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The role of electrostatic interactions in adhesion of *Streptococcus thermophilus* to human erythrocytes in media with different 1:1 and 2:1 electrolyte concentrations

O. I. Gordiyenko[a] and M. O. Barannyk[b]

[a]Department of Low Temperature Preservation, Institute for Problems of Cryobiology and Cryomedicine NAS of Ukraine, Kharkiv, Ukraine; [b]Department of Physics, National University of Pharmacy, Kharkiv, Ukraine

ABSTRACT

The process of bacterial adhesion is usually discussed in terms of a two-stage sorption model. According to this model, at the first stage bacteria rapidly attach to the surface by weak physical interactions, while at the second stage irreversible molecular and cellular adhesion processes take place. An important factor, influencing the adhesion processes, is physical and chemical characteristics of the medium, in particular, the presence of monovalent and bivalent cations. In this work, we assessed the role of electrostatic component of the intercellular interactions in the media with different electrolyte concentrations (namely, 1:1 and 2:1) at the first reversible stage of adhesion and probability of further specific binding. We compared experimental data of lactobacilli *Streptococcus thermophilus* adhesion to human erythrocytes with theoretical Debye radius and surface potential of erythrocytes in the experimental solutions of 1:1 electrolyte. Our results showed that with decreasing ionic strength of the solution, the change in the adhesion index in our experiments is fully in line with the theory DLVO (Derjaguin–Landau–Verwey–Overbeek) predictions. The experimental results were obtained and theoretical calculations of the electrostatic interactions parameters in the experimental solutions of 2:1 electrolyte once again confirmed the acceptability of a two-stage model of sorption and DLVO theory to describe a cell–cell adhesion.

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1. Introduction

The process of the bacterial adhesion has been commonly discussed in terms of a two-stage sorption model that was proposed for the first time by Marshall et al. [1]. According to this model, at the first stage bacteria quickly attach to the surface by weak physical interactions, forming mostly reversible attachment, while at the second irreversible stage molecular and cellular adhesion processes take place, and the aggregates resistant to any washing process

CONTACT M. O. Barannyk Maryano4ka2008@ukr.net
Research was conducted in Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of the Ukraine, 23, Pereyaslavskaya str., 61015, Kharkov, Ukraine.

are formed.[2] However, the impact of the first stage of attachment is very important and certainly affects the final result. Important factors, influencing the adhesion processes, are physical and chemical characteristics of the medium, in particular, the presence of monovalent and divalent cations therein.

The combined force between two interacting surfaces according to Derjaguin–Landau–Verwey–Overbeek (DLVO) theory includes van der Waals attraction forces and the electrical double-layer forces. Potential of van der Waals interaction does not depend on the changes in electrolyte concentration and pH and the first approximation can be considered to be constant. At small distances van der Waals forces exceed the repulsion of electrical double layer, because they depend on the distance by the power law ($W \approx -\frac{1}{D^n}$), while the energy of electric interactions of the double layer at $D \to 0$ is finite or slow growing.

Since total electric charge of the cells is negative, they tend to push away from each other by electrostatic forces. Influenced by the electrolyte concentration and surface charge density, interaction energy depends differently on the distance between the surfaces. There is strong far reaching repulsion between highly charged surfaces in diluted electrolyte (large Debye radius), which reaches a maximum (energy barrier) at a certain distance, usually from 1 to 4 nm. In more concentrated electrolytes, the so-called long-range potential minimum usually appears before the barrier at 3 nm (minimum energy at contact is called near potential minimum).

Unlike van der Waals attractions, repulsive forces of electrical double layer are much more sensitive to the type and concentration of electrolyte, pH and surface charge density (or surface potential). Irrespective of the mechanism of charge origin, surface charge is always compensated by the charge of counterions. Counterions are partially and reversibly associated with the surface forming a Stern–Helmholtz layer, while remaining counterions are in thermal motion near the surface and form diffused electric double layer. The presence of divalent cations causes radical changes of the surface potential and distribution of negatively charged counterions near the surface. It is shown that even at a constant surface charge density a relatively small addition of divalent ions significantly reduces the value of surface potential ψ_0.[3] The value of ψ_0 is largely determined by divalent cations if their concentration is higher than 3% of the concentration of monovalent ions. Moreover, if the concentration of Ca^{2+} in the bulk solution considerably lowers than Na^+ concentration, Ca^{2+} concentration on the surface may be higher. At high concentrations divalent ions often form chemical bonds with negatively charged groups on the surface, thereby reducing the surface charge density σ and further reducing the surface potential ψ_0.[3]

