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Conductometric properties of human erythrocyte membranes: dependence on haematocrit and alkali metal ions of the suspending medium

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Abstract The electrical properties of the cytoplasmatic membrane of human erythrocyte cells have been evaluated by means of dielectric spectroscopy measurements in the radiowave frequency range, using the so-called "suspension method". Measurements have been carried out at different volume fractions of the corpuscular phase (the cell haematocrit) in order to investigate the influence of the cell-cell interactions on the electrical parameters (the membrane permittivity ε and the membrane conductivity σ) of the cell membrane and a set of new values are proposed. Moreover, the influence of different alkali metal ions (Na^+, K^+, Cs^+, Li^+) on the ion permeation properties of the membrane are investigated and the structural alterations in the membrane organized briefly discussed.

Key words Erythrocyte membrane · Conductometric properties · Ion permeation

Introduction

The characterization of the electrical properties of the membrane of human erythrocytes by means of dielectric impedance spectroscopy has a long history (Foster and Schwan 1986, Pethig and Kell 1987) which began almost immediately after precise measurements of electrical alternating currents had been introduced.

This technique, known as the "suspension method", is based on the analysis of the dielectric and conductivity dispersions (changes in both the permittivity ε and the conductivity σ in an appropriate frequency range) occurring in heterogeneous systems, in the presence of an external electric field. These dispersions originate from the frequency-dependent surface polarization at the interface between different media, characterized by different electrical properties.

A biological cell suspension is a typical heterogeneous system where the cytoplasmic membrane separates different electrolyte solutions, i.e., the extracellular medium and the cytosol, with different permittivities and electrical conductivities. The cytoplasmic membrane, displaying a very low conductivity, of the order of 10^{-5}–$10^{-4}\ \Omega^{-1}\,m^{-1}$, enhances the dielectric and conductometric dispersions, re-

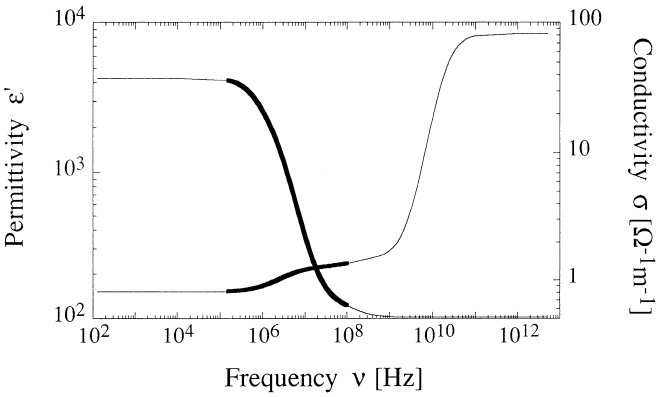

Fig. 1 The calculated dielectric and conductivity spectrum of a typical erythrocyte cell suspension, on the basis of a single-shell ellipsoidal inclusion suspension dielectric model [Eqs. (1) and (2)]. The *thicker line* marks the frequency interval investigated in the present work. The phase parameters of the extracellular medium, the cytoplasmic membrane and the cytosol are $\sigma_m = 1.65\ \Omega^{-1}\,m^{-1}$; $\varepsilon_m = 78.5$; $\sigma_s = 1\times10^{-6}\ \Omega^{-1}\,m^{-1}$; $\varepsilon_s = 5$; $\sigma_p = 0.8\ \Omega^{-1}\,m^{-1}$, $\varepsilon_p = 200$. The fractional volume of the dispersed phase is $\Phi = 0.30$ and the membrane thickness is assumed to be $\delta = 75$ Å. The erythrocyte cell is modelled as an oblate ellipsoid with semiaxes $a_0 = 4.1$ μm; $b_0 = 1.2$ μm

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sulting in a phenomenon known as the β-dispersion, due to the Maxwell-Wagner effect (Takashima 1989). Figure 1 shows the typical dispersion of the electrical conductivity σ and permittivity ε calculated on the basis of the usual dielectric suspension model for an erythrocyte cell suspension. In this figure, the dispersion due to the surface polarization occurs between 10^5 and 10^9 Hz, before that due to the orientational polarization of the aqueous phase. The frequency interval investigated in this work is marked by a thicker line.

Since the pioneering works of Fricke, Cook and Schwan (Fricke 1925; Cook 1952; Schwan 1957), dielectric spectroscopy has received increased interest in the field of interaction of living matter with electromagnetic fields and is now a well-established technique to probe, in a non-invasive way, the electrical structure of living cells and tissues. Various comprehensive reviews have appeared in the last few years (Davey and Kell 1994; Chiabrera et al. 1985; Davey and Kell 1989), dealing with both the static and dynamic properties of the membrane structure and with biomedical and biotechnological applications, including medical diagnostic techniques (Iskkander and Durney 1980).

Among systems of biological relevance, erythrocyte suspensions and whole blood have received particular at-

Table 1 The parameters of the dielectric dispersions of erythrocyte cell suspensions obtained from the fit of two contiguous Cole-Cole relaxation functions characterizing a low-frequency and a high-frequency dielectric dispersion, respectively. $\Delta\varepsilon$, ν and α are the dielectric increment, the relaxation frequency and the spread of the relaxation time, respectively. ε_∞ is the high-frequency permittivity and σ_0 the d.c. electrical conductivity

					Low-freq. dispersion			High-freq. dispersion				
	Φ	T [°C]	σ_m [Ω^{-1}m^{-1}]	ε_∞	$\Delta\varepsilon$	ν [kHz]	α	$\Delta\varepsilon$	ν [MHz]	α	σ_0 [Ω^{-1}m^{-1}]	References
Red blood cells	0.47	37	Low-conductivity medium	70	8270	28.5	0.19	1100	0.692	0.066		Davey and Kell (1989)
Human erythrocyte in 1 mM NaCl, 250 mM sorbitol	0.47	37		70	20900	14	0.16	2400	1.411	0.11		Davey and Kell (1990)
Erythrocyte	peaked cell	20 30						1325 1100	1.98 2.89	0.015 0.015	0.35 0.42	Bao et al. (1994)
Blood	0.50							1500	3.0	0		Schwan (1983)
Erythrocyte ghosts	0.40		0.43						2.5	0		Schwan (1983)
Erytrocyte in saline solution	0.34 0.08	25 25						2000 550	2.5 2.5	0.15 0.15	0.75 1.32	Beving et al. (1994)
Erythrocyte (from mouse) spherocytes		24		55				1950	5.30	0.02		Asami et al. (1989)
Erythrocyte		20						$147\cdot\Phi$	1.4	0.11		Batumbach et al. (1988)
Whole human blood	0.43	25		58				5000[a]	2.15		0.6	Cook (1952)
Erythrocyte in 0.15 M NaCl KCl CsCl LiCl	0.30	25							5.5 4.5 5.0 5.0		0.81 1.02 0.95 0.68	Bordi (1990)
Erythrocyte in 0.15 M NaCl	0.38	20							6.5		0.87	Diociaiuti et al. (1991)
Erythrocyte in 0.14 M NaCl	0.30	15 25 35									0.60 0.68 0.75	Ballario et al. (1984a)
Erythrocyte in 0.15 M NaCl	0.30	15 25 35							6.37 7.6 0.8		0.60 0.70 0.80	Ballario et al. (1984b)

