Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Review

Dielectric measurement of cell death

Pareshkumar Patel[a], Gerard H. Markx[b,*]

[a] School of Chemical Engineering and Analytical Science, The University of Manchester, P.O. Box 88, Manchester M60 1QD, UK
[b] School of Engineering and Physical Sciences, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, UK

ARTICLE INFO

Article history:
Received 9 May 2008
Received in revised form
11 September 2008
Accepted 11 September 2008

Keywords:
Capacitance
Permittivity
Dielectric spectroscopy
Dielectrophoresis
Electrorotation
Viability
Stress

ABSTRACT

Dielectric techniques, which include dielectric spectroscopy as well as AC electrokinetic methods such as dielectrophoresis, electrorotation and electro-orientation, can be provide important information about cell viability. A review is given of the different dielectric techniques that have been used for measuring cell viability and their utility. The changes that occur in the cell dielectric properties during apoptotic and different forms of traumatic cell death are discussed and interpreted in terms of the main parameters involved (membrane capacitance and conductance and internal conductivity).

Contents

* Corresponding author. Microstructures and Microenvironment Research Group, Room NM9, John Coulson Building, School of Engineering and Physical Sciences, Department of Chemical Engineering, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, Scotland, UK. Tel.: +44 131 451 8349; fax: +44 131 451 3129.
E-mail address: g.h.markx@hw.ac.uk (G.H. Markx).

0141-0229/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2008.09.005

1. Introduction

The term "dielectric" was first introduced by William Whewell after a request from Michael Faraday to describe a material through which (greek—"dia" = through) an electric field passes. Dielectric theory was given a firm theoretical foundation by Maxwell [1], and the many contributions by Maxwell to the field of dielectrics include the derivation of an analytical solution for the conductivity of a dilute suspension of spherical particles [1]. Fricke [2,3] proved it was possible to adapt Maxwell's equation so it could be used for the description of the dielectric properties of suspensions of cells by modelling a cell as a conducting spheroid surrounded by a non-conducting membrane. After World War II rapid progress was made in the study of the electrical properties of biological materials. Major contributions to the subject were made by Schwan [4,5], who performed measurements on the electrical properties of tissues and cell suspensions over a much broader frequency range than was previously possible. The study of the movement of particles in AC electric fields was pioneered by Pohl [6], who introduced the term dielectrophoresis in the early 1950's to describe the movement of particles induced by non-uniform electric fields. This was followed by the development of electro-orientation, electrorotation and travelling wave dielectrophoresis techniques, all of which are based on the movement of particles in AC electric fields [6-13].

Nowadays the study of the AC electrical properties of biological material is a very active and continuously expanding field of research. Its application to the study of cells and tissues has been particularly fruitful because of the way all cells are constructed, i.e. they all have a lipid bilayer membrane surrounding the cell interior. The cell membrane's role is to isolate the cell interior from the outside world, and regulate what goes in and out. Because of this barrier function cell membranes have electrical properties that are very different from that of the cell interior or the material outside the cell membrane [9–14]. When cells are exposed to electric fields in the radiofrequency range this causes the cells to be strongly polarised by a Maxwell–Wagner type interfacial polarisation process across the cell membrane. This polarisation is accompanied by the formation of a high electric field across the cell membrane, and the cell itself forming a strong dipole [9–14].

A variety of techniques have made use of this polarisation effect to obtain information about the electrical properties of cells [10]. Measurement of the cell dielectric properties can give important information about a cell's physiology, in particular the properties of the membrane and the cell interior. The major parameters that can be determined are the membrane capacitance C_{mem}, the effective membrane conductance G_{mem}, the cell interior conductivity σ_i. The membrane capacitance C_{mem} determines the amount of charge that can be stored across a membrane when a cell is exposed to an electric field. Its value is strongly dependent on the level of folding of the cell membrane (folds, blebs, microvilli, etc.) and can vary over a 20-fold range for different cell types (0.8–15 $\mu F\,cm^{-2}$) [15]. The value of the effective membrane conductance G_{mem} gives a measure of the permeability of the cell membrane, although movement of ions over the cell surface (surface conductance) also plays a role [16]. For viable cells the value of G_{mem} is very low, in the order of 10–100 $S\,m^{-2}$, for non-viable cells it can be a number of decades higher. The value of the interior conductivity σ_i is to a large extent determined by the mobility and concentration of the ions in the cytoplasm; typically values for viable cells are in the order of 0.2–1 $S\,m^{-1}$. A change in its value may indicate an exchange of material between the cytoplasm and the medium, as may occur when the cell membrane is compromised. As cell death is often accompanied by a loss of the integrity of the cell membrane, significant changes may occur in the value of the dielectric parameters upon cell death, making dielectric techniques some of the most useful techniques for the investigation of cell death. The ability to measure cell death is important in many areas, including the optimisation of fermentations and downstream processing, toxicology studies, drug screening, pathology, food safety, and others.

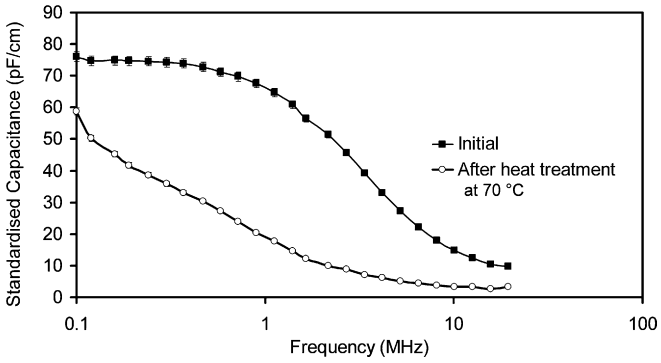

Fig. 1. Dielectric spectra of baker's yeast suspensions before and after killing the cells by heat after their exposure to 70 °C for several hours.

The techniques that have been used for measuring the dielectric properties of cells can broadly be divided into two groups [9]. The first set of techniques are impedance or admittance-based methods, in which the capacitance and conductance of cells in suspension or attached to an electrode are measured. These techniques will be referred to as dielectric spectroscopy. The other set are the AC electrokinetic techniques, which involve the derivation of a cell's dielectric properties from the movement of the cell under the influence of an applied AC electric field. These techniques include dielectrophoresis, electrorotation and electro-orientation. Also relevant, but not discussed here, are methods such as electric cell–substrate impedance sensing (ECIS) [17–19], which do not measure the dielectric properties of cells directly.

