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Dielectric properties of human blood and erythrocytes at radio frequencies (0.2–10 MHz); dependence on cell volume fraction and medium composition

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Abstract. The dielectric properties of human erythrocytes (red blood cells) suspended in whole blood and in isotonic media at various volume fractions (haematocrits) have been studied in the frequency range 0.2–10 MHz, in which the so-called β-dispersion due to the Maxwell-Wagner effect is known to occur. The capacitance and conductance at 25 °C were measured by an instrument interfaced to a computer. The rectangular sample cavity (1 ml volume) contained four pure gold electrode pins, and the sample could be circulated by a roller pump. The frequency-dependence of the permittivity and conductivity were fitted by non-linear least squares regression. Corrections were applied for non-linearity in the dielectric increment at high haematocrit, and for electrode polarisation when diluting the blood in saline. Data were interpreted in terms of a simple equivalent resistor-capacitor circuit. From the measured haematological values the specific membrane capacitance (C_m) and the conductivities internal and external to the cells (σ_i' and σ_o' respectively) were estimated. The conductivities behaved in a predictable manner with a mean of $0.458\ S \cdot m^{-1}$ (s.d. $\pm$ 0.044) for σ_i', whereas the value of C_m (and indeed the actual capacitance of the suspension) was dependent on the amount of plasma present. Hence, in stationary normal (anticoagulated) whole blood samples, C_m was as high as $2.98\ \mu F \cdot cm^{-2}$ (s.d. $\pm$ 0.40), in contrast to about $0.9\ \mu F \cdot cm^{-2}$ in blood diluted more than two-fold (to less than 20% hct) in isotonic media. The high value remained when the diluent was plasma. The C_m value returned to a high value when washed erythrocytes were reconstituted with plasma, provided that this was present at above a critical or threshold concentration of about 30 vol% in the medium, irrespective of the haematocrit in the range studied (15–44%). The C_m remained low in serum. When added to washed cells in saline, purified fibrinogen had no effect. However, high C_m values were obtained by fibrinogen supplementation to serum and diluted plasma. Applying moderate flow to whole blood approximately halved its high C_m value in an exponential manner with flow rate, whilst the C_m of washed cells (31–67% hct) slightly increased, and converged to the value for whole blood under flow. We interpret the high *apparent* C_m value in stationary samples to be a result of rapid cell aggregation in the presence of plasma, where rouleaux formation takes place before visible sedimentation sets in.

Key words: Dielectric spectroscopy – Erythrocyte – Blood – Plasma – Aggregation

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Introduction

The electrical properties of the human erythrocyte, red blood cell (RBC), and whole blood have been a challenge ever since the painstaking studies with alternating current in the frequency domain were pioneered at the beginning of this century (Höber 1910; McClendon 1926; Fricke 1925). Analyses of the fundamental electrical phenomena made it possible to derive the thickness of a biological membrane by a physical method (Fricke 1925). The frequency dependence of the dielectric properties of the RBC in suspension have been pursued by Schwan et al. (Schwan 1957, 1983; Pauly and Schwan 1966; Jenin and Schwan 1980) and others (Fricke 1953a; Gougerot and Foucher 1972; Hanai et al. 1979; Asami et al. 1980; Ballario et al. 1984; Takashima et al. 1988; Bordi et al. 1990; Davey et al. 1990; Zhao et al. 1993). Reviews including the passive electrical effect of blood cells and whole blood have appeared (Cole 1968; Schanne and Ceretti 1978; Schwan 1957, 1983; Trautman and Newbower 1983; Foster and Schwan 1986; Pethig and Kell 1987; Davey and Kell 1994).

The results from the studies on the frequency-dependent dielectric properties of cell suspensions are usually interpreted in terms of simple electrical components giving rise to a β-dispersion due to the Maxwell-Wagner effect (Takashima 1989). In the simplest model the RBC

suspension is treated as an equivalent circuit for the measured capacitance and conductance (Davey and Kell 1989; Kell and Davey 1990). The dielectric properties (admittance) depends on the volume fraction (haematocrit) and the shape of the RBCs (Fricke 1925, 1953 a, b; Hanai et al. 1979). The plasma membrane has a low conductance (Takashima et al. 1988) and its capacitance C_m was early estimated to be 0.8 $\mu F \cdot cm^{-2}$ (Fricke 1925). The membrane insulates the cytoplasm which contains a high concentration of hemoglobin (ca. 5 mM), giving rise to a reduced internal conductivity (Pauly and Schwan 1966; Jenin and Schwan 1980). In the case of whole blood, the outer medium is plasma with a conductivity about three times higher than for the cytoplasm.

When studying the RBC the plasma is usually washed away in order to avoid complications with for instance cell clotting. Although the RBC can retain its discocytic shape in an isotonic buffer this procedure may introduce artefacts. We noticed that some of the values for the dielectric parameters for the RBC in whole blood and in washed cells differed substantially. Time-dependent changes in the phase angle have been related to the sedimentation occurring in blood (Gougerot and Foucher 1972). In the present study we have sought to unravel how the plasma components as well as the cell concentration (haematocrit) influence the results. The plasma contains proteins which may directly or indirectly contribute to the dielectric properties of blood. Experiments with circulating whole blood have also been carried out. The results indicate that rapid aggregation (the formation of rouleaux) in the presence of plasma probably affects the results with stationary whole blood. Suspensions were studied in the frequency range 0.2–10 MHz using an instrument under computer control (Davey et al. 1992).

