Biophysical Chemistry 139 (2009) 8–12

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Influence of ethanol on the high frequency electric polarizability of *E. coli*

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ARTICLE INFO

Article history:
Received 11 August 2008
Received in revised form 19 September 2008
Accepted 19 September 2008
Available online 30 September 2008

Keywords:
Escherichia coli
Maxwell–Wagner polarizability
Charge dependent electric polarizability
Dielectric permittivity
Electric conductivity
Electro-optics

ABSTRACT

The interface electric polarizability of bacteria (charge dependent (ChD) and Maxwell–Wagner (MW) polarizabilities) gives information about their electric charge, determined by the structure and functional state. It is well known that the polarizability could be changed significantly by adding some substances to the suspension, and can be measured using an electro-optical (EO) method. There are some literature data, according to which the adding of ethanol decreases the electric polarizability of the cells. However the reason for the change in this parameter is not clear, as well as which component (ChD and/or MW) of polarizability has the main contribution. Generally the present work shows that the effect of ethanol is connected to the change of the internal (cytoplasm) MW polarizability and is mainly caused by increasing the cell membrane permeability. This results in an ionic flow through the membrane, which velocity and direction depends on the relative values of the inner (cytoplasm) and the outer medium ionic strength.

1. Introduction

The surface charge density and the electric polarizability of bacteria are important parameters giving information about the cells' structure and functional state [1]. These electric properties of bacteria, especially of *E. coli*, have been widely studied by some methods, the main of which are electrophoresis [1,2], dielectric spectroscopy [3,4] and electro-optics [5–8]. The electrophoresis is the best developed method for determination the surface charge density, but it does not give any information about the electric charge inside bacteria, as the dielectric spectroscopy and electro-optics do. The last method has the advantage that it does not require high particle concentration contrary to the dielectric measurements.

The electro-optical methods are based on the orientation of colloid particles in applied electric field [5,6]. The orientation degree is proportional to the electric polarizability, which can be two main types: interface and volume. As a rule, the volume component is negligible in a suspension of particles with micron and submicron dimensions because of the big ratio between the total surface and the volume of all particles. Two basic kinds of interface polarizability are known: Maxwell–Wagner (MW) and surface charge dependent (ChD) polarizability.

The MW polarizability is defined as accumulation of electric charges at the interface between two mediums having different volume electric conductivity and dielectric permittivity under the action of electric field [5,6]. In the case of bacteria this polarization is due to accumulation of ions in the cytoplasm nearby the cytoplasm membrane, which is practically non-conductive. Therefore the MW component increases with the increase in the ionic strength inside the bacteria. MW polarizability does not depend on the particle size and its relaxation usually takes place in the MHz-range [6,9,10].

The ChD polarizability is defined as polarization of the particle electric double layer (EDL) [5], mainly of its diffuse part [11], and it is due to ions' migration parallel to the particle's surface. That is why its value depends on the bacteria long size and the EDL thickness determined by the surface charge density and the medium ionic strength. The ChD polarizability relaxation time depends on the square of the bacteria length and consequently may be observed at relatively low frequencies for cells with micron dimensions.

In the case of charged solid particles the ChD component plays the main role in their orientation, which is proved by the dependence of the electro-optical effect (EOE) on the pH and the ionic strength of the medium: the polarizability practically disappears at the isoelectric point and/or at high electrolyte concentration [5,6]. However in the case of bacteria the MW component should have the most significant contribution, because the cell volume is enclosed by a cytoplasm membrane and a cell wall with different electric parameters than the cytoplasm and the outer medium.

Usually in the electro-optical literature bacteria are treated like homogeneous solid particles [5–8] without considering their more complex structure: liquid cytoplasm inside and cell cover (cytoplasm membrane and cell wall) over it. That is why in most of the cases only the ChD polarizability is taken into account.

Because of the existence of two surfaces of the cell cover — external and internal ones, two EDL must be considered and two ChD polarizabilities — external (EChD) and internal (IChD) ones respectively must exist [12]. By analogy two MW components — external (EMW) and internal (IMW) ones should exhibit.

