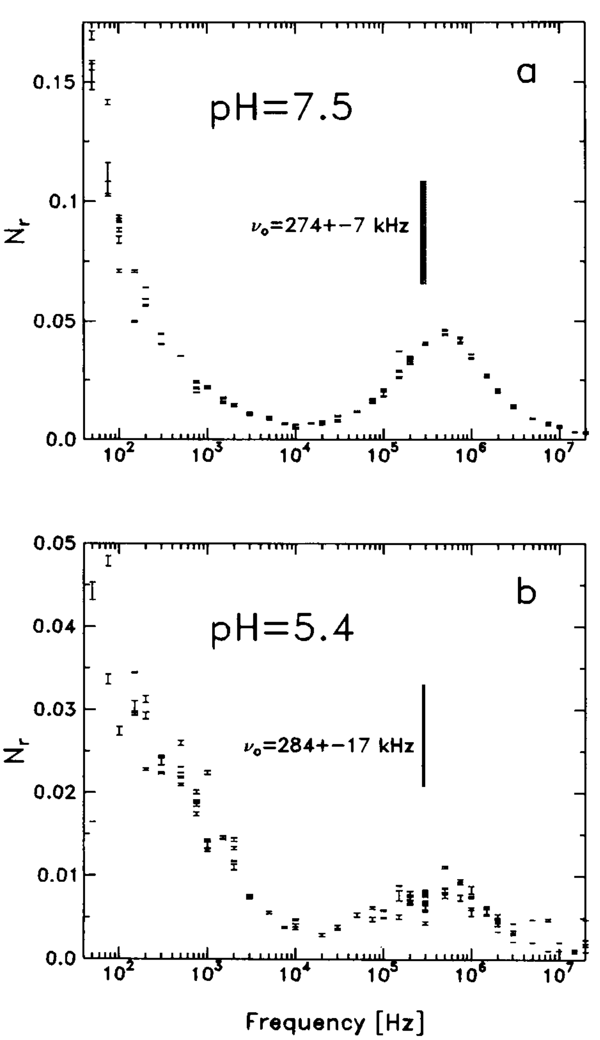

FIGURE 3 Electrorotation spectra of the carboxylated latex particles (Ø9.67 μm, electrophoretic mobility u = (4.86 ± 0.12) × 10^{-4} cm^2/Vs) in a 0.4 mol/liter sucrose solution. (*a*) With bulk pH = (7.47 ± 0.1), obtained by the addition of NaOH. (*b*) With bulk pH = (5.38 ± 0.15), obtained by the addition of HCl. The mean MWF ν_0 and its standard deviation during the experiment are indicated by vertical bars.

DISCUSSION

Conductometric titration

The "parking area" for the closest packing of COOH groups on a two-dimensional surface is 20 Å^2 (Seradyn, Inc., 1992). The surface density of 1.07 neq COOH/cm^2 found in the conductometric titration is equal to a carboxyl group "parking area" of 15 Å^2. This smaller value indicates that the characteristic groups are not only distributed on a two-dimensional surface, but are also buried in a thin three-dimensional subsurface layer. From the ratio of nominal COOH density to the apparent density results follows a thickness of the swollen (water containing) shell of ~100 nm. Normally core sizes and permeability of water vary in a broad range, depending on various factors like polymerization procedure and number of cross-links (El-Aasser et al., 1985). Our results show that the 9.67-μm Polybeads-COOH expose an ion exchanger-like surface layer with a negligible number of sulfate groups, which is thin (2%) compared to the diameter of the sphere. Because water does not seem to exist in the core region of the spheres, the permittivity and conductivity of the solid can be estimated to be similar to that of the pure compound (polystyrene: D_k = 2.5; Saechtling and Zebrowski, 1967). This will not be true in a swollen boundary layer containing water, where an enhanced permittivity and conductivity must be taken into consideration. In addition, this boundary region will expose ion exchanger-like properties; hence apparent electrical surface charge is not necessarily equal to the amount of titrated (chemical) surface groups. In this case charged groups in the solid matrix are partially compensated by counterions in the enclosed liquid phase. For this reason the titration was only used to determine the depth and composition of the water-containing boundary layer, and apparent surface charges and potentials were calculated from the electrophoretic mobility.

