Electrorotation and dielectrophoresis

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SUMMARY

Using microelectrode structures, various forms of electric fields, such as non-uniform, rotating and travelling wave, can be imposed on particles of sizes ranging from proteins and viruses to micro-organisms and cells. Each type of particle responds to the forces exerted on them in a unique way, allowing for their controlled and selective manipulation as well as their characterization. Moreover, particles of the same type but of different viability can be distinguished in a simple, reliable manner. This review outlines the principles that govern the way in which bioparticles respond to these various field types, and how they can be exploited. Examples of current and potential biotechnological and biomedical applications are given, along with a critical comparison of current techniques.

Key words: Dielectrophoresis, electrorotation, travelling wave.

INTRODUCTION

The induced motion or orientation of bio-particles in electrical fields has been observed for over 100 years (e.g. Lortet, 1896). Until comparatively recently, only particle motion or *phoresis*, induced by DC electric fields was studied. From the generic idea of electrophoresis, a whole new branch of novel electrokinetic manipulation methods of bioparticles has arisen, simply by taking advantage of another dimension, the particle response to the frequency of the field. This review describes some kinetic techniques of bio-particles using AC fields.

INNATE ELECTRICAL PROPERTIES OF PARTICLES

In order to understand the interactions of a particle with an electric field one must first consider the innate electrical properties of that particle. A generalized bioparticle suspended in an aqueous solution (weak electrolyte) is represented in Fig. 1 with the relative distribution of innate charges, both bound and free. Many of the molecules that make up biological particles possess ionizable surface chemical groups such as COOH or NH_2. The ionizable head groups of lipids in the plasma membrane are one such example and because of these the particle possesses a net surface charge. An electrostatic potential due to these charges will be present around the particle, the effect of which decreases to that of the bulk medium with increasing distance from the particle (Pethig, 1979). Ions of opposite charge, counter-ions, to those on the surface will be attracted towards the particle by this electrostatic potential. Together, the bound surface charges and the surrounding counter-ion atmosphere, shown as the cation dense region in Fig. 1, form what is termed an electrical double layer.

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APPLICATION OF A DC FIELD TO PARTICLES

On the application of a DC electric field across the bioparticle, all the charges, bound and free, in the system will be attracted to the electrode of opposite polarity, see Fig. 2. Assuming the solution to be more or less neutral only relatively small concentrations of H^+ and OH^- will be present, ions such as Na^+ and Cl^- will carry the bulk of the current. Those ions associated with the electrical double layer will respond to the field forming an asymmetric distribution around the particle (Hartley, 1935), the new equilibrium of which is established by the magnitude of the electric field and the opposing ionic concentration diffusion gradient, which tends to restore the random, symmetrical distribution (Bone & Zaba, 1992). Any motion of the particle towards the electrodes in a DC field is due to the net surface charge. Human erythrocytes, for example, in a standard saline solution under the influence of a DC field of $1\ V\ cm^{-1}$ migrate towards the anode at around $1\ \mu m\ s^{-1}$. Particle separation is therefore possible due to differences in their mobility in an electric field, which may be due to their size, mass or charge. Ruess (1809) was first to use this technique (on clay particles) which, one hundred years later, Michaelis (1909) termed electrophoresis. Later, Coulter (1920) extensively investigated cell surface properties.

Whereas bound charges and polar molecules in the system may orientate in the field, free charge carriers (e.g. ions) will migrate towards the electrodes, that is unless they encounter a material with different electrical properties. Ions encountering the plasma

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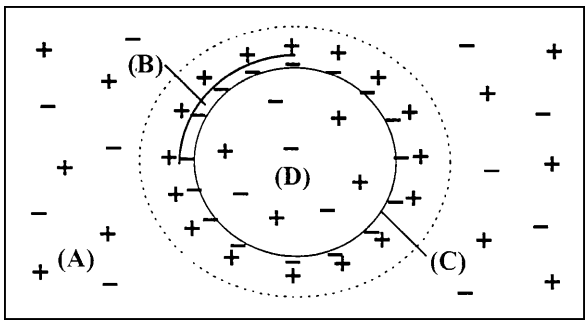

Fig. 1. A simplified cell (solid circle) suspended in an aqueous medium at neutral pH showing the relative distributions of charge, both free and bound. Approximate conductivity (σ = S m^{-1}) and relative permittivity (ϵ_r, where air = 1) of the bulk solution (A) $\sigma = 10^{-4}$, $\epsilon_r = 80$, cell wall (where present) (B) $\sigma = 10^{-2}$, $\epsilon_r = 60$, membrane (C) $\sigma = 10^{-7}$, $\epsilon_r = 9$, and interior (D) $\sigma = 10^{-1}$, $\epsilon_r = 70$ for a typical viable cell.

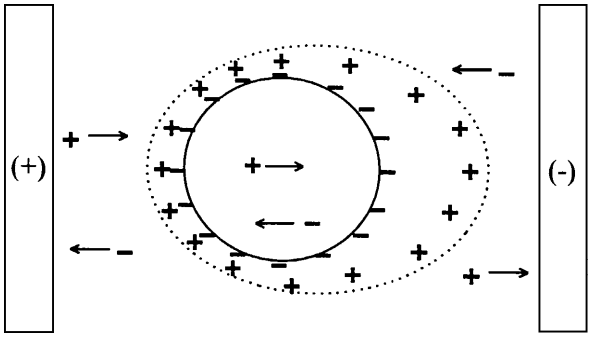

Fig. 2. On the application of a DC electric field to a cell in aqueous solution, charges will experience a force towards the oppositely charged electrode. Ions in the bulk solution are free to migrate to the electrodes, whereas charges associated with the *electrical double layer* are restricted and show a distortion or polarization.

membrane, will be prevented from free motion towards the electrodes by this membrane if it is intact. The membranes of viable cells are only semi- (selectively) permeable to ions and non-lipid soluble molecules (i.e. are relatively non-conducting). The conductivity of the cell membrane tends to be around 10^{-7} S m^{-1}, some 10^7 times less conductive than that of the interior which can be as high as 1 S m^{-1}. For particles the size of erythrocytes, then within about a microsecond after the application of an electric field, the ions will have fully built up on the particle boundary forming an aggregation of interfacial charges. The first description of interfacial charges in heterogeneous materials was given by Maxwell (1891) for DC fields, the theory of which was later elaborated by Wagner (1914) who extended the theory to include AC fields.