Alternative approach is applied by the so-called Electro-Physical (EPh) model of bacteria, based on the MW type of polarization [1]. In

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doi:10.1016/j.bpc.2008.09.017

that case the bacterium is considered like a homogeneous or hollow ellipsoid covered by one or more layers characterized by their specific electric conductivity and dielectric permittivity. The influence of the ionic atmosphere near the cell wall on the polarizability can be estimated by introduction of frequency-dependent values of the conductivity and the permittivity and characteristic frequencies for α and β dispersions [13]. This model shows a presence of few dispersion frequencies and different polarizability values along and across the bacterium [1]. It is possible to fit the theoretical frequency dependence with the experimental one by adjustment of the model parameters. The main disadvantage of this approach is that the EPh-model has too many parameters, which gives practically unlimited possibilities to fit the theoretical results to the experimental ones and consequently it is not clear if the parameters obtained in such a way are real.

We apply an experimental approach to distinguish the different types of surface polarization changing the factors influencing the ChD and the MW components. We use the electro-optical method electric turbidity to obtain the frequency dependence of the polarizability. Generally our approach reduces to investigation of the dependence of the electric polarizability on the bacteria size and the ionic concentration (inside or outside of the cells) at constant other experimental conditions.

In our previous article [14] we have found out that the electric polarizability decreases with the increase in the outer ionic concentration (at constant one inside) in the frequency range from 20 kHz to 20 MHz. Our study has shown that the polarizability depends linearly on the DEL thickness, which is an indication for exhibition of the EChD polarizability even at these high frequencies, where the ChD component would disappear according to the dielectric and the electro-optical literature.

In the present work we distinguish the IMW polarizability from the other components by changing the inner ionic strength. To this purpose we use ethanol, which increases the ion permeability of the cell membrane, possibly by making pores in it [15].

2. Materials and methods

2.1. Materials

Bacteria culture of *E. coli* K12 was cultivated in standard media LB and 1% glucose for 7.5 h at temperature 37 °C and pH 7. The bacteria samples were taken from the medium just before the electro-optical measurements. After that they were washed with distilled water on a 0.8 μm milliporous filter and suspended in aqueous medium with electric conductivity of 5 μS/cm.

To obtain a suspension of fixed (dead) *E. coli* K12, bacteria were suspended in a fresh 3% aqueous solution of formaldehyde after the described filtration. The bacteria were washed from the formaldehyde and suspended in distilled water just before the beginning of the electro-optical measurements.

The experiments connected to increasing in the cytoplasm ionic concentration (Figs. 6–8) were carried out as follow: two suspensions of fixed *E. coli* K12 have been incubated for 24 h in 150 mM KCl in absence and in presence of 5% vol. ethanol respectively. After that bacteria were washed and suspended in distilled water just before the beginning of the electro-optical measurements.

In all the experiments the suspensions of alive or fixed bacteria were adjusted to an optical density 0.1 at 1 cm optical path.

2.2. Electro-optical theory

When electric field is applied to the bacterial suspension, the optical density A is changed due to the particles' orientation. The value of the electro-optical effect (EOE) is determined as $\Delta A = A_E - A$, where the index E is for the applied electric field. In steady-state EOE (ΔA_s) the degree of orientation depends on the ratio between the orientation energy γE^2 and the energy of *random* motion kT of the particles with electric polarizability γ in electric field with intensity E:

$$\Delta A_s/A = G \cdot (\gamma E^2/15kT), \quad (1)$$

where G is an optical function, which depends on the size, form and *refractive* index of the cells.

The EOE decay after the switching off the electric field is defined by the rotational diffusion coefficient D, related respectively to the particle relaxation time $\tau = 1/6D$. The EOE decay of a mono-disperse system is mono-exponential for particles with axial symmetry:

$$\Delta A_t = \Delta A_s \exp(-6Dt) = \Delta A_s \exp(-t/\tau), \quad (2)$$

where ΔA_t and ΔA_s are the values of EOE at the moment t and at the steady-state EOE respectively.

