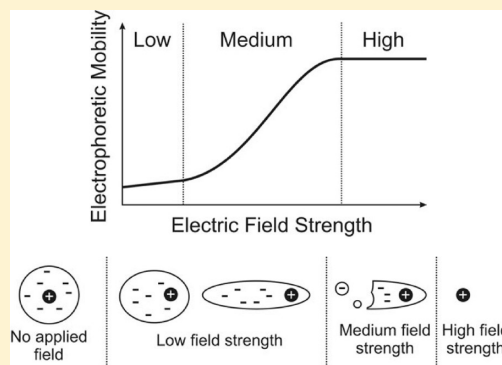


# A Current Opinion on Electrophoretic Deposition in Pulsed and Alternating Fields

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**ABSTRACT:** Electrophoretic deposition (EPD) is a colloidal production process developed in the early 20th century. Industrial scale EPD for the production of electronic components and phosphorescent screens and in the form of cataphoretic painting has known some success. Despite its limited practical applications, the inherent versatility of EPD has never ceased to fuel research into this technique. One of the major drives of this research was to render the method more environmentally friendly by enabling deposition from aqueous suspensions. One particular route, suggested to circumvent the problems caused by the use of water in EPD, is the use of alternating or pulsed fields. Recently, the use of alternating fields in EPD has been investigated for the deposition of biological matter in the form of cells and molecules. With this new avenue of research opening up and coinciding with a rise in biotechnological processes, one can expect a renewed interest in traditional EPD and fundamental research on the use of pulsed and alternating fields in this technique. Hence, this review attempts to summarize a century's worth of both fundamental and applied research for scientists venturing into the field of EPD.



## 1. ELECTROPHORETIC DEPOSITION

In essence, electrophoretic deposition (EPD) is a colloidal production process derived from the transport of charged suspended particles under the influence of an electric field, also known as electrophoresis, and subsequent deposition of this particulate matter on the electrode toward which they are attracted (Figure 1).<sup>1</sup> Due to its simple nature, this technique is

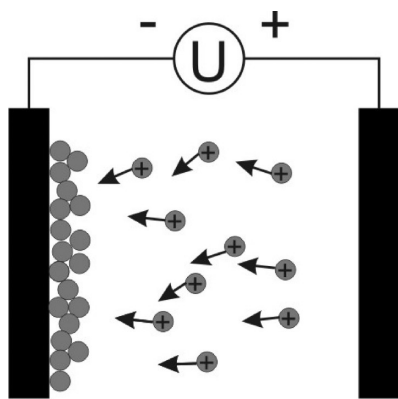


Figure 1. Schematic representation of a traditional EPD cell.

generally considered to be versatile and cost-effective. This results in a broad application range including the production of nanomaterials, biomaterials, and ceramic composites.<sup>2–5</sup> The first EPD experiments were carried out by Bose in 1740.<sup>6</sup> In 1807, the movement of clay particles in an aqueous suspension in an electric field was described by Reuss.<sup>7</sup> It took another 170

years for the first industrial application of EPD, i.e., the elephant process in which ceramic tiles were mass produced by deposition onto zinc drums that transferred the deposit onto a conveyor belt.<sup>8</sup> Thirty-eight years earlier, Hamaker conducted the first fundamental study on the deposition process,<sup>9</sup> resulting in the Hamaker equation (eq 1):

$$\frac{dY}{dt} = f \cdot \mu \cdot C_s \cdot E \cdot A \quad (1)$$

in which  $Y$  represents the deposition yield (g),  $t$ , the deposition time (s),  $\mu$ , the electrophoretic mobility ( $\text{m}^2/(\text{Vs})$ ),  $C_s$ , the solids loading in the suspension ( $\text{g}/\text{m}^3$ ),  $E$ , the electric field strength ( $\text{V}/\text{m}$ ), and  $A$ , the surface area of the electrode ( $\text{m}^2$ ). The factor  $f$  represents the efficiency with which particles that reach the electrode are deposited. Although this equation still forms the theoretical basis for EPD, the basic nature limits the use in practical applications of EPD. It is therefore not surprising that numerous modifications of the Hamaker equation were published. Biesheuvel for instance introduced a suspension concentration correction factor based on the volumetric concentration of particles in the suspension,  $\varphi_s$  ( $\text{m}^3/\text{m}^3$ ), and in the cast or deposit,  $\varphi_c$  ( $\text{m}^3/\text{m}^3$ ), correlating the yield to the particle concentration in the deposit,  $C_c$  ( $\text{g}/\text{m}^3$ ).<sup>10</sup>

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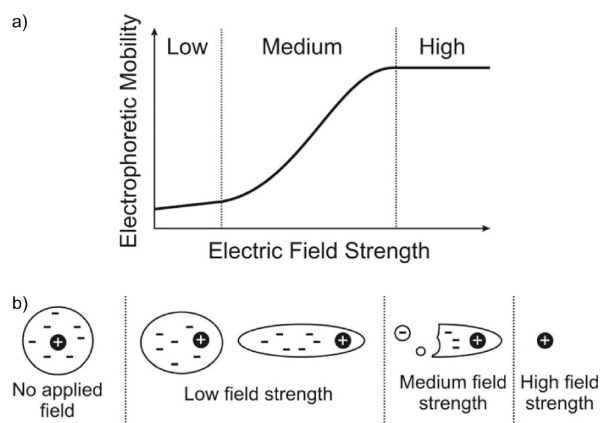
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$$\frac{dY}{dt} = \mu \cdot C_c \cdot \frac{\varphi_c}{\varphi_c - \varphi_s} \cdot E \cdot A \quad (2)$$

The composition of the deposit formed at any time during EPD is directly linked to the composition of the suspension in front of the deposition electrode. This typical characteristic of EPD has been used in the past to create laminates<sup>11</sup> by sequential deposition from different suspensions and functionally graded materials (FGMs)<sup>12</sup> by a continuous variation of the suspension composition during EPD. This inherent versatility has never ceased to fuel research into new applications of the technique. Nonetheless, the number of actual industrial applications is limited.