Importantly these induced charges are not uniformly distributed over the bioparticle surface, forming predominantly on the sides of the particle facing the electrodes. These charges and the distorted electrical double layer lend to the particle the properties of an electrical dipole moment, m. This dipole moment is in the order of $2{\cdot}5 \times 10^5$ debye units (D) for a cell of 5 μm diameter, (*c.f.* 1·84 debye for a water molecule); the cellular dipole moment is therefore described as macroscopic, although the magnitude of the induced charge is still only a fraction, around 0·1 %, of the net surface charge carried by cells and microorganisms (Pethig, 1991).

APPLICATION OF AN AC FIELD TO PARTICLES

If we now consider the application of an alternating field to a particle, we see that various phenomena occur over different frequency ranges of applied field. Starting close to the DC condition, with a field that reverses direction a few times a second, the particle motion is dominated by electrophoretic forces. The particle may follow reversals of the field electrophoretically for frequencies up to a few hundred Hz, where reversals of the field take less than a few milliseconds. Because of the particle's inertia, this electrophoretic motion becomes vanishingly small for frequencies above around 1 kHz.

Other mechanisms can respond to field reversals of much higher frequencies such as the dynamic behaviour of the electrical double layer distortion or polarization around cells. This can follow changes in field direction that take as little as a few microseconds. Any faster than this (i.e. frequencies $>$ 50 kHz) then the counter-ion cloud around cells does not have time to distort. Like the fall off in the electrophoretic motion with increasing field frequency the decrease in response of the double layer to the changing field occurs gradually over a range of frequencies, this is termed a dispersion.

Interfacial polarizations are even more responsive to changing field directions and for sub-cellular sized particles can take as little as tens of nanoseconds to respond to a reversal in field direction, they can therefore exert their influence up to frequencies of 50 MHz and beyond. This is still nowhere near as responsive as small polar molecules such as water to alternating fields. A measure of the ability of molecules in a material to align in an electric field is given by the relative permittivity of that material, which for bulk water molecules at 20 °C in an alternating field less than 500 MHz has a value of 80. At frequencies above 100 GHz the relative permittivity of water falls to that typical of non-polar molecules, around 4. A similar fall in permittivity is seen above about 50 kHz on the freezing of water, because the molecules of the liquid become restricted in a solid lattice and can no longer rotate to align with the field.

On cell death, membrane integrity is lost, it becomes permeable to ions and its conductivity

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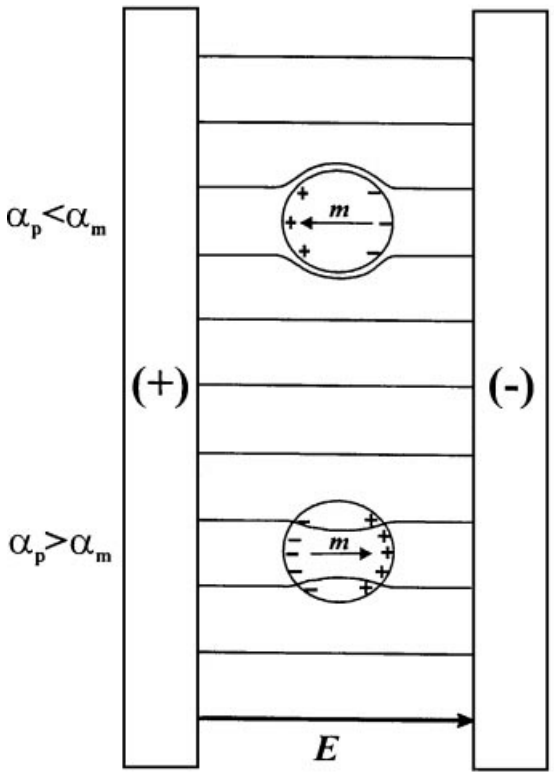

Fig. 3. Two particles of different polarizability are shown in a uniform AC electric field created between parallel electrodes. The alignment of the induced dipole moment m in the particle by the field E depends on whether the polarizability (α) of the particle is greater or less than that of the suspending medium. AC homogenous fields impart no motion on electrically neutral particles, due to the symmetry of forces on the sides of the particles.