2.3. Electro-optical device

The main part of the electro-optical measurements was executed by EloTrace-1.0, developed in Biotronix GmbH (Germany). The device records the EOE as $\Delta A = \Delta A^{//} - \Delta A^{\perp}$, where $\Delta A^{//}$ and $\Delta A^{\perp}$ are the values of the EOE at parallel and perpendicular orientation of two light beams towards the electric field. Because $\Delta A^{//}$ and $\Delta A^{\perp}$ have an opposite sign, the resulting EOE is a sum of both the components. EloTrace-1.0 takes down automatically the dependencies of the EOE on the frequency (20 kHz–20 MHz) and the intensity of the electric field (17–110 V/cm) at wave length 670 nm and optical path 1 cm. It also calculates the average relaxation time of bacteria and their average size.

The rest of the experiments were carried out by EloTrace-2.0 (Biotronix GmbH, Germany), which has the same electrical and optical characteristics, but makes completely automatically all the operations. The device takes a bacteria sample of 1–2 ml from the incubator every 6 min, filtrates and washes it until obtaining a suspension with the definite optical density and electric conductivity mentioned above. After that EloTrace-2.0 takes down and treats the electro-optical data, so that the change in the electric polarizability and the average bacteria size with the growth time can be recorded.

All the frequency dependences of alive and fixed bacteria *E. coli* K12 are taken down at electric field intensity of 78V/cm.

3. Results and discussion

In Fig. 1 are presented the frequency dependencies of the electric polarizability of fixed *E. coli* K12 at different ethanol concentrations.

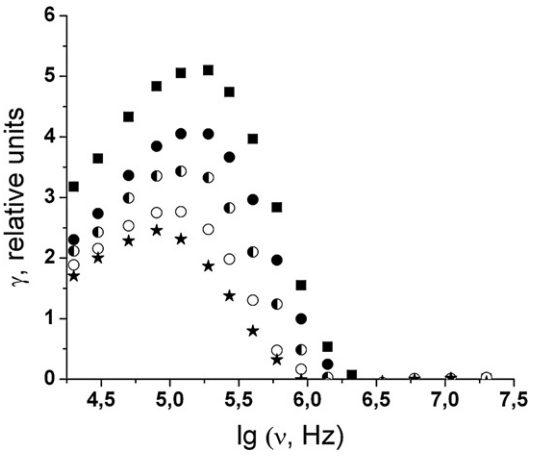

Fig. 1. Frequency dependencies of the electric polarizability of fixed *E. coli* K12: ■ — in absence of ethanol, ● — 5% vol. ethanol, ◐ — 10% vol. ethanol, ○ — 15% vol. ethanol, ★ — 20% vol. ethanol.

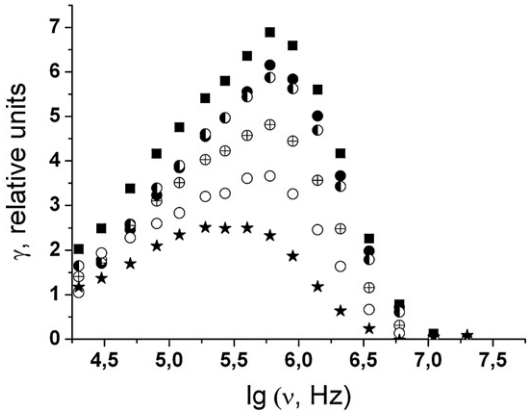

Fig. 2. Frequency dependencies of the electric polarizability of alive *E. coli* K12: ■ — in absence of ethanol, ● — 3% vol. ethanol, ◐ — 5% vol. ethanol, ⊕ — 10% vol. ethanol, ○ — 15% vol. ethanol, ★ — 20% vol. ethanol.

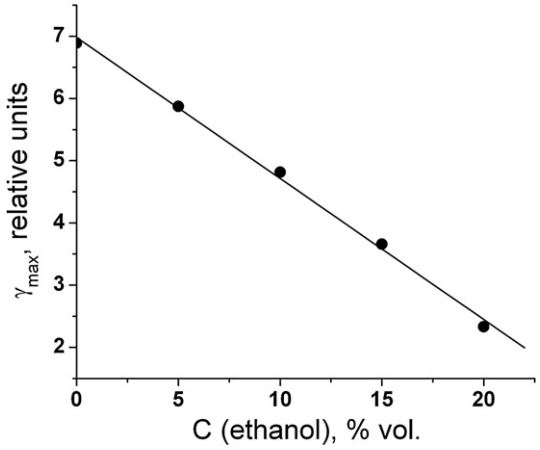

Fig. 4. Dependence of the electric polarizability of the maximum of the frequency dependences of alive *E. coli* K12 (Fig. 2) on the ethanol concentration.