1.1. Impedance/admittance-based methods

Dielectric spectroscopy involves the measurement of the frequency-dependent permittivity and conductivity of a material between a set of electrodes. When this material is a cell suspension or tissue the permittivity of the material in the radiofrequencies shows a decline from low to high frequency as in Fig. 1. If the frequency dependent complex particle conductivity of cells and the dielectric properties of the medium are known, then the frequency-dependent conductivity and permittivity of the suspension can be predicted using Maxwell's equation [1–5]. However, as shown by Schwan [4,5], for most cells the total decline $\Delta\varepsilon$ is linearly correlated with the volume fraction P of (intact) cells present up to high volume fractions (10–20%) [4,5,9,20]:

$$\Delta\varepsilon = \frac{9PrC_{mem}}{4\varepsilon_0} \quad (1)$$

in which C_{mem} is the membrane capacitance per unit area (in $F\,m^{-2}$), r the cell radius and ε_0 the permittivity of vacuum ($8.854 \times 10^{-12}\,F\,m^{-1}$). Eq. (1) has been derived for cells without a cell wall; however, in practice a linear relation between $\Delta\varepsilon$ and P is also found for cells with cell walls. Unlike AC electrokinetic techniques, the use of dielectric spectroscopy directly in growth medium is straightforward. Also, unlike AC electrokinetic techniques [21], it does not matter whether the cells are immobilized or not, and the technique is relatively insensitive to non-cellular matter [22]. The technique has been extensively used for monitoring biomass levels during fermentations [23–27].

The dielectric spectrum of a suspension of dead cells is also shown in Fig. 1. Suspensions of dead cells have a much lower but

measurable capacitance; the actual value depends on the method by which the cells have been killed [28]. Eq. (1) is only valid for cells with an intact membrane which has a low membrane conductivity. Clearly, this is often not the case for dying or dead cells, as the membrane is often compromised. For cells with a high membrane conductivity, instead of Eq. (1) one would have to use [4,5]:

$$\Delta\varepsilon = \frac{9}{4\varepsilon_0}\frac{PrC_{\mathrm{mem}}}{[1 + rG_{\mathrm{mem}}((1/\sigma_{\mathrm{i}}) + (1/2\sigma_{\mathrm{m}}))]^2} \quad (2)$$

in which G_{mem} is the membrane conductance (in S m^{-2}). Using Eq. (1) to describe the changes in the dielectric behaviour of cells when dying, as is often done [29–32], would compel one to draw the conclusion that a fall in the capacitance of a cell suspension upon cell death is mainly caused by drop in the value of C_{mem} (or cell shrinkage), whilst most likely it actually is mainly due to an increase in the membrane conductance G_{mem}.

1.2. AC electrokinetic techniques

AC electrokinetic techniques have in recent years gained tremendously in popularity because they lend themselves well to miniaturisation, with all the advantages that may entail in portability, sample size, cost, etc. A major advantage of AC electrokinetic methods is also that they can be used to measure the properties of single cells one at a time, enabling investigations on very small numbers of cells. A disadvantage compared to dielectric spectroscopy is that high conductivities reduce the electrokinetic forces, and that most experiments are therefore done in low conductivity media.

Although a variety of electrokinetic techniques exist, we will limit the discussion to the two major ones, dielectrophoresis and electrorotation, and only briefly discuss electro-orientation.

1.3. Dielectrophoresis

Dielectrophoresis (DEP) is the movement of particles in non-uniform electric field gradients [6,9–16]. For a particle with equivalent radius r the DEP force is given by:

$$F(\omega) = 2\pi\varepsilon_{\mathrm{m}} r^3 \mathrm{Re}(K^*)\nabla\left|E\right|^2 \quad (3)$$

in which K^* is the Clausius–Mossotti factor.

$$K^* = \left(\frac{\varepsilon_{\mathrm{p}}^* - \varepsilon_{\mathrm{m}}^*}{\varepsilon_{\mathrm{p}}^* + 2\varepsilon_{\mathrm{m}}^*}\right) \quad (4)$$

and

$$\varepsilon^* = \frac{\varepsilon - \mathrm{j}\sigma}{\omega} \quad (5)$$

"Re" stands for "the real part of" and subscripts "p" and "m" for particle and medium, respectively. $\nabla|E|^2$ defines the average local field strength and gradient (in V^2 m^{-3}).

Cells in suspension are also colloidal particles, and when suspended in low-conductivity buffers and exposed to high strength non-uniform electric fields they readily show dielectrophoresis. Theoretical DEP spectra of viable and non-viable (heat-killed) [33] yeast cells at low conductivities are shown in Fig. 2. The differences in the spectra are large, and frequency ranges can be seen in which the dielectrophoretic behaviour of the viable cells is negative and for non-viable cells positive, and *vice versa*. These large differences in the dielectrophoretic behaviour of viable and non-viable cells can be used for their separation [34–37].

Dielectrophoretic spectra can be obtained in various ways, including measuring the force needed to levitate against gravity [6,38], and the measurement of the rate at which suspended cells

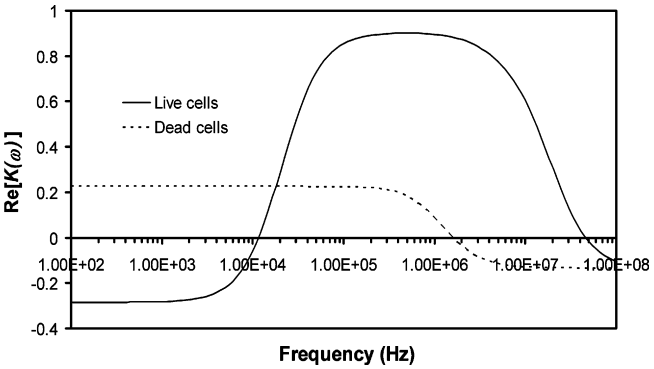

Fig. 2. Theoretical dielectrophoretic spectra of live and dead yeast cells. The spectra were calculated using the multishell model [33]. Parameter estimates were taken from the same reference.

are attracted to (positive DEP) or repelled from (negative DEP) electrodes [39–42]. Particularly useful is the polynomial electrode design which can generate electric field of constant non-uniformity and strength between a set of electrodes [42]. Analysis of spectra can be done by fitting the data to models such as the multishell model [33]. However, for intact (viable) cells without a cell wall (such as mammalian cells) the lower crossover frequency can be used to obtain the specific membrane conductance and capacitance. This is most easily done by deriving these parameters from a plot of the product of the radius and the crossover frequency ($r \times f_{\mathrm{crossover}}$) versus the medium conductivity σ_{m} [16,43,44]. The membrane capacitance C_{mem} can then be obtained from the slope of the line obtained, and the effective membrane conductance G_{mem} from the slope and intercept [16,43,44] (see also Fig. 3). The higher crossover frequency can be used to obtain the internal conductivity, as its value is linearly correlated with the internal conductivity [28,45]. The presence of a cell wall strongly affects the lower crossover frequency, making it more difficult to obtain information about the cell membrane properties. This is less so for the higher crossover frequency, which is only minimally affected by the cell wall conductivity [28].

