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## To what extent is the magnitude of the Cole–Cole $\alpha$ of the $\beta$ -dielectric dispersion of cell suspensions explicable in terms of the cell size distribution?

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### Abstract

The Cole–Cole  $\alpha$  is a number that is often used to describe the divergence of a measured dielectric dispersion from the ideal dispersion exhibited by a Debye type of dielectric relaxation, and is widely assumed to be related to a distribution of the relaxation times in the system involved. The magnitude and relaxation time of the  $\beta$ -dielectric dispersion due to the charging of the plasma membrane capacitance of cell suspensions depend, inter alia, on the cell radius. An investigation was carried out to determine whether there might therefore be a relationship between the Cole–Cole  $\alpha$  of the  $\beta$ -dispersion of yeast cell suspensions and the distribution of cell sizes. Changes in the Cole–Cole  $\alpha$  during the batch culture of baker's yeast were recorded, showing an increase in the Cole–Cole  $\alpha$  during the exponential phase (more than 0.3) relative to those of the lag phase (about 0.28) and the stationary phase (about 0.2). Although the cell size distribution, measured by flow cytometry, also showed an increase in breadth during the exponential phase, this was not strictly related to the changes in the Cole–Cole  $\alpha$  observed. Further, the Cole–Cole  $\alpha$  calculated from the measured cell size distribution was significantly smaller than that obtained experimentally. Simulations in which the internal conductivity or membrane capacitance per unit area of individual cells were allowed to vary substantially did not account for the "excessive" Cole–Cole  $\alpha$ . Thus the magnitude of the Cole–Cole  $\alpha$  of the  $\beta$ -dispersion of yeast cells cannot be ascribed simply to the charging of a static membrane capacitance in cells of differing sizes and/or internal conductivities.

### INTRODUCTION

Dielectric spectroscopy [1–11] has already proven to be a useful tool for the estimation of the biomass levels of many different organisms [12–21]. The simplest

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version of the method, based on an assessment of the magnitude of the  $\beta$ -dielectric dispersion exhibited by all intact cells, gives a signal that is linear with the volume fraction of biomass that is present in suspension, up to very high biomass levels.

In the range of radio frequencies, increases in the capacitance (and decreases in the conductance) occur with decreasing frequency of the exciting field in biological materials in which intact cells are present, this frequency-dependence constituting the  $\beta$ -dielectric dispersion. As charges are unable at low frequencies to cross the membrane insulating the conducting cytoplasm from the conducting medium, a large macroscopic capacitance is observed, due to the molecularly thin nature (and thus large specific capacitance) of the bilayer plasma membrane surrounding the cells.

The capacitance and conductance measured are related to the intrinsic properties permittivity and conductivity according to the relation:

$$\epsilon\epsilon_0 = C(d/A) \quad (1)$$

$$\sigma = G(d/A) \quad (2)$$

where (with their SI units in parentheses)

$\epsilon$  = permittivity

$\epsilon_0$  = permittivity of free space ( $8.854 \times 10^{-12}$  F m<sup>-1</sup>)

$C$  = capacitance (F)

$\sigma$  = conductivity (S m<sup>-1</sup>)

$G$  = conductance (S)

$d/A$  = cell constant (m<sup>-1</sup>), for plane parallel electrodes, each of area  $A$  separated by a distance  $d$

The size of the drop in the permittivity (or "dielectric increment") has been described [22] for spherical cells using the formula:

$$\epsilon_1 = \epsilon_h + 9PrC_m/4\epsilon_0 \quad (3)$$

where (with their SI units in parentheses)

$\epsilon_1$  = low frequency permittivity

$\epsilon_h$  = high frequency permittivity

$P$  = volume fraction of biomass

$r$  = equivalent radius of cell (m)

$C_m$  = membrane capacitance per unit area (F m<sup>-2</sup>)

The dielectric increment of a cell suspension from high to low frequencies is therefore dependent on the volume fraction of biomass, the cell size and the membrane capacitance per unit area. The conductivity of a suspension also has an effect on the permittivity measured at a particular frequency [12], but the effect can be minimised by the choice of the right frequencies (on the plateaus of the dispersion).

