Electrostatic interactions in the adhesion of an ion-penetrable and ion-impenetrable bacterial strain to glass

Abstract

Deposition to glass of *Streptococcus salivarius* HB-C12 and *Staphylococcus epidermidis* 3399 in a parallel plate flow chamber has been studied as a function of ionic strength. Electrophoretic mobility measurements revealed that *S. epidermidis* 3399 possesses a thick ion-penetrable layer, probably associated with its encapsulation, while *S. salivarius* HB-C12 has an ion-impenetrable surface. Streaming potential measurements indicated that also the glass surface was covered with a relatively thin, ion-penetrable layer. Theoretical initial deposition rates of both strains to glass were obtained by numerically solving the convective-diffusion equation, while accounting for the ion-penetrability of the interacting surfaces. Experimentally, the initial deposition rate of the ion-penetrable strain *S. epidermidis* 3399 was found to be higher and less dependent on ionic strength than of the ion-impenetrable *S. salivarius* HB-C12, in accordance with theoretical expectations. Agreement between theoretical and experimental deposition rates could be obtained when glass was considered ion-penetrable when interacting with the ion-penetrable organism *S. epidermidis* 3399, while glass behaved as an ion-impenetrable surface when interacting with the ion-impenetrable *S. salivarius* HB-C12. Probably, interaction with an ion-impenetrable strain drives the diffuse double layer charges into the limited volume of the thin ion-penetrable layer on the glass; readily filling it up and making it appear ion-impenetrable. During interaction of glass with another ion-penetrable surface, as of *S. epidermidis* 3399, diffuse double layer charges move into both ion-penetrable surfaces, resulting in a much lower mobile charge density in the ion-penetrable layer on the glass which consequently continues to behave as ion-penetrable.

Introduction

The DLVO theory, in which adhesion is envisaged as an interplay of Lifshitz-Van der Waals and electrostatic interactions (also referred to as electric double layer interactions), can describe adhesion of colloidal particles. In addition, acid-base interactions are included in the so-called XDLVO theory [1], but acid-base interactions are relatively short ranged and not always operative when structural and chemical heterogeneities as on bacterial cell surfaces maintain a distance between interacting surfaces [2].
The DLVO theory has also been used to describe bacterial adhesion and has proven to be successful when certain collections of bacterial strains and species are considered, in widely different fields of application ranging from meat processing [3] to infection of biomaterials implants [4]. However, the DLVO theory has so far failed to yield a generalised description of all aspects of microbial adhesion valid for each and every strain. Most likely, to achieve this it is necessary to adapt the DLVO theory to account for the wide variety of possible bacterial surface structure [5].

Recently, Ohshima et al. developed a model that describes the electrophoretic mobility of particles for which fixed surface charges are distributed through a porous surface layer in which diffuse double layer ions and water molecules can freely penetrate [6,7]. Since, this model has been successfully applied to describe the electrophoretic mobilities of various particles, such as hydrogel covered latex particles [8], blood cells [9] and also bacteria [10]. Consequently, it can be concluded that bacteria may have an ion-penetrable surface layer, which may result from encapsulation or fibrillation of the bacterial cell surface.

In the Ohshima model, the ion-penetrable layer is characterised by its fixed charge density $\rho$ and a parameter $\lambda^{-1}$, that is referred to as the electrophoretic ‘softness’ of the ion-penetrable layer and that depends on the frictional force exerted on water when it flows through the ion-penetrable layer. Note that this electrophoretic ‘softness’ in principle bears no relation with the mechanical properties of the surface layer, although recently for two streptococcal strains atomic force microscopy has indicated a correspondence between mechanical and electrophoretic softness [11]. Fig. 1 schematically presents the electroosmotic liquid flow around ion-penetrable bacteria with different electrophoretic softness and around and ion-impenetrable bacterium. For ion-impenetrable and ion-penetrable hard (i.e., $\lambda^{-1} \rightarrow 0$) bacterial cell surfaces, electrophoretic liquid flow is zero at the outermost cell surface increasing exponentially with distance from the surface, while for the soft, ion-penetrable cell surface a substantial electrophoretic flow develops already in the ion-penetrable layer. By consequence (see Fig. 1), despite having a similar charge distribution and electric potential, the hard and soft ion-penetrable bacteria demonstrate distinctly different electrophoretic velocities in an applied electric field. The ion-impenetrable bacterium in Fig. 1 has a similar liquid velocity distribution during electrophoresis as the ion-penetrable, hard bacterium, but its charge is located at the cell surface rather than being distributed over the ion-penetrable layer yielding a different electric potential distribution. Traditionally, bacteria have been regarded as ion-impenetrable, but based on the dependence of the electrophoretic mobility on the ionic
Chapter 2a

strength it is possible to distinguish between ion-penetrable and ion-impenetrable bacterial strains.

Upon approach of two similarly charged surfaces, diffuse double layer charges are compressed, which leads to the traditional electrostatic repulsion as accounted for in the DLVO-theory, either under the assumption of constant surface charge or constant surface potential. When the interacting surfaces are ion-penetrable, however, the compression of diffuse double layer ions at constant fixed surface charge density is less and therefore electrostatic repulsion is reduced [12]. Until now, this has not been accounted for in the DLVO-theory of colloidal stability as applied toward bacterial adhesion, because bacteria were generally considered as ion-impenetrable particles [5].

The aim of this paper is firstly to theoretically describe the deposition of an ion-impenetrable and an ion-penetrable bacterial strain to glass in a parallel plate flow chamber on the basis of the DLVO-theory and the convective-diffusion equation and secondly to compare theoretical predictions with experimental results. To this end, two bacterial strains were selected with comparable size, hydrophobicity and Lifshitz-Van der Waals interaction with glass, in order to exclusively study the effects of ion-penetrability upon deposition.
**Figure 1.** Schematic presentation of the surfaces of an ion-penetrable soft and hard bacterium and of a traditional, ion-impenetrable bacterial cell surface. The liquid flow velocity distribution as it occurs during electrophoresis and the electric potential distribution are indicated as a function of distance from the cell surface. Note that for the ion-penetrable bacteria, charge is distributed homogeneously over the surface layer, while for the ion-impenetrable bacterium the charge is located at the surface. The slip plane is assumed to coincide with the ion-impenetrable surface.
Chapter 2a