1.4. Electrorotation

Electrorotation is the rotation of particles in electric fields [7,46–50]. Electrorotation can be induced in cells and other particles by generating rotating electric field by applying phase-shifted electric fields to microelectrodes. Like dielectrophoresis, electroro-

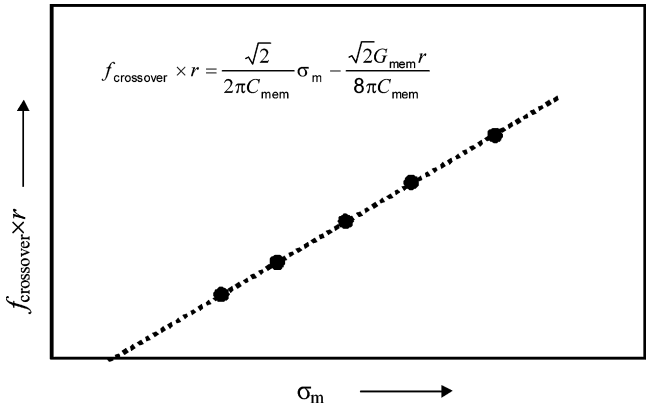

Fig. 3. Determination of the membrane capacitance C_{mem} and membrane conductance G_{mem} from dielectrophoresis experiments from a plot of the product of the lower crossover frequency $f_{\mathrm{crossover}}$ and radius r versus the medium conductivity σ_{m}.

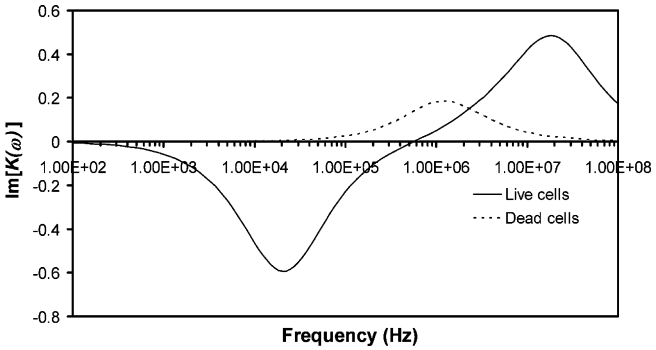

Fig. 4. Theoretical electrorotation spectra of live and dead yeast cells. The spectra were calculated using the multishell model [33]. Parameter estimates were taken from the same reference.

tation is most easily observed in low-conductivity media. Unlike dielectrophoresis, where the rate of movement is determined by the Real part of the Clausius–Mossotti factor, electrorotation rates are determined by the Imaginary part of the Clausius–Mossotti factor K^* as the rotation rate Ω (in rad s^{-1}) is given by [7,46–50]:

$$\Omega(\omega) = -\frac{\varepsilon_m}{2\eta}\mathrm{Im}(K^*)E^2 \quad (6)$$

in which η is the medium viscosity. Measurement of the electrorotation rate as a function of the frequency produces electrorotation spectra as in Fig. 4. The two spectra shown demonstrate the differences between electrorotation spectra of viable and non-viable cells. The large differences that can be seen have been exploited for the creation of assays of cell viability, particularly that of microbial parasites such as *Cryptosporidium*, *Cyclospora* and *Giardia* [50].

Analysis of rotation spectra is similar as that for dielectrophoresis. For more complex cells, including those with a cell wall, the data need to be fitted by a model such as the multishell model [33]. For spherical cells without a cell wall such as animal cells the lower antifield peak f_{c1} can be used to obtain accurate values of the membrane capacitance and conductance, as C_{mem} can be determined from the slope of a straight line through a plot of $f_{c1} \times r$ versus the medium conductivity σ_m, and G_{mem} from the intercept (see Fig. 5).

1.5. Electro-orientation

Electro-orientation is the orientation of particles in electric fields [51–57]. To observe electro-orientation, the particle has to be anisotropic, i.e. its properties have to be different along one of its axes. Typically this means that the particle has to be cylindrical or rod-shaped. Because of this anisotropy the particle shows differences in its orientation at different frequencies, i.e. with its longest axis aligned either with or perpendicular to the electric field.

Because they lack a cell wall many animal cells tend to round up when suspended. Electro-orientation studies have therefore concentrated on (rod-shaped) bacterial [52,53,57] or yeast cells [51,56], although studies have also been performed on disk-shaped blood cells [55]. The frequency at which live cells orient perpendicular to the electric field is significantly different for viable and non-viable cells.

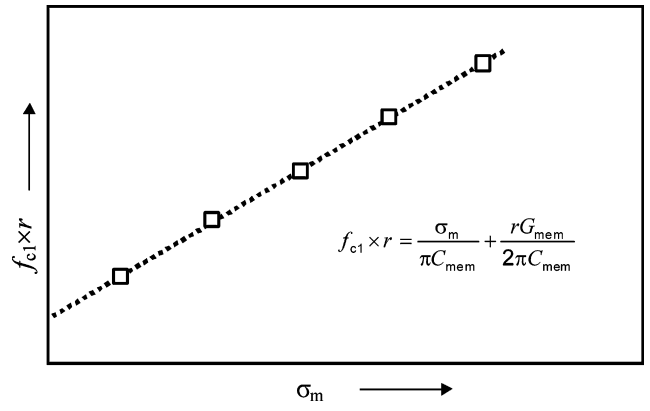

Fig. 5. Determination of the membrane capacitance C_{mem} and membrane conductance G_{mem} from a plot of the product of the lower rotation peak frequency f_{c1} and radius r versus the medium conductivity σ_m.

2. Cell death

A first major problem one encounters when studying cell death using dielectric techniques is that it is not clearly defined what cell death actually is. Many forms of cell death have been described—and the precise distinction between the different terms is still a matter of discussion [58–61]. Some of the difficulties are caused by the fact that dielectric methods are used for many different cell types, and that the definitions of different terms and methods for ascertaining whether a cell is "alive" or "dead" can be significantly different for different branches of biology. For example, the terms "apoptosis" and "necrosis" are commonly used to describe programmed cell death and traumatic cell death in animal or plant cell biology, but rarely in microbiology. Even within a subject area, however, there is often no clarity, as for example the term necrosis has been used for the description of both traumatic cell death, as well as a general term to designate the presence of dead cell or tissue [58,59]. Similarly, in microbiology the distinction between "viable" and "non-viable" cells is disputable [60,61]. The term "viable" usually refers to the ability of an organism to live, develop, or germinate under favorable conditions, and it is often used to describe the ability of microbes to divide and form colonies. This term, however, becomes difficult to use when describing non-culturable or dormant micro-organisms which are unable to form colonies but are otherwise intact [60,61]. Also, the exact moment when a cell can be considered dead is difficult to determine. For example, cells have a strong ability of self-repair, and can come back from the brink of death. Thus, whilst a method may determine that a cell is dead (e.g. because its membranes are broken), because the cell can repair itself the cell is actually not dead—just injured [62].

This brings us to another major problem. Different methods of measuring cell viability give different answers. Plate counts give you a measure of the cells' ability for colony formation, but don't work if the cells are unculturable. Exclusion dyes such as trypan blue or methylene blue only work if cell death is accompanied by membrane injury. Metabolic stains such as formazan give a measure of a cell's metabolic activity, but do not work if a cell is not dead but just resting. Dielectric spectroscopy and AC electrokinetic methods are in this respect not different from other methods- they only report differences if the cell's dielectric properties change. Fortunately, the dielectric properties of cells in the radiofrequencies tend to be dominated by the dielectric properties of the membrane, whilst (the conductivity of) the cytoplasm can play a major role at higher frequencies. Both are dependent on the integrity of the membrane - membrane capacitance and conductance directly, and internal conductivity indirectly as the cell internal conductivity changes when the cell membrane becomes permeable. Both often change when the cell is stressed or is dying. However, not all cell deaths lead to changes in the cell membrane permeability. Examples include toxic chemicals which act at DNA, RNA or protein synthesis level such as metabolic inhibitors. As there are clearly

many issues involved in the measurement of cell death using dielectric techniques, it may be useful to review what is known about the changes that occur in the dielectric properties of cells during different forms of cell death.