The frequency at which the dispersion is half-completed (i.e. at which  $\epsilon = \epsilon_h + \Delta\epsilon/2$ ) is known as the characteristic frequency  $f_c$  [22] and is related to the relaxation time  $\tau$  by  $\tau = (2\pi f_c)^{-1}$ . For the classical type of dielectric relaxation underlying the  $\beta$ -dispersion,

$$\tau = rC_m \left[ (1/\sigma_i) + (1/2\sigma_o) \right] \quad (4)$$

where  $\sigma_i$  and  $\sigma_o$  are respectively the conductivities internal and external to the cells.

The problems of obtaining an accurate measurement of the magnitude of the  $\beta$ -dielectric dispersion in cell suspensions are of two main types: (i) interference by the phenomena collectively referred to as “electrode polarisation” (see refs. 1, 6, 18 and 23) and (ii) divining the best fit to the data obtained in the presence of contaminating signals caused both by electrode polarisation and the overlap of different relaxations not related to the  $\beta$ -dispersion. Four-terminal methods [6,24] can in principle overcome the first type of problem, and we have devised and constructed a four-terminal dielectric spectrometer capable of making accurate measurements in the frequency range 0.1 to 10 MHz [12,15,18]. More recently [25] we have described our solution to the second type of problem, based on the use of substitution and spreadsheet methods for fitting the dielectric spectra of “lossy” biological systems. These methods now permit the registration (and fitting) of very accurate measurements, and have allowed us, for instance, to determine that the temperature-dependence of the magnitude of the  $\beta$ -dispersion possesses, surprisingly and importantly, a positive coefficient [26].

Linear dielectric spectroscopy is subject to the principle of superposition, i.e. the principle that for all cells taking part in a relaxation, each with its own dielectric increment and relaxation time, the overall dielectric spectrum may be obtained by summing the contributions of each cell [22].

In a typical cell suspension, it is improbable that all cells are identical, and differences in their radii and internal conductivities, for instance, will cause a distribution of relaxation times. In biological work, it is usual to describe such effects by means of a modification to the Debye [27] equation, introduced by the Cole brothers [28] in which an additional parameter, the Cole–Cole  $\alpha$ , is used to characterise the fact that the dielectric relaxations typically observed are significantly broader than that of a single Debye-like relaxation. Whilst the Cole–Cole fitting procedure is empirical, it has the merit, since many *types* of distribution of relaxation times give dielectric behaviour indistinguishable from “true” Cole–Cole behaviour [22], of providing a single extra parameter with which accurately to describe a dielectric dispersion [25].

It is not simple to measure the internal conductivities of individual cells, and at all events physiological considerations dictate that it is hardly likely to vary by more than a factor of 2 within a given population in a given medium. However, flow cytometric techniques (see e.g. refs. 29 and 30) permit the rapid acquisition and display of data on *cell size distribution*, judged by the extent of low-angle light scattering. Such data are of interest in biotechnology since they might be used on-line to determine morphological changes associated with the transition to the productive phase of a fermentation (e.g. refs. 31 and 32). As part of a continuing study of the  $\beta$ -dispersion and its application in analytical biotechnology, we therefore wished to establish the extent to which the distribution of cell sizes measured flow cytometrically could account for the magnitudes of the Cole–Cole  $\alpha$  observed during the course of a fermentation. The present article reports the results of this study.

## EXPERIMENTAL

*Yeast*

The yeast used was baker's yeast (*Saccharomyces cerevisiae*) obtained locally. The yeast was grown on the following medium (all w/v): glucose 5%, yeast extract (Lab M, low-salt type) 0.5%, bacterial peptone (Lab M) 0.5%. The pH was set at 4.5 with phosphoric acid before autoclaving the medium.

*Fermentation system*

The fermentor used was a bubble column of a height of 32 cm, a diameter of 5 cm and a working volume of 500 ml. The Bugmeter electrode (see later) was inserted into the bottom of the fermentor. Air was sparged through the medium at a rate of approximately 0.2 vvm. Capacitance at low and high frequency, conductance at low frequency (0.4 MHz) and the state of the pump at the inflow were monitored continuously using a Blackstar 2308 interface (ADC) and an Opus II IBM-compatible PC (and see below).