Despite the fact that the particles in a near potential minimum are at thermodynamic equilibrium, the energy barrier could be so high that the particles are not able to overcome it in a reasonable time. In this case, the particles (cells) are either remain in a distant minimum or will detach and become dispersed in solution. The characteristic distance of distant minimum in solutions close to physiological conditions has been reported to be in the range of 1–3 nm according to [3]. Others estimate secondary minima to be less or equal 7 nm.[4] Such a distance is sufficient for specific interactions between cells. For example, the majority of charges on the red blood cells are found on sialic acids carboxyl groups of surface glycoproteins that may be responsible for the adhesion interaction. These groups are located at some considerable distance (≤10 nm) from the plasma membrane lipid bilayer.[4] At the same time, it should be noted that steric and structural interactions become effective only at short distances (<2 nm).[3] Therefore, the continued presence of

interacting cells in distant potential minimum can lead to the formation of specific bonds between adhesins and ligands.

In previous work [5], we showed that adhesion of lactobacilli *Streptococcus thermophilus* to human erythrocytes depends on the sodium chloride concentration in the medium and reaches its maximum at physiological value. At the same time, the surface charge of both cell types was not affected by the concentration of 1:1 electrolyte in the investigated range. On the other hand, introduction of 2:1 electrolyte (calcium chloride) to the medium reduces intercellular adhesion and surface charge of red blood cells, indicating that the adhesion molecules involved in this process, are not Ca^{2+}-dependent (not activated by these cations). We suggest that blocking of receptors by divalent cations leads to this result. It is also of interest that the largest decrease in adhesion is observed at the lowest concentration of divalent cations Ca^{2+} used (0.01%). Further increase in concentration leads to some increase in adhesion index. In our opinion, the presence of the minimum in the adhesion index dependence on concentration of Ca^{2+} can be explained by the competing influence of these cations at two different stages of adhesion. The appearance of a certain number of divalent cations in the intercellular medium leads to the receptor binding and their partial inactivation and consequently, to reduced adhesion. Further increase in cation concentration leads to their increased impact at the first nonspecific stage of adhesion by reducing electrostatic repulsion and, therefore, to increased probability of adhesion.

In this study, we evaluate the role of electrostatic component of the intercellular adhesion interactions of human erythrocytes and lactobacilli *S. thermophilus.*

2. Mathematical model

Let's consider the distribution of ions near the insulated surface in contact with an electrolyte solution. If the full charge density at any point x is $\sum_i z_i e \rho_{xi}$, and the full concentration of ions (numerical density) is $\sum_i \rho_{xi}$, the Boltzmann's distribution of i ions at x has the form

$$\rho_{xi} = \rho_{\infty i} e^{\frac{z_i e \psi_x}{kT}} \tag{1}$$

while at the surface, at $x = 0$, the density ρ and potential ψ are related by

$$\rho_{0i} = \rho_{\infty i} e^{\frac{z_i e \psi_0}{kT}}, \tag{2}$$

where e – elementary charge, z_i – valence of ion i; ρ_{0i}, $\rho_{\infty i}$ – concentration of ions i on the surface and in the bulk solution (at $x = \infty$), where $\psi_\infty = 0$, respectively.

For example, for solution containing Na^+Cl^- + $Ca^{2+}Cl_2^-$, we may write

$$\begin{array}{ll} \left[Na^+\right]_x = \left[Na^+\right]_\infty e^{-\frac{e\psi_x}{kT}}, & \left[Na^+\right]_0 = \left[Na^+\right]_\infty e^{-\frac{e\psi_0}{kT}}, \\ \left[Ca^{2+}\right]_x = \left[Ca^{2+}\right]_\infty e^{-2\frac{e\psi_x}{kT}}, & \left[Ca^{2+}\right]_0 = \left[Ca^{2+}\right]_\infty e^{-2\frac{e\psi_0}{kT}}, \\ \left[Cl^-\right]_x = \left[Cl^-\right]_\infty e^{+\frac{e\psi_x}{kT}}, & \left[Cl^-\right]_0 = \left[Cl^-\right]_\infty e^{+\frac{e\psi_x}{kT}} \end{array} \tag{3}$$

where the values in brackets, such as $[Na^+]$, are expressed in some convenient concentration units, such as molar concentration (M).