Theory

Mass transport in the parallel plate flow chamber

Transport of colloidal particles such as bacteria by a flowing fluid can be described by the convective-diffusion equation which, expressed in dimensionless form and with parameters adapted for the parallel plate flow chamber configuration [13], reads

\[
Pe f_p (H \times H + 1)[2 - (H + 1)A] \frac{\partial C^*}{\partial x^*} - \frac{\partial}{\partial H} \left[ f_p (H) \left( \frac{\partial C^*}{\partial H} + C^* \frac{\partial \phi^*}{\partial H} \right) \right] = 0
\]

(see nomenclature list for symbols). \( \phi^* \) denotes the interaction potential to which the bacteria are subjected, expressed in \( kT \). A computationally convenient boundary condition to Eq. (1) tailored for the parallel plate flow chamber is

\[
- f_p (H) \left[ \frac{\partial C^*}{\partial H} \bigg|_{H=\delta} + C^* (H = \delta) \frac{\partial \phi^*}{\partial H} \bigg|_{H=\delta} \right] = \frac{C^* (H = \delta)}{\exp(-\phi(\delta))} \int_0^{\delta} \frac{\exp(\phi^*(H))}{f_p (H)} dH
\]

where \( \delta \) denotes the thickness of a layer adjacent to the collector surface over which convection may be neglected. Eq. (2) implies a “perfect-sink” boundary condition, i.e. every bacterium that reaches the substratum surface is immediately, irreversibly bound and disappears from the system, leaving a zero particle concentration at the substratum surface. As a second boundary condition, the first derivative of the bacterial concentration to \( H \) is assumed to be zero at the centre in-between the parallel plates. In this paper, Eq. (1) has been solved numerically [13] using a central difference scheme with a nonuniform mesh for the \( y \) coordinate and explicit discretization with a constant step size for the \( x \) coordinate. In the \( y \) direction, the step size decreases for decreasing \( y \). The step size in the \( x \) direction is decreased by the computer program until a stable solution is obtained.
Ion-penetrability in bacterial adhesion

**Bacterium-substratum interaction potential**

According to the classical DLVO theory, the bacterium-substratum interaction potential occurring in Eq. (1) consists of Lifshitz-Van der Waals and electrostatic interactions. The unretarded Lifshitz-Van der Waals interaction for the sphere-plane configuration is given by [14]

\[ \phi_{LW} = -\frac{A_{132}}{6H} \] (3)

The Hamaker constant \( A_{132} \) depends on the composition of the interacting surfaces and can be calculated from published contact angles on bacterial lawns and glass [15].

The electrostatic interaction term for two ion-impenetrable surfaces assuming constant charge can be taken from Visser [14], and is given in Table 1. Ohshima and Kondo [16] have derived an expression for the electrostatic interaction between two ion-penetrable surfaces (see also Table 1) under the condition of constant fixed surface charge, but no expression yet exists for the interaction between an ion-impenetrable sphere and an ion-penetrable surface with arbitrary dielectric permittivity.

For an infinitesimally thick, homogeneously charged, ion-penetrable plate 1, extending from \( x \leq 0 \) with a fixed charge density \( \rho \) and an ion-impenetrable plate 2 at \( x = h \) with a surface charge density \( \sigma \) interacting in an aqueous solution 3, the linearised Poisson-Boltzmann equation reads

\[ \frac{d^2\psi}{dx^2} = \kappa^2 \psi - \frac{\rho}{\varepsilon_1} \quad \text{for} \quad x < 0 \] (4)

\[ \frac{d^2\psi}{dx^2} = \kappa^2 \psi \quad \text{for} \quad 0 < x < h \] (5)
\[ \begin{align*}
\frac{\varepsilon \frac{\partial \psi}{\partial t}}{\varepsilon \frac{\partial \psi}{\partial t} - \varepsilon \frac{\partial \psi}{\partial t}} &= \alpha \\
\left( \frac{\varepsilon \frac{\partial \psi}{\partial t} + \varepsilon \frac{\partial \psi}{\partial t}}{\varepsilon \frac{\partial \psi}{\partial t} - \varepsilon \frac{\partial \psi}{\partial t}} \right) &= \frac{p}{q} \\
\left( \frac{\varepsilon \frac{\partial \psi}{\partial t} + \varepsilon \frac{\partial \psi}{\partial t}}{\varepsilon \frac{\partial \psi}{\partial t} - \varepsilon \frac{\partial \psi}{\partial t}} \right) &= \frac{p}{q} \\
\left( \frac{\varepsilon \frac{\partial \psi}{\partial t} + \varepsilon \frac{\partial \psi}{\partial t}}{\varepsilon \frac{\partial \psi}{\partial t} - \varepsilon \frac{\partial \psi}{\partial t}} \right) &= \frac{p}{q}
\end{align*} \]

with

The paper [1] expresses

\[ \left( \frac{\varepsilon \frac{\partial \psi}{\partial t} + \varepsilon \frac{\partial \psi}{\partial t}}{\varepsilon \frac{\partial \psi}{\partial t} - \varepsilon \frac{\partial \psi}{\partial t}} \right) \frac{p}{q} \]

2 penetrable

1 penetrable

Table 1. Expressions for the electrostatic interaction potential between various ion-penetrable and penetrable surfaces. For the sphere-plane configuration, 1 and 2 denote the interacting surfaces, \( \varepsilon \) denotes the solution

\[ \begin{align*}
\text{Joules} \\
\text{Interaction Potential \( \Phi \)} \( t \) (1) (2)
\end{align*} \]
where the subscript 1 denotes the ion-penetrable plate and 3 denotes the solution. Three boundary conditions apply

1) \( \sigma = -\varepsilon_1 \frac{d\psi}{dx} \) for \( x = h \), which is Gauss’ law

2) \( \psi \) and \( \varepsilon_i(d\psi/dx) \) need to be continuous at \( x = 0 \)

3) \( \frac{d^2\psi}{dx^2} = 0 \) for \( x \rightarrow -\infty \)

stating that far inside the ion-penetrable layer the fixed surface charge density is fully compensated by the diffuse charge density, yielding a zero net charge.