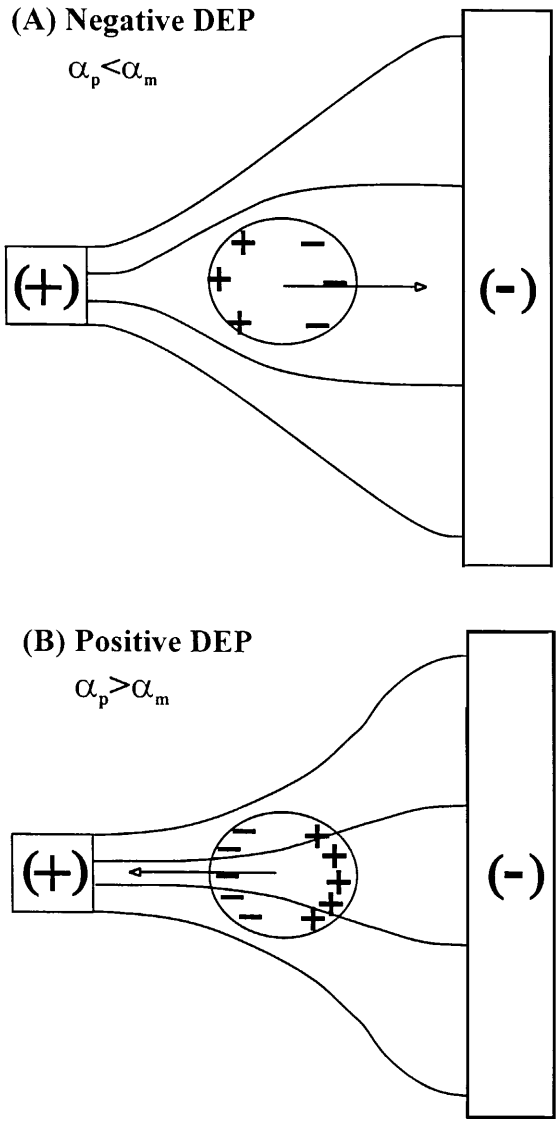

Fig. 4. Two particles of different polarizability in a non-uniform (inhomogenous) electric field. Particle (A) is less polarizable than the surrounding medium and so is directed away from the strong field region, whereas particle (B) of greater polarizability than the medium is attracted towards the strong field at the pin electrode. These effects occur whether or not the field is DC or AC.

increases by a factor of about 10^4 with the cell contents freely exchanging material with the external medium. This transition in the properties of the membrane shows up as a large change in the polarizability of the cell in an electric field. Other causes for particles having different polarizabilities include differences in their morphologies or structural architecture, which may be associated with the cells belonging to different species, different stages of differentiation or physiological state. Two such particles, that differ in polarizability, are shown in Fig. 3 subjected to an alternating homogenous field created between 2 parallel electrodes. The direction of the dipole moment formed by the interfacial charges is shown to depend on the relative polarizabilities of the particle compared with the medium.

PARTICLE MOTION IN AC INHOMOGENOUS ELECTRIC FIELDS, DIELECTROPHORESIS

Homogenous AC electric fields do not induce motions in electrically neutral particles, due to equal forces acting on both sides of the polarised particle. If the particle carries a net charge, it will oscillate back and forth as a result of electrophoresis. As the frequency increases these translational oscillations become vanishingly small. Net translational motion is possible, however, if instead the field is inhomogeneous (see Fig. 4). To distinguish this force from electrophoresis, Pohl adopted the term dielectrophoresis (DEP) (Pohl, 1951) from the term *dielectric* which is used to describe liquid and solid materials of low conductivity. For example, an intact membrane is a dielectric material characterized by having a conductivity 10^{16} times smaller than copper and a dielectric permittivity 3 times that of air. Examples

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Table 1. Examples of particle types investigated by non-uniform AC electric fields (DEP)

Acellular	Virus	Trapping of single virion, *Herpes simplex* type 1 (Hughes & Morgan, 1998)
Prokaryotes	Bacterial	Characterization and separation of bacteria (Markx *et al*. 1994*a*, 1996)
Eukaryotes	Protozoa	Differentiation between normal and *Plasmodium falciparum* infected erythrocytes (Gascoyne *et al*. 1997*b*)
	Yeast	Batch separation of viable and non-viable (heat treated) *Saccharomyces cerevisiae* (Markx *et al*. 1994*b*)
	Plant cells	Batch separation of plant cells from mixture containing yeast and bacteria (Markx & Pethig, 1995)
Mammalian cells	Cell lines	MDA231 human breast cancer cell separation from erythrocytes and T-lymphocytes (Gascoyne *et al*. 1997*a*)
	Lymphocyte	Removal and collection of human leukaemia cells from blood (Becker *et al*. 1994)
Other particles	Proteins	Collection of proteins, e.g. avidin 68 kDa and ribonuclease A 13·7 kDa (Washizu *et al*. 1994)
	DNA	Separation of different sizes of DNA (9–48 kb) using positive DEP with FFF (Washizu *et al*. 1994)
	Liposomes	Alignment of cell size liposomes for subsequent electrofusion (Stiocheva & Hui, 1994)
	Artificial nanoparticles	Separation of latex beads of diameter 93 nm, with differing surface charge (Green & Morgan, 1997)

of some particles investigated with DEP are given in Table 1.

CHANGING THE DEP FORCE WITH THE MEDIUM CONDUCTIVITY

It was illustrated in Fig. 4 that the polarity of the force exerted on the particle depends on the polarity of the induced dipole moment, which in turn is determined by the relative polarizability of the particle and the medium. As a consequence, by altering the polarizability of the medium one can control the direction of motion of a particle. This principle can be exploited to gain particle separations by choosing a suspending medium with an intermediate polarizability, that is between the polarizabilities of two particles in the mixture, so that each particle type will be under the influence of a DEP force of different polarity. Selective manipulation using DEP force has been used to enable separations of various interspecific mixtures such as some Gram +ve and Gram −ve bacteria (Markx *et al*. 1994*a*), as well as the intraspecific separation of live and dead cells (Markx, Talary & Pethig, 1994*b*) or cancerous from normal cells (Becker *et al*. 1994; Gascoyne *et al*. 1997*a*). Differences between normal and parasitized cells within a population, for example erythrocytes parasitized with *Plasmodium falciparum* have also been observed (Gascoyne *et al*. 1997*b*), and so separation of these two by DEP should also be possible. Examples of separations already demonstrated are listed in Table 2, along with the appropriate medium polarizabilities (conductivity) and field frequency. The DEP force imparted on a particle by an electrical field is also proportional to a number of other factors; the particle size (Pohl, 1978; Pethig, 1979) shape (Pethig, 1991) and the magnitude and degree of non-uniformity of the applied electric field.

The electrode geometry is very important in maximizing the forces on the particles. For example, small and sharply pointed electrodes create strong field gradients, and therefore large DEP forces. Microelectrodes and the relatively low conductivity required for these separations both have the advantage of reducing heat production at the electrodes due to electrolysis. Fabricated using standard photolithographic techniques, they typically take the form of thin 0·1 μm layers of gold on chromium, evaporated on glass (microscope slide size) substrates. One design, the interdigitated castellated electrodes (Fig. 5), through their geometry provide an efficient means of repeating regions of high and low field gradient, which, when fabricated over large areas, provide the means of large-scale separations of particles.

