Particle separation by dielectrophoresis

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Abstract

The application of dielectrophoresis to particle discrimination, separation, and fractionation is reviewed, some advantages and disadvantages of currently available approaches are considered, and some caveats are noted.

Keywords

Alternating current electrokinetics; Dielectrophoresis; Field-flow fractionation; Micrototal analysis systems; Particle separation; Review

1 Introduction

When a particle is placed in an electrical field, it experiences a lateral force $\bar{F}_{elec}$ [1-3] given by

$$\bar{F}_{elec} = q\,\bar{E} + \left(\bar{m}\,\nabla\right)\bar{E} + \frac{1}{6}\nabla\left(\overrightarrow{Q}:\nabla\,\bar{E}\right) + \cdots$$

(1)

The first term describes the coulombic interaction between the net charge q of the particle and the electrical field $\bar{E}$ and embodies all electrophoretic phenomena. This vanishes in the absence of a net charge on the particle or in an alternating field whose time average is zero $\left(\left\langle\bar{E}\right\rangle = 0\right)$. The additional force terms arise from the interaction of dielectric polarization components induced in the particle by the electric field with spatial inhomogeneities in that field. These dielectric force terms only vanish if the field is spatially homogeneous $\left(\nabla\,\bar{E} = 0\right)$. Pohl [4] was one of the first to recognize and explore the use of dielectric forces for the manipulation of particles, particularly living cells, in detail and he named the movement of particles induced by them dielectrophoresis (DEP). DEP is the electric analog of magnetophoresis, the familiar force that collects metals at magnet poles (because magnetic monopoles do not exist, there is no magnetic analog of electrophoresis.) Although Pohl identified DEP with the real part of the second term of Eq. (1) (the in-phase, dipole $\bar{m}$ force component), the expressions dielectrophoresis and DEP have since broadened to mean particle translation resulting from all force components embodied in Eq. (1) including quadrupole $\left(\overrightarrow{Q}\right)$ and higher order phenomena as well as traveling wave effects arising from translation of the electric field

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distribution with time. This article reviews the application of dielectrophoresis (DEP) for particle discrimination and fractionation within this broader meaning.

There is currently a high level of interest in developing means to manipulate and discriminate particles and analytes efficiently in microfluidic and micrototal analysis systems (μTAS). DEP enables electrically-controllable trapping, focusing, translation, fractionation and characterization of particulate mineral, chemical, and biological analytes within a fluid suspending medium. Because the dielectric properties of a particle depend on both its structure and composition, dielectrophoretic forces access a much richer set of particle properties than electrophoresis. DEP is particularly well suited to applications at the small scales of microfluidic devices, is amenable to integration by inexpensive fabrication methods, is easily and directly interfaced to conventional electronics, and can reduce or eliminate the need for complex, expensive, and potentially unreliable sample manipulation methods involving microfabricated mechanical pumps and valves. On a larger, preparative scale, DEP methods are applicable to the purification, enrichment, and characterization of a wide range of environmental, biological and clinical components and significant progress has been made in developing technologies in these areas.

2 Theory

From Eq. (1), the time-averaged DEP force $\left\langle \bar{F}\,(t) \right\rangle$ acting on a particle due to an imposed electrical field, $\bar{E}\,(\omega)$ can be approximated in terms of dipole effects as

$$\left\langle \bar{F}\,(t) \right\rangle = 2\pi\varepsilon_m r^3 \left\{ Re\left(f_{CM}\,(\omega)\right) \nabla E_{RMS}^2 + \right.$$
$$\left. + Im\left(f_{CM}\,(\omega)\right)\left(E_x^2 \nabla_{\varphi\xi} + E_\theta \nabla_{\varphi\theta} + E_\zeta \nabla\phi_\zeta\right) \right\}$$

(2)

f_{CM} is the dipolar Clausius-Mossotti factor that embodies the frequency-dependent dielectric properties of the particle and its surroundings that give rise to the induced dipole moment $\bar{m}$ in Eq. (1). r is the particle radius, ω is the angular frequency and E_{RMS} is the root-mean-square value of the applied electric field. E_i and φ_i ($i = x$; y; z) are the magnitudes and phases, respectively, of the electric field components in the principal axis directions. The quadrupole and higher poles in Eq. (1) have been assumed to be negligible as is the case for moderately inhomogeneous electric fields. Force equations analogous to Eq. (2) for higher order poles have been derived elsewhere [1,2]. Equation (2), sufficient for the present discussion, shows there are two independent force contributions to DEP: (i) A field inhomogeneity component: the left hand term depends on the real (in-phase, or capacitive) component $Re(f_{CM})$ of the induced dipole moment in the particle and the spatial nonuniformity, ∇E_{RMS}^2, of the field magnitude. This force pushes particles towards strong or weak field regions, depending upon whether $Re(f_{CM})$ is positive or negative. This is the DEP force identified by Pohl [4]. It allows particles to be attracted to or repelled from electrode edges. (ii) A traveling field component: the right hand term depends on the imaginary (out-of-phase, or lossy) component $Im(f_{CM})$ of the induced dipole moment and the spatial nonuniformity ($\nabla\varphi_x$, $\nabla\varphi_y$ and $\nabla\varphi_z$) of the field phase that describes how the electric field distribution moves with time. This force pushes the particle in the same or the opposite direction to which the field is traveling depending on the sign of $Im(f_{CM})$. It allows particles to be swept along by an electric field that travels over an electrode array. At least three excitation phases must be provided for this force to arise.