## 2. NONLINEARITY OF THE ELECTROPHORETIC MOBILITY

In 1929, Wien showed that the conductivity of an electrolyte and therefore the mobility of ions is related to the strength of the electrical field used to measure this value.<sup>13</sup> Almost 50 years later, Stotz experimentally demonstrated that a nonlinear relation exists between the electrophoretic mobility of particles and the applied field strength in strong fields. Stotz accredited this nonlinearity to a shear driven breakdown of the hydrodynamic influenced sphere around the moving particle which results in three distinctive regimes of electrophoresis (Figure 2a).<sup>14</sup> In low fields, the particle drags along a volume of



**Figure 2.** Schematic representation of (a) the three regimes of electrophoresis and (b) the double layer conformation in these regimes, as suggested by Stotz, adapted with permission from Elsevier.<sup>14</sup>

liquid and ions. The potential at the shear plane, called the zeta potential, determines the electrophoretic mobility and is often used as a measure for suspension stability. However, as the field strength increases, the velocity of the particle and concomitant drag exerted on the fluid sphere dragged along by the particle also increases. This drag causes deformation of the fluid sphere around the particle (Figure 2b). Indeed, one could reasonably assume that this deformation of the fluid sphere causes a minimal reduction in drag in the low field strength or first regime of electrophoresis, and therefore a slight increase in mobility. Although the experimental results of Stotz seem to indicate this slight increase in this regime, this effect was not discussed in his work. Stotz however did suggest that the drag becomes sufficient to cause a gradual breakdown of the fluid sphere beyond a given field strength.<sup>14</sup> The resulting decrease in mass gives rise to an additional increase in particle velocity,

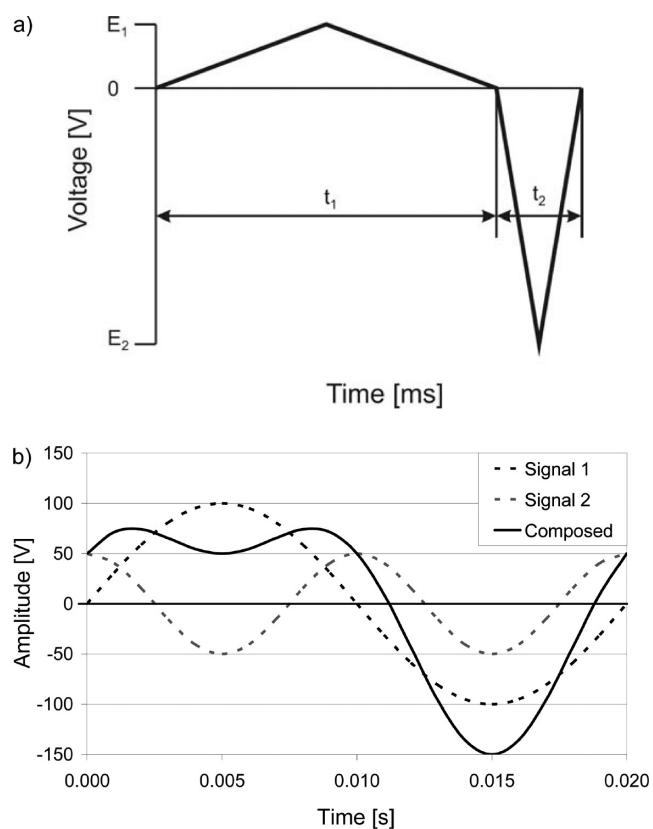
causing a nonlinear relation between field strength and electrophoretic mobility (Figure 2).<sup>14</sup> The particle movement obtained at intermediate field strengths is commonly referred to as electrophoresis of the second order or the second regime. Likewise, as the double layer is eventually broken down to an absolute minimum at a given field strength, a terminal mobility can be reached in strong fields. This results in a third regime of electrophoresis.<sup>14</sup>

Prior to Stotz, Dukhin published experimental proof of this nonlinear effect in a Ukrainian journal<sup>15</sup> and shortly after that in a Russian textbook together with Derjaguin.<sup>16</sup> In recognition of the early work of Wien and Stotz, Dukhin suggested naming this effect of nonlinearity the Stotz–Wien effect.<sup>17</sup>

## 3. UNBALANCED ELECTRIC FIELDS

Unbalanced AC electrical fields are in essence alternating fields with an asymmetric wave shape in which the positive and negative areas are of the same size (Figure 3). In other words, the net field strength,  $E$ , over one period,  $T$ , is zero (eq 3).

$$\int_0^T E \, dt = 0 \quad (3)$$



**Figure 3.** Example of (a) an arbitrary unbalanced signal adapted with permission from Elsevier<sup>35</sup> and (b) an unbalanced signal generated by superimposing two sinusoidal AC signals.

The absence of a net DC component eliminates DC current and associated electrochemical reactions.<sup>17</sup> It also eliminates the directed motion of particles caused by simple electrophoresis. However, as Stotz described, the electrophoretic mobility,  $\mu$ , of particles increases with the field strength in strong fields. This can be theoretically described by adding a third order term to the equation of particle motion,  $\nu$  (eq 4).

$$v = \mu E + \mu_3 E^3 \quad (4)$$

When an unbalanced electric field has a sufficient amplitude, a net drift of particles is obtained. Dukhin denominated this particle motion as aperiodic electrophoresis.<sup>17</sup> Dukhin suggested using this net movement for the separation of biomolecules based on their size,<sup>17</sup> but it might likewise be used to move and subsequently deposit a single biomolecule type. Unbalanced AC fields can be generated arbitrarily using function generators (Figure 3a) or by superimposing two symmetric AC signals which deviate in phase and frequency (Figure 3b).

#### 4. BEHAVIOR OF PARTICLES IN DC AND AC FIELDS

**Deposition in DC Fields.** Although EPD has been studied since 1940, the actual mechanisms active during the deposition of particles are still a matter of discussion. Hamaker suggested that the formation of a deposit during EPD is similar to a sediment formed by gravity.<sup>9</sup> In this model, the speed and pressure exerted by the particles at the electrodes would overcome the interparticle repulsion.

Other deposition mechanisms suggested over the years are based on an ionic strength increase near the electrode giving rise to flocculation effects, neutralization of the particle charge at the electrode and deposit, pH shifts at the electrodes and deformation of the double layer during movement, resulting in thinner double layers at the deposition front (Figure 4).<sup>1,18,19</sup>

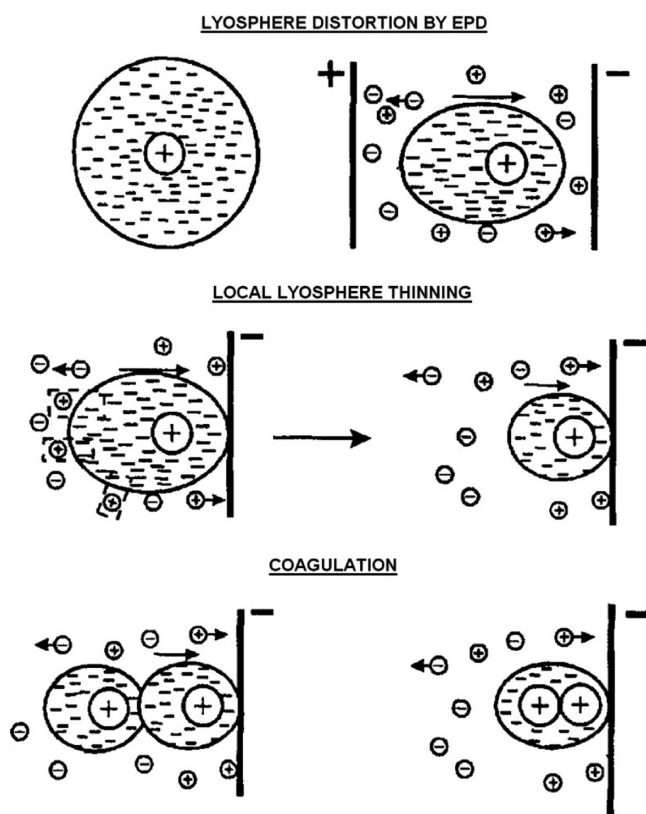
Böhmer showed that the mechanical behavior of particles near an electrode is far more complex than most researchers take into account. Electro-osmotic and hydrodynamic forces

tend to drive particles together, forming densely packed layers.<sup>20</sup> Hence, in addition to ionic, electrochemical, or other effects that allow particles to settle down, mechanical effects also need to be taken into account in the formation of a good deposit.