Materials and methods

Blood samples

Blood samples were withdrawn from an antecubital vein. Vacutainer test tubes no. 606608 (Becton and Dickinson, Cedex) with EDTA as anticoagulant were used. The blood was stored for a maximum of about 5 h at 4 °C. Blood samples referred to as "whole blood" in this paper were taken directly from the Vacutainer tubes without further treatment. Autologous plasma was obtained by centrifugation (at about 500 *g*). Serum was produced by clotting blood at room temperature for about 4 h, in the absence of anticoagulant. The serum was then transferred into an EDTA tube, in order to inhibit any remaining thrombin activity. Washed RBC samples were prepared as follows. The plasma and buffy coat were removed by gentle centrifugation and the RBCs were washed at least three time with four volumes of medium each time. This method removed plasma proteins, as well as most of the white blood cells and platelets. Three alternative media were used all of which were buffered at pH 7.4 with 10 mM HEPES and these isotonic solutions contained saline (9.0 $g \cdot l^{-1}$ NaCl; 154 mM), sucrose (98.3 $g \cdot l^{-1}$), or sorbitol (55.7 $g \cdot l^{-1}$). The samples were freshly prepared and used promptly. In some experiments whole blood was directly diluted with one of these media (see later). Human fibrinogen (Type I, from Sigma) was specified to contain 67% protein (about 92% clottable) with approximately 15% sodium citrate and 20% NaCl. Fibrinogen was dissolved in 0.1 ml distilled water and added to the RBCs, suspended in 2 ml of various media.

Haematocrit (hct; %) and mean cell volume (MCV; fl) were measured in an electronic blood cell counter (Contraves Autolyzer 801, Contraves AG, Zürich) before and after the experiment. Microscopic inspection of the shape of washed RBCs was carried out in a Bürker type chamber in the presence of a minimum amount of albumin (ca. 0.05% w/v) in order to avoid the "glass effect", or inducing stomatocytic cells. Observation of aggregation (rouleaux formation) was done at a relevant haematocrit, usually in a drop without a glass cover.

Dielectric measurements

All the suspensions were analysed using a Biomass Monitor (Model BM 214, Aber Instruments Ltd, Aberystwyth, U.K.). The instrument is a 4-terminal one operating at frequencies between 0.2 and 10 MHz (see e.g. Harris et al. 1987; Kell and Davey 1990; Davey et al. 1992, 1993). The instrument can measure capacitance *C* (Farad) and conductance *G* (Siemens) under full computer control using the "MINISCAN" software (Davey et al. 1992). After an initial delay (15 s) each suspension was measured at 15 logarithmically-spaced frequencies scanned serially, which was repeated (and averaged), usually four times. The duration of each scan was about 2 min, but could probably be reduced somewhat.

The rectangular (15 × 10 mm) measuring cavity was 8 mm deep with four electrodes made of solid 24-carat gold pins, placed symmetrically along the long axis. In the standard configuration the pins had an upright position. The electrodes were cleaned regularly by thoroughly rinsing and were then subjected to electrolytic cleaning pulses from the instrument. The cell was filled with 1.0 ml samples. Measurements were usually carried out at 25 °C but the temperature of the cell contents could be adjusted and stabilised to ±0.2 °C by a regulator in the range 25–45 °C.

Electrode polarisation controls were performed when necessary as described elsewhere (Davey et al. 1990, 1992). Briefly, the conductivity of the cell suspension of interest was noted at the lowest frequency used (0.2 MHz). A sample of cell-free suspending medium was then adjusted with either distilled water, or concentrated aqueous KCl, until its 0.2 MHz conductivity equalled that of the cell suspension. This blank sample constituted the polarisation control for the cell suspension. The control sample was scanned and its permittivity and conductivity were subtracted from the cell suspension scan in the software. This had the effect of massively reducing the electrode polarisations contribution to the cell suspension data. A side effect of this technique is to set the low-frequency conductivity and high-frequency permittivity of the suspension close to zero. To overcome this the conductivity and permittivity of the polarisation control sample at the 7th fre-

quency was added back to the differential cell suspension data. The 7th frequency was chosen because at that frequency the polarisation control data was neither significantly affected by electrode polarisation or cross-talk artefacts.

Experiments were also carried out with the measuring cell equipped with a cover made of Perspex™ and sealed to the top. Two holes in the cover were located in the diagonal corners of the rectangular space. Silicone tubing with 0.9 mm i.d. was fitted in the holes and joined to thicker Tygon™ tubing (1.6 mm i.d.). This allowed circulation of the RBC suspension by means of a peristaltic pump with adjustable speed (Minipulse 2, Gilson, Villiers-le-Bel, France). The electrode pins could either be in the vertical or horizontal position.

The experimental capacitance C and conductance G data were converted into their equivalent relative permittivity ε' (dimensionless), and conductivity σ' ($S \cdot m^{-1}$) at a given frequency (subscript ω) according to

$$\varepsilon'_\omega = C_\omega k/\varepsilon_0 \tag{1}$$

$$\sigma'_\omega = G_\omega k \tag{2}$$

where the constant ε_0 is the permittivity of free space ($8.854 \cdot 10^{-12}$ $F \cdot m^{-1}$). The cell constant k (m^{-1}) reflects the geometry of the electrodes and was calculated from (2) using the measured conductance at 1.0 MHz of 10 mM KCl (the σ' of which is known at a given temperature).