These two force components act independently but, with appropriate electrode array design and electrical signals, can be applied simultaneously to levitate particles above an electrode array while moving them across it, for example [5-8]. Although this dipolar approximation is

adequate in many cases, often it is not. For example, DEP trapping is sometimes accomplished in a quadrupole electrode configuration where the net dipole forces are zero and trapping must be understood in terms of the third term of Eq. (1) whose expansion is different from Eq. (2) [1,9,10]. Even an accurate description of trapping forces on an electrode edge, where the field gradient is very high, involves contributions from higher than dipole order force terms. The underlying principle is that coupling occurs between the 2^n-pole polarization of the particle and the nth derivative of the spatial and temporal distributions of the electric field to yield independent force components [1,9,10]. These higher order force components have different dependencies on the frequency of the applied field than the dipolar components.

As revealed by these frequency-dependent properties of Eq. (1) and (2), far from being restricted to the direct current (DC) fields used in electrophoresis, DEP is the most versatile in alternating (AC) fields. Indeed a major strength of DEP is that it can be used to exploit the frequency-dependent dielectric properties of particles to impose manipulation forces. Particle dielectric properties may reflect several aspects of structure and composition and therefore DEP can enable enhanced particle discrimination that accesses many more properties of the particles than can electrophoresis, which reveals only the ratio of particle charge to hydrodynamic drag. Differences in dielectric properties can be exploited to impose different, even opposing, forces on different particle types in a mixture. The essential characteristic of DEP is the movement of objects with respect to their suspending medium; thus particles can be concentrated to a focal point by negative DEP or trapped by positive DEP, and different particle types can be moved apart from one another all under appropriate field conditions. These basic manipulations can be used to sort, isolate, and trap particles, cells and microbeads [11-30].

3 Particle dielectric properties

The dipolar Clausius-Mossotti factor f_{CM} in Eq. (2) is given by

$$f_{CM}\left(\varepsilon_p^*, \varepsilon_m^*, \omega\right) = \frac{\varepsilon_p^*(\omega) - \varepsilon_m^*(\omega)}{\varepsilon_p^*(\omega) + 2\varepsilon_m^*(\omega)}$$

(3)

$\varepsilon_p^*(\omega)$ and $\varepsilon_m^*(\omega)$ are the frequency-dependent complex permittivities of the particle and its suspending medium, respectively. The complex permittivity of each material depends on its dielectric constant ε and electrical conductivity σ and takes the form $\varepsilon* = \varepsilon - j\frac{\sigma}{\omega}$, where $j = \sqrt{-1}$. If the particle is suspended in a homogeneous fluid, the components of ε_m^* are simply the bulk medium permittivity and conductivity. Electronic, permanent dipolar, and interfacial (or, Maxwell-Wagner) mechanisms of polarization [31] and electrical conductivity may all contribute to the complex dielectric properties. For example, the complex dielectric properties of aqueous media are dominated by dipoles and ionic conductivity, those of latex microspheres arise from the electronic polarizability of the polymer, those of carbon and metal particles from electrical conductivity, and those of living cells from interfacial polarization of ions at cell membrane surfaces.

In the frequency range 5-200 kHz, several groups have shown that the dielectric properties of cells are dominated by Maxwell-Wagner polarization at membrane interfaces governed by cell membrane morphologies, internal conductivities, and size [32-38]. Because different cell types are morphologically distinct they have different dielectric phenotypes [39] making DEP an attractive method for cell manipulation and discrimination. The frequency spectrum of cell dielectric properties can be measured with single-cell discrimination using the method of electrorotation (ROT) [35,36,40-55] in which the rotation of cells resulting from the torque induced by an applied rotating electrical field is measured as a function of the field frequency.

For most purposes, the dielectric properties of cells can be adequately described by the single-shell model [43,56-58] and the cellular dielectric parameters deduced from such an analysis of the ROT spectrum may then be used to predict the DEP properties for both inhomogeneous and traveling fields under any given operating conditions and to explore the optimum conditions for separating dissimilar cell types, for example. This is a sound approach to exploring potential new DEP applications. ROT and DEP analysis may be used to not only characterize but also identify cells and other particle types. An analysis of the accuracy of the ROT method for assessing dielectric properties of particles by cell models has been described [59]. To give some sense of the richness of DEP responses, Fig. 1 shows frequency spectra for the DEP forces calculated using the single-shelled model for cells having several different structures and compositions. Note that at certain so-called crossover frequencies the DEP force traverses zero and its direction reverses.

4 Interfacial effects

Particle complex permittivity ε_p^* is usually more complicated than the particle composition and the bulk properties of the suspending medium suggest because the interface between the particle and its suspending medium introduces an additional "shell" having its own, distinct dielectric properties. In aqueous suspensions, this shell may include surface charge, bound ions (Stern layer), and a diffuse double layer. For a small surface charge density on the particle, the concentration of ions within a diffuse layer falls exponentially with increasing distance from the surface with a characteristic Debye-Hückel [66] screening length, d, given by:

$$d = \left(\frac{\varepsilon_m kT}{8\pi n^0 z^2 e_0^2} \right)^{\frac{1}{2}}$$

(4)

where k is the Boltzmann constant, T is the absolute temperature, n^0 is the bulk concentration of ions in the suspending medium, z is the (assumed balanced) valency of the suspending medium, and e_0 is the electronic charge. The ion concentration near the particle will exceed the medium bulk concentration n^0, increasing the local electrical conductivity. The particle surface may also demonstrate enhanced mobility for ions such as protons. These conductivity effects have been termed surface conductivity. The total charge in the double layer, and hence its capacitance, depends on the particle surface charge. The combined effects of the surface conductivity and capacitance constitute the complex permittivity of the interfacial dielectric shell. Even though this shell resides in the suspending medium, electrokinetic forces acting on it are coupled to the particle and contribute to DEP behavior in a frequency-dependent manner.