[a] assumed

Table 2 Electrical parameters of the erythrocyte cell membrane derived from dielectric measurements

Erythrocytes	T [°C]	σ_s [Ω^{-1} m^{-1}]	ε_s	σ_p [Ω^{-1} m^{-1}]	ε_p	σ_m [Ω^{-1} m^{-1}]	ε_m	Cs [μF/cm^2]	Gs [Ω^{-1} m^{-2}]	References
In saline solutions	25			0.458		1.473		1.0		(Beving et al. 1994)
whole blood	25			0.458		1.22		2.98		
(from mouse blood)	24	$\leq 6 \cdot 10^{-5}$	5.7	0.62	59	0.91		0.72		(Asami et al. 1989)
spherocytes	20			0.61	160	1.134	112			(Batumbach et al. 1988)
	37			0.31		0.004		0.53	0.0625	(Donath et al. 1990)
In										
0.15 M NaCl				0.43 σ_m				0.327	0.91	(Bordi et al. 1990)
0.15 M KCl				0.43 σ_m				0.18	0.19	
0.15 M CsCl	25			0.36 σ_m				0.17	0.23	
0.15 M LiCl				0.72 σ_m				0.36	0.68	
In										
0.15 M NaCl	37	$3.5 \cdot 10^{-5}$	4.25	0.675		1.85		0.80		(Diociaiuti et al. 1991)
In	15	$4.9 \cdot 10^{-5}$	6.0	0.50		1.15				(Ballario et al. 1984a)
0.15 M NaCl	25	$6.1 \cdot 10^{-5}$	6.0	0.65		1.40				
	35	$6.9 \cdot 10^{-5}$	5.9	0.75		1.64				
In	15			0.47		1.16		1.10	1.4	(Ballario et al. 1984b)
0.15 M NaCl	25			0.64		1.38		1.10	2.2	
	35			0.82		1.65		1.08	2.6	

tention and their passive electrical properties have been the object of various comprehensive reviews (Schwan 1983; Davey and Kell 1989; Beving et al. 1994). Moreover, various investigations were concerned with the alterations in the structure and functionality of red blood cells induced by different drugs or by various physico-chemical agents (Diociaiuti et al. 1991; Ballario et al. 1984a, b).

A summary of recent data reported in literature on the dielectric dispersions occurring in erythrocyte suspensions is given in Table 1.

The passive electrical parameters ε and σ of the erythrocyte membrane deduced from dielectric spectroscopy measurements by means of different procedures and using various hypotheses are shown in Table 2.

Owing to the different experimental conditions employed, it is not yet possible to assign unambiguously values to the membrane capacitance (whose value is generally recognized to be of some 0.5–1.5 μF/cm^2), or equivalently to the membrane permittivity ε, and to the membrane conductance (whose value scatters over a wide interval from 0.1 to 10 Ω^{-1} cm^{-2}), or equivalently to the membrane conductivity σ. Attempts to compare these values and to identify the specific mechanisms responsible for the membrane polarization could provide additional information and could offer a deeper insight into the passive transport of ions across biological membranes.

The aim of the present work is twofold. First we suggest a procedure in the analysis of the conductivity dispersion of the whole cell suspension which allows the evaluation of the passive parameters largely independently of the fractional value Φ of the corpuscular phase, i.e., in the absence of cell-cell interactions. We stress that the most widely employed dielectric models hold in the limit of very low Φ, where a simple addition of the interfacial polarizability of the single cell contributes to the observed effect. On the other hand, at very low values of Φ, the dielectric and conductometric dispersions become negligibly small and the evaluation of the membrane parameters may be impossible and therefore the reliability of the method questionable.

Secondly, we want to investigate the effect of different extracellular alkali metal cations (Na^+, Li^+, Cs^+, K^+) on the ionic permeation properties of the cell membrane. To this end, we have varied the composition of the extracellular medium under conditions of constant ionic strength, in order to avoid any change of the cell shape due to osmotic stress, and have observed how different ionic permeation could influence the electrical behaviour of the whole membrane. The presence of the different ions investigated alters the ionic permeation of the erythrocyte membrane resulting, to a first approximation, in a change of the membrane pore distribution.

Theory

a) A review of the dielectric model of the erythrocyte cell

The dielectric model of an erythrocyte cell suspension (Asami et al. 1980a, b) underlying the "suspension method" considers the heterogeneous system as a collection of ellipsoidal particles uniformly dispersed in a continuous medium (the extracellular solution) characterized by a complex conductivity

$$\sigma_m^*(\omega) = \sigma_m + i\,\omega\,\varepsilon_0\,\varepsilon_m$$

The erythrocyte cells are modelled as oblate spheroids (the cytosol, of complex conductivity, $\sigma_p^*(\omega) = \sigma_p + i\,\omega\,\varepsilon_0\,\varepsilon_p$) of

semiaxes a_0, b_0, and $c_0 = b_0$, covered by another confocal ellipsoid of thickness δ (the cytoplasmic membrane of complex conductivity $\sigma_s^*(\omega) = \sigma_s + i\,\omega\,\varepsilon_0\,\varepsilon_s$).

In the above expressions, σ and ε are the electrical conductivity and the permittivity of the different media (external medium, cytosol and cell membrane, indices *m*, *p*, *s*, respectively), ω the angular frequency of the applied electrical field and ε_0 is the dielectric constant of free space. The conductivity σ and the permittivity ε are assumed to be independent of frequency, since our measurements are confined to frequencies (up to 100 MHz) well below those where the dipolar polarization of the aqueous media occurs.

The basic equations of the dielectric model will be briefly reviewed here (Asami et al. 1980a, b). The complex conductivity $\sigma^*(\omega)$ of a suspension of shelled oblate ellipsoids uniformly dispersed in a continuous medium of complex conductivity σ_m^* is given by

$$\frac{\sigma^* - \sigma_m^*}{\sigma^* + 2\sigma_m^*} = \frac{1}{9}\,\Phi\left\{\frac{\sigma_1^* - \sigma_m^*}{A_{01}\,\sigma_1^* + \sigma_m^*(1 - A_{01})} + \frac{2(\sigma_2^* - \sigma_m^*)}{A_{02}\,\sigma_2^* + \sigma_m^*(1 - A_{02})}\right\} \tag{1}$$

where σ_1^*, σ_2^* are the complex conductivities of the shell-covered ellipsoid as seen from the directions of its principal axes, Φ is the fractional volume of the dispersed phase and A_{01} ($i = 1, 2$) the depolarization factors defined as

$$A_{0i} = \frac{1}{2}\,a_0\,b_0^2 \int_0^\infty d\xi \quad \left[(\eta^2 + \xi)\sqrt{(a_0^2 + \xi)(b_0^2 + \xi)(c_0^2 + \xi)}\right]$$

with $\eta = a_0, b_0$, for $i = 1, 2$ respectively.