Computation of data

The data files stored were transferred to the program GraFit 2.0 (Erithacus Software Ltd, Staines, U.K.). Values of the dielectric increment ($\Delta\varepsilon'$), the conductivity increment ($\Delta\sigma'$), the critical frequency f_c (Hz) and Cole-Cole α-value (dimensionless) were fitted to the β-dispersion using the Marquardt non-linear least squares regression method (Davey et al. 1992). The measured frequency dependencies of the relative permittivity (ε'_ω) and conductivity (σ'_ω) of the cell suspensions during the β-dispersion were fitted to the Cole-Cole equations (see Cole and Cole 1941; Cole 1968; Kell and Harris 1985; Foster and Schwan 1986; Pethig and Kell 1987; Davey 1993; Davey and Kell 1994) according to:

$$\varepsilon'_\omega = ([\Delta\varepsilon' \{1+(f/f_c)^{1-\alpha}\sin(\alpha\pi/2)\}]/ [1+2(f/f_c)^{1-\alpha}\sin(\alpha\pi/2)+(f/f_c)^{2(1-\alpha)}]) + \varepsilon'_\infty \tag{3}$$

$$\sigma'_\omega = (\Delta\sigma' + \sigma'_L) + ([-\Delta\sigma' \{1+(f/f_c)^{1-\alpha}\sin(\alpha\pi/2)\}]/ [1+2(f/f_c)^{1-\alpha}\sin(\alpha\pi/2)+(f/f_c)^{2(1-\alpha)}]) \tag{4}$$

where f is the frequency (Hz) of the applied electric field. σ'_L and ε'_∞ are respectively the conductivity and permittivity at frequencies very low and very high relative to the f_c of the β-dispersion. Thus for a given cell suspension the fitting process gives best fit values for the $\Delta\varepsilon'$, ε'_∞, f_c and Cole-Cole α for the ε' spectrum and the $\Delta\sigma'$, σ'_L, f_c and Cole-Cole α for the equivalent σ' spectrum.

The volume fraction P (dimensionless) of cells in suspension was obtained from the measured haematocrit using $P = \text{hct}/100$. Although the RBC has a biconcave form it was treated, conveniently, as an equivalent sphere with a radius r (m) estimated from the mean cell volume determined in the cell counter according to the Coulter method. This approximation has been found to be useful, in the absence of an equation for a true discocytic RBC shape (Schwan 1983). However, as a precaution we inspected that the RBCs retained their shape in the isotonic media. The best fit values for the dielectric spectra combined with the haematological values allowed calculations of the RBCs' *specific* plasma membrane capacitance C_m ($F \cdot m^{-2}$) as well as the conductivities ($S \cdot m^{-1}$) of the suspending medium (σ'_0) and of the RBCs' cytoplasm (σ'_i). The following simplified equations were adopted, based on cases in which the membrane conductance (G_m) can be neglected (Schwan 1983; Foster and Schwan 1986; Davey et al. 1992; Davey 1993).

The plasma membrane capacitance per unit area of membrane (C_m) for the RBC was calculated by:

$$C_m = ([\Delta\varepsilon' \, 4\varepsilon_0]/[9r\,P]) \cdot f(P) \, . \tag{5}$$

Although C_m is calculated in (5) in $F \cdot m^{-2}$ it is usually quoted in the literature in units of $\mu F \cdot cm^{-2}$; this is the convention that will be used in the rest of this paper. The function $f(P)$ in (5) takes care of the non-linearity appearing between $\Delta\varepsilon'$ and P at higher volume fractions ($P > 0.2$). As a compensation factor $f(P) = (1+P/2)^2$ has been introduced by Schwan (Schwan 1957, 1983) and recently tested successfully (Davey et al. 1992). Dilution experiments on RBC washed in saline and then diluted in saline showed that this compensation factor modelled well the effect of dilution (i.e. P) on the $\Delta\varepsilon'$ of RBC suspensions (data not shown).

The conductivity of the medium in which the cells were suspended (σ'_0) was calculated from the best fit low-frequency conductivity σ'_L using Eqs. (6) and (7) (see Bruggeman 1935; Davey et al. 1992).

$$\sigma'_0 = \sigma'_L(1-1.5\,P)^{-1} \, , \tag{6}$$

$$\sigma'_0 = \sigma'_L(1-P)^{-3/2} \, . \tag{7}$$

Dilution experiments on RBCs washed in saline and diluted in saline showed that both (6) and (7) adequately modelled the properties of RCB suspensions (data not shown). Eq. (6) was chosen for the work described in this paper.

The conductivity of the RBC cytoplasm (σ'_i) was calculated by:

$$\sigma'_i = ([\tau/\{r\,C_m\}] - [1/\{2\sigma'_0\}])^{-1} \, . \tag{8}$$

In this equation C_m ($F \cdot m^{-2}$) was calculated from (5) and σ'_0 from (6). The relaxation time τ (sec) was calculated from the best fit f_c value of the ε' spectrum using $\tau = (2\pi f_c)^{-1}$ (see later for a discussion of this).

Results

Experiments with stationary samples

This study has been carried out in the frequency range 0.2–10 MHz, where the β-dispersion of the RBC suspen-