After solving Eqs. (4) and (5) under the condition of constant fixed charge density, the electric potential distribution obtained can be transformed into an electrostatic interaction free energy per unit area \( F(h) \) by

\[
F(h) = -\frac{1}{2} \sigma \psi (x = h) + \frac{1}{2} \int_0^h \rho \psi (x) dx
\]

and consequently the electrostatic interaction potential for the interaction of the two plates \( V_p(h) \) can be obtained from

\[
V_p(h) = F(h) - F(\infty)
\]

In order to obtain an approximate expression for the electrostatic interaction potential for the sphere-plate interaction \( \phi(h) \) the Derjaguin approximation [17] is used

\[
\phi_d(h) = 2\pi a_b \int_h^\infty V_p(h) dh
\]
yielding an expression for the electrostatic interaction energy for the sphere-plate configuration with one of the interacting surfaces being ion-penetrable (see Table 1). In addition to DLVO interactions, bacteria are subjected to the potential due to gravity and buoyancy

$$\phi_{gr} = \frac{4}{3} \pi a_b^3 (\rho_b - \rho_i) g a_b H$$

(9)

**Electrokinetic characterisation of ion-penetrable and impenetrable surfaces**

Calculation of the electrostatic interaction energies as given in Table 1, requires knowledge of the surface potential of an ion-impenetrable surface, and of the fixed charge density of an ion-penetrable surface. Both parameters can be obtained by measuring the electrophoretic mobility. For particles, measurement of the particle velocity $u_E$ in an applied electric field $E$ yields the electrophoretic mobility

$$\mu = \frac{u_E}{E}$$

(10)

while for a plate, the electrophoretic mobility can be derived from the streaming potential $E_{str}$ arising from a forced fluid flow under the influence of a pressure difference $P$ over the surface [18] according to

$$\mu = K \frac{E_{str}}{P}$$

(11)

For ion-impenetrable surfaces, Von Smulochowski’s relation can be used in order to calculate the zeta potential as a measure for the electric potential at a surface [19]

$$\zeta = \eta \mu$$

(12)
For ion-impenetrable surfaces with a low surface potential, the linearised Poisson-Boltzmann equation can be applied, and a relation between the electrophoretic mobility and the inverse Debye length of the solution $\kappa$ can be derived from Eq. (12)

$$\mu = \frac{\sigma}{\eta \kappa}$$  \hspace{1cm} (13)

As the inverse Debye length depends on the ionic strength of the solution, Eq. (13) gives the electrophoretic mobility of an ion-impenetrable surface as a function of ionic strength.

For ion-penetrable surfaces, Eqs. (12) and (13) can no longer be applied, as fluid flow through the ion-penetrable layer contributes to the electrophoretic mobility. Ohshima and Kondo [7] have derived a relation between the electrophoretic mobility of an ion-penetrable surface with a dielectric permittivity equal to the solution permittivity and the inverse Debye length of the solution. However, the dielectric permittivity of an ion-penetrable layer need not necessarily be the same as that of the solution. For a flat ion-penetrable layer with charge density $\rho$, extending from $x \leq 0$ and in contact with a solution, the electric potential is described by Eq. (4) and (5), with boundary conditions 2 and 3. Application of an electric field $E$ parallel to the surface results in a fluid velocity $u(x)$ relative to the surface that is determined by the Navier-Stokes equations

$$\eta \frac{d^2 u}{dx^2} + \rho E = 0 \hspace{1cm} \text{for } x > 0$$  \hspace{1cm} (14)

$$\eta \frac{d^2 u}{dx^2} - \gamma u + \rho E = 0 \hspace{1cm} \text{for } x < 0$$  \hspace{1cm} (15)

subjected to two boundary conditions

1) $u(x)$ and $du(x)/dx$ are continuous at $x=0$

2) $u(\infty) = -u_E$
From this, the electrophoretic mobility can be expressed as

$$
\mu = \varepsilon_j \rho \frac{\kappa_j \lambda \eta - \kappa_j \kappa_i \eta - \kappa_j^2 \eta + \lambda^2 \eta + \frac{\rho}{\eta \lambda^2}}{(\varepsilon_j \kappa_i + \varepsilon_j \kappa_j) \kappa_j (-\kappa_j^2 \eta + \lambda^2 \eta \eta)}
$$

(16)

with $\lambda^{-1} = (\eta/\gamma)^{1/2}$, the ‘electrophoretic softness’ of the ion-penetrable surface.

Eq. (16) was derived assuming an infinitesimally thick ion-penetrable layer, but yields a good approximation for surfaces covered with an ion-penetrable layer with thickness $d$, if $\lambda d$ is comparable to or higher than 1 and if $d$ is in the order of $1/\kappa$. Furthermore, although derived for flat plates, Eq. (16) can be applied to particles if $\kappa a_p \gg 1$, where $a_p$ is the particle radius. If sufficiently low, the surface potential $\psi_0$ of an ion-penetrable surface is given by

$$
\psi_0 = \frac{\rho}{2 \varepsilon_j \kappa_j}
$$

(17)

with $\rho$ derived from Eq. (16).

Measurement of the electrophoretic mobility of surfaces as a function of ionic strength allows application of Eq. (16) or Eq. (13). A distinction can be made between ion-penetrable and ion-impenetrable surfaces on the basis of the quality of the fit. Obtained fit parameters $\sigma$, for ion-impenetrable surfaces, or $\lambda$ and $\rho$, for ion-penetrable surfaces, allow calculation of electrostatic interactions between the electrokinetically characterised surfaces using the expressions in Table 1.
Materials and Methods

Parallel plate flow chamber and data analysis
Deposition of bacteria to the bottom glass plate (5.5 by 3.8 cm) of a parallel plate flow chamber with channel height 0.06 cm [20] was studied. Glass plates were cleaned using a 2% RBS surfactant solution in water (Omnilabo International BV, The Netherlands) followed by thorough rinsing with tap water and demineralized water. The flow chamber was mounted on the stage of a phase contrast microscope (Olympus BH-2) with a x40 objective having an ultralong working distance (Olympus ULWD-CD Plan 40 PL). A charge-coupled device camera (CCD-MX High Technology, Eindhoven, The Netherlands) was linked to an image analyser (TEA image manager, Difa, Breda, The Netherlands), which was installed in a personal computer. This system allowed direct observation of bacterial deposition over a field of view covering about 0.014 mm².

Measurements were carried out with bacteria suspended in potassium phosphate solutions of various ionic strengths up to 60 mM at room temperature. A pulse-free flow (0.019 ml/s) of the suspension was created by hydrostatic pressure, which produced a wall shear rate of 9 s⁻¹ and a Reynolds number of 0.6 (well within the range of laminar flow), while the suspension was recalculated using a peristaltic pump (Multiperpx 2115). Images were grabbed during the experiment and stored in the computer. From the initial, linear increase in the number of adhering bacteria the initial deposition rate \( j_0 \) was determined. Experiments were done in triplicate.