The effective complex conductivity σ_i^* ($i = 1, 2$) of the single shell-covered ellipsoid, along the directions of the semiaxes can be written as

$$\sigma_i^* = \sigma_s^*\,\frac{\sigma_s^* + (\sigma_p^* - \sigma_s^*)\,A_{1i} + \nu(\sigma_p^* - \sigma_s^*)(1 - A_{0i})}{\sigma_s^* + (\sigma_p^* - \sigma_s^*)\,A_{1i} - \nu(\sigma_p^* - \sigma_s^*)\,A_{0i}} \tag{2}$$

where σ_s^* and σ_p^* are the complex conductivities of the shell and the particle interior, respectively, A_{1i} are the depolarization factors defined as

$$A_{1i} = \frac{1}{2}\,a_1\,b_1^2 \int_\xi^\infty d\xi \quad (\eta^2 + \xi)\sqrt{(a_1^2 + \xi)(b_1^2 + \xi)}$$

with $\eta = a_1 \equiv \sqrt{a_0^2 - \delta}$, $b_1 \equiv \sqrt{b_0^2 - \delta}$, for $i = 1, 2$ respectively and the quantity v takes into account the volume occupied by the shell (the cytoplasmic membrane) of thickness δ

$$v = \frac{\sqrt{a_0^2 - \delta}\,(b_0^2 - \delta)}{a_0\,b_0^2}$$

The above equations can be conveniently simplified by assuming a uniform thickness δ of the shell, whose value (of the order of 50–100 Å) is negligible compared with the cell diameter (1–10 μm). This assumption yields, in the case of oblate spheroids ($a_0 < b_0 = c_0$)

$$A_{01} = A_{11} = \frac{1}{2}\,a_0\,b_0^2 \int_0^\infty d\xi/(b_0^2 + \xi)\,(a_0^2 + \xi)^{3/2} = \frac{1}{1 - (a_0/b_0)^2} - \frac{(a_0/b_0)}{(1 - (a_0/b_0)^2)^{3/2}}\arccos(a_0/b_0)$$

$$A_{02} = A_{12} = \frac{1}{2}(1 - A_{01}) = \frac{1}{2}(1 - A_{11})$$

The dielectric and conductometric behaviour of the complete heterogeneous system can be expressed by a combination of Eqs. (1) and (2) and, in the present case, the measured conductivity σ as a function of frequency is given by the real part of Eq. (1)

$$\sigma(\omega) = \mathrm{Re}[\sigma^*(\omega)]$$

Equations (1) and (2) contain six phase parameters (ε_s, σ_s, ε_p, σ_p, ε_m, σ_m), geometrical parameters taking into account the particle shape (in the present case, the two semiaxes a_0 and b_0 and the shell thickness δ) and finally a concentration parameter (the fractional volume Φ).

We have determined from a non-linear least-squares minimization the conductivity of the cytosol (σ_p) and the phase parameters of the cytoplasmic membrane (ε_s, σ_s), whereas the phase parameters of the extracellular medium (ε_m, σ_m) have been measured at the temperature of the experiment (see the experimental section). The thickness of the membrane is assumed to be $\delta = 75$ Å.

Moreover, since the morphological parameters of the cell influence the membrane parameters derived from the "suspension method", knowledge of the cell dimensions is required before the analysis of the conductivity spectra can be carried out meaningfully. In the next section we will analyze in detail the dimensions of the erythrocyte cell as reported in literature.

b) Morphology of the erythrocyte cell

The measurements of sizes of human erythrocytes (the diameter 2a and the thickness 2b of each cell) suspended in their own plasma or serum have been carried out by different authors (Houchin et al. 1958; Canham and Burton 1968; Coleman 1967; Ponder 1971; Evans and Cheng-Fung 1972; Price-Jones 1933) and a summary of some results is shown in Table 3.

When the cells are modelled as oblate spheroids, the surface *S* and volume *V* can be calculated according the expressions

$$S = 2\pi\,a_0^2 + \pi\,\frac{b_0^2}{\chi}\ln\frac{1 + \chi}{1 - \chi}$$

$$V = \frac{4}{3}\,\pi\,a_0^2\,b_0$$

where a_0 and b_0 are the major and minor semiaxes, respectively and $\chi = \sqrt{1 - b_0^2/a_0^2}$ is the eccentricity of the revolving ellipse.

Table 3 Dimensions of human erythrocyte cells

Average diameter 2a [μm]	Minimum thickness 2b [μm]	Average thickness 2b [μm]	Maximum thickness 2b [μm]	Mean corpuscular surface S [μm^2]	Mean corpuscular volume V [μm^3]	References
8.28 ± 0.10		1.71 ± 0.06		134 ± 2.8	82 ± 2.7 (106 ± 4.5)[a]	Houchin et al. (1958)
8.07 ± 0.55				138.1 ± 17.4	107.5 ± 16.8	Canham and Burton (1968)
7.3 ± 0.3						Coleman (1967)
8.5 ± 0.4		1.7 ± 0.2		163	87	Ponder (1971)
7.82 ± 0.62	0.81 ± 0.35	1.70	2.58 ± 0.27	135 ± 14	94 ± 14	Evans and Cheng-Fung (1972)
7.3 ± 0.1						Price-Jones (1933)

[a] Recalculated from the measured diameter and thickness on the basis of an oblate spheroid

Although the ellipsoidal model represents a remarkable improvement in comparison with the single-shell spherical model, it does not accurately model the erythrocyte shape; this can be better accounted for by a flat biconcave object.

To take into account this peculiar cell shape within the single-shell ellipsoidal model, we have established a certain ratio between the maximum and the minimum average thickness, as deduced from the various experimental values listed in Table 3, that could account for the measured mean corpuscular cell volume V, according to the expression (Ponder 1971)

$$V = \frac{4}{3}\pi a_0^2 (0.67 b_0) \quad (3)$$

Since the corpuscular volume of each cell has been directly measured (see the experimental section), Eq. (3) furnishes the "effective" value of the minor axis b_0, once the major axis a_0 is known. In the analysis carried out here, we have assumed a fixed value of $a_0 = 3.94 \pm 0.03$ μm, the weighted average value of those listed in Table 3, for all the samples investigated and a value of b_0 deduced from Eq. (3), the corpuscular volume V being measured (see Fig. 2 of the experimental section). This procedure allows one to take into account the measured variability of the cell volume of each sample investigated, reflected in the semi-axis size, in order to obtain a more accurate result.

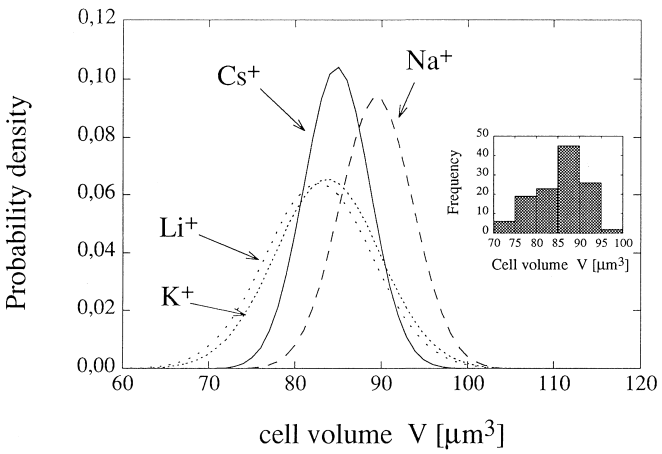

Fig. 2 The cell volume distribution of the erythrocytes in suspensions with electrolyte solutions containing the four different alkali metal ions investigated. The *inset* shows the cell volume distribution in all the cell suspensions studied

Experimental

Fresh blood was drawn by venipuncture from different normal, adult donors and generally only one sample per donor was used. Separation of erythrocytes was obtained by centrifugation at 3.000 rpm for 10 min. Plasma and buffy coat were removed and the red cells were washed three times in various isotonic phosphate-buffered saline solutions containing different monovalent cations, i.e., Na^+, K^+, Li^+, Cs^+.