Separation of particles under positive and negative DEP can be achieved either by gravity or fluid flow over the electrodes. This selectivity removes the less immobilized particles under the influence of negative DEP which can then be collected. Those cells still held, under positive DEP, can be released by turning off the field and collected in a similar manner. Separation chambers based on this mechanism are usually composed of 2 electrode arrays sandwiching a thin layer of fluid. Thin chambers are used because the DEP force decays with distance in a near exponential manner, and an effective DEP force is considered to extend no further than 300 μm from

Table 2. Values of suspending medium conductivity and voltage frequency used to dielectrophoretically separate cell mixtures

	Cell mixture	Conductivity (ms m^{-1})	Frequency	Released cell	Ref.
Escherichia coli (Gram −ve)	*Micrococcus luteus* (Gram +ve)	55	100 kHz	*E. coli*	Markx *et al.* 1994*a*
Erythrocyte	*M. luteus*	10	10 kHz	Erythrocyte	Wang *et al.* 1993
Viable yeast	Non-viable yeast	1	10 MHz	Non-viable	Wang *et al.* 1993; Markx *et al.* 1994*a*
Leukaemic cells	Blood cells	10	80 kHz	Blood cells	Becker *et al.* 1994
Erythrocytes	Breast cancer cells	10	80 kHz	Erythrocyte	Becker *et al.* 1994
Bone marrow	Peripheral blood	1	5 kHz	CD34+	Talary *et al.* 1995; Stephens *et al.* 1996

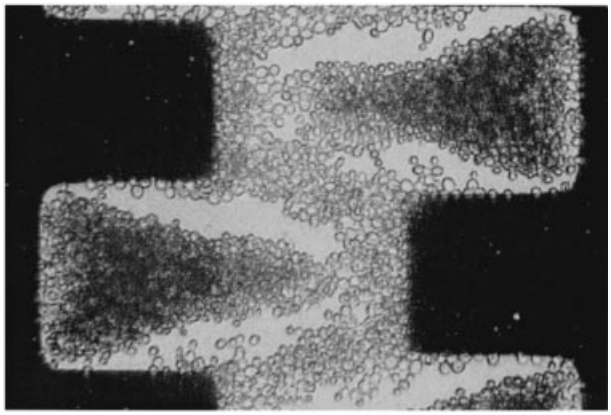

Fig. 5. By applying a 4 MHz signal to a cell suspension on castellated interdigitated electrodes, healthy and non-viable cells can be separated. Non-viable cells stained by a dye, experience a negative force and collect into loosely held triangular formations. The unstained viable cells, experience a positive dielectrophoretic force and collect in chains between opposite castellations (Pethig *et al.* 1992).

the plane of the micro-electrode. Despite this possible limitation, separations of more than 10^4 cells sec^{-1} are still possible.

CONTROLLING THE DEP FORCE WITH FIELD FREQUENCY

The polarizability of a particle changes as a function of the frequency of the applied field. A single particle may therefore exhibit both positive and negative dielectrophoresis as its polarizability changes over a frequency range, for a constant medium conductivity. A typical DEP frequency spectrum with such a transition is shown for a live yeast cell (Fig. 6). Also represented is the DEP spectrum for a dead yeast cell, which only experiences a change in the polarity of DEP force for frequencies greater than a few MHz.

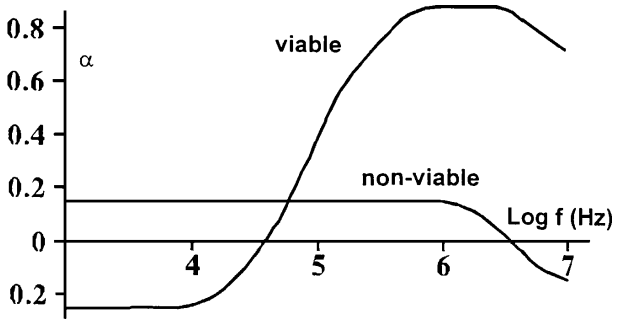

Fig. 6. The variation of the particle polarizability α as a function of the frequency of the applied electric field for viable and nonviable yeast cells in a suspending medium of 8 mS m^{-1}. (Adapted from Talary *et al.* 1996).

LEVITATION OF PARTICLES BY DEP

Contact with the electrode induced by positive DEP may impinge on subsequent removal of the particle (e.g. by fluid flow or gravitational forces). The attractive or repulsive forces on the particles by DEP so far described are for interactions where both the particle and electrode are in the same plane, the particle resting on the substrate. These forces can also be applied to particles to make them levitate above the substrate, either from the result of an attractive high field region presented above the particle in the form of an electrode probe (Kaler & Jones, 1990) or by the repelling action of interdigitated electrodes on the plane of the glass, where the particle can be confined in a stable position above the electrodes (Markx, Rousselet & Pethig, 1997). Particle levitation can be combined with other techniques, for example field flow fractionation (FFF), whereby particles levitated to different heights (up to 100 μm and above) are exposed to different rates of fluid flow (Markx *et al.* 1997). Negative DEP forces can also be exerted from both above and below the particle to trap single particles in a '3D field cage', as was demonstrated for artificial particles by Schnelle *et al.* (1993).

Another application for DEP is the manipulation

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of cells prior to electrofusion. Attractive interactions between the induced dipoles of adjacent cells can result in the formation of chains of cells (pearl chains) of variable length. Close cell contact, followed by a high field strength DC pulse(s) of kV/cm and ms duration can lead to cell fusion of two to several thousand cells, so that giant cells can be formed as well as hybrid cells with two nuclei (Nuemann, Sowers & Jordan, 1989).

ARE CELLS DAMAGED?