Bacterial strains
Experiments were conducted using the bacterial strains *Streptococcus salivarius* HB-C12 and *Staphylococcus epidermidis* 3399. *S. salivarius* HB-C12 was cultured in Todd Hewitt Broth, *S. epidermidis* 3399 was cultured in Brain Heart Infusion, both at 37°C in ambient air. For each experiment, the strain was inoculated from blood agar in a batch culture. This culture was used to inoculate a second culture that was grown for 16 h prior to harvesting. Bacteria were harvested by centrifugation (5 min at 10,000 g), washed twice with demineralized water and resuspended to a concentration of 3 x 10⁸ bacteria/ml in potassium phosphate buffer solutions of different ionic strengths.
**Chapter 2a**

**Microelectrophoresis and streaming potential measurements**

Electrophoretic mobilities were measured at 25°C with a Lazer Zee Meter (PenKem, Bedford Hills, NY, USA) equipped with an image analysis option for tracking and zeta sizing [21]. Streaming potentials of the glass were measured in a home made parallel plate flow chamber [22] and converted into electrophoretic mobilities according to Eq. (11).

**Results**

**Electrokinetic measurements**

Fig. 2 shows the electrophoretic mobilities of *S. salivarius* HB-C12, *S. epidermidis* 3399, and the glass. Note that the electrophoretic mobilities of glass and of *S. epidermidis* 3399 remain

![Figure 2](image-url)

**Figure 2.** Electrophoretic mobilities of glass (●), *S. salivarius* HB-C12 (■) and *S. epidermidis* 3399 (◆) as a function of the ionic strength of a potassium phosphate buffer at pH=6.8. Lines indicates the best fit to an ion-penetrable surface model (Eq. (16)) in the case of glass and *S. epidermidis* 3399, or an ion-impenetrable surface model (Eq. (13)) in the case of *S. salivarius* HB-C12.
negative at high ionic strength while the electrophoretic mobility of \textit{S. salivarius} HB-C12 approaches zero indicating that \textit{S. epidermidis} 3399 and glass possess ion-penetrable surfaces and the dependence of their electrophoretic mobilities upon ionic strength obeys Eq. (16) best (see Table 2).

Least-square fitting shows that the dependence of the electrophoretic mobility of \textit{S. salivarius} HB-C12 on the ionic strength resembles the one of an ion-impenetrable surface (Eq. (13)), as can also be seen in Table 2. Note that in Table 2, the charge density is given per unit volume for the ion-penetrable surface, whereas for the ion-impenetrable surface the charge density is expressed per unit area, as the bacterial charge in this case is concentrated only at the bacterial surface (see Fig. 1). Furthermore, it can be seen from Table 2 that the ion-penetrable layer of the \textit{S. epidermidis} 3399 is electrophoretically softer ($\lambda_{-1} = 3.2$ nm) than the one of glass ($\lambda_{-1} = 1.9$ nm).

\textbf{Table 2.} Electrophoretic properties of glass and the two bacterial strains involved in this study. Ion-penetrable surfaces are characterised by their dielectric permittivity $\varepsilon_I$, electrophoretic softness $1/\lambda$ and fixed charge density $\rho$, and the dependence of their electrophoretic mobility upon ionic strength obeys Eq. (16). Ion-impenetrable surfaces are solely characterised by their surface charge density $\sigma$, according to Eq. (13). Dielectric permittivity of the ion-penetrable layer on glass was taken from ref. [27]. $R^2$ denotes the degree of correspondence between experimental relationships and the equation applied (Eqs. (16) or (13)) with $R^2=1$ denoting full correspondence.

<table>
<thead>
<tr>
<th>Surface</th>
<th>$\varepsilon_I$ ($\times\varepsilon_0$)</th>
<th>$1/\lambda$ (nm)</th>
<th>$\rho$ ($10^6$ C/m$^3$)</th>
<th>$\sigma$ ($10^{-3}$ C/m$^2$)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>15</td>
<td>1.9 ± 0.2</td>
<td>-7.1 ± 0.9</td>
<td>na</td>
<td>0.94</td>
</tr>
<tr>
<td>ion-penetrable model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Eq.16)</td>
</tr>
<tr>
<td>\textit{S. salivarius} HB-C12</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>-4.8 ± 0.2</td>
<td>0.95</td>
</tr>
<tr>
<td>ion-impenetrable model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Eq.13)</td>
</tr>
<tr>
<td>\textit{S. salivarius} HB-C12</td>
<td>80</td>
<td>2.0 ± 0.1</td>
<td>-3.2 ± 0.5</td>
<td>na</td>
<td>0.89</td>
</tr>
<tr>
<td>ion-penetrable model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Eq.16)</td>
</tr>
<tr>
<td>\textit{S. epidermidis} 3399</td>
<td>80</td>
<td>3.2 ± 0.3</td>
<td>-2.0 ± 0.3</td>
<td>na</td>
<td>0.96</td>
</tr>
<tr>
<td>ion-penetrable model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Eq.16)</td>
</tr>
</tbody>
</table>

$^1$not applicable
Deposition experiments

Fig. 3 shows the initial deposition rates measured of *S. salivarius* HB-C12 and *S. epidermidis* 3399 as a function of ionic strength. The initial deposition rate of *S. salivarius* HB-C12 increases with increasing ionic strength, but the initial deposition rate of *S. epidermidis* 3399 is almost constant over the entire ionic strength range used.

Fig. 3 also shows the theoretical initial deposition rates, calculated using the expressions given in Table 1. For the ion-impenetrable *S. salivarius* HB-C12 theoretical deposition rates were calculated assuming that the glass surface was ion-penetrable, as indicated in Table 2, but also taking glass as an ion-impenetrable surface. A comparison of the experimental data for *S. salivarius* HB-C12 with theoretical predictions indicate that in interaction with an ion-impenetrable bacterium, glass behaves as being ion-impenetrable too. For *S. epidermidis* 3399, on the other hand, theoretical predictions based on ion-penetrable glass and bacterial surfaces correspond best with the experimental results. For comparison, theoretical predictions of the traditional model which assumes both an ion-impenetrable substratum and bacterial cell surface, are included in Fig. 3 as well, demonstrating too low theoretical initial deposition rates in comparison with experimental deposition rates for *S. epidermidis* 3399 due to an overestimation of the repulsive electrostatic forces operative between two ion-penetrable surfaces.