In addition, the conductometric titration showed an essential feature of our particles: the acid groups contributing to the surface charge are mainly, if not exclusively, carboxyl

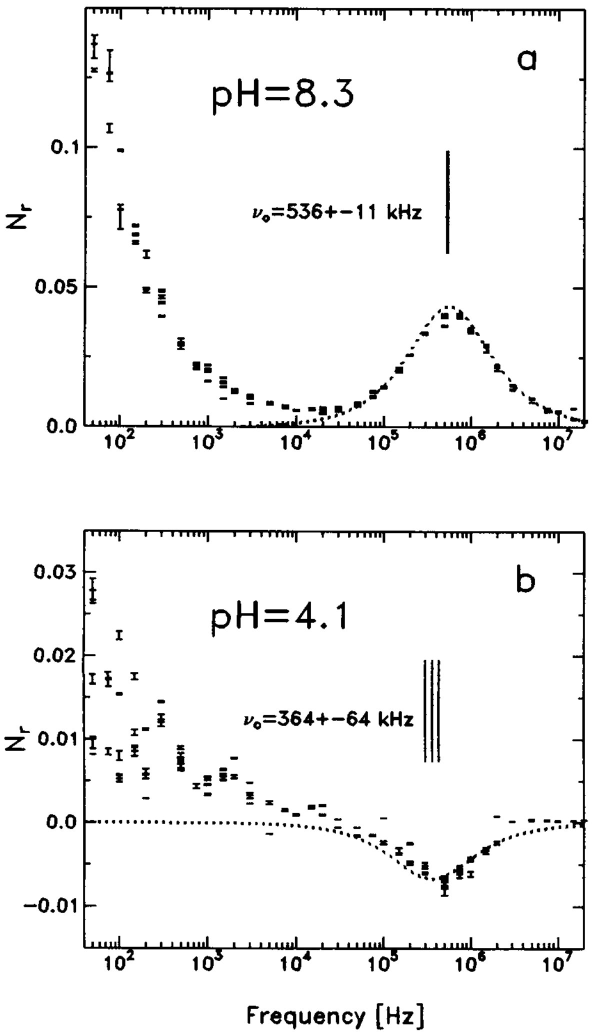

FIGURE 4 Electrorotation spectra of the carboxylated particles (Ø9.67 μm, electrophoretic mobility $u = (4.62 \pm 0.12) \times 10^{-4}$ cm²/Vs) in 0.4 mol/liter sucrose solution. (*a*) With pH = (8.30 ± 0.12), obtained by the addition of NaOH. The mean conductivity was (22.07 ± 0.36) μS/cm. The dashed curve is a least mean square fit of the model without surface charges (Eq. 21) to the high-frequency part (>10 kHz) of the electrorotation spectra. The ratio of the dielectric constants for the sphere and the medium was assumed to be $U = 2.5/74.5$, and the fitting procedure leads to a conductivity ratio of $K = 0.0153$. (*b*) With pH = (4.13 ± 0.3), obtained by the addition of HCl. The mean conductivity was (14.94 ± 2.47) μS/cm. The dashed curve is a fit of the surface charge incorporating model (Eq. 23) to the high-frequency part ($\nu > 10$ kHz) of the data, leading to $A = 9620$ and $p = 59600$ for $U = 2.5/74.5$. The temperature was (27.0 ± 0.1)°C during both experiments. The mean MWF ν_o and its standard deviation during the experiment are indicated by vertical bars.

groups. Generally, strong acid groups (sulfate groups) originating, e.g., from persulfate (which is used as an initiator in the polymerization procedure), are present on nearly all types of such particles. The absence of strong acid groups on our particles makes it possible to suppress surface charges nearly completely in the medium pH range. The titration of strong acid groups would have a pH too low (and a conductivity too high) required to reach an undissociated state.

Electrophoretic experiments

By separating the particles according to their electrophoretic mobilities at a given pH, the effective "electrical surface charge " was determined. Using the Helmholtz equation,

$$\zeta = \frac{\eta u}{\varepsilon_0 \varepsilon_1} \tag{5}$$

the mean electrophoretic mobility u taken over all fractions leads to a ζ-potential of −73.9 mV (η = 1.075 cPoise, water 19°C). Unfortunately, at a pH of the separation medium of 7.2, the weak acid groups were not fully dissociated, as could be seen later in the electrorotation experiments. Correcting this value according to the model function in Fig. 5, a ζ-potential for completely dissociated carboxyl groups of −84.5 mV results.

