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

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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. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Adhesion of colloidal particles can be described by the DLVO (Derjaguin, Landau, Verwey, Overbeek) theory in which adhesion is envisaged as an interplay of Lifshitz–Van der Waals and electrostatic interactions. In addition, acid–base interac-
tions 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 generalized 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 structures [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 characterized 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 an ion-impenetrable bacterium. For ion-impenetrable and ion-penetrable hard (i.e. $\lambda^{-1}\to 0$) 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 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.
2. Theory

2.1. 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

\[ Pe \left( \frac{f_3(H)}{H + 1} \right) \frac{\partial^3 C^*}{\partial X^*} \]

\[ - \frac{\partial}{\partial H} \left[ f_3(H) \left( \frac{\partial C^*}{\partial H} + C^* \frac{\partial \phi^*}{\partial H} \right) \right] = 0 \]  \hspace{1cm} (1)

(see 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

\[ \text{A) Ion-penetrable bacterium, soft} \]

\[ \text{B) Ion-penetrable bacterium, hard} \]

\[ \text{C) Ion-impenetrable bacterium} \]

Fig. 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.
Table 1
Expressions for the electrostatic interaction potential between various ion-penetrable and impenetrable surfaces for the sphere–plate configuration

<table>
<thead>
<tr>
<th>Surfaces</th>
<th>Interaction potential $\phi_{ab}(h)$ (J)</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: impenetrable</td>
<td>$\pi \epsilon_0 \rho_2 \left[ \frac{2 \psi_2}{\psi_1 + \psi_2} \ln \left( 1 + e^{-\kappa_1 h} \right) - \ln(1 - e^{-\kappa_1 h}) \right]$</td>
<td>[9]</td>
</tr>
<tr>
<td>2: impenetrable</td>
<td>$\pi \epsilon_0 \rho_2 \left[ \frac{2 \psi_2}{\psi_1 + \psi_2} \ln \left( 1 + e^{-\kappa_1 h} \right) - \ln(1 - e^{-\kappa_1 h}) \right]$</td>
<td>[11]</td>
</tr>
<tr>
<td>1: penetrable</td>
<td>$\frac{\pi \epsilon_0 \rho_2}{\kappa_1 \kappa_2 \epsilon_0 (\epsilon_1 \kappa_1 + \epsilon_2 \kappa_2) \kappa_1} \left( \frac{2 \rho_1 \rho_2 \kappa_1 \kappa_2 \epsilon_0 (\epsilon_1 \kappa_1 + \epsilon_2 \kappa_2) \kappa_1}{4 \epsilon_0 \kappa_2} e^{-\kappa_1 \kappa_2 (\epsilon_1 \kappa_1 + \epsilon_2 \kappa_2) \kappa_1} \right)$</td>
<td>this paper</td>
</tr>
<tr>
<td>2: penetrable</td>
<td>$\frac{2 \pi \epsilon_0 \beta}{\kappa_1 \kappa_2} \ln \left( 1 - d \times e^{-\kappa_1 h} \right) + \left[ \frac{a}{d} \tan^{-1}(\sqrt{d} \times e^{-\kappa_1 h}) \right]$ for $d &lt; 0$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\left[ \frac{a}{d} \tanh^{-1}(\sqrt{d} \times e^{-\kappa_1 h}) \right]$ for $d &gt; 0$</td>
<td></td>
</tr>
</tbody>
</table>

* 1 and 2 denote the interacting surfaces, 3 denotes the solution. Surface 2, if penetrable, has a dielectric permittivity equal to that of the solution.

$$-f_1(\delta) \left[ \frac{\partial C^*}{\partial H} \bigg|_{H=\delta} + C^*(H = \delta) \frac{\partial \phi^*}{\partial H} \bigg|_{H=\delta} \right] = \frac{C^*(H = \delta)}{\exp(\phi(H))} \int_0^\delta \exp(\phi^*(H)) f_1(H) \, dH,$$

(2)

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. 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.

2.2. 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 Fig. 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...
\[
\frac{d^2 \psi}{dx^2} = \kappa_1^2 \psi - \frac{\rho}{\varepsilon_i} \quad \text{for} \quad x < 0, \tag{4}
\]
\[
\frac{d^2 \psi}{dx^2} = \kappa_3^2 \psi \quad \text{for} \quad 0 < x < h, \tag{5}
\]

where the subscript 1 denotes the ion-penetrable plate and 3 denotes the solution. Three boundary conditions apply:

1. \[\sigma = -\varepsilon_0 \frac{d\psi}{dx} \quad \text{for} \quad x = h\]

which is Gauss’ law

2. \[\psi \quad \text{and} \quad \varepsilon_i (d\psi/dx) \quad \text{need to be continuous at} \quad x = 0\]

3. \[\frac{d^2 \psi}{dx^2} = 0 \quad \text{for} \quad x \to -\infty\]