The value of this parameter decreases at all the frequencies of the experimental range and its relaxation frequency shifts to lower frequencies with the increase in the ethanol concentration. The electric polarizability decrease in the maximum of the dispersion curves is from 20% to 50% at ethanol concentration from 5% vol. to 20% vol. respectively.

The frequency dependencies of polarizability of alive bacteria *E. coli* K12 at different ethanol concentrations are shown in Fig. 2. A similar tendency in the electric polarizability behavior is observed as in the case of dead cells. We note that a comparison between the results for alive and fixed bacteria would not be correct in our case, because they come from different cultivations, and additionally this was not included in the aim of our study.

The decrease in the polarizability at adding of ethanol to *E. coli* suspension has been recorded by some researchers [15,16]. According to Ref. [16] this fact is a proof for the interfacial origin of the dipole moments, however the study of the mechanism of polarizability has not been an aim of the work. The other authors [15] explain the polarizability decrease with the ethanol effect of damaging the cell wall by making pores in it.

In Figs. 3 and 4 are presented the dependencies of the electric polarizability of the maximum of frequency dependences of fixed (Fig. 1) and alive (Fig. 2) *E. coli* K12 on the ethanol concentration. The polarizability decreases linearly with the increase in the ethanol concentration.

The decrease in the electric polarizability (Figs. 1 and 2) may be due to a change in the medium refractive index, the medium dielectric permittivity and the inner ionic strength.

The medium refractive index (n_0) increases from 1.333 to 1.347 at ethanol concentration increase up to 20% vol. [17], which leads to light scattering intense decrease and therefore to the optical density decrease. However the calculations using the theory of Mi [18,19] show that the increase of n_0 by 1% results in an insignificant change of the turbidity [20] at constant particles' dimensions. Furthermore, the change of n_0 does not influence the value of polarizability, which magnitude is determined by the relative value of the EOE: $\Delta A_s/A = A_E/A - 1$, because the optical density at bacteria orientation in electric field A_E and in the case of *random* orientation A are changed in the same way by n_0. The ethanol effect also could be due to the change in the optical function $G(F)$ (Eq. (1)), which depends on the relative particles' size B/λ, where the wave length in the medium λ is defined as $\lambda = \lambda_0/n_0$. However this effect could not explain quantitatively the twice decrease in polarizability values observed in Figs. 1 and 2.

Fig. 5 shows the dependence of the *relative* dielectric permittivity of the medium (ε) on the ethanol concentration. We can see that ε decreases linearly by 15% (from 78 to 67) with the increase in the ethanol concentration from 0 to 20% vol. (at 25 °C) [21]. The comparison between Figs. 5, 3 and 4 results in the supposition that the decrease in the polarizability may be related to the decrease in ε of the medium at presence of ethanol.

The calculations according to the EPh model for a homogenous ellipsoid [1] show that the electric polarizability decreases by 30% in the frequency range from 10^3 to 10^7Hz with the decrease in the medium ε from 80 to 60. This decrease is lower than that in our experiment, but the

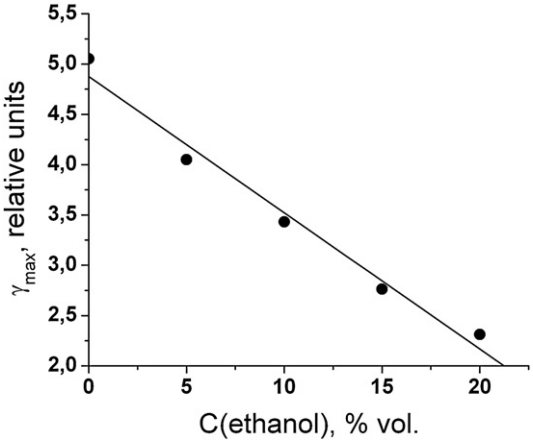

Fig. 3. Dependence of the electric polarizability of the maximum of the frequency dependences of fixed *E. coli* K12 (Fig. 1) on the ethanol concentration.