*Off-line measurements*

Dry weights were measured by filtering a 5 ml culture of the sample using Whatman 0.2  $\mu\text{m}$  (25 mm) filters, washing once with  $1 \times 2.5$  ml distilled water, and drying the filters plus cells in a drying oven at 105 °C overnight.

Methylene blue staining was performed as described previously [16].

Glucose concentrations were measured using procedure 115 from Sigma, which is based on the enzymatic oxidation of glucose.

Alcohol concentrations were measured using procedure 332-UV from Sigma, which is based on the enzymatic oxidation of alcohol by NAD.

*Flow cytometry*

For the flow cytometry 1 ml samples containing 1% glutaraldehyde were stored in the cold room (4 °C). The flow cytometer used was a Skatron Argus 100 flow cytometer (Skatron, PO Box 34, Newmarket, Suffolk). This flow cytometer measures the fluorescence and light scattering of particles. In the present experiments we used only low-angle scattering which gives a measure of particle size (e.g. refs. 33–41). The machine was used with a Photomultiplier Tube voltage of 310 V and an amplification factor of 4. Calibration was performed using 2, 5, 7 and 10  $\mu\text{m}$  diameter latex calibration beads, and the calibration curve was linearised using the procedures and program previously described [42]. Cell sizes are reported as those of the spheres of the equivalent diameter, based upon the calibrating latex beads. As discussed previously [43], for the analysis of normalised cell size distributions, it is not necessary that the *absolute* values of the cell diameters are correct, merely that the apparent diameters recorded are in constant proportion to their true values. This is accomplished by the linearisation program used [42].

### *Dielectric measurements*

The dielectric measurements were performed using a 4-terminal dielectric spectrometer, A Bugmeter<sup>TM</sup>, produced by Aber Instruments, Aberystwyth Science Park, Cefn Llan, Aberystwyth, Dyfed SY23 3DA, UK. This instrument uses a sterilisable probe consisting of four gold pins embedded in a sterilisable epoxy resin probe, and fits a standard 25 mm Ingold-type port. The cell constant of this probe was  $0.803 \text{ cm}^{-1}$ . The Bugmeter was controlled using an Opus II IBM-XT compatible PC and a Blackstar 2308 Interface (ADC). To compensate for drift and fluctuations in the signal caused by outside influences such as changes in the ambient temperature and the efficiency of electrical grounding, the measurements were normally performed by comparison of the signal at a low (0.4 MHz) frequency with that at a high (9.5 MHz) frequency. As drift and fluctuations have the same effect on signals at low and high frequencies, and only the signal at low frequencies contains a major component related to the biomass present, this method allows one to compensate for such artefacts. The method has already been used successfully in the measurement of the dielectric properties of biofilms [19]. The conductance was measured at a frequency of 0.4 MHz.

The Bugmeter contains a unit which allows one to generate gas bubbles on the electrode surface by electrolysis of the medium in order to clean the electrode. This option was not used during the experiments described, since biofilm formation was found to be minimal.

### *Analysis of dielectric spectra*

The Bugmeter permits the frequency of measurement to be controlled externally by a command voltage. Using appropriate signals fed to the Bugmeter via a DAC channel of the Blackstar interface, frequency-dependent dielectric spectra were recorded every half an hour. One hundred datapoints were taken at frequencies spread logarithmically over the frequency range 0.1–10 MHz. The analysis of the dielectric spectra was performed on the spreadsheet-based program "Cole.wks" [25]. Simulations of the behaviour to be expected from systems obeying the equations of the classical  $\beta$ -dispersion were also carried out with spreadsheets written in-house, using the program VP-Planner [25].

### *Chemicals and biochemicals*

These were obtained from the Sigma Chemical Company or BDH Ltd, Poole, Dorset, and were of the highest purity available. Latex beads were from Sigma or from Dyno A/S, Lillestrøm, Norway. Water for the flow cytometer was prepared using a Millipore Milli-Q apparatus.