The suspensions at an haematocrit (HCT) of about 0.5 were incubated at 37 °C for 1 h to equilibrate the cells in the appropriate salt solutions, to avoid transients in the cell shape and in the ion permeation processes. The cells were then resuspended in the different saline solutions NaCl, KCl, LiCl, CsCl, 0.15 M, and the concentration of the cell suspension was adjusted to various values in the range from $\Phi = 0.08$ to $\Phi = 0.75$.

Haematocrit and mean cell volume were measured by means of an electric cell counter (Royco-Cell 920A Coulter) before each conductivity measurement. Figure 2 shows the cell volume distribution for erythrocyte suspensions in different alkali metal ion saline solutions. The inset shows the overall volume distribution for all the samples investigated.

Microscopic inspection of the shape of red blood cells washed in different electrolyte solutions does not reveal any appreciable change in the cell morphology and in particular no transition from discocytes to spherocytes occurs. This control was carried out to see if possible changes in cell shape and dimensions could alter the data analysis and in order to facilitate a direct comparison of electrical parameters of the cell membrane in the presence of different alkali metal ions.

In order to verify the presence of any possible alteration in the intramembranous particles induced by different cations, we performed some scanning electron microscopy measurements. To this end, the cells, washed in the different buffer solutions, were seeded on glass coverslips, pretreated with 0.01 aqueous poly (L-lysine) hydrobromide and allowed to attach for few minutes. An aliquot of 0.1 M Na cacodylate + HCE buffer with 2% sucrose (pH = 7.2) was added to the attached cells. The erythrocytes were then gradually fixed under gently shaking at room temperature by adding 20% glutaraldehyde in the same buffer until a final concentration of 2% was reached within 1 h. No changes in the topography and spatial arrangement of the intramembranous particles were observed.

Electrical conductivity measurements were carried out by means of two Hewlett-Packard Impedance Analyzers (model 4191A in the frequency range from 10 kHz to 10 MHz and model 4192A in the frequency range from 0.5 MHz to 100 MHz) coupled to a computer. At each run, 43 frequency points over the range from 10 kHz to 100 MHz were collected. The conductivity cell, consisting of a short section of a cylindrical waveguide excited far beyond its cut-off frequency, has been calibrated with liquids of known permittivity and conductivity according to the procedure suggested by Bottomley (1978).

The accuracy of the experimental set-up was about 1–2% in the whole frequency range investigated. All measurements were carried out at a temperature of 37.0 °C within 0.1 °C. After each electrical conductivity measurement, the cell suspension was centrifuged, the solid component removed and the resulting supernatant was used in the measurements of the permittivity ε_m and the conductivity σ_m, entering into the dielectric model as the electrical parameters of the extracellular solution.

Results and discussion

Before proceeding further, we want to stress how the "suspension method" allows, in principle, the membrane conductivity σ_s to be properly evaluated. Since the cytoplasmic cell membrane separates aqueous media (the extracellular saline solution and the cytoplasm) with a generally high electrical conductivity (of the order of 0.5–2 $\Omega^{-1}\,m^{-1}$), different authors (Foster and Schwan 1986; Beving et al. 1994; Takashima et Asami 1993; Davey et al. 1993) have stressed, with different reasonings, that the "suspension method" does not allow the membrane conductance to be conveniently evaluated.

The dielectric model underlying the "suspension method" uses the effective medium approximation to describe the electrical properties of the whole heterogeneous system in terms of the electrical parameters (the permittivity ε and the conductivity σ) of the different media involved in the system. In the present case, we have ellipsoidal inclusions (ε_p, σ_p) covered by a thin layer (the cell membrane ε_s, σ_s) uniformly dispersed in an aqueous electrolyte solution (ε_m, σ_m) resulting in an effective medium approximation expression given by Eq. (1). For typical values of the phase parameters involved, Fig. 3 shows the dielectric and conductivity dispersion over a frequency range where β-dispersion occurs, for different values of the membrane conductivity σ_s. The presence of a low-conductivity membrane between high conductivity media yields a conductivity dispersion (or conversely a permittivity dispersion) which produces a well marked decrease of the low-frequency conductivity value, well below that due to the volume of the dispersed inclusions.

The conductivity increment $\Delta\sigma = (\sigma_\infty - \sigma_0)$, i.e., the difference between the high-frequency limit and the low-frequency limit of the conductivity of the whole system, depends on the membrane conductivity σ_s, beside geometrical parameters such as Φ and δ. We stress that the "suspension method" provides information not only on the membrane capacitance $C = \varepsilon_0\varepsilon_s/\delta$, and hence on the membrane permittivity ε_s, but also on the membrane conductance $G_s = \sigma_s/\delta$, and hence on the membrane conductivity σ_s.

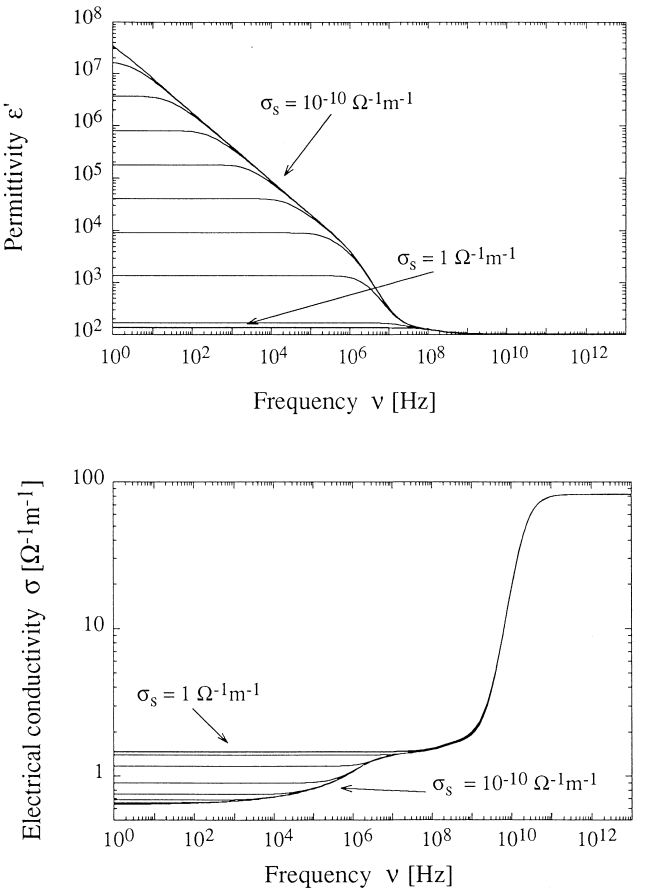

Fig. 3 The permittivity dispersion (*top*) and the conductivity dispersion (*bottom*) of a typical erythrocyte cell suspension, on the basis of a single-shell ellipsoidal inclusion suspension dielectric model [Eqs. (1) and (2)], for different values of the membrane conductivity σ_s from 1 to $10^{-10}\ \Omega^{-1}\,m^{-1}$. The phase parameters of the extracellular medium, cytoplasmic membrane, the cytosol and geometrical factors of the erythrocyte cell are quoted in the legend of Fig. 1

To make this statement more convincing, we show in Fig. 4 the conductivity increment $\Delta\sigma=(\sigma_\infty-\sigma_0)$ as a function of the membrane conductivity σ_s for a volume fraction of $\Phi=0.30$ and typical values of the other parameters involved. As can be seen, both in the limit of low membrane conductivity and high membrane conductivity, Eq. (1), i.e., the effective medium approximation theory, is largely insensitive to σ_s indicating that, in the former case, some kind of saturation occurs and in the latter case the effect itself vanishes. Nevertheless, in a wide range of values, from $\sigma_s=10^{-6}$ to $\sigma_s=10^{-3}\,\Omega^{-1}\,m^{-1}$, the measured conductivity, and hence the conductivity increment $\Delta\sigma=(\sigma_\infty-\sigma_0)$, is strongly influenced by the membrane conductivity σ_s. Unless these condition are fulfilled in the system under investigation, we think that the "suspension method" should furnish an appropriate evaluation of the membrane conductivity σ_s.