Furthermore, the properties of a deposit can change as a function of deposition time. Van Tassel suggested the generation of ion-depleted zones near the electrodes during EPD.<sup>21</sup> Recent work by Stappers experimentally confirmed that such an ion-depleted zone forms near the electrode in deposits obtained by EPD of alumina particles from an ethanol based suspension.<sup>22</sup> The ions in the deposit attracted by the electrode undergo electrochemical neutralization, while the counterions are pushed away toward the counter electrode. The acid–base behavior of the deposit is altered by this mechanism, generating a memory effect in the deposit. According to Stappers,<sup>22</sup> the throwing power of a suspension during EPD as earlier described by Anné<sup>23</sup> is linked to this depletion mechanism.

The large variety in powders, solvents, charging agents, and EPD setups that were successfully or unsuccessfully explored since the conception of EPD as a process suggest that there is no unique deposition mechanism but that the formation of a deposit depends on the used system. Brown for example noticed that aluminum powder in ethanol charged by  $\text{AlCl}_3$  could not be deposited on a membrane in front of the electrode but forms a layer directly on the cathode.<sup>24</sup> Without salt addition, however, deposition on a membrane is possible. Hence, it can be concluded that the charging agent in the case of aluminum is responsible for the need of neutralization at the electrode. The aluminum ions in the particle double layer react with the hydroxyl ions formed at the cathode to form aluminum hydroxide, which in turn glues the deposited particles to the electrode.<sup>24</sup> This mode of EPD, which is called reactive electrophoretic deposition (REPD), can yield exceedingly strong deposits. Furthermore, the properties of the formed deposit can be changed by altering the nature of the reaction product formed at the electrode by using suitable dopants.<sup>25</sup>

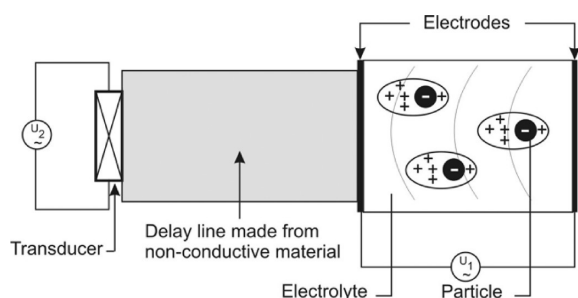
**Particle and Double Layer Behavior in the Bulk Suspension in AC Fields.** In 1933, Debye predicted that, when a sound wave is transmitted through an electrolyte, a potential should be generated due to differences in frictional forces and inertia of hydrated ions.<sup>26</sup> This electroacoustic phenomenon is called the Debye effect, and the generated potential is referred to as the ionic vibration potential (IVP). When a sound wave is passed through a colloidal suspension, a much stronger electroacoustic effect, called the colloidal vibration potential (CVP), can be observed. The combined effect of the IVP and CVP is referred to as the ultrasonic vibration potential (UVP).<sup>27</sup> Likewise, when a sound wave is generated due to particle and ion movement, an alternating field is applied on a suspension. This converse effect was discovered by Oja, Petersen, and Cannon.<sup>28</sup> The sound wave resulting from the alternating field is measured as the electroacoustic sonic amplitude (ESA). This acoustic signal resulting from the applied alternating field is the result of both particle movement and the deformation of the double layers surrounding these particles. In order to differentiate the particle movement induced by the alternating fields from the electrophoretic mobility measured in static conditions, the term dynamic mobility was adopted for the former. The extent of the acoustic contribution of both ion and particle movement depends on the applied frequency and inertia of the particles.



**Figure 4.** Schematic representation of electrical double layer distortion and thinning mechanism suggested as deposition mechanism during EPD, reprinted in part with permission from Wiley.<sup>19</sup>

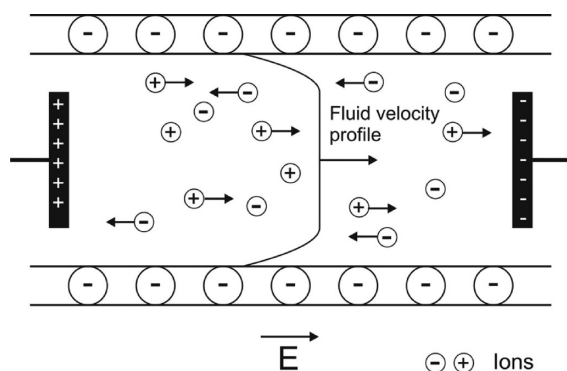


The theory needed to analyze the ESA signal based on these parameters was developed by O'Brien.<sup>29</sup> Soon afterward, this theory yielded a new line of measuring techniques such as the electroacoustic determination of electrophoretic mobilities, particle sizes, and zeta-potentials (Figure 5).



**Figure 5.** Schematic setup for the electro-acoustic zeta potential measuring technique, adapted with permission from Dr. Guy Anné.<sup>82</sup>

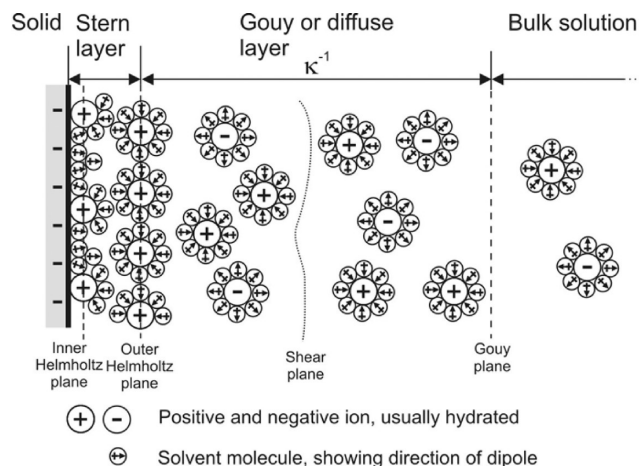
**Particle Behavior at the Electrode in AC Fields.** In electrophoretic deposition, the electrokinetic effects at the electrodes need to be taken into account, since they will influence the formation of the deposit. Whereas electrophoresis governs the movement of the particles in the bulk of the suspension, electro-hydrodynamic flow (EHF) and electro-osmotic flow (EOF) will control the particle movement at the electrodes. EHF is caused by the interaction of the electric field with the diffuse double layer at the electrodes (Figure 7) and