As can be seen in Fig. 2 *b*, the restriction to particles of a smaller range in electrophoretic mobility than in the initial distribution has a pronounced effect on the variation in rotation velocities at field frequencies around the MWF. This reduction can be interpreted as a first hint of the relative importance of surface potentials and surface charges for the rotation in electric fields.

Electrorotation spectra

The results at high pH can be compared with results obtained in earlier experiments (Arnold et al., 1987). This publication includes measurements of particles that are directly comparable to those used in our experiments. Their particles were supplied by the same manufacturer, and were of the same diameter and standard deviation, so that they probably belong to the same batch. ζ-Potentials as measured by the former authors (−84 mV) appear to be comparable to our corrected values. In both investigations, the linear dependence of rotation velocity on quadratic field strength as well as the decrease in rotation velocity at its specific MWF with increasing ionic concentrations is confirmed. When comparing our electrorotation spectra at high pH with those obtained by Arnold et al. (1987), a qualitative and quantitative agreement was apparent. The two maxima were found: at or above the MWF and at low frequencies (<10 kHz). Taking our measurement where the conditions are comparable (Fig. 3 *a*), the ratio between the peak velocities at MWF and at 100 Hz is ~1.9, indicating that the two systems are in a similar state of dissociation. To compare the values of rotational velocity, corrections must be made for the differing viscosities of water (21°C) and the sucrose solution (27°C) (η_1/η_2 = 0.978 cPoise/1.533 cPoise). Further compensation must then be made for the different conductivities and MWF of the two solutions. Taking the model of Arnold et al. (1987) (Eq. 11), direct comparison of the peak rotational velocities at the MWF can be made. Using the parameters 1) sucrose: 11.8 μS/cm, D_k = 74.5; and 2) water: 7 μS/cm, D_k = 79, the peak rotational velocities were equal within 5–10%.

TABLE 2 Initial solutions (0.4 mol/liter sucrose) before the spheres were added (u = (5.33 ± 0.12) × 10^{-4} cm^2/Vs) and particle suspensions used in the pH variation experiment

Experiment no.	Solution pH	Solution σ (μS/cm)	Suspension pH	Suspension σ (μS/cm)	MWF (kHz)	Temp. (°C)
1	9.11	21.7	8.84	22.31 ± 0.06	538 ± 10	26.8
2	7.90	22.1	7.14	23.48 ± 0.07	567 ± 10	26.9
3	6.99	21.2	6.71	22.46 ± 0.05	542 ± 10	27.0
4	6.46	21.4	6.41	22.95 ± 0.04	554 ± 10	27.0
5	5.97	21.2	6.18	22.69 ± 0.12	547 ± 10	27.1
6	5.75	21.7	5.82	22.99 ± 0.05	555 ± 10	27.1
7	5.53	21.3	5.64	22.36 ± 0.04	540 ± 10	27.2
8	5.00	21.5	5.15	22.55 ± 0.09	544 ± 10	27.2
9	4.50	21.4	4.49	22.96 ± 0.11	554 ± 10	27.2
10	4.05	21.6	3.94	21.08 ± 0.14	509 ± 10	27.2

Suspension parameters were determined throughout the experiment.