It must be noted, however, that within this method the conductivity σ_s describes the average behaviour of the whole membrane and it could differ from values influenced by local transport processes due to pores, channels or particular intramembranous protein arrangements, as probed by different experimental techniques.

Once the effectiveness of the "suspension method" in the evaluation of both the membrane permittivity and membrane conductivity has been stated, its accuracy and the influence of different parameters entering into Eq. (1) on the phase parameters of the cell membrane must be further investigated and discussed.

Here, we focus our attention on the fractional volume Φ of the dispersed phase, whose influence is twofold: at high values of Φ, Eq. (1) does not hold, since cell-cell interactions produce deviation from a simple additive hypothesis of the mean field approximation model and at low values of Φ the accuracy is progressively reduced, since the conductivity of the suspension tends to that of the extracellular solution. To overcome this difficulty, we have carried out, for each electrolyte solution investigated, an extensive set of measurements with the fractional volume Φ varying (from 0.08 to 0.70) and for each parameter obtained by the fitting procedure we adopted, we have determined its behaviour as a function of Φ in an interval wide enough that an appropriate empirical functional relationship can be established.

In this way it is possible to find the value of each electrical parameter we have considered, by extrapolating these empirical relationships in the limit of $\Phi\to 0$. This procedure should ensure that the resulting values are largely independent of the fractional volume Φ, i.e., of the cell-cell interaction and should represent the "true" value of the single cell membrane.

Typical electrical conductivity spectra of erythrocyte cells suspended in electrolyte solutions with different alkali metal ions, in the frequency range from 10 kHz to 100 MHz, are shown in Fig. 5. As can be seen, all spectra show the conductivity dispersion due to the interfacial polarization, generally occurring in heterogeneous systems.

The electrical parameters of the erythrocyte membrane, i.e., the membrane conductivity σ_s, the membrane permittivity ε_s and the conductivity of the cytosol σ_p, have been determined for each value of the fractional value Φ by fitting Eq. (1) to the observed conductivity dispersion data by means of a non-linear least-squares minimization procedure with a confidence level of 95%.

The electrical parameters we derived are shown in Figs. 6 to 10 as a function of the volume fraction Φ of the erythrocyte cells. As can be seen, these parameters display a marked dependence on Φ, suggesting that the cell-cell interactions, not accounted for in the dielectric model, influence the conductivity behaviour of the cell suspension.

This behaviour is further demonstrated in Fig. 11, where the uncertainties on the parameters σ_s, ε_s, σ_p as de-

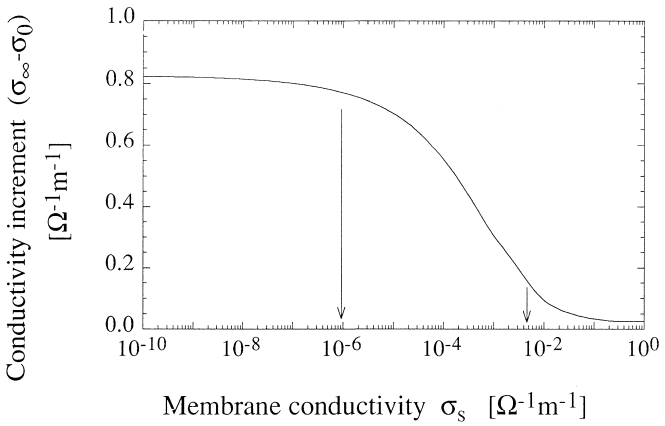

Fig. 4 The conductivity increment, i.e., the difference between the asymptotic values of the electrical conductivity at high-frequency and low-frequency respectively, as a function of the membrane conductivity σ_s. The curve is calculated on the basis of a single-shell ellipsoidal inclusion suspension dielectric model [Eqs. (1) and (2)]. The phase parameters of the extracellular medium, cytoplasmic membrane, the cytosol and geometrical factors of the erythrocyte cell are quoted in the legend of Fig. 1

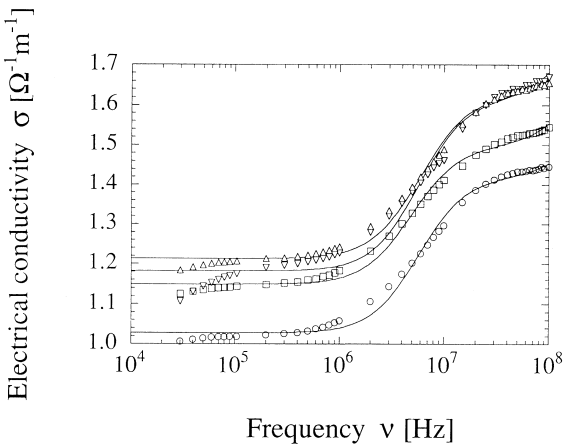

Fig. 5 Typical conductivity dispersions of erythrocyte cell suspensions as a function of frequency in the range from 10 kHz to 100 MHz, for different ions in the extracellular solution: (□): Na^+, (△): K^+; (○): Li^+; (▽); Cs^+. The temperature is (37.0 ± 0.1) °C. The *full lines* represent the calculated values according to Eqs. (1) and (2), with the cell parameters shown in Figs. (6) to (10) and derived from the fitting procedure with 95% confidence level

rived from the fitting procedure we have adopted show, for all the samples investigated, a marked dependence on the fractional volume Φ, both for high and low values of Φ. This behaviour is due to the one hand on the fact that the dielectric model (Eq. (1)) holds in the limits of low particle concentration and then its applicability vanishes at large Φ and, on the other hand, to the progressive decrease of the conductivity increment $\Delta\sigma=(\sigma_\infty-\sigma_0)$ at small Φ.

Figures 6 and 7 show the dependence of membrane conductivity σ_s on the fractional volume Φ for erythrocyte cells suspended in the two different alkali metal ions studied. The curves are non-linear regression fits of the phenomenological function

$$\sigma_s(\Phi) = \sigma_s(\Phi \to 0)\exp(K\Phi) \tag{4}$$

where $\sigma_s(\Phi \to 0)$ should represent the "true" value of the membrane conductivity σ_s, without any effect due to cell concentration (dilute suspension) and K assumes the meaning of an erythrocyte-erythrocyte interaction parameter.