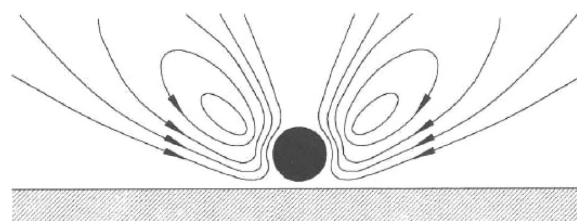
**Figure 6.** Electro-osmotic flow in a glass capillary adapted with permission from Wiley.<sup>31</sup>

causes convection when strong fields are applied. EOF originates from the motion of a polar liquid along charged surfaces under the influence of an electric field and is complementary to the electrophoresis of particles.<sup>30–32</sup> In capillary electrophoresis, this electro-osmotic flow is one of the main forces that drives the particles (Figure 6).<sup>30,31,33</sup>

For particles of which the double layers do not overlap, there is no tendency to move toward or away from each other in the bulk of the suspension. However, long-range attractions have been noticed in both AC and DC fields in the vicinity of the electrodes.<sup>34</sup> In the case of direct current, these clustering effects can be fully explained by EOF around the particles caused by the interaction between the charged electrode and the ions of opposite charge in the particle's diffuse double layer.<sup>34</sup> Between particle and electrode, the flow is directed away from the electrode. At the same time, this flow draws fluid toward the particle from every other direction (Figure 8). If two



**Figure 7.** Schematic representation of a double layer, adapted with permission from Elsevier.<sup>30</sup>



**Figure 8.** Streamlines for EOF around a negatively charged particle in the vicinity of a positively charged electrode. Reprinted with permission from 34. Copyright 1997 American Chemical Society.

particles are depositing in each others vicinity, this effect causes a convective flow which drives the particles together. Typically, the interaction range is of the order of several particle diameters.<sup>20</sup> Similarly, the electro-osmotic driven flow around a particle influences the trajectory of incoming particles, leading to the faster formation of clusters.<sup>34</sup> Due to the continued supply of particles driven toward the edge of the clusters from both the bulk solution and particles depositing in their vicinity, clusters tend to grow rapidly once formed. Particles arriving at an existing cluster tend to move around it until they reach an optimal position in which they can be incorporated in the already existing deposit. In the case of spherical particles with a monomodal size distribution, this can lead to lattice-like structures. As the electro-osmotic forces are strongest in aqueous media, EOF is believed to be the cause for the higher packing rates noticed when depositing from aqueous suspensions.<sup>20</sup>

Upon removal or reversing of the field, clusters tend to deorganize and finally break up.<sup>20</sup> Hence, during electrophoretic deposition using AC fields of moderate frequencies, already deposited particles are subjected to a reversed field which should last long enough to resuspend weakly aggregated particles.<sup>34</sup> These will reposition themselves into a more favorable denser packing when the field is correctly oriented.<sup>35</sup> The process is comparable to the increase of density that occurs during tapping of particle beds. If particles are poured into a container, the density is not particularly high. However, a much higher packing density is obtained by subjecting the particles to an oscillatory force field by tapping.<sup>36</sup>

For AC fields below a few 100 Hz, both EOF and EHF will drive the particle aggregation into dense packed layers.<sup>37</sup> As the frequency increases, the electro-osmotic flow becomes weaker

and the electrohydrodynamic flow stronger.<sup>33,34,38</sup> The electrohydrodynamic flow is believed to be the result of the distortion of the electric field by the presence of particles. As a result, the body force distribution in the electrode charge polarization layer is altered. The interaction of the applied electric field on this charge induces flow, whose direction is independent of the field polarity because both the body forces and the induced charge are proportional to the amplitude of the applied field.<sup>37</sup> Particles entrained in this flow are carried to one another. However, the existence of a critical frequency with a value that is dependent on the particle size and electrolyte composition has been reported for EHF. Above this frequency, the nature of the electrohydrodynamic interaction changes from attractive to repulsive, leading to disaggregation of particle clusters.<sup>33,34,37</sup> The existence of this critical frequency gives rise to the idea that the electrohydrodynamic force is actually the net result of multiple forces acting on the particle.<sup>38</sup> A transition between these forces seems to be linked to the extinction of the faradaic current path to the electrode, which changes the distribution of current around a particle in the vicinity of the electrode.<sup>38</sup>

## 5. ELECTROPHORETIC DEPOSITION OF INORGANIC PARTICLES

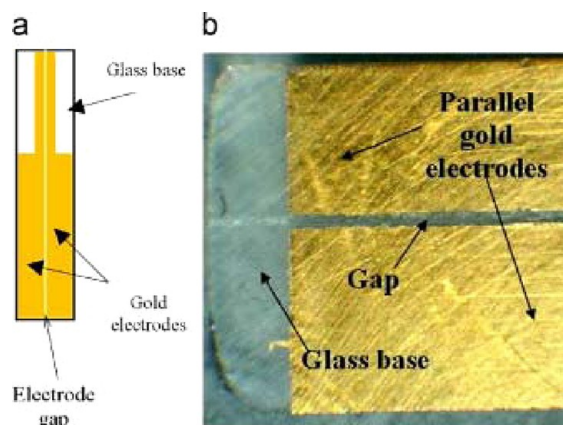
**Pulsed DC Fields.** The electrolysis of water is generally considered to be a slow electrochemical reaction. Bearing this in mind, Uchikoshi investigated whether pulsed DC signals could be used to form gas bubble free deposits from aqueous systems.<sup>39</sup> When the pulse duration is short enough, the slow reaction kinetics could prevent the formation of reaction products. Furthermore, when the relaxation time between pulses is long enough, the formed electrolysis products or half products should be able to diffuse away from the electrodes, hampering bubble nucleation. Uchikoshi was capable of forming damage free deposits by combining pulsed DC signals with hydrogen absorbing palladium electrodes.<sup>39</sup> Besra, in cooperation with Uchikoshi, reported that a narrow pulse width window exists in which deposits without apparent inclusion of gas bubbles can be obtained on any type of electrode surface when using this particular mode of EPD.<sup>40,41</sup> Below this window, no deposit is formed, whereas the deposits are damaged by evolved gas above this critical pulse duration and/or amplitude. The window is widest at low voltages and currents.<sup>40,41</sup> However, even at the conditions where bubble-free deposits are formed, electrolysis of water still occurs. Besra showed that pH shifts can be measured in the vicinity of the electrodes during pulsed DC.<sup>42</sup> As these pH shifts are directed toward the isoelectric point of the particles, they aid in the deposition.

During a study of the deposition of polystyrene particles using conventional DC and pulsed DC signals, Naim noticed a significantly lower number of clusters depositing on the surface in the pulsed mode due to the reduced influence of electroosmotic flow.<sup>43</sup> During further studies using commercial titanium oxide particles, Naim noticed selective deposition of well deagglomerated particles. As single particles have a lower inertia than particle clusters, they can react faster to the applied field. Naim therefore suggested that the use of high frequency DC pulses promotes the deposition of single particles rather than agglomerates, resulting in deposits with a better particle stacking.<sup>44,45</sup> Naim further suggested that the use of pulsed DC-EPD might be used to introduce particle size control during electrophoretic deposition.