## RESULTS

The changes in the capacitance and conductance during a typical batch culture of baker's yeast are given in Fig. 1. The capacitance trace takes the form to be expected

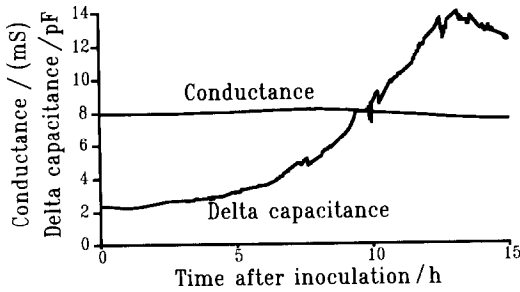


Fig. 1. Changes in the dielectric properties during the growth of *S. cerevisiae* in batch culture. The culture was carried out as described in the Experimental section. The Delta capacitance represents the difference in capacitance between 0.4 and 9.5 MHz, whilst the conductance is that measured at 0.4 MHz.

for an organism growing in batch culture, whilst the conductance changes but little in this particular fermentation [44].

In Fig. 2 the changes in the dry weight, glucose and ethanol concentrations are given. The dry weight increases as expected from the capacitance trace, whilst the complete consumption of glucose and its essentially quantitative conversion to ethanol are clear.

The frequency scans recorded during the culture are given in Fig. 3A. These frequency scans were analysed using the spreadsheet program "Cole.wks" [25], which permits one to obtain the best fit of one or more dispersions in terms of their dielectric increment, characteristic frequency and Cole–Cole  $\alpha$ . The quality of the fit which may be obtained is shown for a representative trace in Fig. 3B, where only at the very lowest frequencies is there any indication of the onset of irremediable electrode polarisation (or, conceivably, an  $\alpha$ -dispersion).

The characteristic frequency of baker's yeast has an almost constant value throughout the batch culture of some 1.1 MHz in the present medium. The effect of the Cole–Cole  $\alpha$  on the (normalised) frequency-dependence of the permittivity for values of the Cole–Cole  $\alpha$  between 0.2 and 0.4 is shown in Fig. 4A, where it may be observed that rather small changes in the Cole–Cole  $\alpha$  lead to substantial changes in the apparent breadth of the dispersion. The magnitudes of the Cole–Cole  $\alpha$  determined from the frequency scans carried out during the fermentation are given in Fig. 4B. The Cole–Cole  $\alpha$  shows a change from 0.27 in the lag phase to a maximum over 0.3 in the exponential phase, declining again to a value of about 0.2 during the stationary phase.

#### *Calculation of the Cole–Cole $\alpha$ from the cell-size distribution observed*

The cell size distributions of representative samples taken from the fermentation and measured using the Skatron flow cytometer are given in Fig. 5. The are broadly similar to those obtained for aerobically grown cells by Alberghina et al. [32] and by Ranzi et al. [45]. The median and modal cell diameters are given in Fig. 6, where it may be observed that they vary but little during the course of the fermentation.

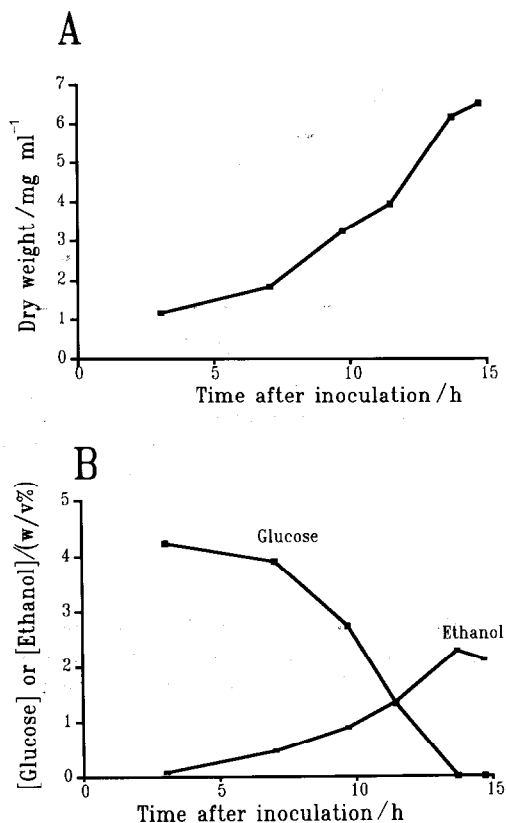


Fig. 2. Changes in various concentrations during the batch culture of *S. cerevisiae*. The experiment was that performed and described in the legend to Fig. 1. (A) Dry weight, (B) glucose and ethanol concentrations.