2.1. Programmed cell death

Apoptosis, or programmed cell death, occurs after a cell has switched on a self destruction programme. The process of apoptosis involves a number of steps, including cell shrinkage and rounding as well as condensation of the cytoplasm and nuclear material, followed by the formation of blebs, and finally complete cell breakdown into vesicles called apoptotic bodies. Considering these large structural changes it is maybe not surprising that apoptosis is accompanied by large changes in the cells' dielectric properties. Cell rounding is accompanied by the loss of microvilli and smoothing of the membrane. This could explain the decrease in the membrane capacitance in HL60 cells and Jurkat cells reported by the many researchers [16,63–64; but see 45]. Labeed et al. [45] also reported that apoptosis in K562 leukaemic cells gives rise to an initial increase in the cytoplasmic conductivity during cell shrinkage, followed by a decrease as cells start to lyse. For human promyelocytic HL-60 and Jurkat E6-1 cells, however, only a decrease was found [16,63–64]

2.2. Traumatic cell death

Unlike apoptosis, in traumatic cell death the cell is the passive victim, and cell death is the direct result of environmental stress, including injury by low and high temperatures, mechanical forces, and chemical or biological challenges.

2.3. Temperature

The effects of elevated yet sublethal temperatures on the dielectric properties of yeast cells were investigated by Zhou et al. [65] using electrorotation. They observed only small effects of increased temperatures, indicating a possible small decrease in membrane or wall conductivity with increasing temperature. More recently, changes in the membrane conductivity were also reported by Sudsiri et al. [66]; it was proposed that these changes may be correlated with changes in the chloride transport rate [66]. Small changes in the dielectric properties of yeast cells with temperature were also observed by Ferris et al. [67] using dielectric spectroscopy; their hypothesis that this may be caused by changes in protein mobility is given credence by the findings that heating does affect the interaction between proteins and phospholipids [68], although other studies have cast doubt of the role of proteins in the dielectric properties of the cell membrane [69]. The effect of lethally high temperatures on the electrorotation and dielectrophoretic behaviour of yeast and other cells have been investigated extensively, as "heat treatment" or "boiling" is often used to obtain non-viable cells for further study of difference in the electrokinetic behaviour of live and dead cells [34–36,46]. The differences between dead and live cells killed by heat-treatment are generally explained in terms of differences in their cell membrane conductivity and interior conductivity; but some changes in the wall properties can also be observed. Huang et al. [33] give useful data on changes in the dielectric properties of baker's yeast after cell death obtained using dielectrophoresis and electrorotation; Kriegmaier et al. [51] give useful data on fission yeast using both electrorotation and electro-orientation. Measurement of the effect of different temperatures on the capacitance of yeast suspensions have shown that the dielectric properties of heat-killed cells depend little on the temperature at which the cells were killed [28].

Dielectric spectroscopy has been used to investigate changes in tissues and organs during storage [70–73]. Freezing of tissues and organs is accompanied by the formation of ice crystals which can damage cell structures [70].

2.4. Osmotic, shear and other mechanical effects

Osmotic stress has been investigated using electrorotation [75–77] and dielectric spectroscopy [78]. The value of the membrane capacitance dropped and the membrane conductance increased when the osmotic pressure of the medium was reduced; this can be explained by the fact that the membrane is stretched as the cells take up water from the medium as the external osmotic pressure is reduced, causing microvilli to unfold. It was also thought that at very low osmotic pressures (below 200 mOsm) the cells could incorporate additional membrane material into the cell surface.

The effects of shear on cells is not well-studied, even though dielectric spectroscopy can give a measure of cell damage by shear on-line and in real time. A study by Markx et al. [29] on plant cells showed that there were significant differences in the susceptibility of the plant cells to shear between cultures, and even within a single culture. Electrorotation was used by Freitag et al. [74] to study the effects of shear on insect cells; they showed that shear lowered the membrane capacitance and increased the membrane conductance, which they explained by the shearing off of microvilli.

2.5. Toxic chemicals

The effect of toxic chemical on the dielectric properties of cells is well-studied, and a large variety of chemicals has been used. We will discuss the effects of organic materials ("solvents") and detergents which insert themselves into the cell membrane and disrupt it, organic agents which chemically react with the cell membrane or other cell components, antibiotics, and heavy metal ions.

Typically, when a solvent is added to a cell suspension, an increase in the suspension capacitance is seen at first, followed by a decrease as the cells lyse [30–32,77]. The cause has been attributed to changes in cell size and membrane capacitance [77,78] as the solvent inserts itself into the membrane and expands it. Recent modelling by Asami, however, has indicated that the presence of approximately 30 nm sized pores in the membrane may lead to an additional α-dispersion-like dispersion at low frequencies [79,80]. It is therefore likely that the initial increase in the suspension capacitance that can be seen after the addition of a solvent is caused by the fact that the solvents produce nanosized pores randomly distributed over the membrane surface. The decrease in the capacitance which the follows may be due to the pores size increasing above 30 nm. Lysis of cells is thought not to be through the sudden breakdown of the membrane, but due to a gradual change in membrane properties [81]. This has also been corroborated by modeling by Asami [80] which has shown that cells that have large pores (up to nearly 50% of the membrane missing) still show a strong dispersion. Whether a solvent has an effect on the cells strongly depends on the polarity of the solvent relative to that of the membrane; very apolar solvents are, because of the large difference in their polarity with that of the membrane, often amongst the most biocompatible solvent for biotransformations [82,83]. Electrorotation measurements by Pauli et al. [84] on the effects of substituted anilines and aliphatic alcohols on yeast drew a similar conclusion; the chemical that had the strongest effect had the highest lipophilicity. The same conclusion was drawn by Arnold et al. [49] during the study of the effects of (chloro)phenols on the electrorotation spectra of yeasts. Experiments by Tileva et al. [32] using dielectric spectroscopy indicated that the susceptibility of the cells to solvents

may be dependent of the history of the cells, including the polarity of the substrate it has previously been grown on. This indicates that cells may alter the composition of their membrane in response to toxic challenges. Markx and Kell [31] described a method by which the addition of a toxic chemical used in biotransformations (benzaldehyde) was controlled using dielectric spectroscopy in order to keep the level of viable biomass in a chemostat constant. Using this method a continuous but sublethal stress could be imposed on the cell population the fermenter, imposing a selection regime (phenotype or genotype) for cells that are highly resistant to the stress.