Combined ion concentration near the isolated surface with the charge density σ is [3]:

$$\sum_i \rho_{0i} = \sum_i \rho_{\infty i} + \frac{\sigma^2}{2\varepsilon\varepsilon_0 kT} \tag{4}$$

where ε_0 – vacuum permittivity, ε – relative dielectric constant of the medium, k – Boltzmann constant, T – absolute temperature.

The ratio between the surface charge density σ and the surface potential ψ_0 for electrolyte mix NaCl + $CaC1_2$ can be obtained from the formula (4):

$$\begin{aligned}\sigma^2 &= 2\varepsilon\varepsilon_0 kT\left(\sum_i \rho_{0i} - \sum_i \rho_{\infty i}\right) = \\ &= 2\varepsilon\varepsilon_0 kT\left\{\left[Na^+\right]_\infty e^{-\frac{e\psi_0}{kT}} + \left[Ca^{2+}\right]_\infty e^{-\frac{2e\psi_0}{kT}} + \left[Cl^-\right]_\infty e^{+\frac{e\psi_0}{kT}} - \left[Na^+\right]_\infty - \left[Ca^{2+}\right]_\infty - \left[Cl^-\right]_\infty\right\}\end{aligned} \tag{5}$$

Since $[Cl^-]_\infty = [Na^+]_\infty + 2[Ca^{2+}]_\infty$, this expression can be written as follows:

$$\sigma^2 = 2\varepsilon\varepsilon_0 kT\left\{\left[Na^+\right]_\infty\left(e^{-\frac{e\psi_0}{kT}} + e^{+\frac{e\psi_0}{kT}} - 2\right) + \left[Ca^{2+}\right]_\infty\left(e^{-\frac{2e\psi_0}{kT}} + 2e^{+\frac{e\psi_0}{kT}} - 3\right)\right\}, \tag{6}$$

Therefore the final result can be written as follows:

$$\sigma = \left(8\varepsilon\varepsilon_0 kT\right)^{\frac{1}{2}} \sinh\left(\frac{e\psi_0}{2kT}\right)\left\{\left[Na^+\right]_\infty + \left[Ca^{2+}\right]_\infty\left(2 + e^{-\frac{e\psi_0}{kT}}\right)\right\}^{\frac{1}{2}} \tag{7}$$

or at 37 °C, where concentration $[NaCl] = [Na^+]_\infty$, and $[CaCl_2] = [Ca^{2+}]_\infty$ expressed in units of M, charge density σ in C m^{-2} and potential ψ_0 in mV:

$$\sigma = 0.119 \sinh\left(\frac{\psi_0}{53.4}\right)\left\{\left[NaCl\right] + \left[CaCl_2\right]\left(2 + e^{-\frac{\psi_0}{26.7}}\right)\right\}^{\frac{1}{2}} \tag{8}$$

Equation (7), known as Grahame equation, allows to calculate the value of surface potential ψ_0, if we know the surface charge density σ, whereas the concentration of individual ions ρ_0 on the surface can be calculated by the formula (3).

For erythrocyte the surface charge density $\sigma = -1.31 \times 10^{-2}$ C m^{-2}.[6] Or 1.31×10^{-2} C m^{-2} / 1.602×10^{-19} C = 0.82×10^{17} m^{-2} = 0.082 nm^{-2}, that is one elementary charge per 12.2 nm^2. The erythrocyte surface area is ~140×10^{-12} m^2 (or 14×10^7 nm^2); therefore, the surface of a red blood cell contains ~10^7 charges.

The concentrations of NaCl used in our experiments on the influence of ionic strength on adhesion rate were: 0.15, 0.1, 0.05, 0.025 M. The surface potential was calculated on the assumption that the density of surface charges in NaCl solutions with varying ionic strength remained unchanged. This assumption is confirmed by our data on binding of Alcian blue cationic dye (AB) by erythrocytes, which did not differ significantly in the investigated solutions.[5] The values of erythrocyte surface potential calculated by the formula (8) in NaCl solutions with different ionic strength are given in Table 1.