To describe the breadth of a cell-size distribution, it is convenient to characterise it by the peak width at half-height, i.e. the difference in the two cell sizes at which the cell number is one half of the modal cell number. The value of the Cole–Cole  $\alpha$  determined above is plotted in Fig. 7 versus the peak width at half-height, where it may be observed that it does not appear to depend significantly upon the latter over the range of samples studied.

It was therefore of interest to enquire in detail as to the extent by which the Cole–Cole  $\alpha$  reflected the cell-size distribution in *more absolute* terms.

The extent of low-angle light scattering recorded in an instrument of this type is not usually linear with the size of the scattering particle [36,42]. The data file from the Skatron of the sample taken 13.7 h after inoculation was therefore loaded into the program FLOWTOVP [42] and converted to a data file accurately reflecting the cell size distribution. The frequency scan of the  $\beta$ -dispersion of a suspension of a sample taken at the same time was loaded into the program Cole.wks [25]. After

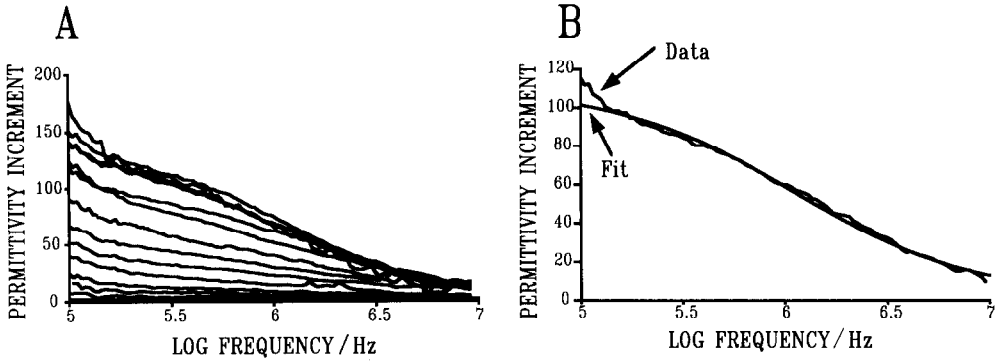


Fig. 3. Frequency-dependence of the dielectric permittivity of *S. cerevisiae* during the course of a batch culture. Measurements were performed as described in the Experimental section and in the legend to Fig. 1. (A) Frequency-dependence of the culture permittivity; increasing values of the low-frequency permittivity represent samples taken at later times. (B) Typical fit of the data of (A) to the Cole-Cole equation, using the spreadsheet program Cole.wks [25].

subtraction of the frequency scan of the medium, which allows one to correct for electrode polarisation [25], the following dielectric parameters were obtained: Cole-Cole  $\alpha = 0.22$ , characteristic frequency = 1.1 MHz and dielectric increment = 127 permittivity units.