The effect of detergents on cells is similar to that of solvents. For example, using dielectric spectroscopy it has been shown [85] that the membrane capacitance of yeast cells was increased by the presence of a surfactant during surfactant-induced cell shrinkage. Further addition of surfactant caused the capacitance to drop due to membrane solubilisation by the additional surfactant causing cell lysis. The observed effects of antibiotics on the dielectric properties of cells is also similar [57,86–88], and can usually most readily be explained by the fact that the cell membrane becomes compromised and the cell lyses.

Whilst solvents and detergents can be expected to insert themselves directly into the lipid membrane and physically disrupt it, other agents can be expected to directly chemically attack the membrane. Treatment of insect cells with trypsin caused only relatively small changes in their rotation spectra [74]. Ratanachoo et al. [44] used dielectrophoresis to detect changes in the specific membrane conductance and capacitance of a human cultured leukemia cell line (HL-60) after exposure to paraquat, styrene oxide, *N*-nitroso-*N*-methylurea (NMU) and puromycin. Paraquat and styrene oxide directly attack the cell membrane by lipid peroxidation; the action of NMU and puromycin is intracellular. All chemicals led to a time-dependent decrease in the membrane capacitance and an increase in the membrane conductance. However, because dielectric measurements are more sensitive to agents that affect the membrane, the effects of paraquat and styrene oxide were detected earlier. No increase in the membrane capacitance was seen with any of the chemicals used, as none of the chemicals in these experiments had a solvent or detergent-like effect.

The effects of various heavy metal ions such as copper, silver and mercury on the cell dielectric properties have been investigated using various dielectric measurements techniques including dielectric spectroscopy [89], electrorotation [90,91] and electro-orientation [52,53]. The presence of heavy metal ions in the suspending medium strongly affected the membrane integrity even at low concentrations as the heavy metals bound to membrane components. The effects of the heavy metals depended not only on the metal involved but also on the presence of other ions in the medium, as well as medium pH. This may be at least partly correlated with the number of negative groups on the cell surface [52,53].

2.6. Viruses

Changes in the cell's dielectric properties after its infection by viruses have been investigated using a number of different techniques [92–97]. Electrorotation measurements on baby hamster kidney fibroblasts after infection with herpes simplex virus showed a small decrease in the membrane capacitance as the surface morphology changed [92]; also a decrease in the internal permittivity was observed as membrane vesicles and enveloped viral capsids were formed in the cytoplasm. Changes in cell properties after infection by viruses have also been observed using dielectric spectroscopy [94–97], providing potentially an effective method for following monitoring and controlling virus-based recombinant protein production in cell cultures. Phages can be highly selective towards specific cells; phage technology combined with electro-orientation has also been show to be an effective method to selectively assay the viability of microbial cells [54].

3. Conclusions and future trends

A survey of the literature on the dielectric measurement of cell death has shown that changes that occur in the dielectric properties of cells when they die depend to a large extent on the method by which cell death is induced, but some general trends can be observed.

Cell death is nearly always accompanied by changes in the cell membrane permeability, a fact that is exploited in many viability stains. This change in the membrane permeability can be expected to be mirrored by the change in the membrane conductance G_{mem}. An increase in the cell permeability also leads to an increase in the exchange of material between the cytoplasm and the medium, which can cause change (typically a decrease) in the internal conductivity σ_i. The decline in the capacitance of cell suspensions that is often seen when cell death is induced is most likely mainly due to this increase in the membrane conductance. The large differences in the dielectrophoretic behaviour that can be see in many cells (e.g. yeast above 2 MHz [28,35]) is most likely due to this change in the internal conductivity.

The situation with the membrane capacitance is less clear. Analysis of the literature is complicated by the fact that many researchers, including ourselves [29–32], have previously reported changes in the dielectric properties of cells based on capacitance measurements on cells without measuring the cell size or referral to the fact that the membrane had become permeable. Where actual values of C_{mem} have been obtained during cell death some researchers find a decrease in C_{mem}, indicating a loss of membrane structures and the rounding of the cell membrane [45,16,63,64]. Some researchers, however indicate an increase in C_{mem}, which has been attributed to factors such as increased folding of the membrane, for example when the cell shrinks as the cell contents leaks out [45,96,97], a change in membrane permittivity [89], and an increase in membrane fluidity [98]. Asami [79,80] reported the appearance of an α-dispersion-like dispersion when nanosized pores are formed in the membrane. This would manifest itself as an apparent increase in C_{mem}, and may explain the temporary increase in suspension capacitance often observed when solvents, detergents or other compounds are added to cell suspensions [29–32].

The application of dielectric techniques in the study of biological systems enjoys increasing attention, exemplified by the act that the numbers of papers on the use of dielectrophoresis has increased form a handful per year in the 1980, to a few tens in the 1990, to several hundreds per year currently. New techniques are constantly being developed, which are likely to impact on our ability to study cell death using dielectric techniques. In dielectric spectroscopy there is a clear trend from single or dual point measurements to frequency scanning. Combined with improved data analysis and chemometric methods [96,97,99,100] this would allow one to obtain better and additional estimates of cell parameters on-line and in real time. Also, the modeling of the dielectric properties of cells is constantly improving, in particular the modelling of the dielectric properties of cells with more complicated shapes [101–103] and conductive membranes [80,104]. This will help in the analysis of both electrokinetic as well as dielectric spectroscopy measurements. Impedance-based methods for the analysis of the electrical properties of single cells are currently under also development [105,106]. This promising development may result in techniques that combine the advantages of AC elec-

trokinetic techniques such as its ability to measure the properties of single cells with the advantage dielectric spectroscopy of being able to measure directly in growth medium. In AC electrokinetics the development of new electrode systems and methods of field application such as the application of multiple frequencies simultaneously and electrodeless DEP is particularly rapid [107–110]. At the same time the dielectric techniques are becoming more easy to use and compatible with modern standard analyses equipment such as microplate readers [111–115]. Thus one can expect to the increasing use of dielectric techniques in the study of cell death and associated applications such as process monitoring and improvement, drug screening, etc. to keep its momentum in future years.

Acknowledgements

We wish to thank Aber Instruments for an Industrial Scholarship to P.M. Patel. We also wish to thank Prof Richard Snook, SCEAS, University of Manchester, for his help with project supervision, and Aditya Bhat for his assistance with some of the experiments. Thanks are long overdue to Dr. Mike Arnold for many useful and encouraging discussions.