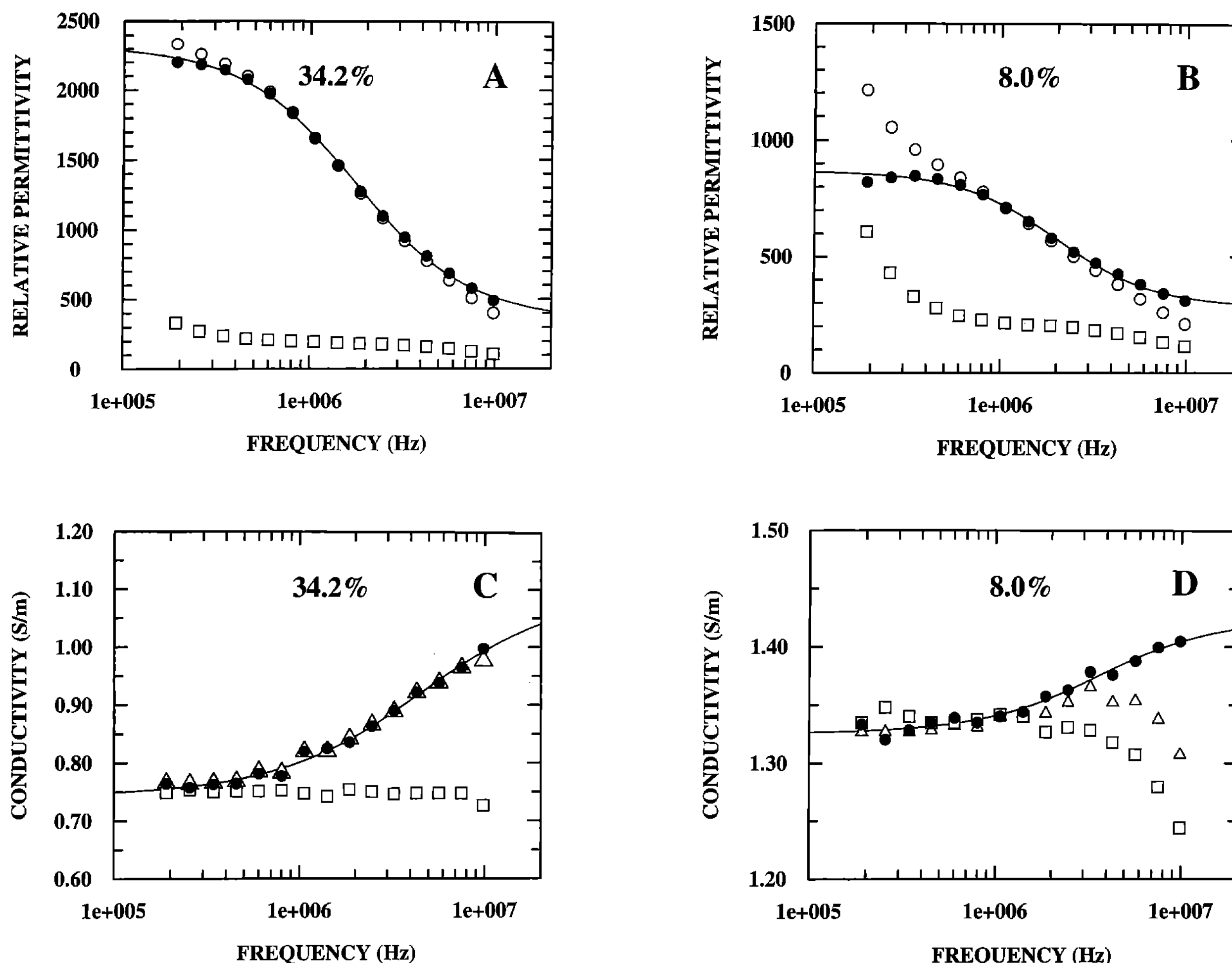

Fig. 1 A–D. The frequency dependence of the relative permittivity (ε') and conductivity (σ') of suspensions of washed RBCs suspended in saline/HEPES at two different haematocrit values. Each set of ε' and σ' data belong to a single scan (the 4th scan of 4) at each haematocrit value and were carried out at 25 °C. Both the original uncorrected values (○ for ε' and the △ symbol for σ') and the corresponding contributions from the polarisation controls done with cell-free medium (□) are demonstrated. The conductivities of the polarisation control samples were adjusted to exhibit the same values as the suspensions at low frequency (0.2 MHz). The corrected differential data (●) have been used to fit the Cole-Cole equations by non-linear regressions (*full lines*). *Permittivity* with 34.2% **A** and 8.0% haematocrit **B**. *Conductivity* with 34.2% **C** and 8.0% haematocrit **D**

sion occurs at around 2 MHz. The low-frequency α-dispersion will appear at frequencies below the present range (Schwan 1983; Davey et al. 1990) and no evidence of its presence was seen during these experiments. The instrument was optimised to perform measurements at the high cell concentrations occurring in whole blood. Working with samples at high haematocrit values will introduce non-linearity effects, whereas any electrode polarisation will be minimised. The observations in the low-frequency region of the spectrum were influenced by electrode polarisation when working with highly conducting samples. This was especially critical when using washed RBC in saline at reduced haematocrit. At low haematocrit ($< 20\%$) electrode polarisation (if uncorrected) would become very significant, giving rise to a substantial increase in the calculated parameter values. The effect was reduced when using isotonic sucrose (or sorbitol) as a medium, since the effect of polarisation is strongly dependent on the conductivity of the suspension, but only weakly on the nature of the ions contributing to it (Davey et al. 1990).

When required, corrections for the polarisation were carried out by performing separate measurements on the media alone. This is illustrated in Fig. 1, where the relative permittivity and conductivity data of a single scan presented for samples of (washed) RBCs in saline at two different haematocrit values. With low hct (8%) the contributions from the polarisation are severe, in particular at low frequencies, and have to be subtracted. At the higher hct (34.2%), as in whole blood, the polarisation has much less influence. After subtraction of the polarisation background the differential data allowed non-linear fits to the Cole-Cole equations.