Figures 8 and 9 show the analogous dependence of the membrane permittivity ε_s on the fractional volume Φ and the curves are calculated on the basis of a linear relationship

$$\varepsilon_s(\Phi) = \varepsilon_s(\Phi \to 0) + \eta\Phi \tag{5}$$

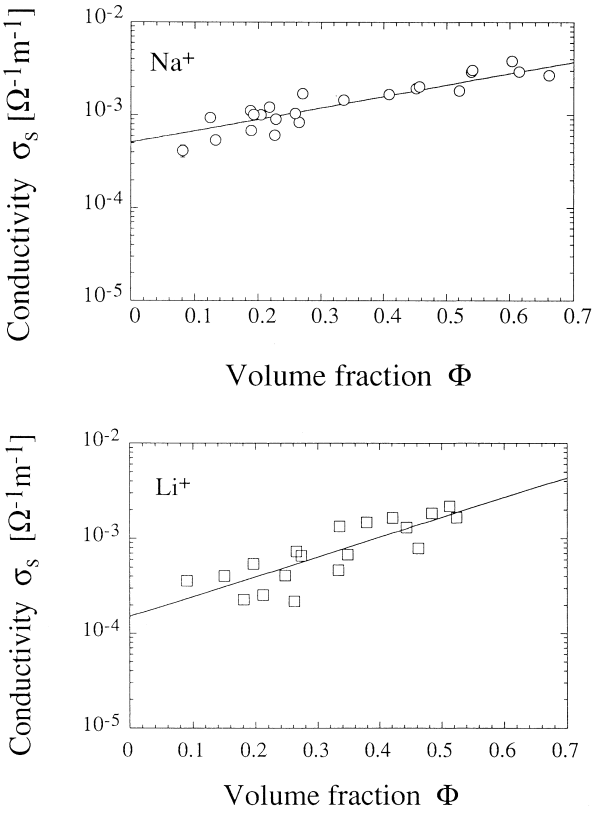

Fig. 6 The membrane conductivity σ_s as a function of the fractional volume of the corpuscular phase Φ for two different ions in the extracellular solution: (○): Na^+; (□): Li^+. The *full line* is a weighted fit according the functional relationship $\sigma_s(\Phi)=\sigma_s(\Phi\to 0)\exp(K\Phi)$

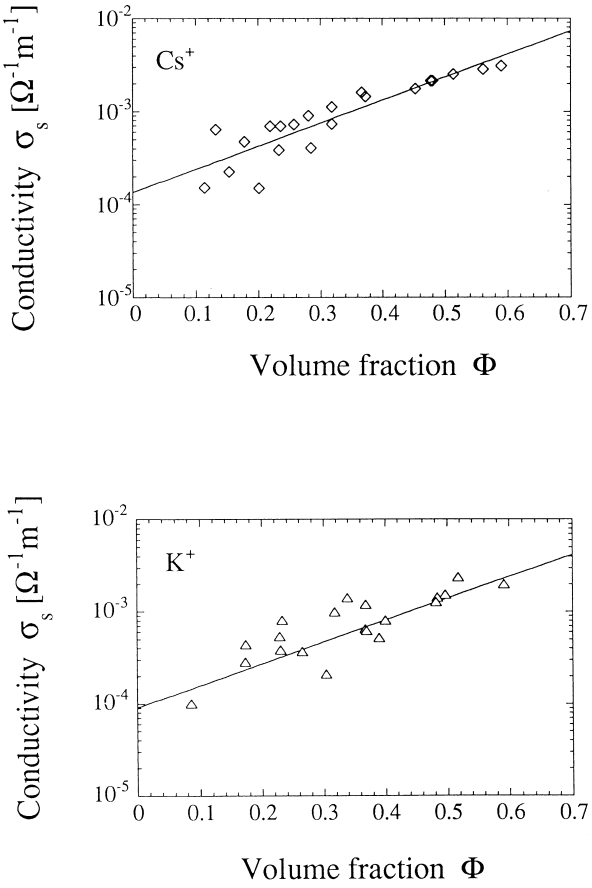

Fig. 7 The membrane conductivity σ_s as a function of the fractional volume of the corpuscular phase Φ for two different ions in the extracellular solution: (◇): Cs^+; (△): K^+. The *full line* is a weighted fit according the functional relationship $\sigma_s(\Phi)=\sigma_s(\Phi\to 0)\exp(K\Phi)$

where η again assumes the meaning of an interaction parameter.

Finally, Fig. 10 shows the cytosol conductivity σ_p for all the suspensions investigated as a function of Φ and in this case also the straight line

$$\sigma_p(\Phi) = \sigma_p(\Phi \to 0) + \zeta\Phi \tag{6}$$

with the ζ interaction parameter represents the data.

For simplicity, error bars in Figs. 6 to 10 were not plotted. For all points, the average error was about 10% of their absolute value. In each plot, the full line represents the calculated values on the basis of a weighted fit of Eqs. (4) to (6), respectively.

The values of the electrical parameters ε_s, σ_s, σ_p, extrapolated to $\Phi \to 0$ obtained by fitting Eqs. (4) to (6), are shown in Table 4. As stated above, owing to the particular procedure adopted, these values should represent the "true" passive electrical parameters of the human erythrocyte membrane. As can be seen, these values differ a little from those reported in Table 2 and moreover different ionic species (in the extracellular solution) produce significant effects both in the ion permeation across the membrane it-

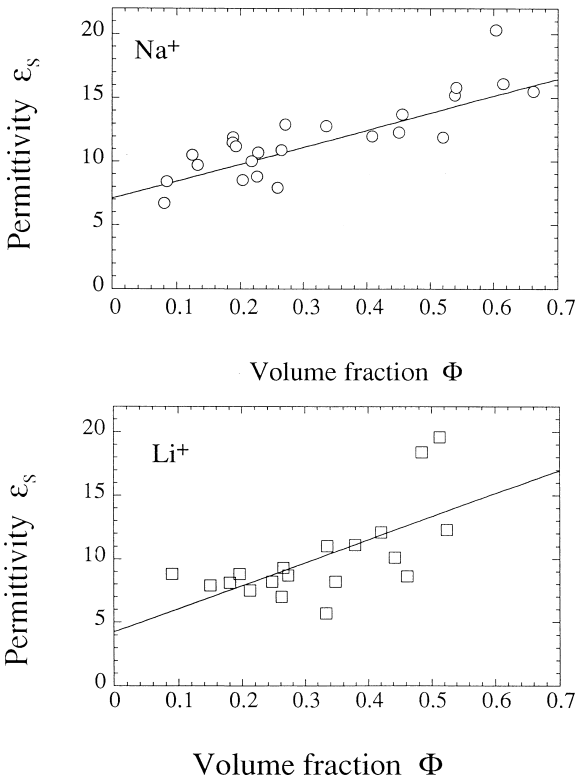

Fig. 8 The membrane conductivity ε_s as a function of the fractional volume of the corpuscular phase Φ for two different ions in the extracellular solution: (○): Na^+; (□): Li^+. The *full line* is a weighted fit according the functional relationship $\varepsilon_s(\Phi)=\varepsilon_s(\Phi\to 0)+\eta\Phi$