References

[1] Maxwell JC. A treatise on electricity and magnetism. Oxford: Clarendon Press; 1873.
[2] Fricke H. The electrical capacity of suspensions with special reference to blood. J Gen Physiol 1925;9:137–52.
[3] Fricke H. A mathematical treatment of the electrical conductivity and capacity of disperse systems. II. The capacity of a suspension of conducting spheroids surrounded by a non-conducting membrane for a current of low frequency. Phys Rev 1925;26:678–81.
[4] Schwan HP. Electrical properties of tissue and cell suspensions. In: Lawrence JH, Tobias CA, editors. Advances in biological and medical physics, vol. 5. New York: Academic Press Inc.; 1957. p. 147–209.
[5] Schwan HP, Foster KR. RF-field interactions with biological systems: electrical properties and biophysical mechanisms. Proc IEEE 1980;68:104–13.
[6] Pohl HA. Dielectrophoresis. Cambridge: Cambridge University Press; 1978.
[7] Arnold WM, Zimmermann U. Electrorotation—development of a technique for dielectric measurements on individual cells and particles. J Electrostat 1988;21:151–91.
[8] Fuhr G, Fiedler S, Muller T, Schnelle T, Glasser H, Lisec T, et al. Particle micromanipulator consisting of two orthogonal channels with travelling-wave electrode structures. Sens Actuators A: Phys 1994;41:230–9.
[9] Markx GH, Davey CL. The dielectric properties of biological cells at radiofrequencies: applications in biotechnology. Enzyme Microb Technol 1999;25:161–71.
[10] Pethig R, Kell DB. The passive electrical properties of biological systems: Their significance in physiology, biophysics and biotechnology. Phys Med Biol 1987;32:933–70.
[11] Hughes MP. Nanoelectromechanics in engineering and biology. Boca Raton: CRC Press; 2002.
[12] Jones TB. Electromechanics of particles. Cambridge: Cambridge University Press; 1995.
[13] Morgan H, Green NG. AC Electrokinetics: Colloids and Nanoparticles. Baldock: Research Studies Press; 2001.
[14] Pethig R. Dielectric and Electronic Properties of Biological Materials. Chichester: Wiley; 1979.
[15] Gascoyne PRC, Vykoukal JV. Dielectrophoresis-based sample handling in general-purpose programmable diagnostic instruments. Proc IEEE 2004;92:22–42.
[16] Pethig R, Talary MS. Dielectrophoretic detection of membrane morphology changes in Jurkat T-cells undergoing etoposide-induced apoptosis. IET Nanobiotechnol 2007;1:2–9.
[17] Xiao C, Lachance B, Sunahara G. Assessment of cytotoxicity using electric cell–substrate impedance sensing: concentration and time response function approach. Anal Chem 2002;74:5748–53.
[18] Xing JZ, Zhu L, Jackson JA, Gabos S, Sun XJ, Wang XB, et al. Dynamic monitoring of cytotoxicity on microelectronic sensors. Chem Res Toxicol 2005;18:154–61.
[19] Spegel C, Heiskanen A, Skjolding LHD, Emneus J. Chip based electroanalytical systems for cell analysis. Electroanalysis 2008;20:680–702.
[20] Harris CM, Todd RW, Bungard SJ, Lovitt RW, Morris JG, Kell DB. Dielectric permittivity of microbial suspensions at radio-frequencies—a novel method for the real-time estimation of microbial biomass. Enz Microb Technol 1987;9:181–6.
[21] Falokun CD, Markx GH. Electrorotation of beads of immobilized cells. J Electrostat 2007;65:475–82.
[22] Salter GJ, Kell DB, Ash LA, Adams JM, Brown AJ, James R. Hydrodynamic deposition: a novel method of cell immobilisation. Enz Microb Technol 1990;12:419–30.
[23] Kell DB, Markx GH, Davey CL, Todd RW. Real-time monitoring of cellular biomass: methods and applications. Trends Anal Chem 1990;9:190–4.
[24] Ducommun P, Kadouri A, von Stockar U, Marison IW. On-line determination of animal cell concentration in two industrial high-density culture processes by dielectric spectroscopy. Biotechnol Bioeng 2002;77:316–23.
[25] Markx GH, Davey CL, Kell DB. The permittistat: a novel type of turbidostat. J Gen Microbiol 1991;137:735–43.
[26] Carvell JP, Dowd JE. On-line measurements and control of viable cell density in cell culture manufacturing processes using radio-frequency impedance. Cytotechnology 2006;50:35–48.
[27] Negrete A, Esteban G, Kotin RM. Process optimization of large-scale production of recombinant adeno-associated vectors using dielectric spectroscopy. Appl Microbiol Biotechnol 2007;76:761–72.
[28] Patel PM, Markx GH. A comparative study of cell death using electrical capacitance measurements and dielectrophoresis. Enzymol Microb Technol 2008;43:523–30.
[29] Markx GH, ten Hoopen HJG, Meijer JJ, Vinke JL. Dielectric spectroscopy as a novel and convenient tool for the study of the shear sensitivity of plant cells in suspension culture. J Biotechnol 1991;19:145–58.
[30] Stoicheva NG, Davey CL, Markx GH, Kell DB. Dielectric spectroscopy: a rapid method for the determination of solvent biocompatibility during biotransformations. Biocatalysis 1989;2:245–55.
[31] Markx GH, Kell DB. The use of dielectric permittivity for the control of the biomass level during biotransformations of toxic substrates in continuous culture. Biotechnol Prog 1995;11:64–70.
[32] Tileva FP, Yotova LK, Markx GH. Dielectric measurement of the resistance of *Trichosporon cutaneum* against toxic chemicals. African J Microbiol Res 2007;1:117–20.
[33] Huang Y, Hölzel R, Pethig R, Wang XB. Differences in the AC electrodynamics of viable and non-viable yeast-cells determined through combined dielectrophoresis and electrorotation studies. Phys Med Biol 1992;37:1499–517.
[34] Pethig R. Dielectrophoresis: Using inhomogeneous AC electrical fields to separate and manipulate cells. Crit Rev Biotechnol 1996;16:331–48.
[35] Markx GH, Talary MS, Pethig R. Separation of viable and non-viable yeast using dielectrophoresis. J Biotechnol 1994;32:29–37.
[36] Markx GH, Pethig R. Dielectrophoretic separation of cells: continuous separation. Biotechnol Bioeng 1995;45:337–43.
[37] Li HB, Bashir R. Dielectrophoretic separation and manipulation of live and heat-treated cells of *Listeria* on microfabricated devices with interdigitated electrodes. Sens Actuators B Chem 2002;86:215–21.
[38] Kaler KVIS, Xie JP, Jones TB, Paul R. Dual-frequency dielectrophoretic levitation of Canola protoplasts. Biophys J 1992;63:58–69.
[39] Broche LM, Labeed FH, Hughes MP. Extraction of dielectric properties of multiple populations from dielectrophoretic collection spectrum data. Phys Med Biol 2005;50:2267–74.
[40] Fatoyinbo HO, Hoeftges KF. Hughes MP Rapid-on-chip determination of dielectric properties of biological cells using imaging techniques in a dielectrophoresis dot microsystem. Electrophoresis 2008;29:3–10.
[41] Gascoyne PRC, Noshari J, Becker FF, Pethig R. Use of dielectrophoretic collection spectra for characterizing differences between normal and cancerous cells. IEEE Trans Ind Appl 1994;30:829–34.
[42] Huang Y, Pethig R. Electrode design for negative dielectrophoresis. Meas Sci Technol 1991;2:1142–6.
[43] Gimsa J, Marszalek P, Loewe U, Tsong TY. Dielectrophoresis and electrorotation of neurospora slime and murine myeloma cells. Biophys J 1991;60:749–60.
[44] Ratanachoo K, Gascoyne PRC, Ruchirawat M. Detection of cellular responses to toxicants by dielectrophoresis. Biochim Biophys Acta: Biomembr 2002;1564:449–58.
[45] Labeed FH, Coley HM, Hughes MP. Differences in the biophysical properties of membrane and cytoplasm of apoptotic cells revealed using dielectrophoresis. Biochim Biophys Acta: Gen Subj 2006;1760:922–9.
[46] Hölzel R. Non-invasive determination of bacterial single cell properties by electrorotation. Biochim Biophys Acta 1999;1450:53–60.
[47] Hölzel R, Lamprecht I. Dielectric properties of yeast cells as determined by electrorotation. Biochim Biophys Acta 1992;1104:195–200.
[48] Wang XB, Huang Y, Gascoyne PRC, Becker FF, Hölzel R, Pethig R. Changes in Friend murine erythroleukemia cell-membranes during induced-differentiation determined by electrorotation. Biochim Biophys Acta: Biomembr 1994;1193:330–44.
[49] Arnold WM, Zimmermann U, Pauli W, Benzing M, Niehns C, Ahlers J. The comparative influence of substituted phenols (especially chlorophenols) on yeast cells assayed by electro-rotation and other methods. Biochim Biophys Acta 1988;942:83–95.
[50] Dalton C, Goater AD, Drysdale J, Pethig R. Parasite viability by electrorotation. Colloid Surf A: Phys Eng Aspects 2002;195:263–8.
[51] Kriegmaier M, Zimmermann M, Wolf K, Zimmermann U, Sukhorukov VL. Dielectric spectroscopy of Schizosaccharomyces pombe using electrorotation and electroorientation. Biochim Biophys Acta: Gen Subj 2001;1568:135–46.
[52] Ivanov AY, Fomchenkov VM, Khasanova LA, Kuramshina ZM, Sadikov MM. Effect of heavy-metal ions on the electrophysical properties of *Anacystis nidulans* and *Escherichia coli*. Microbiology 1992;61:319–26.