We noticed that the electrical parameters derived were affected by the presence of plasma, even at a low volume fraction of RBCs. The effects on the dielectric properties at 25 °C of the RBCs by dilution with isotonic media or with plasma are shown in Fig. 2. We observed that the specific membrane capacitance value (C_m) derived for the RBC with stationary *whole* blood samples from normal (healthy) donors was as high as 2.98 μF · cm^{-2}. The stan-

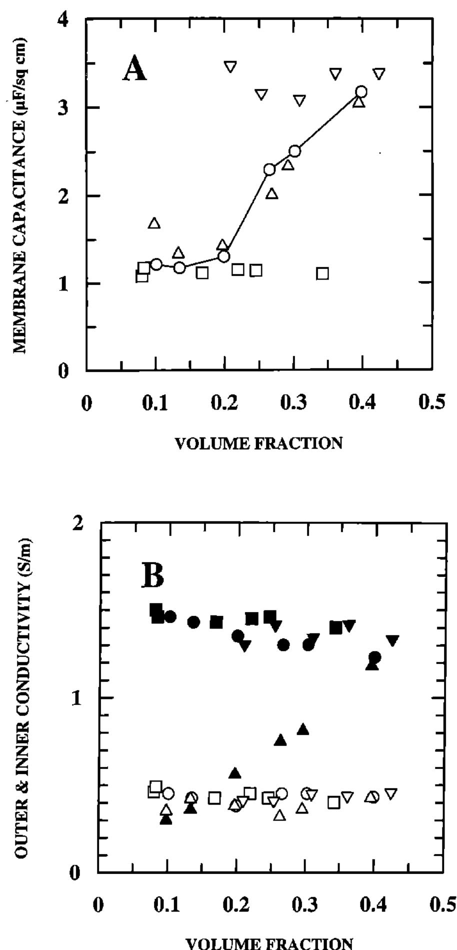

Fig. 2 A, B. The effect of volume fraction (P = hct/100) and suspension medium on the dielectric properties of the RBC as measured with stationary samples and with the electrodes in an upright position. The values have been corrected for electrode polarisation. Temperature about 25 °C. **A** Calculated membrane capacitance C_m ($\mu F \cdot cm^{-2}$): Whole blood diluted with saline/HEPES (○, connected points), sucrose/HEPES (△), or autologous plasma (▽). RBCs *washed* and diluted with saline (□). **B** Calculated conductivities ($S \cdot m^{-1}$) on the inside σ'_i (*open symbols*) and the outside σ'_0 (*filled symbols*): Whole blood diluted with saline (○, ●), sucrose (△, ▲), or plasma (▽, ▼). RBCs *washed* and diluted with saline (□, ■)

dard deviation was $0.40\ \mu F \cdot cm^{-2}$, and takes into account both any variation between blood samples ($n = 39$) and the precision of the method. Washed RBCs in isotonic buffered saline had C_m values about $1\ \mu F \cdot cm^{-2}$ in the haematocrit range covered. However, at a hct < 20% this value appeared substantially higher if the electrode polarisation correction was not applied. Just diluting whole blood by saline (1:1) was enough to reduce the estimated membrane capacitance dramatically. The same effect was also observed with buffered isotonic sucrose (or sorbitol). In contrast, the C_m values remained constantly high (about $3\ \mu F \cdot cm^{-2}$) when diluting the cells (down to 20% haematocrit) using autologous plasma. The conductivity at 25 °C of saline medium alone (σ'_0 for washed cells in saline) derived using Eqs. (6) and (7) was 1.473 (s.d. 0.018) and 1.455 (s.d. 0.014) $S \cdot m^{-1}$ respectively. The conductivity of plasma in whole blood was only 1.22 $S \cdot m^{-1}$ (s.d. 0.13). The internal conductivity (σ'_i) of RBCs in fresh whole blood was 0.458 $S \cdot m^{-1}$ (s.d. 0.044). This low internal conductivity was also found for washed RBCs as long as they were able to remain discocytic and maintain their cell volume. The outer conductivities and to some extent the inner ones as well, reflected the dilution of the RBCs (Fig. 2 B) with the media (when corrected for the polarisation). As one would expect diluting the whole blood with the sucrose medium continuously reduced σ'_0, in this case down to about 0.3 $S \cdot m^{-1}$. Extensive washing with buffered isotonic sorbital resulted in about 0.2 $S \cdot m^{-1}$ for σ'_i as well as σ'_0. The medium contained 10 mM HEPES neutralised to pH 7.4 which maintained the RBCs discocytic.

The critical frequencies (f_c) as well as the Cole-Cole α-values were to some extent sensitive to the volume fraction values even when the cells were diluted in the medium in which they were originally suspended (i.e. σ'_0 was roughly constant). The α-values from the ε' and σ' data were approximately the same (about 0.15) for whole blood and washed RBCs at the same haematocrit. The f_c values from the ε' data were lower than for the σ' data, as is usually observed with biological cells. Hence with whole blood the f_c was about 0.6 and 1.5 MHz, and with saline washed RBCs 1.7 and 3.0 MHz for the permittivity and conductivity fittings, respectively. The somewhat higher external conductivity (σ'_0) existing in samples of saline washed RBC is from (8) indeed expected to result in a shift of the f_c value to a higher frequency.

Suspending washed RBCs in plasma and serum

In order to determine the level of plasma which is critical to the capacitance values we replaced the saline medium with autologous plasma/saline mixtures. The RBCs were carefully washed in saline and concentrated. The concentrate was then suspended in the appropriate saline/plasma mix and the haematocrit measured and adjusted to keep the value as constant as possible between samples. The plasma concentration is given as volume% in the cell-free medium, or $100\ V_{plasma}/(1-P)\ V_{total}$, where $V_{total} = V_{plasma} + V_{saline}$. Suspending the washed RBCs in media with an increasing amount of plasma resulted in a rise in C_m (Fig. 3), eventually approaching the high values that had been observed with whole blood merely diluted in plasma (cf. Fig. 2 A). The capacitance remained constant below a critical concentration of plasma of about 30 volume%, irrespective of the RBC concentration in the haematocrit range 15–44%.