**Symmetric AC Fields.** Electrochemical decomposition of water is unlikely to occur in alternating fields with a sufficient frequency. In view of this, Hirata suggested the use of symmetric AC signals to form deposits from aqueous suspensions.<sup>46</sup> In his experiments, no deposition was observed when using fields with a frequency below 1 kHz. At 10 kHz, a deposit was formed but the deposition rate was extremely low and seemed to be controlled by the diffusion of alumina in the suspension. Hence, it can be concluded that no net electrophoresis occurs under the influence of symmetric AC fields and the term electrophoretic deposition does not apply for the production of deposits in this way.

During experiments using suspensions of polystyrene particles in a symmetric AC field, Zhou noticed that particles with a low zeta-potential tend to cluster and particles with a high zeta-potential remain mutually repellant in low frequency field.<sup>47</sup> Furthermore, over time, the clustering particles form a homogeneous monolayer over the entire electrode depending on the applied frequency. A similar frequency dependence was noticed by Song who deposited metal nanoparticles in symmetric AC fields.<sup>48</sup> Song argues that the AC fields induce EHD flows around the charged nanoparticles that induce the packing on the electrode. The frequency dependence of the deposition hence is the result of the frequency dependence of these flows. By optimizing the frequency, hexagonal dense packed monolayers can be obtained.<sup>48</sup> Zhou allowed particles to sediment to the bottom electrode under the influence of gravity prior to rearranging them using the AC field.<sup>47</sup> It can therefore be argued that the electrode-particle contact might be obtained by gravity, whereas the stacking is an effect of electrohydrodynamic flow patterns. As both Zhou and Song performed their experiments in a similar small horizontal cell mounted on a microscope for the visualization of the particle behavior, gravity might also play a role in the deposition of the monolayer formed by Song.

Recently, a group of scientists at the University of Tehran demonstrated the formation of deposits by means of symmetric sinusoidal AC fields with frequencies ranging from 0.01 Hz to 1 kHz.<sup>49</sup> During these experiments, gold electrodes located on the same side of a single sheet of insulating material, such as glass, were used (Figure 9).<sup>50</sup> In this manner,  $\text{SnO}_2$ ,<sup>49,50</sup>  $\text{WO}_3$ ,<sup>51</sup> and multiwalled carbon nanotubes were successfully deposited.<sup>52</sup> In most of the presented results, however, the deposit grows in the gap between the electrodes instead of on



**Figure 9.** Electrode setup as used by Gardeshzadeh and Raissi, reprinted in part with permission from Elsevier.<sup>50</sup>

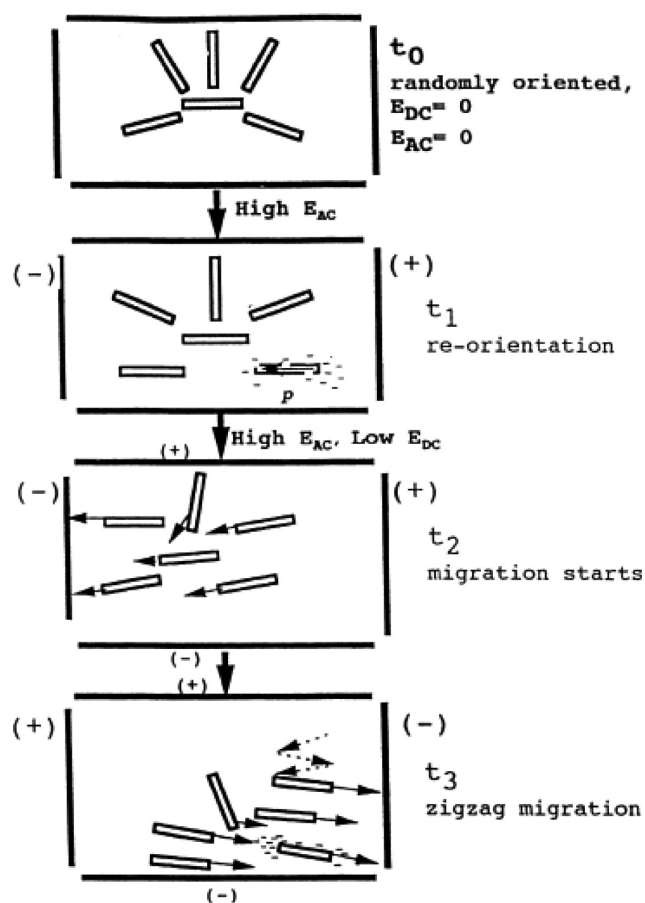
top of the electrodes as in traditional EPD. Most probably, these deposits are not formed by electrophoretic deposition, as described by Hamaker,<sup>9</sup> but are the result of dielectrophoresis due to the inhomogeneous distribution of the field lines. For instance, the chain-like structures formed using this technique are similar to the pearl chains formed during dielectrophoresis.<sup>51,53</sup> Localized destabilization of the suspension due to local heating is another possible cause for the formation of deposits using this technique. Because the setup used consists of two electrodes on a dielectric material, it acts as a basic capacitor. Hence, the impedance of the setup will decrease as a function of the field frequency, leading to increasing both the current flowing through the electrode gap and local Joule heating.

**AC Field Assisted Electrophoretic Deposition.** In one particular application, the use of alternating fields during electrophoretic deposition has been suggested as a means to texture materials. Yue described the use of a symmetric AC field oriented perpendicular to a DC field for the production of Ag-sheathed  $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_x$  thick films with improved microstructural and superconducting properties.<sup>54</sup> The AC field causes the platelet superconductor particles to move back and forth in a plane parallel to the deposition electrode. Due to drag, the platelets align themselves into the plane of movement. Upon applying a DC field directed toward the deposition electrode, the particles migrate in a zigzag way, resulting in deposition with a preferentially aligned *c*-axis.<sup>54</sup> The alternating field does however not contribute to the actual deposition but only serves to direct the orientation of the particles in the deposit (Figure 10). As the main deposition mechanism is controlled using a DC field, this method cannot be considered to be true AC-EPD. Instead, Yue aptly named this technique AC-electric field assisted electrophoretic deposition.<sup>54</sup>

**Asymmetric AC Fields.** The use of alternating fields superimposed on DC fields for improving EPD efficiency has been suggested in patents as early as 1969.<sup>55,56</sup> Unfortunately, patents are often overlooked by the scientific community as a valuable source of information. It is therefore not surprising that research executed behind closed doors in industrial laboratories is unknowingly repeated in an academic setting.