**Figure 3.** (see next page) Initial deposition rates to a glass collector surface measured 3 cm from the inlet of a parallel plate flow chamber as a function of the ionic strength for ion-impenetrable *S. salivarius* HB-C12 (*Pe* = 0.005; *a*_b = 550 nm) and ion-penetrable *S. epidermidis* 3399 (*Pe* = 0.007; *a*_b = 600 nm) and *A*_12 = 0.4 x 10\(^{-20}\) J for both bacteria as calculated from measured contact angles [15]. Error bars indicate the standard deviation over 3 separate experiments. Theoretical predictions are given by the lines, based on solving the convective-diffusion equation for an ion-impenetrable bacterium (top) interacting with ion-penetrable and ion-impenetrable glass and for an ion-penetrable bacterium (bottom) interacting with ion-penetrable glass. To illustrate the effect of ion-penetrability, theoretical predictions of the deposition of *S. epidermidis* 3399 have also been given when both interacting surfaces are taken impenetrable. To account for surface heterogeneity, lines were calculated assuming a normally distributed surface charge of the glass [30] with the average equal to the value derived from streaming potential measurements and a standard deviation of 50% of the average.
Figure 3. (caption on previous page)
Discussion

In this paper, the role of ion-penetrability of surfaces in bacterial deposition to a glass collector was studied experimentally in a parallel plate flow chamber, while experimental data were compared with theoretical solutions of the convective-diffusion equation. Differences between the deposition rate of the ion-penetrable and the ion-impenetrable strain used can not be explained in terms of the XDLVO theory as both bacteria have a comparable hydrophobicity and thus have a similar acid-base energy of interaction with glass ($\Delta G_{AB} \approx 50 \text{ mJ/m}^2$). Hence, differences between the deposition behaviour of both strains will be due to their different ion-penetrability as determined by electrokinetic measurements at different ionic strengths.

Electrokinetic measurements

The bacterial cell surfaces involved in this study represent extremes with regard to their ion-penetrability. *S. epidermidis* 3399 possesses an infinitesimally thick ion-penetrable surface layer, while *S. salivarius* HB-C12 can be described as an ion-impenetrable strain. *S. epidermidis* 3399 is an encapsulated strain [23] with a capsular thickness reportedly in the order of 100 nm [24], which validates the use of Eq. (16), derived for an infinitesimally thick ion-penetrable layer. Likely, the capsule can be associated with the ion-penetrable layer. Alternatively, *S. salivarius* HB-C12 is devoid of any encapsulation or proteinaceous surface structures [25], which enforces the conclusion based on the electrokinetic model proposed that its cell surface is ion-impenetrable.

Analysis of the electrokinetic data of glass, indicates that its surface is ion-penetrable. This was first suggested by Lyklema [26] in order to explain the unexpectedly high values of the measured surface charge density of glass. Estimates of the thickness of the ion-penetrable layer present on glass range from 0.7 nm [27] to 4.0 nm [28], which partly invalidate the use of Eq.(16) because the glass surface is only ion-penetrable over a relatively short distance.

Influence of ion-penetrability on deposition

Ion-penetrable layers on interacting surfaces experience less electrostatic repulsion than ion-impenetrable surfaces with a similar surface potential, which leads to higher deposition rates at low ionic strengths. Theoretical deposition rates correspond with experimental ones for the ion-penetrable strain *S. epidermidis* 3399, when the glass surface is taken as an ion-penetrable
surface, but when interacting with the ion-impenetrable *S. salivarius* HB-C12, good correspondence could only be obtained when the glass surface was taken ion-impenetrable. Likely, whether or not glass should be considered as ion-penetrable or ion-impenetrable depends on the penetrability of the opposing surface. In a 20 mM potassium phosphate solution, the Debye length inside the ion-penetrable layer of the glass can be calculated to be 0.8 nm, which is in the order of the thickness of the ion-penetrable layer (estimated to be in-between 0.7 nm [27] and 4.0 nm [28]). Consequently, when glass interacts with an ion-penetrable bacterium (Figs. 4a and b), their electric double layers commence to overlap, but diffuse double layer charges are driven into the ion-penetrable layers causing an effective decrease in surface potential and electrostatic repulsion. As the ion-penetrable layer on glass is relatively thin, it can be anticipated that most of the diffuse double layer charges will be accommodated in the relatively thick ion-penetrable layer of the bacterium. However, when the bacterial cell surface is ion-impenetrable (Figs. 4c and d), upon approach, all diffuse double layer charges have to be accommodated in the thin ion-penetrable layer of the glass and the diffuse double layer charge density inside the ion-penetrable layer of the glass increases stronger during interaction with an ion-impenetrable bacterium than during interaction with an ion-penetrable bacterium to the extent that the glass surface readily presents itself as ion-impenetrable.

*Implications for electrophoretic characterisation of bacteria and their adhesion*

Electrophoretic softness of ion-penetrable bacterial cell surfaces may yield an overestimation of bacterial surface potentials if Von Smoluchowski’s equation is used to convert electrophoretic mobilities into surface potentials. For two ion-penetrable bacteria with the same surface potential, an electrophoretically soft bacterium attains a higher electrophoretic velocity in an applied electric field than an electrophoretically hard bacterium, as can be seen from Fig. 1. Consequently, if the electrophoretic mobility of an electrophoretically soft bacterium is analysed using Von Smulochowski’s equation, the bacterial surface potential is overestimated. For *S. epidermidis* 3399 with a measured electrophoretic mobility of $-3.3 \times 10^{-8}$ m² V⁻¹ s⁻¹ in 20 mM potassium phosphate buffer, for instance, using the ion-penetrable model (Eq. (16)) yields a surface potential of $-5$ mV (from Eq. (17)), whereas Von Smulochowski’s equation (Eq. (12)) yields a zeta potential of $-38$ mV.
Figure 4. Distribution of diffuse double layer charges during interaction of glass, possessing a relatively thin ion-penetrable layer, with an ion-penetrable bacterium (a and b) and an ion-impenetrable bacterium (c, d). Only counter ions are shown.
Figure 4. (continued) Electric potential distributions are calculated from the equations given in [12], assuming a 2 nm thick ion-penetrable layer on the glass surface in 25 mM potassium phosphate buffer for two flat interacting surfaces, a relative dielectric permittivity of glass equal to 80 and both bacterial surface potentials equal to -11 mV.
Because diffuse double layer charges can move into ion-penetrable surface layers during interaction, an ion-impenetrable and an ion-penetrable bacterium with equal surface potential and maintaining a constant charge density experience different repulsion upon approach of a similarly charged surface. This is shown in Fig. 5. If the organism is taken ion-impenetrable, overestimation of its surface potential by Von Smoluchowski’s equation and neglect of the effect of ion-penetrability on electrostatic interactions lead to a potential energy barrier of several thousand $kT$. Alternatively, if in the electrokinetic characterisation of the organism its cell surface softness is accounted for, yielding a much smaller surface potential, but ion-penetrability is not incorporated in the calculation of the interaction energy, a smaller potential energy barrier of around 455 $kT$ is calculated. Finally, accounting for both cell surface softness during electrophoresis and ion-penetrability during electrostatic interactions, it can be seen in Fig. 5 that the potential energy barrier has almost completely disappeared in the calculations.