According to our work [5] addition of 0.9×10^{-3} M $CaCl_2$ in 0.15 M NaCl solution decreases the surface charge by ~18%, and for concentrations 1.8×10^{-3} M, 2.7×10^{-3} M, 3.6×10^{-3} M by ~11%. We assumed that in the first solution $\sigma \approx 1.074 \times 10^{-2}$ C m^{-2}, in others – $\sigma \approx 1.166 \times 10^{-2}$ C m^{-2}. Computer calculations of erythrocyte surface potential by the formula (8) in NaCl isotonic solutions with the addition of the indicated concentrations of $CaCl_2$ (close to the concentrations in blood plasma) give the values given in Table 2.

Table 1. The erythrocyte's surface potential and the Debye radiuses in solutions with different concentrations of NaCl.

Concentration of NaCl, M	Adhesion index [5]	Quantity of bound AB by erythrocytes, ng/10^6 er. [5]	Surface potential, mV	Debye radius at 37 °C, nm
0.025	$0.95 \pm 0.63^*$	226.8 ± 9.3	−34.4	1.98
0.05	$1.52 \pm 0.85^*$	226.8 ± 9.8	−25.4	1.40
0.1	$1.44 \pm 0.94^*$	228.2 ± 7.7	−18.4	0.99
0.15	2.21 ± 0.87	220.8 ± 4	−14.7	0.81

Notes.
*data significantly differ from data for blank test, $p < 0001$.
The correlation between values of the adhesion index and the Debye radius is $r = 0.86$.
The correlation between values of the adhesion index and the surface potential is $r = 0.88$.

Table 2. Effect of $CaCl_2$ concentration in the medium on the erythrocyte surface potential, on Ca^{2+} concentration on its surface and on the Debye radius.

Ca^{2+} concentration 10^{-3} M	Adhesion index [5]	Quantity of bound AB by erythrocytes, ng/10^6 er.[5]	Surface potential, mV	Debye radius at 37 °C, nm
0.00	2.21 ± 0.87	220.8 ± 4	−14.7	0.81
0.9	$0.97 \pm 0.84^*$	$180.98 \pm 11.5^+$	−12.2	0.80
1.8	$1.57 \pm 0.96^*$	$195.1 \pm 6.3^+$	−13.2	0.79
2.7	$1.4 \pm 0.84^*$	$199.9 \pm 9.7^+$	−13.1	0.79
3.6	$1.17 \pm 0.86^*$	$196.3 \pm 12.5^+$	−13.0	0.78

$^{*,+}$ -data significantly differ from data for blank test, $p < 0.01$.

Concentrations of individual ions ρ_{0i} on the surface of red blood cells can be calculated by formula (3) by substituting the calculated values of surface potential for our solutions.

The data for one-component solutions of sodium chloride only and two-component solutions with the addition of calcium chloride are given in Table 3.

For small values of potential, approximately less than 25 mV, which is true in the case of red blood cells, Grahame equation (9) simplifies to the form [3]:

$$\sigma = \varepsilon\varepsilon_0\kappa\psi_0 \tag{9}$$

where

$$\kappa = \left(\frac{\sum \rho_{\infty i} e^2 z_i^2}{\varepsilon\varepsilon_0 kT}\right)^{\frac{1}{2}} \mathrm{m}^{-1} \tag{10}$$

Then Debye radius ($1/\kappa$) can be defined as follows:

$$\frac{1}{\kappa} = \left(\frac{\varepsilon\varepsilon_0 kT}{\sum \rho_{\infty i} e^2 z_i^2}\right)^{\frac{1}{2}} \tag{11}$$

The Debye radius value depends on the properties of the solution and is independent of surface properties such as charge or potential.