When the suspension of washed RBCs was carried out in serum, the capacitance and conductance values remained close to their values in the saline medium.

Effect of fibrinogen addition

Washed RBCs were suspended in saline, serum, as well as plasma diluted with saline. To the suspensions (27–39% hct), purified fibrinogen was added. No effect was measured due to the presence of about 10 $g \cdot l^{-1}$ fibrinogen in

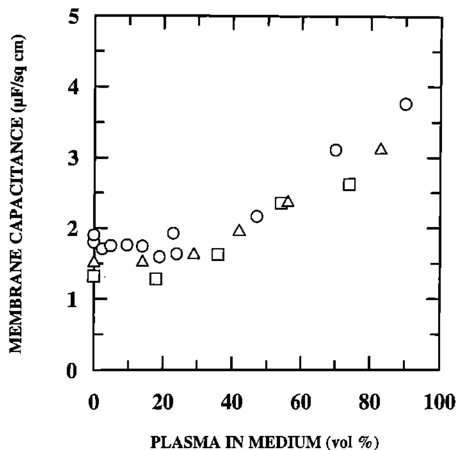

Fig. 3. Effects on the membrane capacitance (C_m) of reconstitution of saline-washed RBCs with plasma. The plasma was diluted with saline/HEPES and constant haematocrit values were maintained. The haematocrit values in the samples were approximately 15% (○), 28% (△) and 44% (□). The plasma level is given as volume% in the cell-free medium. The electrodes in the measurement cell were positioned vertically and no flow was applied. The values are uncorrected for electrode polarisation. Temperature about 25 °C

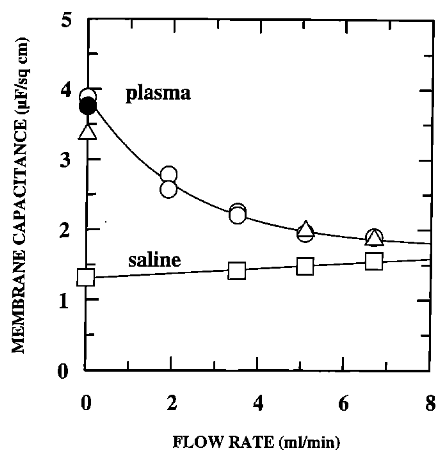

Fig. 4. The effect of flow on the membrane capacitance (C_m). Whole blood and washed RBCs in saline/HEPES were circulated by means of a peristaltic pump. Two *separate* samples with whole blood at haematocrits of 42% (○) and 43% (△) are shown. After stopping the flow the capacitance returned to the starting value (●). The full line is an exponential fit to the whole blood values (○). The haematocrits of the samples with *washed* RBCs in saline covered the range 31–67%, and the corresponding mean values (□) are indicated on their fitted line. No corrections for electrode polarisation were carried out. The electrode pins were positioned horizontally. Temperature about 25 °C

the saline. With fibrinogen added ($9\ g \cdot l^{-1}$) to the serum, a strong increase in C_m was observed. Supplementing the diluted plasma (30 vol%; cf. Fig. 3) with extra fibrinogen ($9\ g \cdot l^{-1}$) resulted in $C_m > 3\ \mu F/cm^2$, or a value closer to that with pure plasma (or whole blood). No effects of fibrinogen additions on the internal conductivities were observed.

Experiments with flow

Measurements were usually started immediately after filling the sample compartment. However, it was noticed that with stationary samples the ε' (and C_m) values from the first frequency scan, in particular, were usually significantly lower than those in the subsequent scans which were all very similar to each other. These trends were also the same in blood samples that had been compensated for electrode polarisation. After the initial ε' (and C_m) increase had occurred gently stirring the electrode contents (with a plastic rod) caused the values to return to their original lower ones before increasing once again. This stirring effect could be repeated over-and-over again on the same sample. The initial transient effect was most pronounced with whole blood. The effect also did not occur with suspensions of non-blood cells (such as yeast).

In order to find out if RBC sedimentation or aggregation might contribute to the high value C_m we applied flow, using a peristaltic pump. As the flow rate was increased, there was a decrease in the apparent membrane capacitance observed with whole blood (Fig. 4). The C_m value dropped exponentially from a large value with no flow, until it was about halved when applying the highest pumping rate, corresponding to a flow-through of a bout $7\ ml \cdot min^{-1}$. When stopping the pump the capacitance immediately returned to the higher value observed with no flow. The measured inner and outer conductivities were *not* sensitive to the flow. When flow was applied to washed RBCs the C_m values were less affected. However, RBCs in saline exhibited a small linear increase in their C_m value approaching the asymptotic value of whole blood (Fig. 4). The slope of the increase was insensitive to the haematocrit in the range studied, 31–67%. The peristaltic pump did cause a weak haemolysis, especially with washed blood at the highest pump speed; however, the haemolysis was not nearly enough to influence the measured haematological values.

The orientation of the electrode pins relative to the direction of the flow was also tested. No significant differences were observed in the data obtained with the pins in a vertical position with horizontal flow *versus* those with the pins pointing horizontally (either in row or column) with vertical flow.