To induce cell fusion, or indeed electrical breakdown of the cell membrane, a field strength of at least 10 times more than is typically used in DEP separations is required. Hybrid cells from electrofusion are viable, which suggests that cells having undergone exposure to normal DEP forces are not damaged. Further evidence was given for dielectrophoretically separated erythrocytes which excluded trypan blue (Becker *et al*. 1994) and for yeast cells (Markx *et al*. 1994*b*) and CD34+ cells (Stephens *et al*. 1996) which were successfully cultured following exposure to DEP separation. Damage by the fluid flow has also been considered, the maximum shear stress exerted on the cell is around 3 dyn cm^{-2} (Pethig, 1996). Erythrocytes have been reported to be able to withstand a shear stress 500 times this value (Leverett *et al*. 1972), and T-lymphocytes over 50 times (Chittur, McIntire & Rich, 1988), therefore, almost insignificant levels of shear stress are experienced by these cells in DEP chambers. The conductivity of suspending medium used is normally much below that of a normal physiological medium, however, as long as the osmolarity is of the right value, osmotically-sensitive cells can be investigated. This is achieved by additives such as sucrose at 280 mM, which has little effect on the conductivity. An alternative approach has been that of Fuhr *et al*. (1994) who, using sub-micron electrodes which minimize heating effects, have been able to use normal physiological strength media.

DEP: CONCLUDING REMARKS

The method is non-invasive and does not require knowledge of cell surface antigens or the use of antibodies, or other reporter molecules to label bioparticles, although in some applications the use of specific markers or dielectric labels may be an advantage (Burt *et al*. 1996). DEP can be employed at either the single-cell or multicell (more than 10^4 cells sec^{-1}) level, and it has already been demonstrated for a variety of applications, notably: the purification of cell cultures by DEP separation of non-viable or contaminating species, the isolation or enrichment of cell sub-populations, also the rapid isolation of toxic micro-organisms in water and food. Separation of micro-organisms from faeces is seen as a potential for this technique, for example the isolation of trophozoites of pathogenic protozoa from stools prior to culture. DEP can also be used to manipulate cell orientation prior to electroporation and to juxtapose cells prior to electrofusion. Finally, manipulation of sub-micrometre particles such as single virions of the *Herpes simplex* virus (type-1) both in enveloped and in capsid form, gives an indication of the potential for sub-micron applications, such as studying single virion-host cell interactions or virus harvesting. Rapid biopolymer (DNA or protein) fractionation has also been described in a method termed DEP chromatography (Washizu *et al*. 1994).

PARTICLES SUBJECTED TO ROTATING FIELDS

Whereas conventional DEP utilizes stationary fields, 2 closely related techniques utilize moving fields, more specifically either of rotating or travelling wave form. The investigation of particle motion in these moving fields has led to the development of some slightly different applications. In 1978, Pohl stated that non-uniform (inhomogeneous) fields can sometimes induce a torque on a particle, causing it to spin (Pohl, 1978). Four years later a reliable method was described to induce cellular spin: that of subjecting the cell to a uniform (homogeneous) rotating electrical field (Arnold & Zimmermann, 1982; Mischel, Voss & Pohl, 1982) termed electrorotation (ROT). An uniform rotating electric field can be generated by energizing 4 electrodes with sinusoidal voltages, with 90° phase difference between adjacent electrodes.

Creation of the dipole moment in a particle takes a characteristic time to reach its maximum value, equally, when the field changes direction, the dipole will respond and decay at a rate determined in part by the passive electric properties of the particle and suspending medium that appertain to the frequency of the applied voltage. Torque resulting in cellular spin is induced by the interaction between the rotating electric field and the remnant dipole. Interestingly, as shown in Fig. 7, the torque created can result in spin of the particle in the opposite direction to the field as well as in the same direction as the field (not shown). For a more mathematical approach see the review of Sauer & Schlögel (1985).

For a given particle, there is a unique rotation rate for each frequency of applied voltage. This variation in rotation rate is shown in Fig. 8 for a viable and nonviable oocyst of *Cryptosporidium parvum* suspended in a 5 μS cm^{-1} solution, whose viability had been confirmed using the fluorogenic vital dye technique of Campbell, Robertson & Smith (1992). Although the field may be rotating at rates greater than 10^7 s^{-1}, the induced particle rotation rate, which is dependent on the square of the field strength, remains measurable by the human eye. Depending

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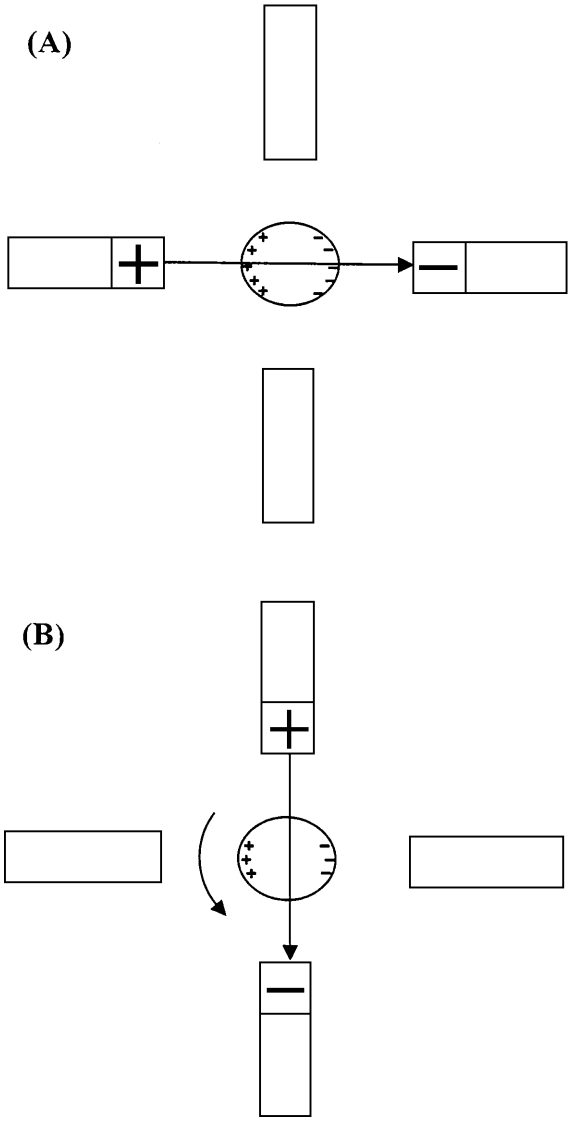