Equation (2) shows that the DEP force on an object depends upon its volume. The thickness d of the double layer increases with decreasing ionic concentration of the suspending medium but is independent of the particle size. Therefore, for a given ionic strength, the volume of the double layer will increase with the square of the particle radius while the volume of the particle itself increases with its cube. Thus charge double layer effects become more prominent for small particles at low conductivities. Interfacial dielectric effects in low conductivity media are apparent in the DEP properties of polymer microspheres and cells at low frequencies [60, 61] and, indeed, the DEP properties of small particles and molecules may be dominated by such effects. For this reason, while the DEP properties of μm- and larger-sized particles at sufficiently high frequencies depend sensitively on particle composition and structure, surface effects limit the expected ability of DEP to discriminate between nanoparticles or molecules on this basis. For example, the DEP properties of DNA molecules in solution are essentially sequence-independent because they are controlled by charge double-layer and surface conductivity effects that depend on the gross structure of the molecules.

Charge double layer effects are most important in low-conductivity media typically at frequencies in the low kHz range where they can cause a positive DEP force to be exerted on a particle of otherwise low polarizability [62]. The manner in which the sign of the DEP force for a dielectric particle behaves with frequency is constrained by dielectric theory [63]. Because the additional force terms introduced by charge double layer effects may allow these constraints to appear to be violated for a dielectric model that neglects them, positive DEP at low frequencies for cells was initially referred to as anomalous DEP. Early attempts were made to explain this in terms of nonlinear biological dielectric properties [64] but similar low frequency DEP responses have been observed for simple, nonbiological particles composed of linear dielectric materials in low-conductivity media and these responses now seem to be a universal characteristic of small particles at low frequencies arising from the interfacial dielectric shell [60,61]. If not recognized, such interfacial dielectric effects may mislead newcomers to the field. However, the effects are useful if understood and they may be overcome under appropriately chosen frequency and conductivity conditions allowing small particles to be stably trapped using negative DEP field cages, for example [65].

Depending on the particle surface and suspending medium properties, particle interfacial effects may be highly sensitive to the suspending medium parameters including conductivity, electrolyte valency, pH, and the presence of reagents that can be adsorbed on the particle surface. These dependencies offer an untapped and potentially powerful approach to particle surface analysis in well-defined media and to fluid and analyte characterization using well-defined particles. Such an approach may conceivably be adapted to the detection of the binding of extremely low concentrations of molecular analytes that might be useful for high sensitivity assays. However, the interfacial dielectric properties are derived from the combined characteristics of the charge double layer surrounding the particle, the bound charge, and the particle surface conductivity; therefore, a significant proportion of the particle surface charge may need to be modified before detectable changes in DEP behavior occur.

The surfaces of the electrodes used to apply electric fields in DEP devices are also susceptible to interfacial effects in the form of electrode polarization. This arises because of the discontinuity in charge carrier species between the metal and liquid phases and the development of a contact potential and corresponding charge double layer. According to widely accepted models [5,66,67], the effective impedance of the electrode-solution interface has a frequency dependency of $\omega^{-\frac{1}{2}}$ and has similar dependencies on the solution ion concentration and valency as d in Eq. (4). Electrode polarization becomes particularly problematical for suspension conductivities below 100 mS/m and field frequencies below about 15 kHz. At such low frequencies and conductivities, electrode polarization tends to obscure the field penetrating the aqueous phase to particles in suspension. Since the DEP force depends on the square of the field strength felt by the particles, this screening effect can lead to a profound reduction in particle manipulation capabilities. Furthermore, when a voltage drop occurs because of electrode polarization, dielectric loss occurs in the double layer that results in local heating and in a phenomenon known as AC electroconvection. Although the mechanisms underlying these effects are still being elucidated [68-71], both tend to drive convection currents in the suspending medium that rise from electrode edges and fall at electrode gaps (see Fig. 2). Particles caught in convection cells tend to be swept across electrode edges towards the center of electrodes where they may be deposited at a node in the fluid motion. In an additional imposed fluid flow (see later), such deposition may not occur and instead these convection cells may lead to particle levitation away from the electrode plane. In both cases the particle displacement, although electrokinetic in nature, is brought about by fluid motion caused by energy coupling to the suspending medium, not by dielectrophoresis. Again, this is a potentially rich area for theoretical development, experimental research, and practical applications [72], but, unfortunately, another potential confusion if particle translation caused by fluid motion is mistaken for DEP.

One last low frequency effect that needs to be considered is charge injection from the electrodes. This occurs when the applied voltage is sufficient to cause electrochemical reactions that may produce potentially disruptive chemical species including free radicals. At high frequencies, electrochemically-produced species do not have time to diffuse away from the electrodes and are eliminated on the next field reversal [73]. As a result, mammalian cells, which are particularly sensitive to free radical species, are not damaged even if grown on electrodes with high frequency fields applied [74]. At low frequencies, electrochemical species have time to diffuse away from the electrodes into the suspending medium where they may alter the chemistry, pH and other experimental conditions and can, for example, damage cells. The frequency ranges where damage occurs for some mammalian cells, and steps that can be taken to eliminate cell damage under these conditions, have been studied [75].

5 Spatial particle separation by differential DEP affinity

Particles experiencing positive DEP forces move to local electrical field maxima. In the near-field regime used in DEP (the wavelength of the applied frequency is much greater than the electrode spacing), all field maxima are at electrode edges. Once particles arrive at electrode edges, steric forces prevent further movement and they remain trapped by positive DEP. While DEP trapping may be used to concentrate particles from a suspension as demonstrated for yeasts, bacteria, mammalian, and other cell types [76-81], the present article focuses on the more complex problem of fractionating a mixture of two or more different particle types. In this case particles must be discriminated by subjecting them to differential forces.