Recently, Nold demonstrated the use of asymmetric alternating block waves, created using a waveform generator, for the localized deposition of alumina particles by means of microcounter electrodes.<sup>57</sup> By moving this counter-electrode, patterns can be deposited, yielding a simple yet effective printing technique. Furthermore, Nold found that the resolution obtained using alternating fields is significantly higher than with DC fields and less sensitive to damage by gas evolution. The resolution difference between deposition using DC and AC fields can possibly be linked to a difference in the generation of the ion-depleted zone noticed by Stappers.<sup>22,58</sup> The alternating nature of the asymmetric fields used by Nold might retard formation of this ion depleted zone, as ions are not continuously repulsed or attracted by a single electrode. In addition, the pH shift at the electrodes due to the electrolysis of water is reduced. Hence, the locally formed deposit imposes a smaller resistance against the applied field, preventing spreading of the electric field and subsequent throwing power effects. Theoretically speaking, the resolution should only depend on the geometry of the induced electric field when the deposit forms no resistance against the applied field.

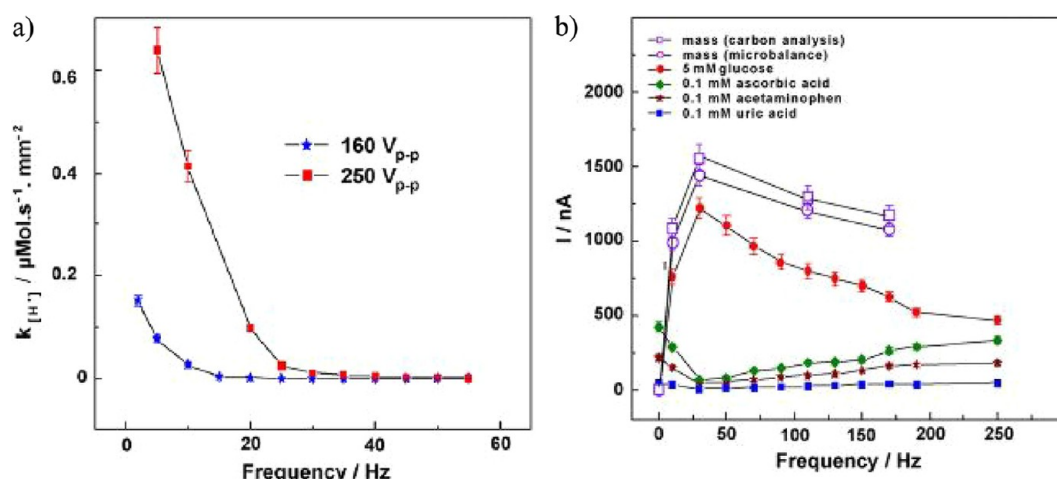
On the other hand, Neirink deposited the same alumina powder from aqueous suspensions using the unbalanced



**Figure 10.** Schematic representation of AC field assisted electrophoretic deposition as used for the texturing of materials by oriented deposition of platelets, reprinted in part with permission from Elsevier.<sup>54</sup>

alternating fields as described by Dukhin.<sup>17</sup> Neirink observed during these experiments that, next to the absence of defects caused by gas bubble evolution, a generally higher green density was obtained than possible when using this powder in traditional DC-EPD from alcohol based suspensions.<sup>35</sup> Furthermore, Neirink noted the absence of the self-limiting behavior characteristic of DC-EPD from alcohol based suspensions.<sup>35</sup> In traditional electrophoretic deposition of insulating materials from polar solvent suspension, the deposit resistance increases with deposit thickness. This results in the self-limiting effect, described as throwing power, when using constant voltage deposition.<sup>23</sup> As the deposit resistance increases, the voltage drop over the suspension driving the deposition decreases, resulting in a limited deposit thickness. The increase in deposit resistance is linked to the formation of ion-depletion zones near the electrodes.<sup>22,58</sup> Neirink proposed that these depletion zones are less prone to form in an alternating field, hence preventing a gradual increase in deposit resistance and suggesting the possibility of unlimited deposit thickness.<sup>35</sup> In subsequent work, Neirink expanded these experiments toward the deposition of particle stabilized emulsions in order to form macroporous ceramics with controlled porous properties.<sup>59</sup> These experiments show that entities with sizes up to 50  $\mu\text{m}$  can be deposited in unbalanced fields.





**Figure 11.** (a) Proton production rate during the deposition of glucose oxidase using asymmetric AC fields as a function of signal frequency and voltage and (b) deposit yield and response of glucose sensors prepared by AC-EPD of glucose oxidase at  $160 V_{p-p}$  as a function of frequency, reprinted in part with permission from Elsevier.<sup>62</sup>

## 6. DEPOSITION OF BIOMOLECULES

Electrophoresis by means of DC fields is widely used in biotechnology for the separation and characterization of biomolecules.<sup>60</sup> However, DC-EPD might also be used in large scale production of biotechnological products. Novak for instance was able to separate inclusion bodies from *E. coli* bacteria by selecting a pH located between the isoelectric point of the inclusion bodies themselves and that of the bacteria fragments remaining after mixing a bacterial suspension. Upon application of a DC field, the inclusion bodies were deposited on an electrode.<sup>61</sup>

For the actual functional deposition of biomolecules thus far, only unbalanced AC fields have been used. Amman deposited glucose oxidase,<sup>62</sup> beta-galactosidase,<sup>63</sup> and glutamate oxidase<sup>64</sup> on a platinum wire with the intention of producing biosensors for the detection of glucose, lactose, and glutamate, respectively. In all cases, a thin polyurethane coating was needed to improve the stability of the biosensor. This indicates that the biomolecules do not strongly adhere to the noble wire to form a permanent coating. Amman however demonstrated that the produced sensors were highly sensitive and provided a stable signal despite this additional barrier.<sup>62–64</sup> Furthermore, Amman demonstrated that the electrolysis of water still occurs despite the use of balanced asymmetric AC fields during these experiments. The rate at which the protons are formed is linked to the amplitude and frequency of the applied field (Figure 11a).<sup>62</sup> Above a given frequency threshold, no measurable amount of protons is formed. For lower amplitudes, this threshold occurs at lower frequencies (Figure 11a). The deposit yield and sensor activity is maximal for a frequency just above this threshold (Figure 11b). Amman suggests that the deposition of the glucose oxidase enzyme might be caused by precipitation of the molecule due to the pH decrease at the electrode.<sup>62</sup>

## 7. MANIPULATION OF CELLS WITH ALTERNATING FIELDS

It stands to reason that each living organism has a specific but limited tolerance toward an electrical current flowing through it or potential applied over it. As Tesla demonstrated in public using his own body, this tolerance is typically higher for AC than for DC signals but nevertheless is still limited.<sup>65,66</sup> In fact,

the use of high field strength pulsed and alternating fields is investigated as a method for the sterilization of liquid foods and clinical waste waters.<sup>67,68</sup> The pulsed electric field (PEF) treatments specifically target cell membranes by dielectric breakthrough of the phosphorus lipid bilayer. Due to the formation of pores and ruptures in the membrane, cell viability is compromised. Because of their cell wall composition, gram positive bacteria are significantly less sensitive to this form of sterilization than gram negative types. The electric energy density,  $Q$ , used to achieve the desired effect for a volume of liquid,  $V$ , can be calculated as a function of the applied potential,  $U$ , current,  $I_A$ , and time,  $t$ .<sup>67</sup>