Fig. 7. A particle in the ROT chamber. In a stationary field (A) the induced dipole moment for a particle that is less polarizable than the medium is directed against the field. On turning the field in a clockwise direction (B), the field interacts with the decaying charges to produce a torque and spin of the particle that opposes the direction of the moving field, termed *anti-field electrorotation*. Conversely for a particle that is more polarizable than the surrounding medium the torque induced results in a spin in the same direction as the field or *co-field rotation* (not shown) (Huang, 1993).

on the frequency, typical rotation rates observed are between -3 and $+1{\cdot}5$ rotations per second for a viable *C. parvum* oocyst subjected to a rotating field of around 10 kV m^{-1}, with negative rotation rates indicating antifield rotation of the particle. There is a frequency (around 800 kHz for this conductivity) in the ROT spectra of Fig. 8 where the viable and non-viable oocysts rotate in opposite directions, providing a convenient, single frequency, viability check on individual oocysts.

Oocysts for observation in a ROT chamber, (which can be manufactured on a reusable glass slide

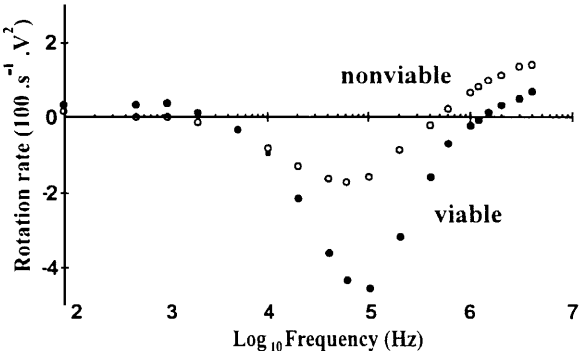

Fig. 8. ROT spectra of live and dead *Cryptosporidium parvum* oocysts. Viability was confirmed with the fluorogenic dyes 4′,6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) according to Campbell *et al.* (1992).

or as a cheap 'use once – throw away' device) require to be partially purified and resuspended in a medium of known conductivity. Fluorogenic dyes in contrast, such as the fluorogenic vital dye assay (Campbell *et al*. 1992), the fluorogen exclusion test (Belosevich *et al*. 1997), have several disadvantages: apart from a similar requirement for partial purification, there is often a requirement for an incubation stage, specialist storage and handling, as well as analysis by epifluorescence microscopy. PI, for example, causes a list of acute toxicological effects, is carcinogenic, and requires refrigeration. ROT observation of a sample using bright field microscopy requires less than 15 min, which is also faster than a reverse transcription polymerase chain reaction (RT-PCR) test, which amplifies a sequence of mRNA from a *C. parvum* heat shock protein (Stinear *et al*. 1996) that can determine the viability of individual oocysts. Although the particle suspension requires sufficient purification to avoid particle-debris interactions, ROT has found many applications both with biological and synthetic particles (see Table 3).

ROT demonstrates several features, the most important of which is the real-time assessment of viability of individual cells, which for *Cryptosporidium parvum* oocysts (Goater, Burt & Pethig, 1997) has been shown to correlate closely with the fluorogenic vital dye technique of Campbell *et al.* (1992) and the maximized *in vitro* excystation assay of Robertson, Campbell & Smith (1993). As well as the rapid (a few seconds per cell) straightforward assessment of the viability of individual cells, the viability of larger numbers of cells (e.g. 30 cells in a field of view at a magnification of $\times 400$) can also be assessed simultaneously. To assist the analyst, automatic measurement of the rotation rate or for a full spectrum is also possible (Schnelle, Glasser & Fuhr, 1997; Zhou, Burt & Pethig, 1998). A full frequency ROT spectrum, which can be thought of as a 'fingerprint' for heterogeneous particles like oocysts, provides information not only about the viability of the particle, but also the conductivity and

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Table 3. Examples of particle types investigated by rotating electric fields (ROT)

	Particle type	Examples
Acellular	Virus	Virus erythrocyte interaction (Gimsa, Pritzen & Donath, 1989)
Prokaryotes	Bacteria	Investigation of the flagellar motor torque of *E. coli* (Berry & Berg, 1996)
		Biocide treatment of bacterial biofilm (Zhou *et al*. 1996)
Eukaryotes	Protozoa	*Plasmodium falciparum* infected erythrocytes (Gascoyne *et al*. 1997*a*)
		Cryptospordium spp. oocysts (Goater *et al*. 1997)
	Yeast	*Saccharomyces cerevisiae* comparison of wild type and vacuole deficient mutant (Asami & Yonezawa, 1996)
	Algae	*Neurospora* slime (Gimsa *et al*. 1991)
	Plant cells	Barley mesophyll protoplasts (Arnold & Zimmermann, 1982)
	Insect cell line	Effect of osmotic and mechanical stresses and enzymatic digestion on IPLB-Sf cell line of the fall armyworm (*Spodoptera frugiperda*, Lepidoptera) (Freitag *et al*. 1989)
Mammalian cells	Cell lines	MDA-231 human breast cancer cells (Gascoyne *et al*. 1997*a*)
	Lymphocyte	Influence of membrane events and nucleus (Ziervogel *et al*. 1986)
	Erythrocyte	Erythrocytes parasitized by *Plasmodium falciparum* (Gascoyne *et al*. 1997*a*)
	Platelet	Influence of activators (Egger & Donath, 1995)
Other particles	Liposomes	Liposomes with 1–11 bilayers (Chan *et al*. 1997)
	Latex bead	Effect of surface conductance (Arnold, Schwan & Zimmermann, 1987)