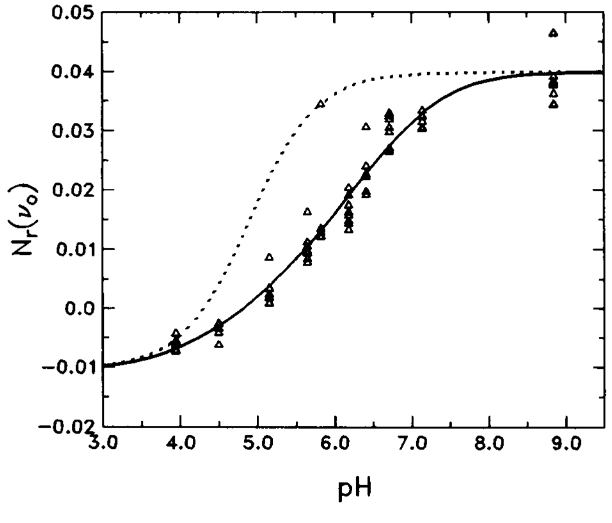

FIGURE 5 Dependence of the rotation velocity at the MWF (ν_0 = 500 kHz) on the pH of the surrounding medium (0.4 mol/liter sucrose). Carboxylated latex particles (∅ 9.67 μm, electrophoretic mobility u = (5.33 ± 0.12) × 10^{-4} cm^2/Vs at pH = 7.2) having a maximal ζ-potential of −93.6 mV according to the Helmholtz equation (Eq. 5) when fully dissociated. Conductivity was kept constant (22.58 ± 0.43 μS/cm) while the pH was varied between 3.94 and 8.84. The "best fit" procedure of the model in Eq. 22, assuming a surface charge of Γ = 11.87, leads to pK 4.91 with a lower limit of $N_r^0 = -0.0103$ and a span of $\Delta N_r = 0.0502$ for $N_r(\nu_0)$. For comparison, the "normal" symmetrical titration behavior (Henderson-Hasselbalch) for pK 4.91 is shown, taking no surface charges into account (*dashed line*). Measurements were performed at ν = 500 kHz field frequency and a field strength of ~100 V/cm.

To quantify the pronounced dependence of the rotation rate at the MWF on surface charge, the following model was suggested. According to the mass balance equation, the dissociation $\alpha = C_{H+}/C_{COOH}$ for a given concentration of protons is given by

$$K = C_{H^+}\frac{\alpha}{1-\alpha} \tag{6}$$

For a charged surface the concentration of charges at the surface and in the Gouy-Chapman double layer are not equal to the bulk concentrations. In case of a small Debye length δ_D (small compared to the radius A of the sphere), the potential at a distance r from the center of the sphere is given by the solution of the one-dimensional Poisson-Boltzmann equation:

$$2\frac{d^2\Phi}{d\xi^2} = C_- - C_+ \tag{7}$$

with the dimensionless variables

$$\Phi = \frac{\varphi F}{RT} \quad C_\pm = \frac{c_\pm}{c_0} \quad \xi = \frac{r-A}{\delta_D} \tag{8}$$

where φ is the electrical potential; c_+, c_- are the ion concentrations; and c_0 is the bulk concentration of the ions (assuming a 1–1 electrolyte and electroneutrality at infinity). F denotes the Faraday constant, R the gas constant, and T the absolute temperature. Ion concentrations are given by the electrochemical potential equilibrium:

$$\ln C_\pm \pm \Phi = \text{const.} \tag{9}$$

leading with $r \rightarrow \infty \Rightarrow C_\pm \rightarrow 1$ to

$$\frac{\partial^2\Phi}{\partial\xi^2} = \sinh\Phi \tag{10}$$

The solution of this differential equation is given by Sherwood (1979 (unpublished Ph.D. thesis cited in Chew and Sen, 1982b)):

$$\Phi = \ln\left(\frac{1+te^{-\xi}}{1-te^{-\xi}}\right)^2 \tag{11}$$

with

$$t = \tanh\left(\frac{\Phi_0}{4}\right) \qquad \delta_D^2 = \frac{\varepsilon_l RT}{2F^2c_0} \tag{12}$$

where Φ_0 is the dimensionless electrical potential at the surface (ζ-potential). This result is valid for all Φ_0 and leads in the approximation for $t < 1$ to the well-known Gouy-Chapman equation. Substituting the proton concentration given by Eqs. 9 and 11 and using the dimensionless concentration at infinity $C_\pm^\infty$,

$$C_\pm = C_\pm^\infty e^{\mp\Phi} = C_\pm^\infty\left(\frac{1 \mp te^{-\xi}}{1 \pm te^{-\xi}}\right)^2 \tag{13}$$

In Eq. 6 a modified Henderson-Hasselbalch equation on a charged surface results:

$$\mathrm{pH} = \mathrm{pK} + \log\left(\frac{\alpha}{1-\alpha}\left(\frac{1-t}{1+t}\right)^2\right) \tag{14}$$

The additional term incorporates the influence of a surface potential on the dissociation of surface groups. The relationship between the surface charge and the surface potential is given by the first Maxwell equation, $\vec{\nabla} \cdot \vec{D} = \sigma$, yielding with the surface charge γ (mol/cm^2),

$$\varepsilon_l \frac{\partial\varphi}{\partial r} = -F\gamma \tag{15}$$

and in its dimensionless form for one dimension,

$$\frac{\partial\Phi}{\partial\xi} = -\frac{\Gamma}{2} \tag{16}$$

with the dimensionless surface charge $\Gamma = \gamma/c_o\delta_D$.