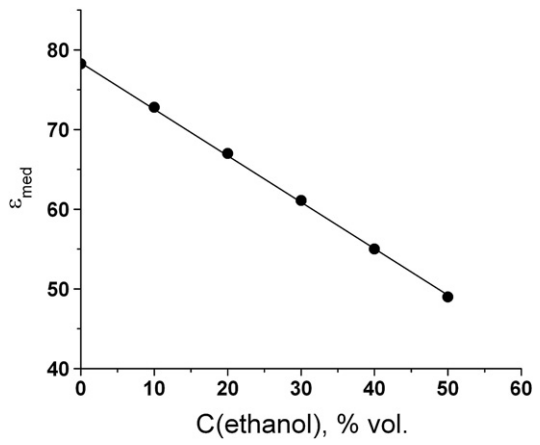

Fig. 5. Dependence of the *relative* dielectric permittivity of the medium on the ethanol concentration.

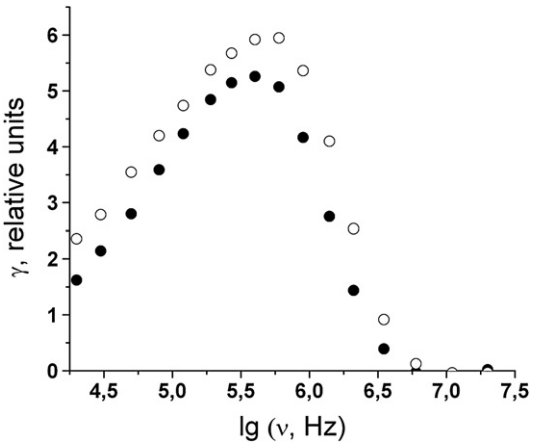

Fig. 6. Frequency dependencies of the electric polarizability of fixed *E. coli* K12 after the bacteria have incubated for 24 h in: ● — 150 mM KCl; ○ — 150 mM KCl and 5% vol. ethanol.

estimation presented above leads to the conclusion that the adding of ethanol influences significantly the value of the polarizability.

However the study of Ref. [15] shows that there is a kinetics of the decrease in the polarizability with the time in presence of ethanol. The fact cannot be explained by change in ε, because the decrease in this parameter of the medium happens at once at the moment of adding of ethanol, consequently the value of the polarizability should be decreased at once too, if it is only due to the change in ε. As it was mentioned, the authors suggest that the ethanol makes pores in the cell wall, through which an ionic flow appears. Because the ionic concentration inside the bacterium is higher than that in the outer medium in the case of Figs. 1 and 2, this flow should be directed out of the cell and would lead to decrease in the cytoplasm electrolyte concentration. The inner ionic concentration influences both the IMW and the IChD components.

According to the EO literature the components of the SChD polarizability should not exhibit at such high frequencies at such a large particle size [5]. However our previous study [14] shows that the contribution of this component can be observed at the range from 20kHz to 20MHz, which means that the factor of the frequency is not enough for recognizing the polarizability mechanism. An additional criterion is the change in the ionic strength, which influences the different polarizability components in a different way, as it was described above. In our case the IChD polarizability should decrease strongly to disappearance because of the high electrolyte concentration of the cytoplasm. However the EChD component exists in the low ionic strength of the outer medium and maybe the ionic flow through the damaged membrane could increase the outer ionic strength. The following calculations help us to find if that supposition is possible.

The volume of a cell with average length 3 μm and diameter 1 μm is equal to 2.1 μm^3. The bacteria concentration in our experiment is 10^7 cells/cm^3 (at 0.1 OD), so the total volume of all the cells is $2.1 \cdot 10^7 \mu m^3$ in 1 cm^3 suspension. Even if all the ions flow out of the bacteria, the electrolyte concentration would be $5 \cdot 10^4$-times lower than that in the cytoplasm (150mM), therefore the outer medium concentration should become $3 \cdot 10^{-6}$M. So, this estimation shows that the ionic flow through the ethanol damaged membrane is not able to change significantly the outer electrolyte concentration at the experimental conditions. Therefore in our case the magnitude of the EChD component remains almost constant and does not contribute to the decrease in the electric polarizability.