**Figure 5.** DLVO interaction potential as a function of separation distance for *S. epidermidis* 3399 ($a_0$=600 nm) interacting with ion-impenetrable glass in 20 mM potassium phosphate buffer with $A_{12} = 0.4 \times 10^{-20}$ J:

- Curve 1: ion-impenetrable bacterium with a surface potential of −38 mV, derived from electrophoretic mobility measurements neglecting bacterial electrophoretic softness.
- Curve 2: ion-impenetrable bacterium with a surface potential of −5 mV, derived from electrophoretic mobility measurements while accounting for bacterial electrophoretic softness.
- Curve 3: ion-penetrable bacterium with a surface potential of −5 mV.
Summary of conclusions

In this work, we have distinguished ion-penetrable and ion-impenetrable bacterial cell surfaces on the basis of electrophoretic mobility measurements. Theoretically, the presence of an ion-penetrable surface layer decreases electrostatic repulsion during deposition to negatively charged collector surfaces, which has been confirmed experimentally by comparing the deposition rate of the ion-penetrable strain *S. epidermidis* 3399 and of the ion-impenetrable strain *S. salivarius* HB-C12 in a parallel plate flow chamber to a glass surface. The thin ion-penetrable layer present on glass was demonstrated to behave as an ion-impenetrable surface when opposed to an ion-impenetrable bacterial cell surface.

List of symbols

\( a_b \)  
- bacterial radius

\( A \)  
- bacterial radius divided by half the distance between the parallel plates

\( A_{132} \)  
- Hamaker constant for the interaction of a bacterium 1 in medium 3 with a planar surface 2

\( b \)  
- half the distance between the plates in the parallel plate flow chamber

\( C^* \)  
- bacterial concentration expressed as a fraction of the bulk concentration

\( C_0 \)  
- bulk bacterial concentration

\( E \)  
- applied electric field

\( E_{str} \)  
- streaming potential

\( f_i \)  
- universal hydrodynamic correction functions [29]

\( F \)  
- electrostatic interaction free energy per unit area

\( g \)  
- acceleration of gravity

\( h \)  
- bacterial surface-collector surface separation distance

\( H \)  
- bacterial surface-collector surface distance expressed in bacterial radii

\((H = y/a_b) - 1\)

\( k \)  
- Boltzmann constant

\( K \)  
- electric conductivity

\( P \)  
- pressure difference

\( Pe \)  
- Péclet number

\( T \)  
- absolute temperature

\( u \)  
- fluid velocity
Chapter 2a

$u_E$  electrophoretic velocity

$V_p$  electrostatic interaction potential for plate-plate interaction

$x$  co-ordinate parallel to the flow plates with its origin at the place where the laminar flow becomes fully developed

$x^*$  $x$ divided by half the distance between the parallel plates

$y$  distance between the centre of the bacterium and the substrate

$\gamma$  frictional coefficient

$\varepsilon$  dielectric permittivity

$\varepsilon_0$  vacuum permittivity

$\kappa$  inverse Debye length

$\lambda$  electrophoretic ‘softness’ of an ion-penetrable layer

$\eta$  viscosity

$\mu$  electrophoretic mobility

$\rho$  charge density

$\rho_b$  bacterial density, taken to be $1.1 \cdot 10^3$ kg/m$^3$

$\rho_l$  liquid density, taken to be $1.00 \cdot 10^3$ kg/m$^3$

$\sigma$  surface charge density

$\psi$  electric potential

$\phi_{ed}$  electrostatic interaction potential

$\phi_{LW}$  Lifshitz-Van der Waals interaction potential

$\phi_{gr}$  potential due to gravity and buoyancy

$\zeta$  zeta potential
References

Chapter 2a


Lack of effect of an externally applied electric field on bacterial adhesion to glass

Abstract

Deposition to glass of *Streptococcus salivarius* HB-C12 and *Staphylococcus epidermidis* 3399 in a parallel plate flow chamber in the absence and presence of an externally applied electric field has been studied experimentally. No effect on bacterial adhesion, including initial deposition rates, numbers of adhering bacteria after 4 hours, spatial distributions of adhering bacteria and air bubble induced detachment, was found. A theoretical analysis shows that electric fields applied over a 150 µm thin glass substratum do not have a sufficiently strong effect on its surface potential to influence bacterial adhesion.

Introduction

The formation of a biofilm occurs in several sequential steps, starting with the formation of a conditioning film, followed by bacterial transport and initial adhesion, anchoring and eventually growth [1]. Several authors have claimed to be able to influence biofilm formation by using electric fields. For example, Costerton and co-workers reported that they could enhance the efficacy of antibiotics in killing biofilm bacteria by using only low electric field strengths (1.5 to 30 V/cm) and current densities (15 µA/cm² to 2.1 mA/cm²) [2,3]. Others [4] have demonstrated inhibition of bacterial colonisation of surfaces by using an electric current of only 10 µA and medical devices such as catheters provided with an electric field generator are claimed to inhibit bacterial attachment [5,17]. In all cases, the exact mechanism by which the electric field influences biofilm formation is not clear.

In this chapter, we study the effect of an applied electric field on bacterial adhesion to glass. Adhesion of bacteria is determined by physico-chemical interactions [6] and the classical DLVO approach describes bacterial adhesion as an interplay of Lifshitz-Van der Waals and electrostatic interactions, with a possible influence of an external electric field. Experimentally, a distinction must be made between the application of an electric field with and without electrical charge transfer. In most studies, adhesion to an electrode surface is studied, which enables charge transfer. Morisaki *et al.* [7] determined the applied electric field at which the initial deposition rate of *Pseudomonas syringae* to indium tin oxide (ITO) coated glass was essentially zero and from this value derived the microbial attachment force to be 5.0 $\times$ 10^{-11} N per bacterium. Charge transfer during deposition of *Leuconostoc mesenteroides* and
*Streptococcus thermophilus* to stainless steel was studied by Boulangé-Petermann *et al.* [8]. Adhesion of bacteria from an aerated 0.15 M NaCl solution was accompanied by a change in rest potential of the stainless steel in contact with the solution from 50 mV with respect to a saturated calomel electrode (SCE) to 170 mV/SCE and caused changes in the current present under potentiostatic conditions. Similar experiments were done with *Escherichia coli* adhering to carbon-cloth electrodes [9,10], demonstrating that application of an electrode potential of 0.74 V/SCE resulted in the death of cells attached to the electrode due to oxidation of the intracellular coenzyme A. To our knowledge, no reports have been made on the effects of an applied electric field on bacterial adhesion in the absence of charge transfer, i.e. when the substratum is not used as an electrode. It has therefore hitherto been unclear if effects on bacterial adhesion of electric field application result from the applied electric potential or whether they are caused by charge transfer between the electrode and bacteria, electrophoresis or other electrochemical reactions.