Discussion

Although dielectric spectroscopy has been applied to the study of erythrocyte suspensions for many years, by exploiting the relatively rapid analyses possible with modern, automated instrumentation (Kell 1987; Kell and Davey 1990; Davey et al. 1992) we have observed a number of features which have not previously been explicitly reported. In particular, we have studied the time-, frequency- and medium-dependence of the electrical properties of erythrocytes in order to test the reliability of the method to the study of anticoagulated whole blood. Most

previous work seems to have been devoted to the study of *washed, diluted* RBCs; whilst this may be expected to eliminate some problems, it may instead introduce artefacts due to the effects of the diluents on the erythrocytes' properties themselves. Biological cells, including the RBC, are generally believed to have intrinsic (specific) plasma membrane capacitance values, C_m, close to $1\ \mu F \cdot cm^{-2}$ ($0.01\ F \cdot m^{-2}$) along with a low plasma membrane conductance (G_m). Using a micropipet technique with a single RBC the C_m was found to be weakly frequency-dependent (below 10 MHz), and a limiting value of the membrane conductance (G_m) was estimated to be $< 10\ \mu S \cdot cm^{-2}$ (Takashima et al. 1988). However, when applying the suspension technique to RBCs in isotonic solutions of various alkali chlorides a membrane conductance of the order of $1\ S \cdot cm^{-2}$ was reported (Ballario et al. 1984; Bordi et al. 1990). The reason for the discrepancy is not at all clear, although the analytical technique used in the latter case may have failed to take account of a surface conductance of the erythrocytes, such that the lower values may be much more reasonable. Nevertheless, the measurement of a small membrane conductance in a cell suspension with a conducting external medium is not possible by dielectric spectroscopy, due to the insensitivity of the permittivity increment to normal G_m values (Forster and Schwan 1986; Davey 1993).

The radio frequency range covered here allowed the study of the β-dispersion with a minimum of interference from the α-dispersion at lower frequencies. The nonlinearity between the dielectric increment and P at high volume fractions of RBCs observed earlier (Fricke 1953 b) was taken care of numerically. We have adopted the most simple equivalent circuit for the RBC suspensions, neglecting the membrane conductance. The specific capacitance (C_m) of a (single shell) membrane is frequency-independent and in series with the cytoplasmic conductivity (σ_i'), and both these elements are in parallel with the medium conductivity (σ_0'). In the present work we have tried to compare results with whole blood and isolated RBCs in various media and at different cell concentrations. The performance of the instrument allowed measurements on samples with relatively high haematocrit. However, at a hct below 15% the strong electrode polarisation in a highly conducting medium will hamper the method.

Only freshly prepared samples have been used and the haematological parameters were controlled during the time course of the experiments. For practical reasons, and in order not to stress the samples most of the measurements were carried out at 25 °C. However, some tentative temperature studies have been carried out in the range 25–45 °C. As expected, the two conductivities were temperature dependent with σ_i' being only about 40% of the plasma (outer) conductivity in whole blood at 25 °C. Suspending the RBCs in a low-conducting medium, in order to reduce the electrode polarisation effect, resulted in a further lowered value for σ_i'. The most striking parameter was found to be C_m, in particular with whole blood. The literature did not indicate that one should expect such a high C_m value as that of around $3\ \mu F \cdot cm^2$ observed at 25–37 °C for the RBCs in the presence of plasma. The shape and volume of the RBCs did not change as a function of whether they were suspended in plasma or in saline. Only by careful washing of the RBCs, and the use of polarisation controls, was it possible to obtain $C_m < 1\ \mu F \cdot cm^2$. The lowest value observed was 0.94 $\mu F \cdot cm^{-2}$ in isotonic saline. This value is in agreement with reported values (Takashima et al. 1988; Bao et al. 1992). Dilution of RBCs in autologous plasma down to 20% hct did not lower the C_m value. That the presence of sufficient plasma is responsible for the high C_m values was also demonstrated by the reconstitution of washed RBCs in plasma/saline mixtures at fixed haematocrit (Fig. 3), where it was found that there is a critical plasma concentration required to promote a high C_m in stationary samples.

Changes in the phase angle, in the admittance plane, as a function of the time and the composition of the medium in RBC suspensions have been studied and aggregated cells were discussed as a complication (Gougerot and Foucher 1972; Schanne and Ceretti 1978). The influence of albumin on the Cole-Cole plot has been briefly reported (König et al. 1986). Recently the impedance of stationary blood, as measured by a simple triplet-frequency (0.1, 0.8 and 1.2 MHz) method has been reported (Zhao et al. 1993; Zhao and Locker 1993). A cylindrical cell with four circular silver electrodes, positioned to reduce any effects due to sedimentation, was employed. The same equivalent circuit as used here was applied by Zhao et al. and in (5) they assumed $r = 2.6\ \mu m$ and performed no nonlinearity compensation. The value derived for the membrane capacitance (C_0 according to their notation) was about $1.3\ \mu F \cdot cm^{-2}$ with so-called normal blood, and around $2\ \mu F \cdot cm^{-2}$ from patients with a high measured erythrocyte sedimentation rate (ESR). The capacitance was reported to be independent of the temperature (25–40 °C).