Solving this differential equation, one obtains

$$t^2 + \frac{8t}{\Gamma} - 1 = 0 \tag{17}$$

and in the case of negative surface charges ($\Gamma < 0$), the results

$$t = -\frac{4}{\Gamma} - \left(1 + \left(\frac{4}{\Gamma}\right)^2\right)^{1/2} \tag{18}$$

Now substituting t in Eq. 14, an algebraic equation of fourth-order results, giving the relationship between dissociation α and pH for a given surface concentration of dissociable groups:

$$\alpha^4 - \alpha^3 + \frac{a^2(1+b)}{4b}\alpha^2 - \frac{a^2(1+b)}{2}\alpha + \frac{a^2b}{4} = 0 \tag{19}$$

with

$$a = \frac{4}{\Gamma} \quad b = 10^{(\mathrm{pH}-\mathrm{pK})} \tag{20}$$

This equation may be solved analytically by the method of L. Ferrari (1522–1565), but it is more comfortable to solve it numerically with the Newton method. Introducing Eq. 17 in Eq. 14 leads to an inverse function pH(α) with a single solution, which allows us to distinguish between several possible solutions of Eq. 19 by choosing $|\mathrm{pH} - \mathrm{pH}(\alpha(\mathrm{pH}))| < \epsilon$ as a criterion for convergence to the "right" solution. From a "best-fit" procedure of the model of Sauer and Schlögl (1985) for a noncharged sphere,

$$N_r = \frac{3(K-U)}{(K+2)(U+2)}\frac{X/X_r}{1+(X/X_r)^2} \tag{21}$$

with

$$K = \frac{\sigma_s}{\sigma_l};\quad U = \frac{\varepsilon_s}{\varepsilon_l};\quad X = \frac{\nu}{\nu_0};\quad X_r = \frac{K+2}{U+2}$$

to the low pH data (Fig. 4 *b*), it follows that the conductivity of the solid is small compared to that of the liquid. This must not be true in general, especially in a water-containing "swollen" state with fixed surface charges. Using the model of Arnold et al. (1987) leads to a similar result. Under the condition of low conducting spheres, the approximation of the model used in this source results in a linear dependence of rotation on surface conductivity. With this working hypothesis in mind, the following "best fit" procedure of a linear dependence of rotational velocity (at the MWF ν_0) on surface charge was applied:

$$N_r(\nu_0) = N_r^0 + \Delta N_r \cdot \alpha(\mathrm{pH}) \tag{22}$$

The surface potential of fraction 24 used in this experiment was calculated from the electrophoretic mobility with the Helmholtz equation (Eq. 5). It yields $\zeta = -81.9$ mV, $\Gamma = 9.32$, and $\zeta = -93.6$ mV, $\Gamma = 11.87$ if the correction for partially dissociated surface groups were taken into account. The result of the curve fit is shown as the solid line in Fig. 5, showing an excellent approximation of the experimental data. It results further in an independent pK of 4.91 ($\zeta = -93.6$ mV) for the carboxyl groups. This value is much more realistic with respect to carboxyl groups in this kind of chemical binding than the value of the half-maximum effect, which is ~6.0 (for comparison: benzoic acid: pK = 4.22; 2-phenyl-propionic acid: pK = 4.64; $CH_3(CH_2)_nCOOH$: pK = 4.82–4.95 (n = 2–7), which are all below a pK of 5). In addition, the "normal" symmetrical titration behavior in the absence of a surface potential is not able to approximate the much broader and asymmetrical dependence on the bulk pH. For comparison, this normal titration behavior of surface groups in the absence of a surface potential is shown as a dashed line in Fig. 5, assuming a pK = 4.91. From this model it becomes apparent that the assumption of a linear dependence of rotational velocity on surface charge is adequate to describe the rotational velocity at the MWF maximum. The model function implies, furthermore, that the lowest investigated pH still leads to a dissociation of ~10%. To estimate the residual rotational velocity at low frequencies, the ratios between maximum (at high pH) and residual mean velocity (at low pH) at the five lowest frequencies (50–200 Hz) were calculated (Fig. 4, *a* and *b*). The residual rotational velocity at these low frequencies is 13% on average, with a standard deviation of 2%. In the case of a linear dependence, this correspondence indicates that the low-frequency rotation is mostly or totally due to the surface charge system. This analysis shows that the models of Sauer and Schlögl (1985)