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 Eq. (4) and Eq. (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

\[
F(h) = -\frac{1}{2} \sigma \psi(x = h) + \frac{1}{2} \int_{-\infty}^{0} \rho \psi(x) \, dx \tag{6}
\]

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

\[
V_p(h) = F(h) - F(\infty). \tag{7}
\]

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

\[
\phi_p(h) = 2 \pi a_0 \int_{h}^{\infty} V_p(h) \, dh \tag{8}
\]

yielding an expression for the electrostatic interaction energy for the sphere–plate configuration with one of the interacting surfaces being ion-penetrable (see Fig. 1). In addition to DLVO interactions, bacteria are subjected to the potential due to gravity and buoyancy

\[
\phi_g = \frac{4}{3} \pi a_0^3 (\rho_p - \rho) g a_n H. \tag{9}
\]

2.3. Electrokinetic characterization of ion-penetrable and impenetrable surfaces

Calculation of the electrostatic interaction energies as given in Fig. 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

\[
\mu = \frac{u_E}{E} \tag{10}
\]

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

\[
\mu = K \frac{E_{str}}{P}. \tag{11}
\]

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

\[
\zeta = \frac{\eta}{\varepsilon_i} \mu. \tag{12}
\]

For ion-impenetrable surfaces with a low surface potential, the linearized Poisson–Boltzmann equation can be applied, and a relation between the electrophoretic mobility and the inverse Debye length of the solution \(\kappa_3\) can be derived from Eq. (12)

\[
\mu = \frac{\sigma}{\eta \kappa_3}. \tag{13}
\]
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, Eq. (12) and Eq. (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 $x = 0$ and in contact with a solution, the electric potential is described by Eq. (4) and Eq. (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

$$\frac{d^2u}{dx^2} + \rho E = 0 \quad \text{for } x > 0,$$

$$\eta \frac{d^2u}{dx^2} - \gamma u + \rho E = 0 \quad \text{for } x < 0$$

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

$$\mu = \varepsilon_0 \rho \frac{\kappa_3 \lambda \eta - \kappa_3 \kappa \eta - \kappa_2 \eta + \lambda \eta + \rho \eta \lambda^2}{(\varepsilon_0 \kappa_1 + \varepsilon_0 \kappa_2)(-\kappa_2 \eta + \lambda \eta) \eta} + \frac{\rho \eta \lambda^2}{(\varepsilon_0 \kappa_1 + \varepsilon_0 \kappa_2)(-\kappa_2 \eta + \lambda \eta) \eta}$$

with $\lambda = (\gamma/\eta)^{1/2}$, the ‘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

$$\psi_0 = \frac{\rho}{2\varepsilon_0 \kappa \eta}$$

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 characterized surfaces using the expressions in Fig. 1.

3. Materials and methods

3.1. 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 × 40 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 mm$^2$. 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$^{-1}$) 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 recirculated 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.

3.2. 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 \( \times \) g), washed twice with demineralized water and resuspended to a concentration of \( 3 \times 10^8 \) bacteria ml\(^{-1} \) in potassium phosphate buffer solutions of different ionic strengths.

3.3. 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 homemade parallel plate flow chamber [22] and converted into electrophoretic mobilities according to Eq. (11).

4. Results

4.1. 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 negative at high ionic strength while the electrophoretic mobility of *S. salivarius* HB-C12 approaches zero indicating that *S. epidermidis* 3399 and glass possess ion-penetrable surfaces and the dependence of their electrophoretic mobilities upon ionic strength obeys Eq. (16) best (see Fig. 2). Least-square fitting shows that the dependence of the electrophoretic mobility of *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 *S. epidermidis* 3399 is electrophoretically softer (\( \lambda^{-1} = 3.2 \) nm) than the one of glass (\( \lambda^{-1} = 1.9 \) nm).

4.2. 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
Table 2
Electrophoretic properties of glass and the two bacterial strains involved in this study. Ion-penetrable surfaces are characterized by their dielectric permittivity $\varepsilon_1$, electrophoretic softness $1/\lambda$ and fixed charge density $\rho$, and the dependence of their electrophoretic mobility upon ionic strength obeys Eq. (16)\(^a\)

<table>
<thead>
<tr>
<th>Surface</th>
<th>$\varepsilon_1$ ($\times \varepsilon_0$)</th>
<th>Electrophoretic properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass ion-penetrable model</td>
<td>15</td>
<td>$1/\lambda$ (nm) $\rho$ ($10^6$ C m(^{-3})) $\sigma$ ($10^{-3}$ C m(^2)) $R^2$</td>
</tr>
<tr>
<td>$S.\text{salii}r\text{us}$ HB-C12 ion-impenetrable model</td>
<td>na</td>
<td>na $-7.1 \pm 0.9$ na $-4.8 \pm 0.2$ 0.94 (Eq. (16))</td>
</tr>
<tr>
<td>$S.\text{salii}r\text{us}$ HB-C12 ion-penetrable model</td>
<td>80</td>
<td>$2.0 \pm 0.1$ na $-3.2 \pm 0.5$ na 0.95 (Eq. (13))</td>
</tr>
<tr>
<td>$S.\text{epidemi}r\text{dis}$ 3399 ion-penetrable model</td>
<td>80</td>
<td>$3.2 \pm 0.3$ na $-2.0 \pm 0.3$ na 0.96 (Eq. (16))</td>
</tr>
</tbody>
</table>