The aim of this paper is to determine experimentally the effect of an externally applied electric field on the deposition of two bacterial strains to glass in a parallel plate flow chamber.

**Materials and Methods**

*Parallel plate flow chamber and data analysis*

Deposition of bacteria to glass in the presence and absence of an externally applied electric field was studied using a parallel plate flow chamber [11] with image analysis. The top and bottom glass plates of the flow cell (5.5 by 3.8 cm) were separated by two Teflon spacers of 0.06 cm thickness. Bacterial deposition was determined to a 150 µm thick bottom glass plate, partially coated with a gold layer (transmittance of 80% for white light and resistivity of 100 Ω per square, coated area of about 1.6 cm²) with the gold layer at the outside of the glass plate, i.e. not in contact with the solution. Consequently, no electric current or charge transfer was possible during bacterial adhesion as experimentally verified. A partial coating was applied to enable adhesion studies in the presence and absence of the electric field in a single experiment. The electric potential (−4 kV, 0 kV or +4 kV) was applied to the gold coating with respect to a platinum (Pt) electrode in contact with the bacterial suspension (see also Fig.1). Measurements were conducted with bacteria suspended in potassium phosphate buffer.
olutions of various ionic strengths up to 60 mM at room temperature. A pulse-free flow (0.019 ml/s) was created by hydrostatic pressure, which produced a shear rate of 9 s\(^{-1}\) (Reynolds number of 0.6), while the suspension was recirculated using a peristaltic pump.

Both the initial deposition rate and the number of deposited bacteria after 4 hours (\(n_{4h}\)) were determined. Analysis of the spatial arrangement of adhering bacteria was done by calculating radial pair distribution functions [14] which indicate the relative density of the adhering bacteria as a function of distance. Then, the suspension was drained from the system, which allowed an air-liquid interface to pass over the substratum (i.e. exposure to a high shear force [12,13]) and detachment was quantitated by comparing the number of adhering bacteria in pre- and post-draining images as an indication of the strength of adhesion.

**Bacterial strains**

Experiments were conducted using the bacterial strains *Streptococcus salivarius* HB-C12 and *Staphylococcus epidermidis* 3399. Bacteria were cultured and harvested as described by Poortinga et al.[15] and subsequently resuspended to a concentration of 3 x 10\(^8\) bacteria/ml in potassium phosphate buffer solutions of different ionic strengths. Zeta potentials of the bacterial strains and the glass vary from -68 mV to -35 mV (glass), -20 mV to -4 mV (*S. salivarius* HB-C12) and -51mV to -32mV (*S. epidermidis* 3399) for 5 mM to 60 mM potassium phosphate solutions, respectively.
Results and Discussion

Fig. 2 shows the measured initial deposition rates of *S. salivarius* HB-C12 and *S. epidermidis* 3399 to glass as a function of the potassium phosphate concentration in the absence of an applied electric potential. Initial deposition rates in the presence of an applied electric field (-4 and +4 kV) were not significantly different (for all cases p>0.1, Student's t-test) as can be seen in Table 1 were the average measured paired differences in the initial deposition rate in the presence and absence of an applied electric field are given. Table 2 also shows the average measured paired differences in the number of adhering bacteria after 4 hours in the presence and absence of an applied electric field. Again, no significant difference was measured (for all cases p>0.1, Student's t-test). Fig. 3 gives examples of radial distribution functions for both strains, which are seen to be invariant upon application of an electric field. *S. epidermidis* 3399 shows a minor preferential adhesion (*g*(r)=1.25) and a different, more oscillatory distribution than *S. salivarius* HB-C12 at larger separation distances, because of the higher density of adhering bacteria for *S. epidermidis* 3399. For both strains, a significant part of the adhering bacteria (on average about 50 %) detached upon passage an air-bubble through the flow chamber, irrespective of the externally applied field and whether it was positive or negative.

Summarising, no effect of the applied electric field on bacterial adhesion has been found which raises the question up to what extent the electric field at the glass surface had actually been altered by the application of 4 kV. Fig. 4 shows the electric potential distribution resulting from an applied electric field. Assuming a diffuse double layer inside the solution, it can be derived that the applied electric potential V equals

\[ V = \frac{\sigma}{\varepsilon_s \kappa} + \phi_o + \frac{\sigma}{\varepsilon_s} d \]  

(1)

where \( \sigma \) is the charge density stored at the gold coating upon application of the electric field, \( \kappa \) is the inverse Debye length inside the solution, \( \varepsilon_s \) and \( \varepsilon_g \) are the dielectric constant of the glass and the solution, respectively, \( d \) is the thickness of the glass and \( \phi_0 \) is surface potential of glass (see Fig. 4). The first term in Eq. (1) represents the change in surface potential of the glass upon application of the electric field, i.e. charging of the gold layer. This change is most
pronounced at low ionic strength and is calculated to be as high as $+\text{ or } -12\, \text{mV}$ at $5\, \text{mM}$ potassium phosphate concentration. By solving the convective-diffusion equation [15] on the basis of these changes in surface potential of the glass upon application of the electric field, the initial deposition rate in the absence and presence of the electric field can be calculated (see lines in Fig. 2). A maximal effect on initial deposition rates of the applied electric field of $260\, \text{cm}^2\, \text{s}^{-1}$ can be calculated for the adhesion of *S. epidermidis* 3399 from $20\, \text{mM}$ potassium phosphate buffer in the presence of an applied electric potential of $+4\, \text{kV}$ (see also Table 1), comparable with the experimental errors.