It is possible to apply a field having a frequency between the respective crossover frequencies of two dissimilar particle types. In this case, one fraction of particles experiences negative DEP and moves towards local field minima at the center of, or between, electrodes while the other fraction is trapped by positive DEP at electrode edges. This method has been termed differential DEP affinity separation. This method can be extremely effective if the dielectric properties, and therefore the crossover frequencies, of the particle types are greatly dissimilar. For example, DEP has been applied to the beneficiation of ores [82] and dewatering of aqueous dispersions of clays [28], bacteria have been separated from mammalian cells [14], blood cells from cancer cells [83,84], normal from malaria-infected blood cells [85], CD 34[+] stem-cells from blood [86], live cells from dead cells [15], and cells from debris.

If the dielectric properties of the different particle types are not significantly different, as is the case in many mammalian mixed cell systems, for example, their DEP crossover frequencies will be close. Because particles experience weak DEP forces close to their crossover frequencies, the differential driving forces acting to separate the particles will be very small. Particles of one type may become entrapped within rafts of the other, especially when the particle loading is high, and a low separation efficiency will be achieved. This problem can be relieved somewhat by using a slowly frequency-modulated field that provides negative DEP to all the particles for part of each modulation cycle [83]. This relieves particle packing and encourages particles to migrate to their low energy positions on the electrode array. However, when in close proximity, similar particles undergoing DEP experience attractive dipole-dipole interactions [87] and therefore like particles tend to cluster making it harder to release the entrapments. This constrains the particle loading density for differential DEP affinity separations. As a result of these complications, it has been found impractical to separate particles having less than a 50% difference in their crossover frequencies using DEP differential affinity alone. This is sufficient for separating such distinctly different particle types as bacteria from blood cells but is inadequate for many mammalian cell applications.

Traveling wave dielectrophoresis (twDEP), the dielectric equivalent of a linear motor, may be used to increase particle discrimination. twDEP is dependent on the out-of-phase component

of particle polarization and this has a different frequency dependency from the in-phase component responsible for DEP trapping and repulsion [88-91]. By judicious choice of frequencies, DEP trapping and repulsion may be combined with twDEP. In this way, particles [92-94], tumor cells [95], yeast [96], and malarially-parasitized cells [85] have been focused at the center of spiral electrodes or transported laterally. To increase the flexibility of DEP for sample separations, it is useful to remove at least one particle type from the DEP electrode for subsequent analysis. One way is to flush the untrapped particles from the chamber. To understand the best approach to this, the hydrodynamic principles inherent in fluid flow will now be considered in the microfluidic context.

6 Field-flow fractionation

Field-flow fractionation (FFF) is a family of methods [97] in which force fields are applied to particles to position them characteristically within the velocity profile of a fluid flow stream. Particles having different characteristics are carried at different velocities in accordance with their positions in the stream. In this way, a sample plug containing a mixture of particle types can be introduced at one end of a flow channel and fractionated as they are carried through it at different rates, causing the different particle types to emerge at different times. Giddings [97] and others [98-100] elucidated three primary modes of FFF: normal, steric, and hyperlayer. Normal FFF, the first to be studied, involves thermal diffusion profiles of sub-µm-sized particles. As a rough guide, Brownian motion and thermal diffusion are negligible for particles above about 1 µm in diameter at room temperature. We will confine ourselves to larger particles for which DEP coupled to FFF has been studied. In steric FFF, the applied force causes the particles to impact one side of the separation chamber causing them to experience steric hindrances that diminish their velocity in the flow stream. In hyperlayer FFF, particles are positioned away from the chamber walls at an equilibrium height in the flow stream and are carried at the velocity of the fluid at that height.

6.1 Steric DEP-FFF

Particle trapping in a fluid flow stream using positive DEP forces is a form of steric FFF. The particle behavior can be understood in terms of three main forces, the DEP force, the particle sedimentation force, and the hydrodynamic lift force (see Fig. 3). The latter force is present whenever a particle is placed in a flow velocity gradient and tends to move the particle away from the wall into the faster flow. In microfluidic systems, where the characteristic dimensions are small, the Reynolds number for flow velocities used in particle separations is correspondingly small and laminar flow profiles occur spontaneously in microchannels when fluid flow is present. At viscosities associated with aqueous samples, the flow velocity profile $v_p(h)$ is almost perfectly parabolic,

$$v_p(h) = 6 \langle v \rangle \frac{h}{H} \left(1 - \frac{h}{H} \right)$$

(5)

where h is the height above the chamber bottom, $\langle v \rangle$ is the mean fluid velocity, and H is the chamber height. The net force resulting from the interaction of positive DEP, hydrodynamic lift and sedimentation forces for a horizontal separation chamber configured with the DEP electrodes on the chamber floor is shown in Fig. 3. Under conditions where the positive DEP force is sufficiently strong, there is only one equilibrium condition (where the net force crosses zero), for particles trapped on the electrode plane. In this stable equilibrium particles experience a strong local DEP force balanced by steric forces from the chamber floor. As the DEP force decreases (as may occur when particles are exposed to fields close to their crossover frequency) there is a regime under which the net force includes up to three equilibrium positions. The first corresponds to DEP trapping on the electrode plane as before (Fig. 3A). However, particles

just above the electrode plane experience a second, unstable equilibrium position where DEP and lift forces balance (Fig. 3B). Further above the electrode plane, particles experience yet a third equilibrium position where hydrodynamic lift and sedimentation forces balance (Fig. 3C).