\(^a\) Ion-impenetrable surfaces are solely characterized by their surface charge density $\sigma$, according to Eq. (13). Dielectric permittivity of the ion-penetrable layer on glass was taken from [22]. $R^2$ denotes the degree of correspondence between experimental relationships and the equation applied (Eq. (16)) or Eq. (13)) with $R^2 = 1$ denoting full correspondence.

\(^b\) not applicable

with increasing ionic strength, but the initial deposition rate of $S. \text{epidemi}r\text{dis}$ 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 Fig. 1. For the ion-impenetrable $S. \text{salii}r\text{us}$ 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 $S. \text{salii}r\text{us}$ HB-C12 with theoretical predictions indicate that in interaction with an ion-impenetrable bacterium, glass behaves as being ion-impenetrable too. For $S. \text{epidemi}r\text{dis}$ 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 $S. \text{epidemi}r\text{dis}$ 3399 due to an overestimation of the repulsive electrostatic forces operative between two ion-penetrable surfaces.

5. 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 cannot 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 behavior of both strains will be due to their different ion-penetrability as determined by electrokinetic measurements at different ionic strengths.

5.1. Electrokinetic measurements

The bacterial cell surfaces involved in this study represent extremes with regard to their ion-penetrability. $S. \text{epidemi}r\text{dis}$ 3399 possesses an infinitesimally thick ion-penetrable surface layer, while $S. \text{salii}r\text{us}$ HB-C12 can be described as an ion-impenetrable strain. $S. \text{epidemi}r\text{dis}$ 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, $S. \text{salii}r\text{us}$ 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, indicate 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 [25] to 4.0 nm [27], which partly invalidate the use of Eq. (16) and indicates that the glass surface is only ion-penetrable over a relatively short distance.

5.2. Influence of ion-penetrability on deposition

Ion-penetrable layers on interacting surfaces experience less electrostatic repulsion than similarly charged ion-impenetrable surfaces, which leads to higher deposition rates at low ionic strengths. Theoretical deposition rates correspond with experimental ones for the ion-penetrable strain \textit{S. epidermidis} 3399, when the glass surface is taken as an ion-penetrable surface, but when interacting with the ion-impenetrable \textit{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 [25] and 4.0 nm [27]). Consequently, when glass interacts with an ion-penetrable bacterium (Fig. 4a and Fig. 4b), 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 (Fig. 4c and Fig. 4d), 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.

Fig. 3. 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 \textit{S. salivarius} HB-C12 (Pe = 0.005; \(a_b = 550\) nm) and ion-penetrable \textit{S. epidermidis} 3399 (Pe = 0.007; \(a_b = 600\) nm) and \(A_{105} = 0.4 \times 10^{-20}\) 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 \textit{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 [29] with the average equal to the value derived from streaming potential measurements and a standard deviation of 50% of the average.
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.

5.3. Implications for electrophoretic characterization 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

![Diagram of electric potential distribution](image)

Fig. 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). The 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 $-11 \text{ mV}$. 


using Von Smoluchowski’s equation, the bacterial surface potential is overestimated. For \textit{S. epidermidis} 3399 with a measured electrophoretic mobility of $-3.3 \times 10^8$ m$^2$ 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 Smoluchowski's equation (Eq. (12)) yields a zeta potential of $-38$ 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 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 characterization 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...
5.4. 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 [28]
- \( F \) 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
- \( u_E \) 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 substratum
- \( \gamma \) frictional coefficient
- \( \varepsilon \) dielectric permittivity
- \( \varepsilon_0 \) vacuum permittivity
- \( \kappa \) inverse Debye length
- \( \lambda \) ‘softness’ of an ion-penetrable layer
- \( \eta \) viscosity
- \( \mu \) electrophoretic mobility
- \( \rho \) charge density
- \( \rho_p \) bacterial density, taken to be \(1.1 \times 10^3\) kg m\(^{-3}\)
- \( \rho_l \) liquid density, taken to be \(1.0 \times 10^3\) kg m\(^{-3}\)
\( \sigma \)  
\( \psi \)  
\( \phi_{el} \)  
\( \phi_{LW} \)  
\( \phi_{gr} \)  
\( \zeta \)  

Surface charge density
Electric potential
Electrostatic interaction potential
Lifshitz–Van der Waals interaction potential
Potential due to gravity and buoyancy
Zeta potential

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