[53] Ivanov AY, Fomchenkov VM. Electrophysical analysis of the damage to *Escherichia coli* by silver ions. Microbiology 1992;61:326–32.
[54] Guliy OI, Bunin VD, O'Neil D, Ivnitski D, Ignatov OV. A new electro-optical approach to rapid assay of cell viability. Biosens Bioelectron 2007;23:583–7.
[55] Miller RD, Jones TB. Electro-orientation of ellipsoidal erythrocytes—theory and experiment. Biophys J 1993;64:1588–95.
[56] Markx GH, Alp B, McGilchrist A. Electro-orientation of *Schizosaccharomyces pombe* in high conductivity media. J Microb Methods 2002;50:55–62.
[57] Guliy OI, Ignatov OV, Markina LN, Bunin VD, Ignatov VV. Action of ampicillin and kanamicin on the electrophysical characteristics of *Escherichia coli cells*. Int J Environ Anal Chem 2005;85:981–92.
[58] Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: Mechanistic description of dead and dying eukaryotic cells. Infect Immun 2005;73:1907–16.
[59] Schwartz SM, Bennett MR. Death by any other name. Am J Pathol 1995;147:229–34.
[60] Kell DB, Kaprelyants AS, Weichart DH, Harwood CR, Barer MR. Viability and activity in readily culturable bacteria: a review and discussion of the practical issues. Antonie van Leeuwenhoek 1998;73:169–87.
[61] Kaprelyants AS, Gottschal JC, Kell DB. Dormancy in non-sporulating bacteria. FEMS Microb Rev 1993;104:271–86.
[62] McNeil PL, Terasaki M. Coping with the inevitable: how cells repair a torn surface membrane. Nature Cell Biol 2001;3:E124–9.
[63] Wang XJ, Becker FF, Gascoyne PRC. Membrane dielectric changes indicate induced apoptosis in HL-60 cells more sensitively than surface phosphatidylserine expression or DNA fragmentation. Biochim Biophys Acta: Biomembr 2002;1564:412–20.
[64] Huang CJ, Chen AL, Wang L, Guo M, Yu J. Electrokinetic measurements of dielectric properties of membrane for apoptotic HL-60 cells on chip-based device. Biomed Microdev 2007;9:335–43.
[65] Zhou XF, Burt JPH, Pethig R. Automatic cell electrorotation measurements: studies of the biological effects of low-frequency magnetic fields and of heat shock. Phys Med Biol 1998;43:1075–90.
[66] Sudsiri J, Wachner D, Gimsa J. On the temperature dependence of the dielectric membrane properties of human red blood cells. Bioelectrochemistry 2007;70:134–40.
[67] Ferris LE, Davey CL, Kell DB. Evidence from its temperature-dependence that the beta-dielectric dispersion of cell-suspensions is not due solely to the charging of a static membrane capacitance. Eur Biophys J 1990;18:267–76.
[68] Morita S, Shimanouchi T, Sasaki M, Umakoshi H, Kuboi RJ. Detection of a heat stress-mediated interaction between protein and phospholipid membrane using dielectric measurement. Bioscience Bioeng 2003;95:252–6.
[69] Stoneman M, Chturvedi A, Jansma DB, Kosepma M, Zeng C, Raicu V. Protein influence on the plasma membrane dielectric properties: In vivo study utilizing dielectric spectroscopy and fluorescence microscopy. Bioelectrochemistry 2007;70:542–50.
[70] Kent M, Knochel R, Daschner F, Schimmer O, Oehlenschlager J, Mierke-Klemeyer S, et al. Intangible but not intractable: the prediction of fish 'quality' variables using dielectric spectroscopy. Meas Sci Technol 2007;18:1029–37.
[71] Querido D. Temperature-correction of abdominal impedance: improved relationship between impedance and postmortem interval. Forensic Sci Int 2000;109:39–50.
[72] McRae DA, Esrick MA, Mueller SC. Changes in the noninvasive, in vivo electrical impedance of three xenografts during the necrotic cell-response sequence. Int J Radiat Oncol Biol Phys 1999;43:849–57.
[73] Raicu V, Saibara T, Irimajiri A. Multifrequency method for dielectric monitoring of cold-preserved organs. Phys Med Biol 2000;45:1397–407.
[74] Freitag R, Schügerl K, Arnold WM, Zimmermann U. The effect of osmotic and mechanical stresses and enzymatic digestion on the electro-rotation of insect cells (*Spodoptera frugiperda*). J Biotechnol 1989;11:325–36.
[75] Sukhorukov VL, Arnold WM, Zimmermann U. Hypotonically induced changes in the plasma-membrane of cultured mammalian cells. J Membr Biol 1993;132:27–40.
[76] Davey CL, Kell DB, Kemp RB, Meredith RWJ. On the audio- and radio-frequency dielectric behaviour of anchorage-independent, mouse L929-derived LS fibroblasts. Bioelectrochem Bioenerg 1988;20:83–98.
[77] Davey CL, Markx GH, Kell DB. On the dielectric method of monitoring cellular viability. Pure Appl Chem 1993;65:1921–6.
[78] Seeman P. Membrane actions of anesthetics and tranquillizers. Pharmacol Rev 1972;24:583–655.
[79] Asami K. Dielectric dispersion of erythrocyte ghosts. Phys Rev E 2006;73:052903.
[80] Asami K. Effects of membrane disruption on dielectric properties of biological cells. J Phys D: Appl Phys 2006;39:4656–63.
[81] Zhou XF, Markx GH, Pethig R. Effect of biocide concentration on electrorotation spectra of yeast cells. Biochim Biophys Acta 1996;1281:60–4.
[82] Salter GJ, Kell DB. Solvent selection for whole-cell biotransformations in organic media. Crit Rev Biotechnol 1995;15:139–77.
[83] Laane C, Boeren S, Vos K. On optimizing organic solvents in multi-liquid-phase biocatalysis. Trends Biotechnol 1985;3:251–2.
[84] Pauli W, Berger S, Kohler M, Gies A. Detecting membrane impairment caused by xenobiotics. Environ Toxicol Water Qual 1993;8:173–89.