Table 4. Examples of particle types investigated by travelling wave electric fields

	Particle type	Examples
Eukaryotes	Protozoa	*Cryptosporidium parvum* oocysts (Goater *et al*. 1997)
	Yeast	*Saccharomyces cerevisiae* (Huang *et al*. 1993)
	Plant cells	Membrane covered pine polls (Hagedorn *et al*. 1992)
Mammalian cells	Blood cells	Erythrocytes (Masuda, Washizu & Kawabata, 1988)
		Separation of components of whole blood (Morgan *et al*. 1997)
Other particles	Artificial spheres	Cellulose spheres (Hagedorn *et al*. 1992)

permittivity of the various 'compartments' within its structure (Chan *et al*. 1997). After ROT analysis, as with DEP, the particle remains intact and unchanged, and because ROT is a non-invasive method the particle can be subjected to further holistic or destructive analytical methods.

A variety of particle types, including the transmissive stages of parasites can be investigated by this technique. Indeed, whereas there is often the need to develop viability stains, molecular methods or excystation protocols for determining the viability of newly investigated cell types, ROT probes a common difference between all dead and viable cells, namely membrane integrity. Potential applications also include distinguishing between sub-types or strains of particles, whose surface or membrane properties differ, for example distinguishing between the cysts of the non-invasive *Entamoeba dispar* from the potentially invasive *E. histolytica* in which species specific exposed cyst wall antigens have recently been identified (Walderich *et al*. 1998).

PARTICLES SUBJECTED TO TRAVELLING WAVE ELECTRIC FIELDS

Like ROT, a third dielectric technique also uses moving fields, instead of rotating they are in the form of linear travelling waves, made simply by applying AC voltages in phase sequence to a linear array of electrodes. At low frequencies (< 100 Hz) translational motion is induced in the particles by largely electrophoretic forces, associated with surface charge characteristics. Early studies took this low frequency approach (e.g. Masuda, Washizu & Iwadare, 1987) however, a problem encountered in these initial studies were erroneous particle trajectories and motion caused by the convection of the suspending fluid.

Later, studies using higher frequencies (e.g. Fuhr *et al*. 1991) at which DEP forces have the strongest effect on the motions of particles, were described, see Table 4 for a summary. Unlike DEP, the motion of particles in travelling waves or travelling wave

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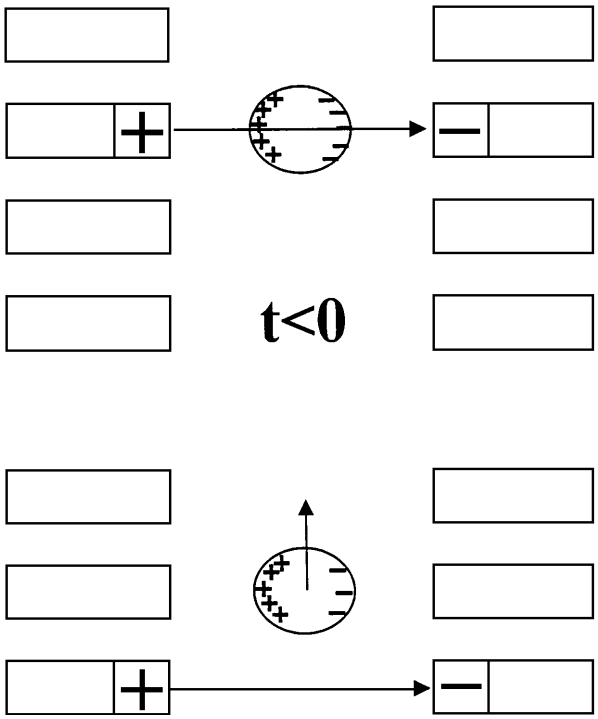

Fig. 9. Travelling-wave dielectrophoresis for a particle that is less polarizable than the medium ($\alpha_p < \alpha_m$). In the instant before voltage switching between electrodes occurs ($t < 0$) the dipole moment induced is opposed to the direction of the field. On switching the electrode voltages ($t = 0$) the interaction between the remnant dipole and the field induces a translational force in the particle in the opposite direction to the travelling field, termed counter-field *travelling wave dielectrophoresis* (TWD).

dielectrophoresis (TWD) is achieved in a stationary supporting fluid: without the need for fluid flow there is no dilution of particle density. Indeed, the concentration of particles without the use of a centrifuge may be important for certain delicate particles which may be distorted or damaged. Centrifugation can also incur a loss of particles through adhesion to the centrifuge tube.

Selective retention or transportation of sub-populations from a suspension is 1 application of TWD. Talary *et al*. (1996) demonstrated the separation of yeast cells using TWD, both by retaining viable cells at 5 MHz and moving non-viable cells, and at a higher conductivity by moving viable cells while retaining non-viable cells. It was shown that for successful translational motion of particles by TWD, the particle must be under conditions of negative DEP (or negligible positive DEP) and at a frequency where ROT of the particle would be expected. The sense and magnitude of rotation expected indicates the direction and magnitude of the TWD force on the particle in the travelling wave. Electrode geometries were found to influence the TWD force, the optimum electrode gap was found to be similar to the effective particle size. The particles can be made to move over lines of electrodes (of the appropriate geometry, spacing width and voltage) as demonstrated by Fuhr *et al*. (1991) or for more convenient viewing, in the gap between the tips of many rows of electrodes (Huang *et al*. 1993; Talary *et al*. 1996) as shown in Fig. 9. Transparent electrodes manufactured from ITO (indium titanium oxide) are also an option. Particles travelling over lines of electrodes do so at a small distance from the surface. In this contact-free manner, particle adhesion to the substrate is avoided, which may be important for 'sticky' particles such as undecorticated *Ascaris* spp. ova (Dalton, personal communication).