In practice, it is found that because of vibration, uneven fluid flow, and field imperfections on large electrode arrays, particles will move between equilibrium states as they progress through a separation channel, resulting in a broad, ill-defined elution profile. This analysis reveals that different particle types may be separated effectively if one type is trapped strongly by positive DEP while the other experiences a negative DEP force but that for particles having similar crossover frequencies care must be taken when operating in this multi-equilibrium regime. This is most problematical if separation is conducted by first trapping particles and then attempting to flush one fraction of them from the chamber by decreasing the DEP force or by increasing the flow rate. In this case, many particles may remain trapped that can later become stably levitated by lift forces under the very same conditions during a separation. The effect can be overcome if particles are always trapped from a levitated position in the moving fluid, ensuring that they enter a stable trapping regime where the DEP force is strong enough to overcome the hydrodynamic lift force at all heights (see Fig. 3). The corollary is that to ensure good separation of particles by DEP trapping, a particle mixture should never be trapped and then partially released by increasing the flow rate, lowering the applied voltage, or reducing the field-frequency. If sequential release of multiple particle types from a trapped mixture is desired, it should be accomplished by releasing and levitating the entire population of trapped particles and then by retrapping all but the next fraction to be eluted under the flow and field conditions to be used for the separation. The equilibrium resulting from a balance of hydrodynamic lift and sedimentation forces may be eliminated by orienting the electrode plane vertically so that the sedimentation force is orthogonal to the lift force. This has been successfully accomplished, and the sedimentation forces exploited to improve the separation characteristics [101]. Nevertheless, this method does not eliminate the metastable equilibrium between hydrodynamic lift and positive DEP forces. A lack of appreciation for the existence of multiple equilibria represents a major caveat for the DEP-affinity method.

The electrode configuration is another important consideration for optimizing separation efficiencies when DEP trapping is used. An early and important configuration introduced by Pethig's group was the castellated, interdigitated electrode geometry [102]. This popular configuration has the advantage that it provides multiple field maxima and minima with a range of magnitudes that are ideal for particle manipulations in the absence of fluid flow and it has led to significant advances in the understanding of particle manipulations by DEP. In flow-based separations, however, the presence of regions in the unit geometry that have different trapping characteristics is a disadvantage because similar particles may be released from those regions under different field and flow conditions. A simpler electrode array comprising two interlaced combs of plane-parallel fingers provides uniform trapping characteristics in the fringing fields at the electrode edges because all electrode edges, electrode surfaces and electrode gaps have identical respective characteristics. The force characteristics for periodic electrode geometries have been modeled by rapid methods [103,104].

6.2 Hyperlayer DEP-FFF

In order to overcome the disadvantages of particle-particle interactions and multiple equilibrium heights inherent in particle separations employing steric FFF with positive DEP trapping, hyperlayer DEP-FFF was developed [97]. In this mode, particle separation is accomplished by balancing a levitating negative DEP force against gravity or another force that directs particles towards the electrode array. In sedimentation DEP-FFF, the levitating effects of hydrodynamic lift forces and negative DEP are balanced against the particle sedimentation force (Fig. 4). The DEP force has been shown to fall exponentially with height

above the electrode array [105]. Particles are carried through the chamber at velocities given by Eq. (5) according to their respective equilibrium levitation heights. The force balance equation is always single-valued for hyperlayer DEP-FFF, yielding a unique equilibrium position and elution time for all particles of the same type. The differential velocities of different particle types are maintained as the particles traverse the length of the chamber yielding a spatial separation that is cumulative - greater separation can be obtained by increasing the chamber length. In contrast, separation by DEP trapping is effectively a single-shot effect.

An especially important characteristic of the hyperlayer DEP-FFF separation mode is that the discriminating ability is a function of the levitation height. This is because the flow velocity gradient is height-dependent. Since particle height can be adjusted by altering the applied frequency and voltage, the discrimination can be electrically programmed. Furthermore, as the DEP crossover frequency is approached, the DEP levitation force also decreases. Therefore, in contrast to the differential DEP affinity method, the discriminating power in the hyperlayer DEP-FFF separation mode increases as the crossover frequency is approached [105,106].

In practice, steric and hyperlayer DEP-FFF may be used simultaneously to separate complex particle mixtures. Particles having a low crossover frequency can be firmly trapped while particles having higher crossover frequencies may be separated with high discrimination by hyperlayer DEP-FFF in the same channel. Later, the field frequency may be adjusted to release the initially trapped particle type and subject it to its own high discrimination separation. In this way complex particle mixtures may be fractionated in a sequence of electronically programmed steps. In such sequential separations it is important to briefly release the trapped particles by negative DEP before the frequency is adjusted to its new value in order to avoid the multi-equilibrium problem discussed above for steric FFF. A number of groups have successfully applied DEP-FFF to particle separations, including latex microspheres [105, 107,108], stem cells from tumor cells [109,110] and blood cell subpopulations [111].

In a variation of the hyperlayer DEP-FFF method, twDEP has been combined with DEP levitation to allow lateral displacement of particles based on the imaginary part of their dielectric permittivity [112]. The DEP force has the advantage that it falls exponentially with height above the electrode plane and can be used to balance any opposing force whether or not that force exhibits spatial dependence. Therefore, additional forces may be applied to facilitate DEP-FFF fractionation. For example, a balance of positive magnetophoretic and negative DEP forces has been used to separate mixtures of magnetic-antibody labeled cells in a technique called MAG-DEP-FFF [113].

7 Particle handling by DEP deflection

As already discussed, negative DEP forces can be used to spatially confine particles. This is not limited to confinement in a static reservoir, but can also be used to focus particles in a moving fluid. For example, DEP has been used to focus diffuse particle suspensions into a well-ordered streams of particles for subsequent trapping [114,115] and this method has been used to focus particles prior to impedance sensing [116]. Unbalanced DEP forces may also be used to deflect, rather than focus, the trajectories of particles in a flow stream in accordance with particle dielectric properties.