The value of Debye radius in aqueous solutions of NaCl is:

$$\frac{1}{\kappa} = \frac{\left(\varepsilon\varepsilon_0 kT\right)^{\frac{1}{2}}}{\left\{\left(\rho_{\mathrm{Na}} e^2 z_{\mathrm{Na}}^2 + \rho_{\mathrm{Cl}} e^2 z_{\mathrm{Cl}}^2\right) \times \mathrm{N_A} \times 10^3\right\}^{\frac{1}{2}}} \tag{12}$$

where ρ_i – numerical density of ions (number of charges in m^3); N_A – Avogadro's number. Multiplication on $N_A \times 10^3$ is for translation in concentration from the numerical density to mol l^{-1}.

Then for the temperature t = 37 °C (310 K):

$$\frac{1}{\kappa} = \frac{0.313}{\left([\mathrm{NaCl}]\right)^{\frac{1}{2}}} \tag{13}$$

For two-component solution 0.15 M NaCl + $CaCl_2$ the Debye radius is:

$$\frac{1}{\kappa} = \frac{\left(\varepsilon\varepsilon_0 kT\right)^{\frac{1}{2}}}{\left(\rho_{Na}e^2z_{Na}^2+\rho_{Cl}e^2z_{Cl}^2+\rho_{Ca}e^2z_{Ca}^2+2\rho_{Cl}e^2z_{Cl}^2\right)^{\frac{1}{2}}} = \\ = \frac{\left(\varepsilon\varepsilon_0 kT\right)^{\frac{1}{2}}}{e\{2[\mathrm{Na}]+6[\mathrm{Ca}]\}^{\frac{1}{2}}\times\left(N_A\times10^3\right)^{\frac{1}{2}}} = \frac{0.443}{\{0.3+6[\mathrm{Ca}]\}^{\frac{1}{2}}} \tag{14}$$

The calculated values of the Debye radius at 37 °C in solutions with different concentrations of NaCl (formula (13)) presented in Table 1 and in two-component solutions with $CaCl_2$ concentration ranging from 0.9 to 3.6 mM in 0.15 M NaCl (for formula (14)) – in Table 2.

In the case of two-component solutions based on our measurements, the calculated Debye radius does not change significantly after addition of $CaCl_2$ close to physiological concentrations (Table 2). The concentration of calcium ions on the surface of red blood cells with a relatively small potential exceeds its concentration in bulk solution ~1.6 times. At the same time, the concentration of sodium and chlorine on the surface of red blood cells differs insignificantly from those for 0.15 M solution of NaCl (Table 3). Thus, the most changes occur in the cell surface potential and calcium concentration at the surface of red blood cells.

When evaluating the probability of adhesion of lactobacilli *S. thermophilus* to human erythrocytes depending on the concentration of Ca^{2+} ions, we take into account the effect of Ca^{2+} ions on electrostatic repulsion force of cells and the probability of establishing specific adhesive bonds by binding Ca^{2+} ions to specific receptors, causing their inactivation. Therefore, following,[4] we assume that the formation of adhesive bonds between two cells is carried out in two stages. To assess the probability, we used the concept of the collision complex. According to this approach, the reaction is conceptually divided into two steps. At the first stage, the reagents collide with each other, i.e. they diffuse to the distance close

Table 3. The concentration of ions in the Stern–Helmholtz layer on the surface of red blood cells in solutions with different content of 1:1 and 2:1 electrolytes.

	Concentration of ions on the surface of a red blood cell, M		
Medium composition, M	$[Na^+]_0$	$[Cl^-]_0$	$[Ca^{2+}]_0$
0.025 NaCl	0.09	0.0069	–
0.05 NaCl	0129	0.019	–
0.1 NaCl	0.199	0.05	–
0.15 NaCl	0.26	0.086	–
0.15NaCl+0.9·10^{-3} $CaCl_2$	0.237	0.096	1.42·10^{-3}
0.15NaCl+1.8·10^{-3} $CaCl_2$	0.246	0.094	2.95·10^{-3}
0.15NaCl+2.7·10^{-3} $CaCl_2$	0.245	0.095	4.4·10^{-3}
0.15NaCl+3.6·10^{-3} $CaCl_2$	0.244	0.097	5.85·10^{-3}

enough that allows the second stage of the reaction to occur. In our case, we consider the first (reversible) reaction stage to take place when cells approach to the distance of the distant potential minimum.

Thus the reaction can be written as follows:

$$A + B \leftrightarrow AB \leftrightarrow C \tag{15}$$

where AB – encounter complex, C – formation from AB-bound state.