[85] Raicu V, Gusbeth C, Anghel DF, Turcu G. Effects of cetyltrimethylammonium bromide (CTAB) surfactant upon the dielectric properties of yeast cells. Biochim Biophys Acta: Gen Subj 1998;1379:7–15.
[86] Johari J, Hubner Y, Hull JC, Dale JW, Hughes MP. Dielectrophoretic assay of bacterial resistance to antibiotics. Phys Med Biol 2003;48:N193–8.
[87] Hoettges KF, Dale JW, Hughes MP. Rapid determination of antibiotic resistance in *E. coli* using dielectrophoresis. Phys Med Biol 2007;52:6001–9.
[88] Hölzel R. Nystatin-induced changes in yeast monitored by time-resolved automated single cell electrorotation. Biochim Biophys Acta 1998;1425:311–8.
[89] Bai W, Zhao KS, Asami K. Effects of copper on dielectric properties of *E. coli* cells. Colloid Surf B: Biointerfaces 2007;58:105–15.
[90] Arnold WM, Geier BM, Wendt B, Zimmermann U. The change in the electro-rotation of yeast cells effected by silver ions. Biochim Biophys Acta 1986;889:35–48.
[91] Geier BM, Wendt B, Arnold WM, Zimmermann U. The effect of mercuric salts on the electro-rotation of yeast cells and comparison with a theoretical model. Biochim Biophys Acta 1987;900:45–55.
[92] Archer S, Morgan H, Rixon FJ. Electrorotation studies of baby hamster kidney fibroblasts infected with herpes simplex virus type 1. Biophys J 1999;76:2833–42.
[93] Gimsa J, Pritzen C, Donath E. Characterization of virus-red-cell interaction by electrorotation. Stud Biophys 1989;130:123–31.
[94] Zeiser A, Elias CB, Voyer R, Jardin B, Kamen AA. On-line monitoring of physiological parameters of insect cell cultures during the growth and infection process. Biotechnol Prog 2000;16:803–8.
[95] Zeiser A, Bedard C, Voyer R, Jardin B, Tom R, Kamen AA. On-line monitoring of the progress of infection in Sf-9 insect cell cultures using relative permittivity measurements. Biotechnol Bioeng 1999;63:122–6.
[96] Ansorge S, Esteban G, Schmid G. On-line monitoring of infected Sf-9 insect cell cultures by scanning permittivity measurements and comparison with off-line biovolume measurements. Cytotechnology 2007;55:115–24.
[97] Carvell J, Bhat A, Patel PM, Markx GH. Recent developments in using scanning radio-frequency impedance measurements on cell culture processes. Bioprocess J 2007;4:24–7.
[98] Bonincontro A, Di Ilio V, Pedata O, Risuleo G. Dielectric properties of the plasma membrane of cultured murine fibroblasts treated with a nonterpenoid extract of *Azadirachta indica* seeds. J Membr Biol 2007;215:75–9.
[99] Cannizzaro C, Gugerli R, Marison I, von Stockar U. On-line biomass monitoring of CHO perfusion culture with scanning dielectric spectroscopy. Biotechnol Bioeng 2003;84:597–610.
[100] Maskow T, Röllich A, Fetzer I, Ackemann JU, Harms H. On-line monitoring of lipid storage in yeast using impedance spectroscopy. J Biotechnol 2008;135:64–70.
[101] Green NG, Jones TB. Numerical determination of the effective moments of non-spherical particles. J Phys D: Appl Phys 2007;40:78–85.
[102] Gimsa J. A comprehensive approach to electro-orientation, electrodeformation, dielectrophoresis, and electrorotation of ellipsoidal particles and biological cells. Bioelectrochem 2001;54:23–31.
[103] Asami K. Characterization of biological cells by dielectric spectroscopy. J Non Cryst Solids 2002;305:268–77.
[104] Pavlin M, Miklavcic D. Effective conductivity of a suspension of permeabilised cells: a theoretical analysis. Biophys J 2003;85:719–29.
[105] Morgan H, Tao S, Holmes D, Gawad S, Green NG. Single cell dielectric spectroscopy. J Phys D: Appl Phys 2007;40:61–70.
[106] Gawad S, Cheung K, Seger U, Bertsch A, Renaud P. Dielectric spectroscopy in a micromachined flow cytometer: theoretical and practical considerations. Lab Chip 2004;4:241–51.
[107] Lapizco-Encinas BH, Simmons BA, Cummings EB, Fintschenko Y. Dielectrophoretic concentration and separation of live and dead bacteria in an array of insulators. Anal Chem 2004;76:1571–9.
[108] Demierre N, Braschler T, Muller R, Renaud P. Focusing and continuous separation of cells in a microfluidic device using lateral dielectrophoresis. Sens Actuators B: Chem 2008;132:388–96.
[109] Hawkins BG, Smith AE, Syed YA, Kirby BJ. Continuous-flow particle separation by 3D insulative dielectrophoresis using coherently shaped, dc-biased, ac electric fields. Anal Chem 2007;79:7291–300.
[110] Urdaneta M, Smela E. Multiple frequency dielectrophoresis. Electrophoresis 2007;28:3145–55.
[111] Chin S, Hughes MP, Coley HM, Labeed FH. Rapid assessment of early biophysical changes in K562 cells during apoptosis determined using dielectrophoresis. Int J Nanomed 2006;1:333–7.
[112] Pethig R, Bressler V, Carswell-Crumpton C, Chen Y, Foster-Haje L, Garcia-Ojeda ME, et al. Dielectrophoretic studies of the activation of human T lymphocytes using a newly developed cell profiling system. Electrophoresis 2002;23:2057–63.
[113] Burt JPH, Goater AD, Menachery A, Pethig R, Rizvi NH. Development of microtitre plates for electrokinetic assays. J Micromech Microeng 2007;17:250–7.
[114] Hoettges KF, Hubner Y, Broche LM, Ogin SL, Kass GEN, Hughes MP. Dielectrophoresis-activated multiwell plate for label-free high-throughput drug assessment. Anal Chem 2008;80:2063–8.
[115] Talary MS, Burt JPH, Pethig R. Future trends in diagnosis using laboratory-on-a-chip technologies. Parasitology 1998;117:S191–203.