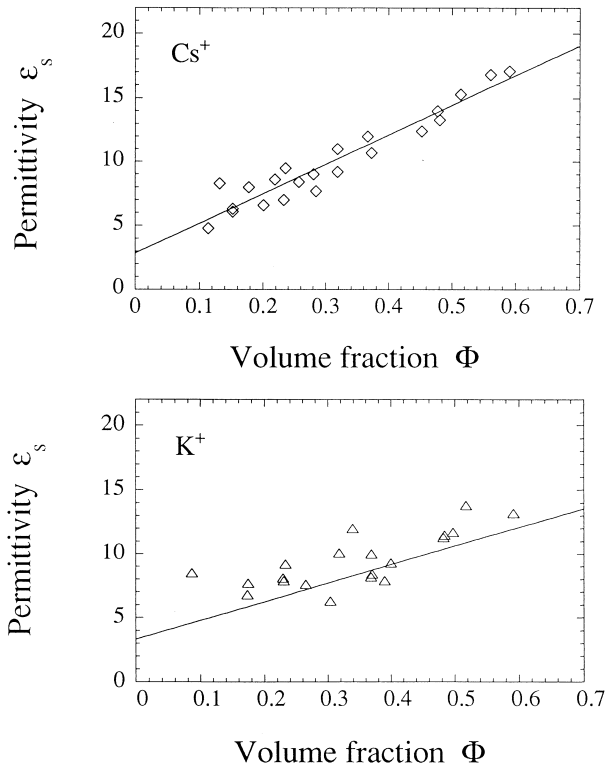

Fig. 9 The membrane conductivity ε_s as a function of the fractional volume of the corpuscular phase Φ for two different ions in the extracellular solution: (◇): Cs^+; (△): K^+. The *full line* is a weighted fit according the functional relationship $\varepsilon_s(\Phi)=\varepsilon_s(\Phi\to 0)+\eta\Phi$

self and in the arrangement of the polar groups in the phospholipid bilayer.

This set of data may be used to calculate the equivalent pore density in the erythrocyte membrane. The concept of a membrane perforated by uniformly distributed cylindrical pores can only be offered as a model by which the ion permeation may be described.

A common approach to obtaining a rough estimate of the pore density N_p is to assume that the conductivity of the hydrocarbon phase of the lipid bilayer is negligibly small compared to that of the pore phase bathed by the electrolyte solution. The equation relating the aqueous pore conductivity σ_{sp} to the membrane conductivity σ_s can be written as

$$\sigma_s = N_p \sigma_{sp} \pi (D/2)^2 \qquad (7)$$

Table 4 Passive electrical parameters of erythrocyte cells in different alkali metal salt solutions. The quoted values are those extrapolated to $\Phi\to 0$ by means of a weighted fit in the dependences shown in Figs. 6 to 10

Ions	σ_s [Ω^{-1} m^{-1}]	ε_s	σ_p [Ω^{-1} m^{-1}]	$N_p\times 10^{10}$ [pore/cm^2]
Na^+	$5.09\pm0.08\cdot10^{-4}$	7.1 ± 0.3	0.699 ± 0.003	3.69 ± 0.06
Li^+	$1.45\pm0.04\cdot10^{-4}$	4.3 ± 0.3	0.699 ± 0.003	0.43 ± 0.01
Cs^+	$1.46\pm0.03\cdot10^{-4}$	2.8 ± 0.5	0.699 ± 0.003	0.83 ± 0.02
K^+	$0.91\pm0.03\cdot10^{-4}$	3.3 ± 0.2	0.699 ± 0.003	0.47 ± 0.01

where D is the pore diameter. Assuming that the conductivity σ_{sp} is that of the electrolyte solution bathing the erythrocyte membrane, the pore density N_p can be evaluated from Eq. (7). As can be seen in Table 4, there is a marked decrease of N_p when the Na^+ ion is replaced with different ions, in the order $Na^+ > Cs^+ > K^+ \approx Li^+$. This

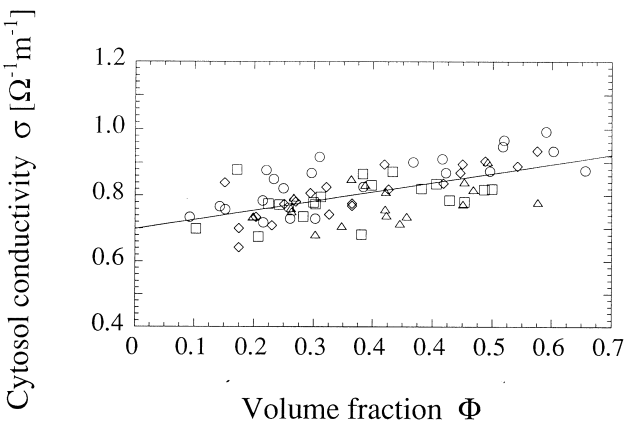

Fig. 10 The cytosol conductivity σ_p as a function of the fractional volume of the corpuscular phase Φ for four different ions in the extracellular solution: (○): Na^+; (□): Li^+; (◇): Cs^+; (△): K^+. The *full line* is a weighted fit according the functional relationship $\sigma_p(\Phi)=\sigma_p(\Phi\to 0)+\zeta\Phi$

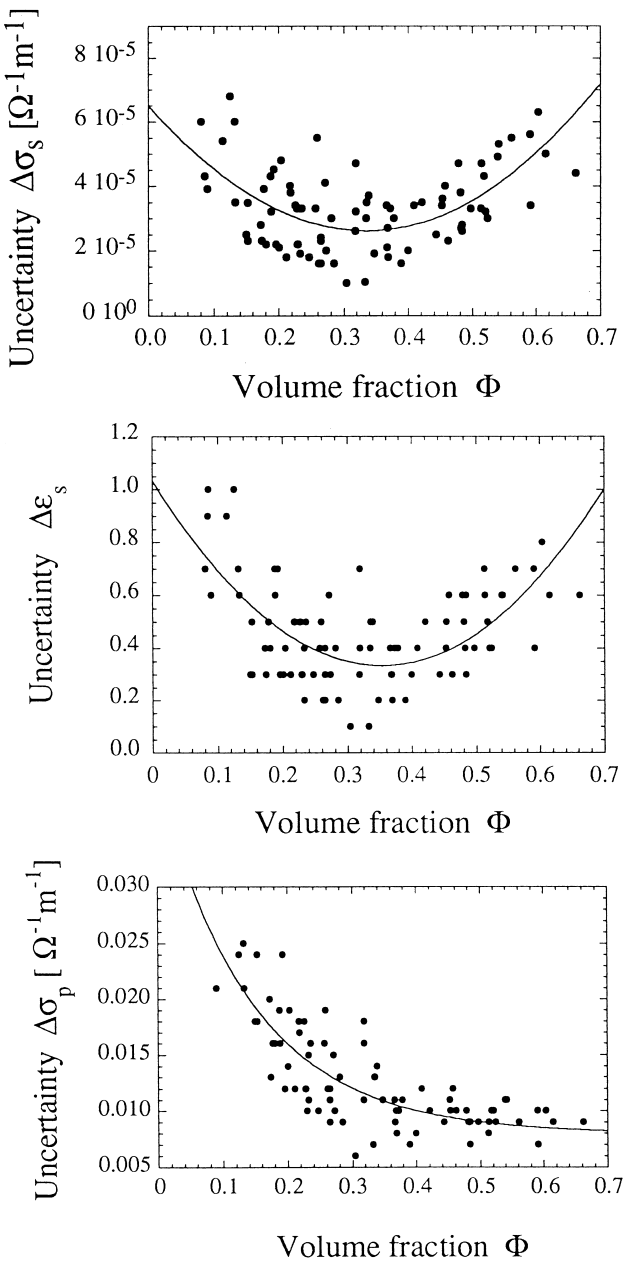

Fig. 11 The uncertainties $\Delta\sigma_s$, $\Delta\varepsilon_s$, $\Delta\sigma_p$ on the membrane conductivity, membrane permittivity and conductivity of the cytosol as a function of the fractional volume Φ of the corpuscular phase, as deduced from the fitting procedure adopted

order differs from that of the usual lyotropic series $Li^+ > Na^+ > K^+ > Cs^+$, according to the ion mobility and ion hydration. This means that some specific effects on the ion permeation should occur. Although the values of the pore density are subject to relatively large uncertainties, which may arise from the rough model employed, the difference induced by different alkali metal cations is still obvious.