We assume that cells form the collision complex whenever they are at the collision distance D_{AB}, i.e. the distance of the distant potential minimum.

In the approximation of Derjaguin [3], the force of interaction between two spheres can be expressed through the interaction energy per unit area of two flat surfaces at a distance D.

$$F(D) \approx 2\pi\left(\frac{R_1R_2}{R_1+R_2}\right)W(D) \tag{16}$$

where W – free energy of interaction.

This formula is applicable to any type of force, attraction, repulsion, or variable interaction if the interaction radius and the distance D are much less than the sphere's radiuses. In the case of cell interaction at a distance of ~ distant minimum (3–4 nm), this requirement is satisfied. If one sphere is very large, so that $R_2 >> R_1$, one can obtain $F(D) = 2\pi R_1 W(D)$ that is consistent with the limiting case of sphere near the flat surface. The diameter of lactobacilli *S. thermophilus* is ~1 micron, while the diameter of erythrocytes is ~7–8 microns. However, we can accept provided approximations. Although erythrocyte shape is quite complex, most of its surface can be considered flat relative to the almost spherical *S. thermophilus* cells (radius of curvature of the erythrocyte surface << radius of curvature of lactobacillus).

At small surface potentials, less than approximately –25 mV, which is consistent with our case, formulas for energy and interaction forces can be simplified. For a sphere near a flat surface, the electrostatic repulsion force is

$$F \approx 2\pi R\varepsilon\varepsilon_0\kappa\psi_0^2 e^{-\kappa D} = \frac{2\pi R\sigma^2 e^{-\kappa D}}{\kappa\varepsilon\varepsilon_0} \tag{17}$$

In these equations, ψ_0 and σ are related by expression $\sigma = \varepsilon\varepsilon_0\kappa\psi_0$, which is valid for small potentials.

Expressions (16 and 17) for the interaction force between two electrical double layers are accurate only at distances between the surfaces that are larger than approximately one Debye radius. Our calculations (Table 2) show that this is true for the interaction of cells at distances of a distant minimum and larger. The interaction of the electric double layer between a spherical particle and a surface exponentially decreases with distance; moreover, the characteristic scale of the damping equals the Debye radius of screening.

Thus, the probability of *S. thermophilus* approaching red blood cells at the distance of distant minimum is inversely proportional to the electrostatic repulsion force:

$$P_1 \sim \frac{1}{F} = \frac{1}{\left(2\pi R\varepsilon\varepsilon_0\kappa\psi_0^2 e^{-\kappa D}\right)} = \frac{A}{\kappa\psi_0^2 e^{-\kappa D}} \tag{18}$$

where $A = \frac{1}{2\pi R\varepsilon\varepsilon_0}$.

Accepted that $D \approx 4$ nm [3, p.583], $R \approx 0.5$ micron = 0.5×10^{-6} m (radius of *S. thermophilus* cells).

In many cases, when the properties of the solution change, neither σ nor ψ_0 remain constant. This happens only in exceptional cases when the ionized groups on the surface are completely dissociated, and usually they are partially neutralized by the ions in solution. For example, in our case, we assume that calcium ions bind negatively charged receptors on the surface of red blood cells. Then the equilibrium concentration of receptors on the surface is given by the expression:

$$Re^{2-} + Ca^{2+} \Leftrightarrow ReCa \tag{19}$$

The concentration of calcium on the surface is denoted as $[Ca^{2+}]_0$, the concentration or surface density of Ca^{2+} receptors on the surface as $[Re^{2-}]_0$ and density of the receptors associated with Ca^{2+} as $[ReCa]_0$. The value $[Re^{2-}]_0$ is related to σ by the ratio $\sigma = -b\left[Re^{2-}\right]_0$, where b – fraction of receptors that are responsible for adhesion with lactobacilli in respect of all negatively charged groups on the surface of red blood cells. $b\sigma_0 = N$ – initial surface density of receptors, and α – part of the receptors not associated with Ca^{2+}. The value of α is calculated from our experimental data on the change of the surface charge based on binding cationic dye in solutions after adding 2:1 electrolyte $CaCl_2$ (Table 2).