$$N_r = \frac{3X(PX^2(A - PU) - A - U}{X^2(X + 2 + A + 2P)^2 + (2 - (U + 2)PX^2)^2} \quad (23)$$

with

$$U = \frac{\varepsilon_s}{\varepsilon_l}; \quad A = \frac{\Delta\varepsilon}{\varepsilon_l}; \quad X = \frac{\nu}{\nu_0}; \quad P = 2\pi\nu_0\tau_a$$

hold for the presence and absence of surface charges (see also Fig. 4, *a* and *b*) around the MWF. It is also probable that this model describes the behavior at low frequencies for noncharged particles, but does not hold for surface charged particles in this frequency range.

CONCLUSION

The behavior of colloids in electrorotation experiments is dominated by the presence of surface charges. This includes the high-frequency range around the MWF as well as the low-frequency region (<10 kHz). The high-frequency (MWF) rotation may be approximated by a surface-charge-dependent linear term. A model for surface-bound chemical groups incorporating the surface potential was developed that describes the experimental data in an adequate manner. In the case where the conductivity of the particle is small compared to that of the surrounding liquid, it was shown that the effect may be so drastic that it can invert the direction of the rotation. The rotation rate at low frequencies is mainly or perhaps totally determined by the surface charge system. The influence of the surface charge may be described in the high-frequency domain either directly or as an enhanced surface conductivity in the sense of G. Schwarz. This need not to be true for the low-frequency region.

The importance of surface charge systems may be emphasized not only for solid particles but also for multishell systems as spherical cells. A further implication arises from the Kramers-Kronig integral relationship, so that major influences can also be expected in particle-particle interactions as well as in dielectrophoretic experiments.

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REFERENCES

Arnò, R. 1896. Sulla isteresa dielettrica viscosa. *Rend. Linc.* 5(5):262.

Arnold, W. M., H. P. Schwan, and U. Zimmermann. 1987. Surface conductance and other properties of latex particles measured by electrorotation. *J. Phys. Chem.* 91:5093–5098.

Arnold, W. M., and U. Zimmermann. 1982. Rotating-field induced rotation and measurement of the membrane capacitance of single mesophyll cells of *Avena sativa*. *Z. Naturforsch.* 37:908–915.

Bangs, L. B. 1984. Uniform Latex Particles. Seradyn, Indianapolis, IN.

Becker, F. F., X.-B. Wang, Y. Huang, R. Pethig, J. Vykoukal, and P. R. C. Gascoyne. 1995. Separation of human breast cancer cells from blood by differential dielectric affinity. *Proc. Natl. Acad. Sci. USA.* 92:860–864.

Blum, G., H. Maier, F. Sauer, and H. P. Schwan. 1995. Dielectric relaxation of colloidal particle suspensions at radio frequencies caused by surface conductance. *J. Phys. Chem.* 99:780–789.

Born, M. 1920. Über die Beweglichkeit der elektrolytischen Ionen. *Z. Physik.* 1:315–336.

Chew, W. C., and P. N. Sen. 1982a. Dielectric enhancement due to the electrochemical double layer: thin double layer approximation. *J. Chem. Phys.* 77:4683–4693.

Chew W. C., and P. N. Sen. 1982b. Potential of a sphere in an ionic solution in thin double layer approximation. *J. Chem. Phys.* 77(4): 2042–2044.

Dukhin, S. S., and V. N. Shilov. 1974. Dielectric Phenomena and the Double Layer in Dispersed Systems and Electrolytes. Halsted, Jerusalem.

El-Aasser, M. S., F. V. Loncar, Jr., and J. W. Vanderhoff. 1985. Swelling of carboxyl-containing copolymer latexes. *Makromol. Chem. Suppl.* 10/11:335–357.

Fischer, J. P., and E. Nölken. 1988. Correlation between latex stability data determined by practical and colloid chemistry-based methods. *Prog. Colloid Polym. Sci.* 77:180–194.