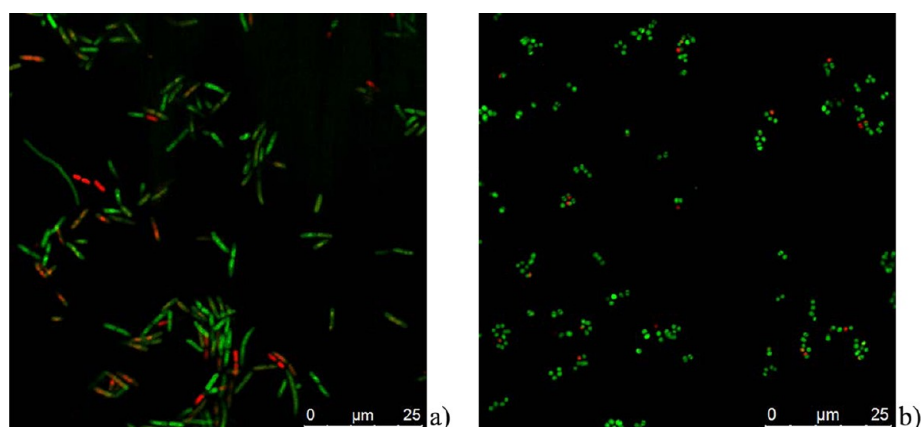
$$Q = \frac{U \cdot I_A \cdot t}{V} \quad (5)$$

Block pulses with field strengths ranging from 30 to 80 kV/cm in a frequency range of 10–300 Hz are typically used to ensure complete inactivation of the bacteria present in a matter of milliseconds to seconds.<sup>67–69</sup> Compared to DC signals, these high field strength PEFs do not cause deterioration of the liquid matrix, the generation of toxic byproduct, or a significant increase in temperature, making them suitable for the sterilization of food.

The EPD experiments with living cells reported in the literature are typically carried out using a fraction of the field strength used for PEF treatments but over a much longer period of time. Hence, it is not exactly clear what effect these fields have on the cell membrane.

### Dielectrophoresis versus Electrophoretic Deposition.

The use of electric fields to move bacteria and even deposit them on a surface or organize them in a structure is not new. Direct current electrophoretic deposition has been suggested and investigated as a method to produce cell coatings and to remove and/or inactivate bacterial films growing on conductive surfaces.<sup>70,71</sup> Since most biological systems are aqueous, the crucial parameter for the use of DC fields is the applied voltage. When the voltage is too high, the electrochemical decomposition of water will cause pH shifts and even gas evolution at the electrode. Near the cathode, the pH shifts toward more basic values, whereas the system becomes more acidic at the anode. Depending on the resilience of the cell being deposited, this may lead to cell death. In fact, the use of DC fields is under



**Figure 12.** Confocal microscope images of (a) the deposition electrode after 2400 s deposition of *E. coli* from demineralized water and (b) the deposition electrode after 2400 s deposition of *S. aureus* from 300 mM sucrose solution. In the images, green represents living cells, while the dead cells are colored red. Cells stained by both colorants have a compromised cell wall and are to be considered dead. All experiments were carried out using a 100 V peak-to-peak field with a frequency of 25 Hz and an asymmetry of 4. Reprinted in part with permission from Elsevier.<sup>80</sup>

investigation as a method to remove or inactivate bacterial films.<sup>72</sup>

In recognition of this problem, many researchers switched to the use of high frequency alternating fields to move cells. However, cell movement in the proposed systems is due to dielectrophoresis instead of electrophoresis. Dielectrophoresis is the movement of particles in a nonuniform field due to polarization effects in the particles or cells induced by the nonuniform field. Contrary to electrophoresis, this implies that the particles do not need to be charged. When the permittivity of the particle is higher than for the medium, the particle is prone to locate itself in the regions where the field lines are concentrated. Lower permittivity particles move to places where the electric field is weaker.<sup>73</sup> In principle, dielectrophoresis occurs both in AC and DC fields. When living cells need to be moved, high frequency AC fields are used to prevent electrochemical poisoning of the cells.

Because the cells locate themselves as a function of the electric field concentration, patterns can be obtained.<sup>73,74</sup> In most cases, these patterns build up between electrodes instead of on them, whereas the particles move toward an electrode in electrophoresis. The patterns in most cases also break up after the nonuniform alternating field is removed. Dielectrophoresis alone does not give rise to cell fixation. Instead, the cells are usually trapped by cross-linking of a hydrogel in the system.<sup>75,76</sup>

**Electrophoretic Deposition in Symmetric AC Fields.** In 1999, Poortinga described the formation of clusters of spherical and rod-shaped bacteria on an electrode during electrophoretic deposition using DC fields.<sup>70</sup> The interbacterial distance in these clusters was related to the ionic strength and the DC field density. The clusters could be immobilized by applying a sufficiently high current. In AC fields with frequencies up to 2 MHz and amplitudes up to 13 V, Poortinga did not observe the same bacterial clustering. However, the rod-shaped *A. naeslundii* 147 aligned instantaneously parallel to the AC field in fields with frequencies below 1 MHz.<sup>70</sup> This effect is similar to the alignment of platelet particles using AC fields as described by Yue.<sup>54</sup>

Unfortunately, the extent of Poortinga's work on alternating fields is not clear from his papers. Brisson argues that the studied frequencies might have been too high to induce bacterial clustering on electrodes.<sup>77</sup> Indeed, the frequencies at which these clustering effects occur depend on particle size,

electrolyte mass transport properties, and the rate of electrode reactions.<sup>20,34,78,79</sup> Furthermore, Brisson states that, given the importance of the electrode reactions, using a buffer such as the bicarbonate system to mitigate the electrode reactions might be crucial for the occurrence of clustering in AC fields. Poortinga only used KNO<sub>3</sub> to control the ionic strength of the cell suspensions.<sup>77</sup> Brisson observed the formation of dense two-dimensional cell clusters from extremely dilute *Saccharomyces cerevisiae* suspensions in a matter of minutes.<sup>77</sup> The forming rate of these clusters is inversely related to the ionic strength and applied frequency.<sup>77</sup> The steady-state density of the cell clusters on the other hand appeared to be directly related to the applied field strength and to be independent of both frequency and ionic strength.<sup>77</sup> Furthermore, the formation of the clusters in AC fields was reversible. Upon removal of the field, the cells dissipated by diffusion. Adhesive contact however could be achieved by a 3 min application of a modest 50  $\mu$ A DC current.<sup>77</sup> Brisson suggested that the presented technique could be used to produce patterned cell arrays for biotechnological applications and that low ionic strengths yield the highest production rates. Brisson also proposed that nonionic osmotic pressure balancing solutes should not impair EPD performance and might be used for manipulating cells which lack rigid cell walls.<sup>77</sup>