8 Conclusions

DEP is a very versatile force than can be used to manipulate and discriminate between different particle types in fluid suspensions. It accesses a wide range of particle characteristics through the frequency-dependent dielectric properties of particle materials and structures and can be modified by the choice of suspending medium. The field gradients needed to produce DEP

forces can be readily produced by small electrodes produced inexpensively by lithographic methods. The structures involved are extremely simple and require no microelectromechanics. The voltage levels typically required for DEP manipulations are sufficiently low to be produced by microelectronic circuits that can be interfaced directly to the electrode arrays. DEP devices are typically simple flow-through chambers that are easy to integrate with other elements in μTAS. Despite the versatility of DEP and the ease with which the necessary structures can be made, this review makes clear that the successful application of DEP to separation problems does demand thought and awareness of a number of confounding factors including charge double layer, electrode polarization, thermal convection and ac electrokinetic effects. In addition, the apparently simple approach of trapping one particle type by DEP while repelling another demands attention to detail if the dielectric properties of the different particle types to be separated are not greatly different. Nevertheless, when these issues are understood, it is possible to control or avoid them, usually simply by choosing different operating conditions for the separation. Figure 5 is offered as a hopefully useful guide to the typical frequency ranges in which different DEP phenomena are helpful and in which certain caveats appear. The diagram applies to separation applications in an aqueous suspending medium having a conductivity of 50 mS/m which is applicable to many existing cell studies. Charts of this type may be constructed for other operating conditions to assist in planning particle separation characteristics.

Finally, although charge double layer and electroconvective effects have been discussed here as caveats, this is so only because these phenomena have yet to be fully understood and incorporated into the design process. It is to be anticipated that once they can be adequately modeled and predicted these phenomena will take their place in the toolbox of useful electrokinetic effects for separation applications alongside electrophoresis, electroosmosis, and dielectrophoresis.

Abbreviations

AC, alternating current; DEP, dielectrophoresis; FFF, field-flow fractionation; μTAS, micro total analysis systems; twDEP, traveling-wave dielectrophoresis.