The present experimental results and theoretical analysis show that in our set-up, in the absence of electric current, the application of a high electric potential results in only a relatively small change in electric potential at the glass surface with no significant effect on bacterial adhesion. Often, also metals are covered with a poorly conducting oxide layer and when these metals are used as simultaneous electrode and substratum for bacterial adhesion, there will be little effect on the electric potential at the substratum surface, similar as in the present study [16]. However, unless special precautions are taken, it can not be ruled out for a simultaneous electrode and substratum set-up that a small electric current will be present. Therefore, reported effects of applied electric fields on bacterial adhesion and biofilm formation to electrodes [2-5,7, 9,10,17] must likely be attributed to the electric current rather than to the effects of the electric potential.
Figure 2. Initial deposition rates for *S. salivarius* HB-C12 (a) and *S. epidermidis* 3399 (b) to glass as a function of the ionic strength of the potassium phosphate buffer used. Points indicate experimentally measured initial deposition rates in the absence of an applied electric field, with the error bars indicating the standard deviation over 3 separate experiments. Lines indicate theoretically calculated initial deposition rates for external potentials of 0kV: drawn line, +4kV: dashed line (short dashes) and -4kV: dashed line (long dashes).
**Table 1.** Difference between initial deposition rates in the presence and absence of an electric field expressed as a paired difference with SD (n=2 separate experiments) $\Delta j_{0,+4kV}$ and $\Delta j_{0,-4kV}$ for used bacterial strains and ionic strengths. Subscripts refer to the potential applied to the gold coating. $\text{EXP} =$ measured , $\text{NUM} =$ numerically calculated.

<table>
<thead>
<tr>
<th>I (mM)</th>
<th>$\Delta j_{0,+4kV}^{\text{EXP}}$ (cm$^{-2}$s$^{-1}$)</th>
<th>$\Delta j_{0,-4kV}^{\text{EXP}}$ (cm$^{-2}$s$^{-1}$)</th>
<th>$\Delta j_{0,+4kV}^{\text{NUM}}$ (cm$^{-2}$s$^{-1}$)</th>
<th>$\Delta j_{0,-4kV}^{\text{NUM}}$ (cm$^{-2}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. epidermidis 3399</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>$1 \pm 101$</td>
<td>$-70 \pm 102$</td>
<td>2</td>
<td>-32</td>
</tr>
<tr>
<td>10</td>
<td>$-160 \pm 69$</td>
<td>$-130 \pm 78$</td>
<td>70</td>
<td>-90</td>
</tr>
<tr>
<td>20</td>
<td>$12 \pm 64$</td>
<td>$-32 \pm 114$</td>
<td>260</td>
<td>-194</td>
</tr>
<tr>
<td>30</td>
<td>$-99 \pm 145$</td>
<td>$-33 \pm 68$</td>
<td>116</td>
<td>-137</td>
</tr>
<tr>
<td>40</td>
<td>$74 \pm 139$</td>
<td>$54 \pm 103$</td>
<td>0</td>
<td>-35</td>
</tr>
<tr>
<td><strong>S. salivarius HB-C12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>nd$^1$</td>
<td>$-10 \pm 64$</td>
<td>2</td>
<td>-4</td>
</tr>
<tr>
<td>25</td>
<td>nd</td>
<td>$-9 \pm 20$</td>
<td>32</td>
<td>-19</td>
</tr>
<tr>
<td>40</td>
<td>nd</td>
<td>$14 \pm 45$</td>
<td>56</td>
<td>-55</td>
</tr>
</tbody>
</table>

$^1$ not determined

**Table 2.** Difference in the number of adhering bacteria after 4 hours in the presence and absence of an electric field expressed as a paired difference with SD (n=2 separate experiments) $\Delta n_{4h,+4kV}$ and $\Delta n_{4h,-4kV}$ for used bacterial strains and ionic strengths. Subscripts refer to the potential applied to the gold coating. $n_{4h}$ denotes the number of adhering bacteria after 4 hours in the absence of an applied electric field with SD (n=4 separate experiments).

<table>
<thead>
<tr>
<th>I (mM)</th>
<th>$\Delta n_{4h,+4kV} ($10$^6$ cm$^{-2}$)</th>
<th>$\Delta n_{4h,-4kV} ($10$^6$ cm$^{-2}$)</th>
<th>$n_{4h}$ ($10^6$ cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. epidermidis 3399</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>$-0.7 \pm 1.2$</td>
<td>$0.4 \pm 1.2$</td>
<td>$23.3 \pm 2.0$</td>
</tr>
<tr>
<td>10</td>
<td>$-0.2 \pm 1.2$</td>
<td>$1.6 \pm 1.2$</td>
<td>$24.0 \pm 2.1$</td>
</tr>
<tr>
<td>20</td>
<td>$-0.5 \pm 1.2$</td>
<td>$0.5 \pm 1.2$</td>
<td>$23.8 \pm 3.3$</td>
</tr>
<tr>
<td>30</td>
<td>$0.6 \pm 1.2$</td>
<td>$0.1 \pm 1.2$</td>
<td>$25.0 \pm 1.5$</td>
</tr>
<tr>
<td>40</td>
<td>$0.1 \pm 1.2$</td>
<td>$0.4 \pm 1.2$</td>
<td>$28.0 \pm 2.0$</td>
</tr>
<tr>
<td><strong>S. salivarius HB-C12</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>nd$^1$</td>
<td>$0.2 \pm 0.4$</td>
<td>$2.9 \pm 1.0$</td>
</tr>
<tr>
<td>25</td>
<td>nd</td>
<td>$-0.1 \pm 0.2$</td>
<td>$3.9 \pm 1.5$</td>
</tr>
<tr>
<td>40</td>
<td>nd</td>
<td>$0.1 \pm 0.6$</td>
<td>$4.7 \pm 0.8$</td>
</tr>
</tbody>
</table>

$^1$ not determined
Figure 3. Radial pair distribution functions g(r) (centre to centre distances) of *S. salivarius* HB-C12 (a) and *S. epidermidis* 3399 (b) in 25 mM potassium phosphate buffer on glass in the presence (dashed line) and absence (drawn line) of an applied potential of -4kV. Each analysis included approximately 1500 adhering bacteria. Average bacterial densities were 3.2 x 10^6 bacteria/cm² (*S. salivarius* HB-C12) and 10.0 x 10^6 bacteria/cm² (*S. epidermidis* 3399).
Figure 4. Potential distribution in the presence (drawn line) and absence (dotted line) of a positive potential applied to a gold coating on glass in contact with an aqueous solution with respect to a reference electrode given as a function of the distance from the gold coating z.

References

Lack of effect of an electric field on bacterial adhesion