It is well known that ions greatly influence a variety of biochemical processes at the cell membrane level, such as the conformation of proteins inserted in the lipid bilayer, the packing of the lipids in the membrane organization or finally the structure of the water in contact with the membrane itself. It is noteworthy (Cunningham et al. 1986) that the main transition in fully hydrated dipalmitoylphosphatidylcholine (DPPC) bilayers in the presence of different cations occurs at temperatures in the order $Na^+ > Cs^+ > K^+ > Li^+$, showing a reasonable correlation with the order observed in the ion permeation. This finding provides support for the idea that the influence of ions on phospholipid bilayers is mainly related to specific head group binding rather than to a modification of the water structure surrounding the head groups. On the other hand, the pore density is sufficiently small not to affect the basic structure of the cell membrane and the values we quoted completely justify the measured behaviour of the ionic permeation.

References

Alison JM, Sheppard RJ (1993) Dielectric properties of human blood at microwave frequencies. Phys Med Biol 38:971–978

Asami K, Hanai T, Koizumi N (1980a) Dielectric approach to suspensions of ellipsoidal particles covered with a shell in particular reference to biological cells. Jap J Appl Phys 19:359–365

Asami K, Hanai T, Koizumi N (1980b) Dielectric analysis of *Escherichia coli* in the light of the theory of interfacial polarization. Biophys J 31:215–225

Asami K, Takahashi Y, Takashima S (1994) Dielectric properties of mouse lynfocites and erythrocytes. Biochim Biophys Acta 1010:49–55

Ballario C, Bonincontro A, Cametti C, Rosi A, Sportelli L (1984a) Effect of extracellular alkali metal salts on the electric parameters of human erythrocytes in normal and pathological conditions. Z Naturforsch 39C:1163–1169

Ballario C, Bonincontro A, Cametti C, Rosi A, Sportelli L (1984b) Conductivity of normal and pathological human erythrocytes (homozygous beta – thalassemic) at radiowave frequencies. Z Naturforsch 39C:160–166

Bao JZ, Davis CC, Swicord ML (1994) Microwave dielectric measurements of erythrocyte suspensions. Biophys J 66:2173–2180

Batumbach S, Bogus P, Novak J, Wielgosz A, Rutkowski B, Terlecki J, Manitius A (1988) Dielectric properties of blood and erythrocytes of patients with chronic renal failure. Stud Biophys 124:51–59

Beving H, Eriksson LEG, Davey CL, Kell DB (1994) Dielectric properties of human blood and erythrocytes at radio frequencies (0.2–10 MHz); dependence on cell volume fraction and medium composition. Eur Biophys J 23:207–215

Bordi F, Cametti C, Di Biasio A (1990) Determination of cell membrane passive electrical properties using frequency domain dielectric spectroscopy technique. A new approach. Biochim Biophys Acta 1028:201–204

Bottomley PA (1978) A technique for the measurements of tissue impedance from 1 to 100 MHz using vector impedance meter. J Phys E Sci Instrum 11:413–414

Canham PB, Burton AC (1968) Distribution of size and shape in populations of normal human red cells. Circ Res 22:405–422

Chiabrera A, Nicolini C, Schwan HP (1985) Interactions between electromagnetic fields and cells. NATO ASI Series, Series A, Life Science, Vol 97

Coleman PN (1967) Measurements of red cell diameter by image shearing. J Clin Path 20:915–917

Cook HF (1952) A comparison of the dielectric behaviour of pure water and human blood at microwave frequencies. Br J Appl Phys 3:249–255

Cunningham BA, Shimotake JE, Tamura-Lis W, Mastran T, Kwok MW, Kaufmann JW, Lis LJ (1986) The influence of ion species

on phosphatidylcholine bilayer structure and packing. Chem Phys Lipids 39:135–143
Davey CL, Kell DB (1994) The low-frequency dielectric properties of biological cells. In: Berg H (ed) Treatise on Bioelectrochemistry, Vol VI. Birkhäuser, Zürich
Davey CL, Kell DB (1989) In: Paris R (ed) Electrical field phenomena in biological systems, IOP, Vol 21. Institute of Physics, London
Davey CL, Markx GH, Kell DB (1993) On the dielectric method of monitoring cellular viability. Pure Appl Chem 65:1921–1926
Davey CL, Kell DB (1990) In: O'Connor ME, Bentall RHC, Monahan JC (eds) Emerging electromagnetic medicine. Springer, Berlin Heidelberg New York
Diociaiuti M, Molinari A, Calcabrini A, Arancia G, Isacchi G, Bordi F, Cametti C (1991) Alteration of the passive electrical properties of adriamycin-treated red cell membrane deduced from dielectric spectroscopy. Bioelectrochem Bioenerg 26:177–192
Donath E, Egger M, Patsushenko VPh (1990) Dielectric behavior of the anion-exchange protein of human red blood cells. Bioelectrochem Bioenerg 23:337–360
Evans E, Cheng-Fung Y (1972) Improved measurements of erythrocyte geometry. Microvasc Res 4:335–347
Foster KR, Schwan HP (1986) Dielectric permittivity and electrical conductivity of biological materials. In: Polk C, Postow E (eds) CRC Handbook of biological effects of electromagnetic fields. CRC Press, Boca Raton
Fricke H (1925) The electrical capacity of suspensions with special reference to blood. J Gen Physiol 9:137–152
Houchin DH, Munn JI, Parnell BL (1958) A method for the measurement of red cell dimensions and calculation of mean corpuscular volume and surface area. Blood 13:1185–1191
Iskkander MF, Durney CH (1980) Electromagnetic techniques for medical diagnosis: a review. Proc IEEE 68:126–132
Pethig R, Kell DB (1987) The passive electrical properties of biological systems: their role in physiology, biophysics and biotechnology. Phys Med Biol 32:933–970
Ponder E (1971) Hemolysis and related phenomena, Chap II: shape changes unaccompanied by volume changes. Grune and Stratton, New York
Price-Jones C (1933) Red blood cell diameters. Oxford Medical Publications, London
Schwan HP (1957) Electrical properties of tissue and cell suspensions. Adv Biol Med Phys 5:147–209
Schwan HP (1983) Electrical properties of blood and its constituents: alternating current spectroscopy. Blut 46:185–197
Takashima S (1989) Electrical properties of biopolymers and membranes. Adam Hilger, Bristol
Takashima S, Asami K (1993) Frequency domain studies of impedance characteristics of biological cells using micropipet technique. Pure Appl Chem 65:1921–1926