Zhou noticed the formation of large clusters of yeast cells on an electrode after 10 min of application of a low frequency AC field.<sup>47</sup> Furthermore, Zhou noticed a phase separation upon application of similar fields in binary systems containing both yeast cells and polystyrene particles.<sup>47</sup> This phase separation yields regions rich in yeast cells surrounded by a shell of polystyrene particles. Upon continued application of the field, an Ostwald type ripening of the yield clusters was observed, resulting in large yeast assemblies edged by polymer particles. Zhou concluded that the two-dimensional sorting is the result of the difference in dielectric properties of polymers and cells.<sup>47</sup>

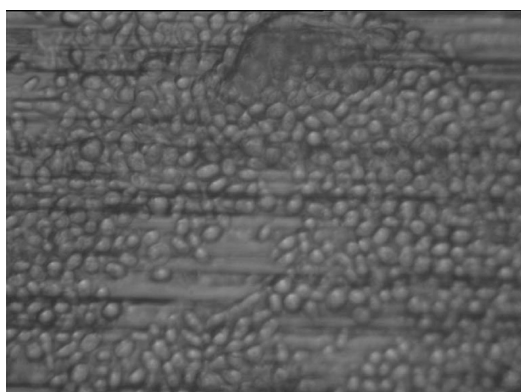
#### **Electrophoretic Deposition in Asymmetric AC Fields.**

The results of Brisson show that symmetric AC fields can be used to form cell clusters but that an additional short DC current is needed to adhere them. The latter will push the cells toward the electrode, ensuring a mechanical contact which on its own might be sufficient to cause the cells to stick to the electrode. Hence, asymmetric AC fields, with a net DC or the unbalanced AC signals as described by Dukhin<sup>17</sup> and



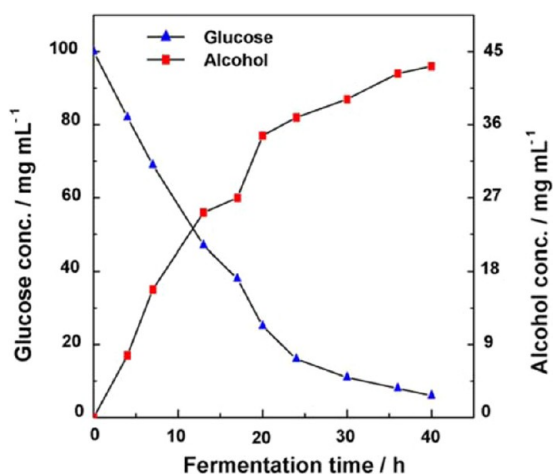
Neirinck,<sup>35</sup> which cause a net force toward one electrode, might allow both the clustering and permanent cell adherence to be achieved simultaneously. With this in mind, Neirinck expanded his work from inorganic matter to bacterial cells, more specifically *Staphylococcus aureus* and *Escherichia coli*.<sup>80</sup> Neirinck's results showed that, in unbalanced AC fields, the bacteria can be deposited selectively from dilute suspensions in demineralized water. Furthermore, the contact between electrode and bacteria is strong enough to prevent removal upon washing in medium. Live-dead analysis revealed that a significant number of bacteria survive 40 min of exposure to a 100 V peak-to-peak field in demineralized water (Figure 12). Using a 300 mM sucrose solution with *S. aureus* reduces the number of deposited cells, whereas the fraction of surviving cells increased (Figure 12b).<sup>80</sup>

Ammam showed that yeast cells immobilized using the same type of field as described by Neirinck<sup>80</sup> (Figure 13) are still



**Figure 13.** Micrograph of yeast cells deposited on a stainless steel foil electrode using a field of 50 V<sub>p-p</sub> and a suspension of 0.1 g of yeast in 3 mL of demineralized water. The mean size of the used yeast cells is 6–7  $\mu\text{m}$ .<sup>83</sup>

capable of fermenting sugar into alcohol after immobilization (Figure 14).<sup>81</sup> The produced yeast arrays even exhibited a higher fermentation efficiency than free floating yeast cells, a result which is further improved when the cell arrays are additionally stabilized using a polyurethane coating.<sup>81</sup> These



**Figure 14.** Fermentation of alcohol by *Saccharomyces cerevisiae* cells immobilized on stainless steel after AC-EPD, reprinted in part with permission from Elsevier.<sup>81</sup>

results demonstrate that immobilization of metabolizing species by means of electrophoretic deposition might be used to produce bioreactors. With bioethanol as a potential fuel of the future, this evolution may have a worldwide impact on daily life.

## 8. CONCLUSIONS

Electrophoretic deposition is a scientifically interesting technique because of its inherent versatility. Nonetheless, EPD has known only limited success in industrial applications. This is partly due to the need to use expensive and potentially hazardous organic solvents in traditional electrophoretic deposition. This disadvantage has fueled research into methods which enable the use of aqueous suspensions in electrophoretic depositions. Recently, taking advantage of fundamental knowledge which has been available for decades, a significant effort is directed toward the use of alternating fields. This review provides a perspective in both these renewed efforts and the original research which serves as the foundation of these efforts.

Due to the less invasive nature of aqueous systems and alternating fields, this new form of electrophoretic deposition is particularly interesting for the manipulation of biological materials and production of biotechnological devices. This is already reflected in an increased amount of papers published on this subject, which are summarized in this review and which provide promising results toward the production of biosensors and bioreactors.

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### Notes

The authors declare no competing financial interest.

### Biographies

Bram Neirinck obtained the degree of Master in Chemical Engineering at KU Leuven in 2004. He subsequently started his career in research at Department of Metallurgy and Materials Engineering (MTM) of KU Leuven with his Ph.D. on the production of porous materials by electrophoretic deposition (EPD) of emulsion templates. During this research, he developed an alternating current electrophoretic deposition (AC-EPD) technique which enabled him to deposit emulsion droplets from aqueous systems. Currently, Dr. Neirinck is working on using AC-EPD for the deposition of proteins and cells.

Prof. Omer Van der Biest holds a degree of Master in Metallurgical Engineering of the KU Leuven (1970) and a Ph.D. in Materials Science and Engineering at the University of California, Berkeley (1974). His past and present research interests include high temperature materials, ceramic composites, powder metallurgical processes, processing of materials in strong magnetic fields, and electrophoretic deposition, a field in which he is considered to be one of the leading authorities. Omer Van der Biest is currently connected to the Department of Metallurgy and Materials Engineering (MTM) of KU Leuven as Professor Emeritus.

Jozef (Jef) Vleugels obtained an engineering degree in chemistry and agricultural industries at KU Leuven in 1988. He joined the Department of Metallurgy and Materials Engineering (MTM) of KU Leuven, obtaining the degree of Doctor in the Applied Biological Science in 1995. He became associate professor at the Faculty of Engineering at KU Leuven in 2001 and full professor in 2012, and is currently teaching courses on ceramic materials and powder metallurgy. Keywords describing his research are powder metallurgy,

processing of ceramics, ceramic composites, graded materials, cutting tools, and biomaterials.

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