The EPh-model for homogeneous ellipsoid shows that the MW polarizability exhibits at electric conductivity values above 10^{-2}S/m, which corresponds to 1mM KCl. The outer electrolyte concentration in our experiment is less than 10^{-5}M, therefore the EMW component does not exhibit. Anyway if it would exhibit, it would not be changed because the medium ionic strength is practically constant.

Generally we got to the conclusion that the decrease in the electric polarizability resulting from the ethanol effect of damaging the cell membrane is due to the decrease of the IMW component because of the progressively lower values of the cytoplasm electrolyte concentration.

Let us now turn back to Figs. 1 and 2. As it was mentioned, the relaxation frequency of the polarizability shifts to lower frequencies with the increase in the ethanol concentration. The decrease in the ionic strength leads to relaxation frequency shift to lower frequencies in the case of exhibition of ChD polarizability [22], as well as of the MW one [23]. However, as it was discussed above, The IChD and EMW components practically disappear at the conditions of our experiment and the EChD one does not change. Consequently the relaxation frequency shift to lower values is only due to the change in the IMW polarizability.

So, our study shows that there are two main reasons for the decrease in the electric polarizability as an ethanol effect — the decrease in the medium dielectric permittivity and the ionic flow out of the cells. However both the factors lead to decrease in the polarizability and it is difficult to compare their contributions. Therefore, to separate them we had to change the experimental conditions in such a way that the two ethanol effects lead to opposite results.

To find out the relative contribution of the two ethanol effects, we made the following experiment. The bacteria *E. coli* K12 had been suspended in distilled water for a week in order to decrease strongly

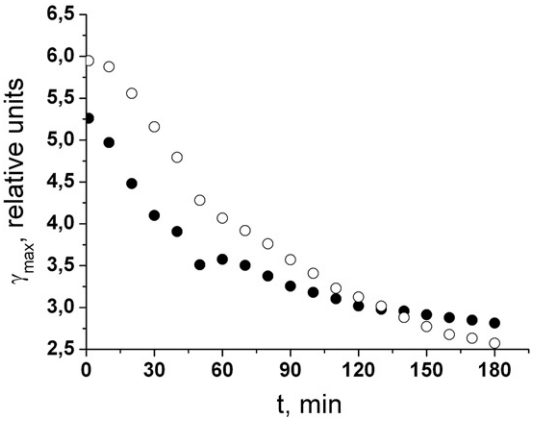

Fig. 7. Kinetics of the change of the electric polarizability of the maximum of the frequency dependences of fixed *E. coli* K12 (Fig. 6) with the time after the bacteria have incubated for 24 h in: ● — 150 mM KCl; ○ — 150 mM KCl and 5% vol. ethanol.

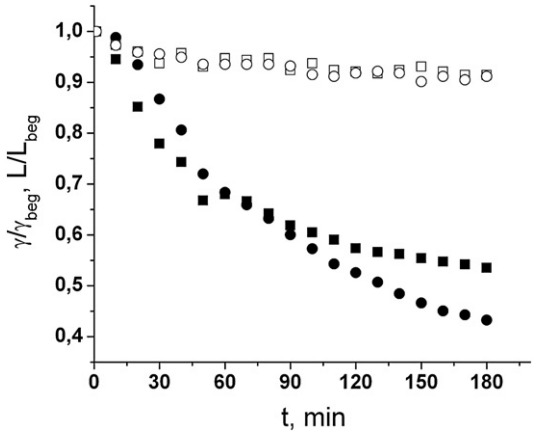

Fig. 8. Relative change of the electric polarizability (γ/γ_{beg}) of the maximum of the frequency dependences of fixed *E. coli* K12 (Figs. 6 and 7) and their average size (L/L_{beg}) with the time after the bacteria have incubated for 24 h in 150 mM KCl: ■ — γ/γ_{beg}, □ — L/L_{beg}; and in 150 mM KCl and 5% vol. ethanol: ● — γ/γ_{beg} and ○ — L/L_{beg}; where γ_{beg}=5.9 rel. units, L_{beg}=2.94 μm.

the inner ionic strength and then the ionic concentration of the suspension was increased up to 150mM KCl (in ethanol absence). Another suspension was prepared and treated in the same way, but in presence of 5% vol. ethanol. After the incubation under these conditions for 24h both the suspensions were washed and suspended in aqueous medium and then investigated electro-optically (in ethanol absence).