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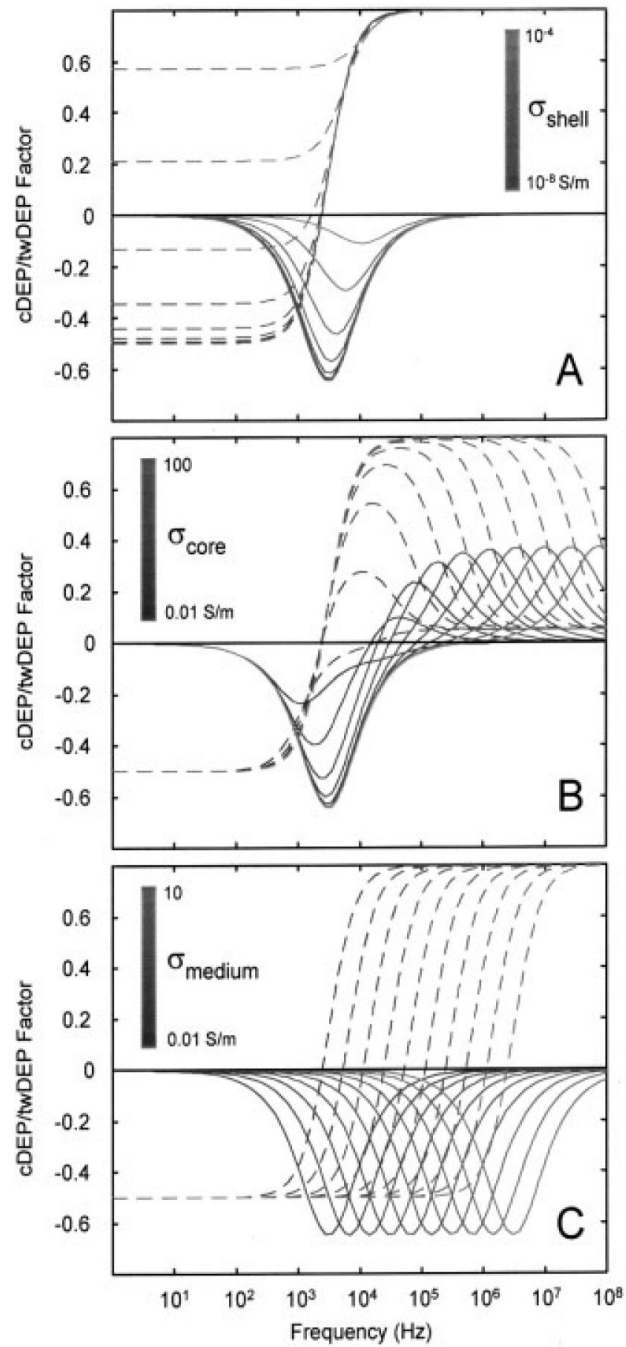
Figure 1.
DEP responses of a model particle comprising a thin insulating shell over an electrically conductive core. The DEP response was calculated from the shell model (see text) as a function of (A) varying the conductivity of the thin outer shell from 10^{-8}-10^{-4} S/m, (B) of the core from 0.01-100 S/m, and (C) varying the conductivity of the suspending medium from 0.01-10 S/m.
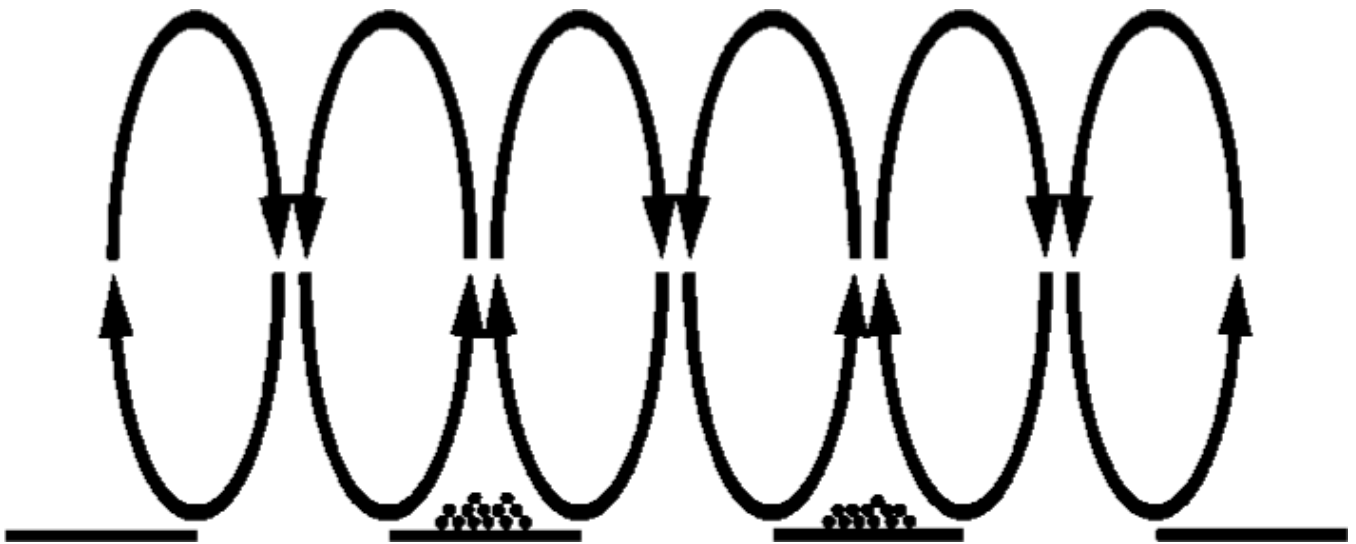
Figure 2.
Particle trajectories in convection cells observed over a periodic electrode array. Such effects result at low frequencies from energy coupling between the applied electric field and the suspending medium (see text).
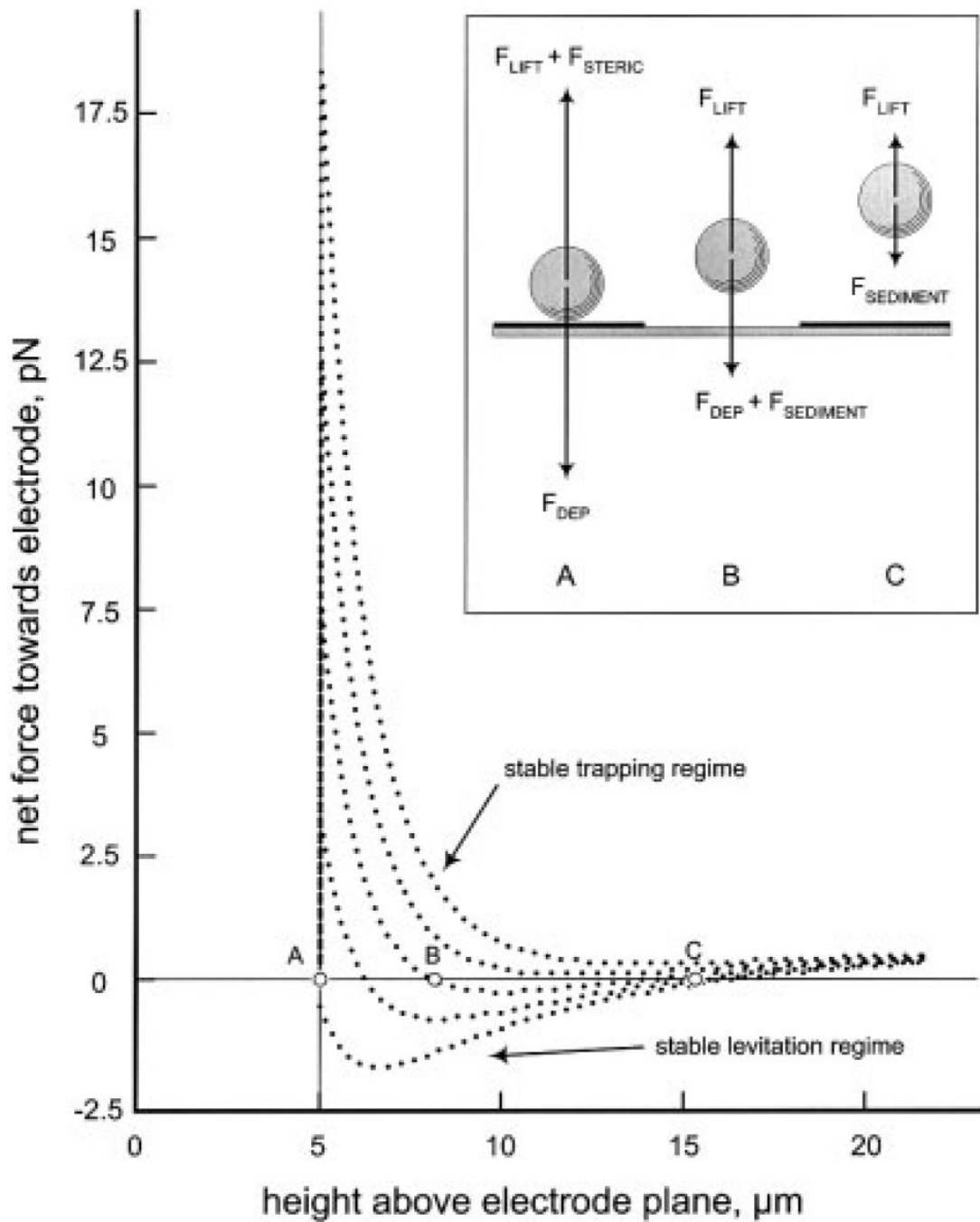
Figure 3.
The sums of hydrodynamic lift, positive DEP and sedimentation forces acting on a particle in a fluid flow stream are shown at five frequencies close to the crossover frequency. Between a stable trapping regime and a stable levitation regime, for which there are unique equilibrium positions, lies a regime characterized by multiple equilibrium positions. For example, the middle profile possesses the three equlibria that correspond to the balances of forces shown in the inset.
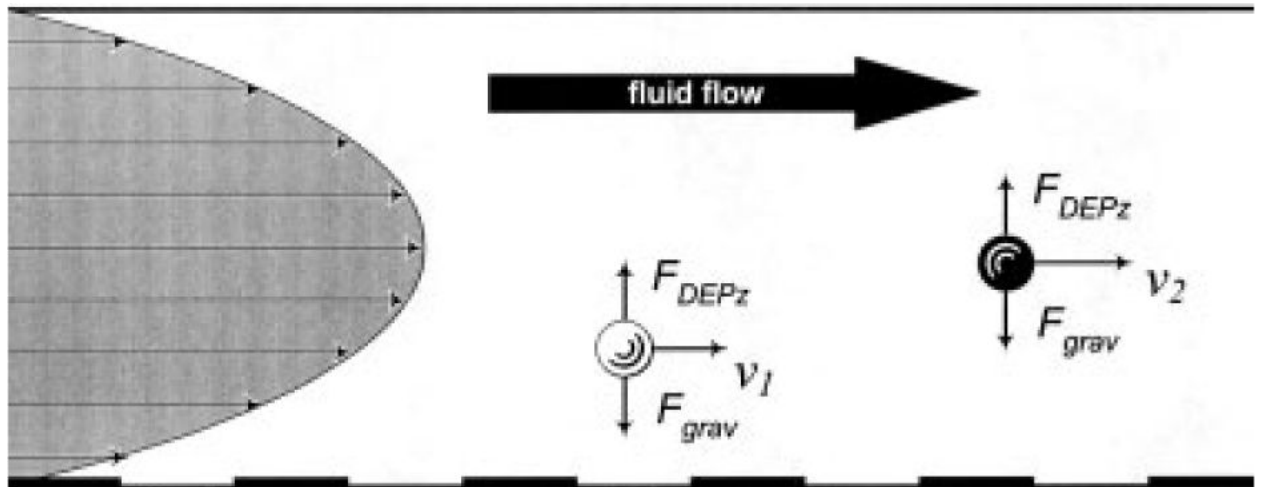
Figure 4.