We noticed that when filling the electrode compartment with whole blood there was a short lag period (< 1 min) before stable (and higher ε') values were obtained from the frequency scans. Hence, the first scan usually had to be rejected. With washed RBCs the lag was less obvious, and when applying sufficient flow to the blood this lag period was absent. The apparent C_m value of whole blood decreased with the flow rate in an exponential way (Fig. 4), whilst in contrast the C_m of washed RBCs, over a relatively wide range of haematocrits, very slightly increased with flow rate. It seems as if the membrane capacitance values of RBCs in whole blood and in washed cell suspensions, both under flow, converge. The reason why the asymptotic value remained $> 1\ \mu F \cdot cm^{-2}$ with flow is unclear. Electrode polarisation should not be the cause at high hct. Nevertheless, with a laminar flow, alignment (and deformation) of the RBCs can take place. This will influence the dielectric parameters and the form factor becomes anisotropic and dependent on shape of the particles (Velick and Gorin 1940). At present we have no *direct* indication of such an orientational effect on the RBCs in the (prismatic) measuring compartment used. The conductance of moving RBC suspensions has been investigated using two-electrode techniques (Velick and Gorin 1940; Frewer 1972). A different sensitivity to flow was experienced with the electrodes disposed longitudinally compared to when they were laterally disposed. In

our study, the orientation of the electrodes with respect to the flow was found not to be critical. However, our electrode configuration might be more responsive to stationary blood, where the RBCs will aggregate due to the rouleaux formation (Rampling 1988). In the rouleaux many RBCs are in close contact in a formation resembling a pile of coins, where the repulsive force due to the negative charges is balanced. Artificial sphering of the RBCs (at constant volume) prevents aggregation. The tendency of the RBCs to aggregate should be diminished when applying enough flow to the blood. The aggregation forces are rather weak in normal human blood and the cells should be dispersed by low shear forces (e.g. by mixing or pumping the electrode contents). The fact that stationary blood on addition to the electrode compartment produced stable $C_m(\varepsilon')$ values, after the initial transient increase itself suggests aggregation, rather than a continuous settling process. It would be interesting to characterize the recovery time for the measured capacitance of whole blood, after the interruption of the flow.

The existence of a *threshold* requirement for an adequate concentration of plasma, before the apparent membrane capacitance is raised, is intriguing. It is reminescent of the percolation phenomena widely observed in the studies of the electrical properties of a variety of mixtures (e.g. Clerc et al. 1990), as well as in a number of dielectric studies of biological systems (e.g. Rupley et al. 1988; Careri and Consolini 1991). Our reconstitution experiment (Fig. 3) demonstrates that the plasma content in the medium must be above about 30 volume% before the apparent C_m value becomes influenced by the rouleaux formation, and it is known that a critical concentration of fibrinogen in the suspending medium is required before it is able to produce intercellular adhesive forces, sufficient to effect the rouleaux formation (Rowlands 1988; Rampling 1988). Undiluted normal plasma contains about 3 g $\cdot$ l^{-1} fibrinogen. Fibrinogen is a long phosphorylated protein, mainly responsible for this adherence phenomenon, but globulins and other factors may also participate. Albumin is considered to be less active. This is in agreement with our results, where fibrinogen was found to be an essential, but not sufficient component for the existence of a high C_m value. The capacitance values reported by Zhao et al. for anticoagulated normal blood are closer to our low C_m value derived for washed RBCs, where no aggregation exists. They did not mention the rouleaux phenomenon, and instead tried to explain their observations by a double layer polarisation effect of fibrinogen (counter-ion) molecules (Zhao and Lockner 1993). However, such a phenomenon should mainly contribute to the α-dispersion, but at low frequencies (Takashima 1989). Nevertheless, the sedimentation rate may be indirectly related to the rouleaux tendency. When present, the rouleaux formation is rapid and precedes a discernible gravitational sedimentation.

The human erythrocyte is a biconcave discoid of about 8 μm diameter and 2.4 μm maximal thickness. However, our estimated capacitance values are based on an equivalent spherical RBC (with an isotropic form factor of 2) with a volume, exposed to the electric field, corresponding to the *measured* mean haematological values. With a mean cell volume of e.g. 90 fl this should correspond to $r = 2.8$ μm in (5). The results based on this approach seem to be reliable, as demonstrated by a C_m value of about 0.9 μF $\cdot$ cm^{-2} with *washed discocytic* RBCs. RBCs have also been treated as ellipsoids in dielectric studies (Velick and Gorin 1940; Fricke 1953a; Cole 1968; Asami et al. 1980; Ballario et al. 1984; Takashima 1989; Bordi et al. 1990). The choise of geometry (and form factor) should not be very critical as long as the RBCs are suspended at random, and not oriented with respect to the electrical field (Velick and Gorin 1940). Nevertheless, how the RBCs in the aggregated (rouleaux) state respond to the external electrical field is not trivial. Thus a more complex equivalent circuit than that considered here will be required in a Maxwell-Wagner type theory to account for the complicated dielectric behaviour of RBCs and their interfacial polarisation in loose aggregates.

Conclusion

From the biological point of view, one might consider the erythrocyte with no organelles to be a fairly primitive cell, but its physical behaviour in blood is complex; we have shown that this can strongly influence the results from dielectric spectroscopy. Whereas the derived membrane capacitances exhibit large variations, this is not the case for the cytoplasmic conductivity of intact discocytic cells. Many factors might contribute to the high capacitance value observed for the erythrocyte in normal blood. The value calculated should be considered as an apparent one, reflecting the dielectric response of red blood cells interacting weakly *via* rouleaux formation, where fibrinogen is an essential but not sufficient component in the plasma. Moderate dilution of blood with an isotonic medium is the easiest way to weaken the rouleaux formation. Whilst electrode polarisation will become a complication when using blood diluted with saline, blood samples at normal haematocrit and plasma concentration can be studied under flow without this problem. However, the flow by itself was not able to reduce the measured value of membrane capacitance down to that observed with stationary washed RBCs, which might indicate a remaining orientational effect of the flow on the cells.

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