Then, the probability of establishing adhesion bond with the bacterial cell, located at a distance of potential minimum is proportional to the number of receptors not associated with calcium

$$P_2 \sim \alpha b\sigma_0 = \alpha N \tag{20}$$

Therefore, the probability of establishing specific adhesion bond taking into account the probability of cells approaching to the distance of a distant potential minimum will be

$$P = P_1 \times P_2 \sim \frac{\alpha \mathrm{NA}}{\kappa\psi_0^2 e^{-\kappa D}} \sim \frac{\alpha \mathrm{NA}}{\kappa\psi_0^2 e^{-4\kappa}} \tag{21}$$

Experimental and theoretical data regarding the probability of establishing a specific adhesive bond between *S. thermophilus* and human erythrocytes are summarized in Table 4.

Estimations of the probability of establishing a specific adhesive bond (in relative units) are presented in Figure 1.

The graph presented in Figure 1 shows that obtained values of adhesion index of lactobacilli *S. thermophilus* to human erythrocytes are adequately explained by the proposed model.

Table 4. Effect of $CaCl_2$ concentration in the medium on the erythrocyte surface characteristics and the probability of the formation of a specific adhesive bond.

Ca^{2+} concentration, 10^{-3} M	α	κ, nm^{-1}	ψ_0, mV	P, relative units
0.0	1	1.23	−14.7	1
0.9	0.82	1.25	−12.2	0.51
1.8	0.89	1.26	−13.2	0.62
2.7	0.89	1.27	−13.1	0.58
3.6	0.89	1.28	−13.0	0.54

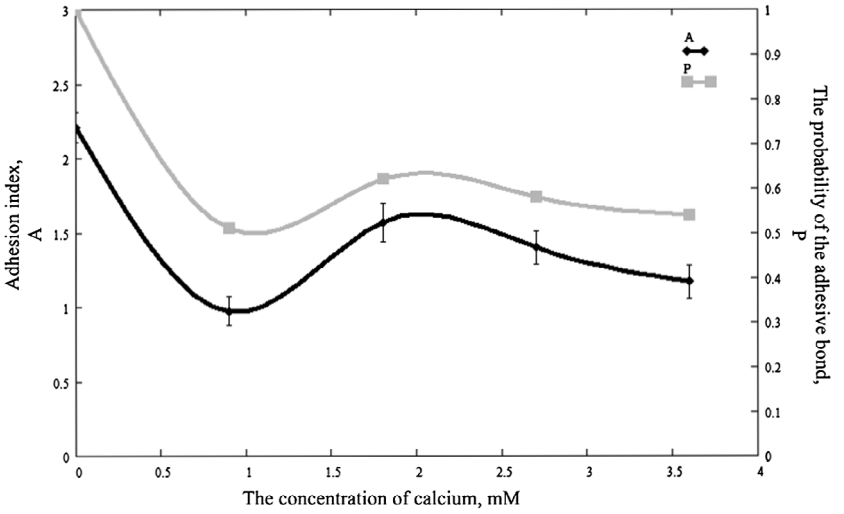

Figure 1. Value of adhesion (♦) and the probability of the specific bond formation (■) between lactobacilli S. thermophilus and human erythrocytes depending on the $CaCl_2$ concentration.

3. Conclusions

Here, we compared the experimental data on adhesion of lactobacilli *S. thermophilus* to human erythrocytes with theoretically defined Debye radius and erythrocyte surface potential in solutions of 1:1 electrolyte with varying ionic strength. Our data show that changes in adhesion index in solutions with decreasing ionic strengths are fully consistent with the predictions of DLVO theory. Therefore, the dominant factor that affects the adhesion index of lactobacilli *S. thermophilus* to human erythrocytes in solutions with varying sodium chloride concentrations is electrostatic interactions at the first reversible stage of adhesion.

As we suggested in previous work [5], the obtained dependence of adhesion index on the Ca^{2+} concentration in the medium can be explained by blocking specific receptors by divalent cations binding. At the same time electrostatic interactions play a major role in the process of intercellular adhesion. The experimental results obtained and theoretical calculations of electrostatic interaction parameters once again confirmed that a two-stage sorption model and DLVO theory are acceptable for description of intercellular adhesion.

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