Unless spiral electrode geometries are utilized, whose area can be increased *ad infinitum* by adding further helical turns, there are limits to the size of planar monolayer electrode arrays as there are spatial limitations for the connections to the individually addressed electrodes. Multilayer electrode fabrications only require 4 connections to energize 1 or more TWD arrays of any length. Recently multilayer TWD devices have been constructed and tested for their bioparticle manipulation and separation characteristics (e.g. Talary *et al*. 1996; Morgan *et al*. 1997). Theoretically, multilayer TWD devices can be built up (like a model railway track) so that particles may be taken to many investigative units such as ROT chambers, in a single integrated device. Separation, manipulation and characterization of a particle in a single device, a sort of 'laboratory-on-a-chip device' has been proposed (see Ward, 1997 for a general review) and is under development.

A monolayer device has already been designed and constructed that combines TWD with ROT. This spiral electrode device was demonstrated for concentrating and testing the viability of cells (Goater *et al*. 1997). A travelling wave produced perpendicular to the spiral electrodes, directs and concentrates particles into the central region of the microelectrode structure, essentially a ROT chamber, where they are then subjected to a rotating electric field. The viability of a cell is then obtained by observation of the sense of induced rotation at a single frequency. One application has been for the concentration and viability determination of the water-borne transmissive stage of *C. parvum* (Goater *et al*. 1997). A forecast of the potential of this particular device is its ability to handle particle concentrations of 10 ml^{-1} and less.

Although there is a current trend towards the miniaturization of electrokinetic devices, along with investigations into submicron sized particles, it should be possible to investigate larger particles. Electrode geometries can be modified to suit particles up to 100 μm in size or larger. Detection, identification and viability determination of parasitic helminth ova, from faecal samples or urban sludge that is reused in agriculture, is one possibility.

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Table 5. Summary of potential applications in parasitology

	Approach	Comments
Research	Selective concentration, separation and manipulation of particles between ~ 60 nm to 100 μm and above	General research tool
	Removal of contaminating bacteria from *in vitro* cultures of parasites	Leaving the culture unaffected, unlike chemical methods
	Real time detection of cell physiological state during interactions with chemicals, toxins or parasites	E.g. effect of anti-malarial agents on the permeation pathway, or for studying merozoite inhibition
	Monitor parasite induced membrane alterations	Membrane architecture changes or ion channel activity
	Determination of viability in infectious doses which may be combined with testing of disinfection/drug sensitivity	Various obligate parasites, water, soil and food transmitted parasites
	Isolation of individual parasite from sample for subsequent cloning/molecular analysis, etc.	General research tool
Diagnostic	Monitor antibody coating of microbeads, fractionate using DEP	Quality assurance, improve consistency of diagnostic assays such as latex agglutination and immuno-magnetizable separation
	Specific identification and viability tests for bio-particles from samples including: blood, urine, sputum, faeces, cerebrospinal fluid for deciding appropriate treatment, source of environmental contamination or risk assessment of water	Protozoans and helminth ova
	Differentiate closely related species or sub-species	E.g. distinguishing between the morphologically similar cysts of *E. histolytica* from *E. dispar*, *Giardia*, *Ascaris*, etc.
	Manipulation of single bio-particles to specific antibody test sites or to combine with coated microbeads	Laboratory-on-a-chip applications
Treatment	Removal of parasites/parasitized cells from body fluids e.g. blood/CSF for transfusion	Removal of *Plasmodium* stages including infected erythrocytes or trypanomastigotes from blood

Presently, culture-based viability techniques for *Ascaris* ova take more than one week (Gaspard, Wiart & Schwartzbrod, 1996) but rapid determination of viability (< 15 min) can be predicted if this analysis is made using ROT.

Detection of environmental contaminates in food and water are of increasing public concern. Also, rapid automatic detection and enumeration of pathogenic micro-organisms such as those from patient samples; blood, sputum, urine and faeces, could improve diagnosis. Methods for the early identification of pathogens are limited. Conventional microbiological methods are time consuming, largely because their culture *in vitro* is required to generate detectable concentrations of cells, which can take up to 2 days. The rapid concentration, separation and identification of small numbers of micro-organisms (and the determination of viability) present in clinical samples would avoid delays required to amplify, selectively, small numbers of pathogens prior to conventional detection. The novel dielectric methods of DEP, ROT and TWD may offer such a solution.

Table 5 summarizes some potential applications of the techniques described, which either alone, in combination or in conjunction with conventional methods may assist in the fields of parasitological research, diagnostics and treatment of disease. Whereas some have already been demonstrated on bench-scale experiments, for the majority of these predictions to be realized as robust field techniques further data must be collected on the electrokinetic responses of a wider range of bio-particles. These analyses include non-target animal, vegetable and mineral particles, non-pathogenic organisms and other bioparticles, so the specificity of the tests can be optimized. Particles from different sources must also be investigated. For example, there are subtle differences between preserved and fresh whole blood including lower cholesterol levels and a decrease in the level of phosphorylation of membrane proteins in the preserved erythrocytes, both of which may alter the electrokinetic response.

For these technologies to proceed and compete with current techniques they must be more specific, sensitive, reliable, rapid or more competitively priced. One simple way of achieving many of these requirements is to make the tests fully automated by integrating them onto a single disposable device. Following the introduction of micro-electrodes into this field of study (Price, Burt & Pethig, 1988; Masuda, Washizu & Nanba, 1989; Fuhr *et al.* 1991), using photolithography and associated semi-conductor micro-fabrication technologies, and more recently the development of multilayer fabrication

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techniques, dielectrophoresis, electrorotation and travelling wave have all developed into techniques that can be incorporated onto a single 'bioprocessor-chip' device (Pethig *et al*. 1998). The potential for incorporating all 3 techniques described, into 1 device, are outlined further in the article by Talary *et al*. (1998, see in this supplement).

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