The frequency dependencies of the electric polarizability of both the suspensions are presented in Fig. 6. The polarizability of the bacteria treated with KCl and ethanol is higher than that incubated only in KCl. In this experiment (in both the suspensions described) the outer electrolyte concentration was higher than the inner one, so the concentration gradient was directed from the medium to the cells. This ionic flow leads to increase in the cytoplasm ionic strength and therefore to higher value of the IMW component at the time of electro-optical measurement. On the other hand the presence of ethanol results in lower medium dielectric permittivity and decrease in the electric polarizability. Fig. 6 shows that the ethanol effect of increasing the inner ionic strength predominates over that of decreasing the medium dielectric permittivity.

Fig. 7 presents the kinetics of the change in the polarizability in the two suspensions with the time. We note that this experiment was carried out in aqueous medium (in ethanol absence). The cytoplasm electrolyte concentration of the bacteria treated by 5% vol. ethanol is higher than that of the untreated bacteria. That is why the electric polarizability of the cells of the first suspension is higher in the beginning of the measurement. However the speed of the polarizability decreasing is different, because the ionic flow is faster in the case of ethanol-treated bacteria, whereas the ions' transport through the non-damaged membrane is slower.

Alternative explanation is that the decrease in the electric polarizability (Fig. 7) may be due to a possible decrease in the bacteria size with the time. That causes a decrease in the ChD component of polarizability, which still appears in the investigated frequency range [14].

The relative change in the electric polarizability and the size of the cells with the time are compared in Fig. 8. Little changes in the bacteria size (which do not exceed 10 %) are observed. They result from a decrease in the osmotic pressure caused by the ionic transport out of the cells. However Fig. 8 shows that the discussed change in bacteria size corresponds to twice decrease in the value of the polarizability. Such an insignificant decrease cannot cause a decrease in the polarizability like that observed in Fig. 7. So, the reason for the change in this parameter remains the decrease in the cytoplasm electrolyte concentration.

4. Summary

We found out that the bacteria electric polarizability decreases linearly with the increase in the ethanol concentration. The presented results can be explained by the influence of ethanol on the electric polarizability of bacteria in two ways — by decreasing the medium *relative* dielectric permittivity and increasing the membrane permeability (possibly by damaging the cell wall). The second factor causes gradual decreasing of the cytoplasm ionic strength because of appearance of an ionic flow through the membrane, which leads to corresponding change in the internal MW polarizability. Additionally, the ionic flow out of the bacteria practically does not influence the ionic strength of the outer medium, therefore the OChD polarizability does not depend on the ionic flow. So, the presence of ethanol can change only the IMW component.

The influences of the medium dielectric permittivity and the inner electrolyte concentration are distinguished by an investigation of the electric polarizability of bacteria previously treated by ethanol at high electrolyte concentration, but measured in distilled water in absence of ethanol. In this case the polarizability is higher and its kinetics is faster compared to that of the ethanol-untreated bacteria. The conclusion is that the ethanol effect of the ionic flow predominates over that one of the decrease in the medium dielectric permittivity. Consequently the ethanol influences the high frequency electric polarizability of bacteria *E. coli* mainly by changing the cytoplasm ionic strength, which gives a possibility for studying the contribution of the IMW component of the polarizability.

Acknowledgments

We thank Dr. V. Bunin and Dr. A. Angersbach for the possibility, which they gave us to make the experiments in the laboratory of Biotronix GmbH, Germany, using the automatic electro-optical devices EloTrace 1.0 and EloTrace 2.0 developed in the same company.

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