Fixman, M. 1983. Thin double layer approximation for electrophoresis and dielectric response. *J. Chem. Phys.* 78:1483.

Foster, K. R., F. A. Sauer, and H. P. Schwan. 1992. Electrorotation and levitation of cells and colloidal particles. *Biophys. J.* 63:180–190.

Fricke, H. 1932. The theory of electrolytic polarization. *Philos. Mag.* 14:310–318.

Fuhr, G. R. 1985. Über die Rotation dielektrischer Körper in rotierenden Feldern. Dissertation, Humboldt Universität Zu Berlin, Berlin.

Fuhr, G., H. Glasser, T. Müller, and Th. Schnelle. 1994. Cell manipulation and cultivation under a.c. electric field influence in highly conducting culture media. *Biochim. Biophys. Acta.* 1201:353–360.

Fürth, R. 1924. Eine neue Methode zur Bestimmung der Dielektrizitätskonstanten guter Leiter. *Z. Phys.* 22:98–108.

Gimsa, J., T. Müller, Th. Schnelle, and G. Fuhr. 1995. Electrorotation of particles measured by dynamic light scattering—a new dielectric spectroscopy technique. *Colloids Surf. A.* 98:243–249.

Hannig, K., and H. G. Heidrich. 1990. Free-Flow Electrophoresis. GIT Verlag, Darmstadt, Germany.

Kaler, K. V. I. S., and T. B. Jones. 1990. Dielectrophoretic spectra of single cells determined by feedback-controlled levitation. *Biophys. J.* 57: 173–182.

Kirchhoff, G. 1877. Vorlesungen über mathematische Physik. Mechanik. Teubnen, Leipzig.

Labib, M. E., and A. A. Robertson. 1980. The conductometric titration of latices. *J. Colloid Interface Sci.* 77:151–161.

Landolt, B. 1959. Band II: Elektrische Eigenschaften I. 6,783. Springer Verlag, Heidelberg.

Lertes, P. 1921. Untersuchungen über Rotationen von dielektrischen Flüssigkeiten im elektrostatischen Drehfeld. *Z. Physik.* 4:315–336.

Prüger, B., P. Eppmann, E. Donath, and J. Gimsa. 1997. Measurement of inherent particle properties by dynamic light scattering: introducing electrorotational light scattering. *Biophys. J.* 72:1414–1424.

Saechtling, H., and W. Zebrowski. 1967. Kunststoff-Taschenbuch. Hanser, Munich.

Sauer, F. A., and R. W. Schlögl. 1985. Torques exerted on cylinders and spheres by external electromagnetic fields: a contribution to the theory of field induced cell rotation. *In*: Interaction between Electromagnetic Fields and Cells. A. Chiabrera, C. Nicolini, and H. P. Schwan, editors. Plenum, New York. 203–251.

Schnelle, Th., R. Hagedorn, G. Fuhr, S. Fiedler, and T. Müller. 1993. Three-dimensional electric field traps for manipulation of cells—calculation and experimental verification. *Biochim. Biophys. Acta.* 1157: 127–140.

Schwan, H. P. 1967. Determination of biological impedances. *Phys. Tech. Biol. Res.* 6:323–407.

Schwan, H. P. 1985. EM-field induced force effects. *In* Interaction between Electromagnetic Fields and Cells. A. Chiabrera, C. Nicolini, and H. P. Schwan, editors. Plenum, New York. 371–389.

Schwan, H. P., G. Schwarz, J. Maczuk, and H. Pauly. 1962. On the low-frequency dielectric dispersion of colloidal particles in electrolyte solution. *J. Phys. Chem.* 66:2626–2635.

Schwarz, G. 1962. A theory of the low-frequency dielectric dispersion of colloidal particles in electrolyte solution. *J. Phys. Chem.* 66:2636–2642.

Seradyn, Inc. 1992. Carboxylate-modified microparticles (CM-MP) defining parking area and acid content. *Particle Technol. News.* 3:3–4.

Sherwood, J. D. 1979. Ph.D. thesis. Trinity College, Cambridge University.

Shirahama, H., and T. Suzawa. 1984. Surface characterization of soap-free carboxylated polymer lattices. *Polym. J.* 16:795–803.