Principle of hyperlayer DEP-FFF. An array of parallel electrodes on the lower surface of the DEP-G/FFF channel is used to generate an inhomogeneous, fringing electric field above the electrode edges. The magnitude and frequency of the field are chosen such that negative dielectrophoretic forces F_{DEPz1} and F_{DEPz2} act to levitate the two particles in the system. Both the electric field strength and field inhomogenity decrease with increasing height above the electrode plane and the DEP force decreases approximately exponentially with height. In addition to the DEP force, a sedimentation force F_{GRAV} acts on each particle. Particles are driven to an equilibrium height h where the sedimentation and levitation forces are balanced. Particles possessing different density or dielectric properties are levitated to different characteristic heights in the flow-velocity profile and attain different velocities V_1 and V_2 in the chamber, and are thereby fractionated.
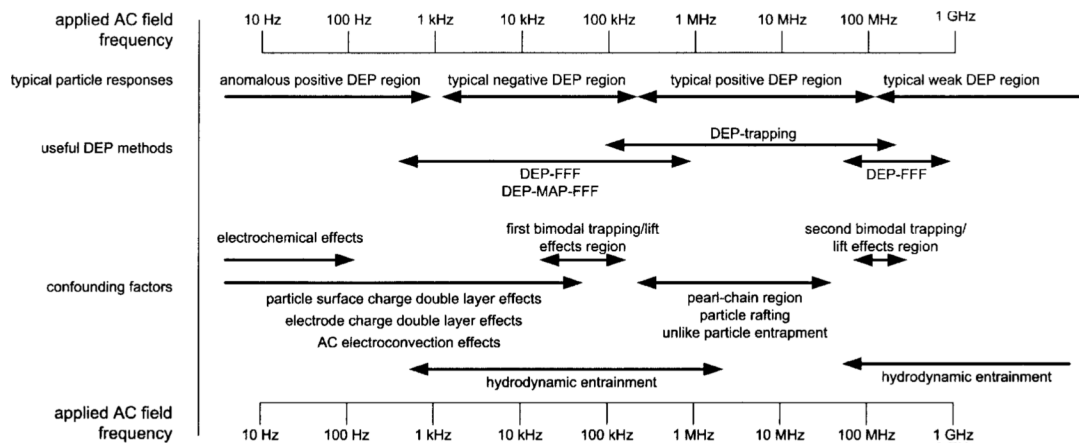
Figure 5.
Guide to frequency bands in which various DEP phenomena occur, some DEP methods are applicable, and a number of confounding effects may occur. The guide applies to aqueous media having a conductivity of the order of 50 mS/m. The particle responses shown would be typical of mammalian cells. Similar guides may be constructed for other conditions.