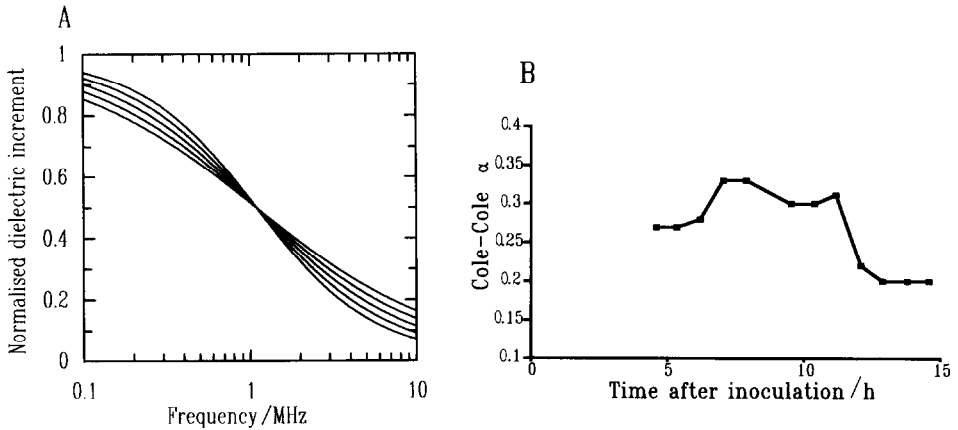


Fig. 4. (A) The effect of the Cole-Cole  $\alpha$  on the shape of a dielectric dispersion. Simulations were performed for a dielectric dispersion having a characteristic frequency of 1.1 MHz, and for values of the Cole-Cole  $\alpha$  ranging from 0.2 to 0.4 in steps of 0.05, the broader dispersions reflecting the larger values of the Cole-Cole  $\alpha$ . (B) Changes in the Cole-Cole  $\alpha$  during the batch culture of *S. cerevisiae*. Measurements were performed exactly as described in the legend to Fig. 3.



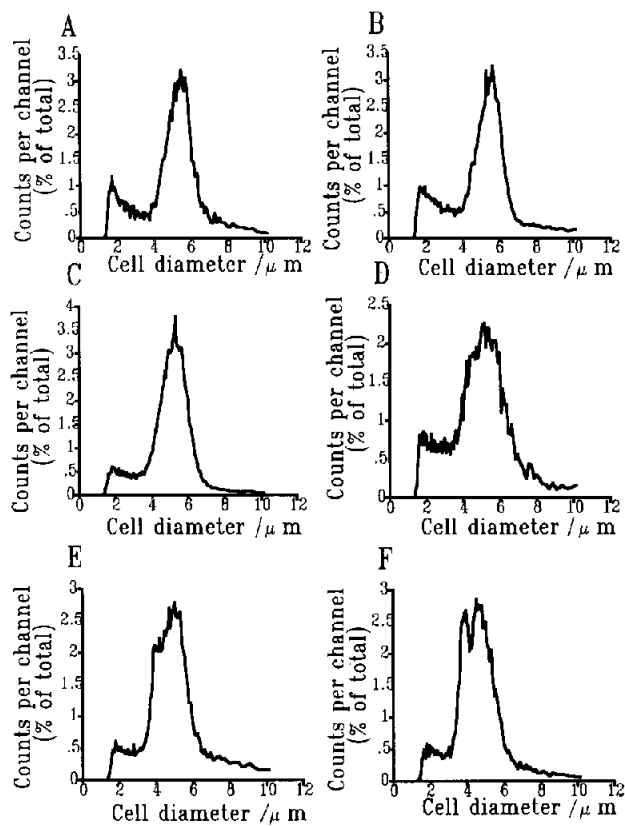


Fig. 5. Cell size distributions during the batch culture of *S. cerevisiae*. Samples were taken from the run displayed in Fig. 3, and analysed as described in the Experimental section at (A) 3.05, (B) 7.05, (C) 9.7, (D) 11.5, (E) 13.7 and (F) 14.7 h following inoculation.

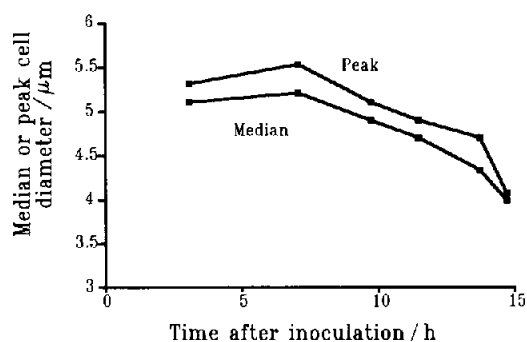


Fig. 6. Changes in the median and peak (modal) cell size (diameter) during batch culture of *S. cerevisiae*. Experiments were performed as described in the legend to Fig. 5.

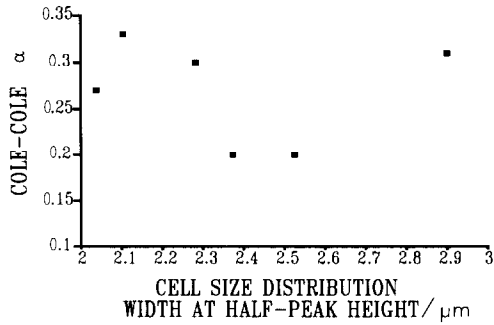


Fig. 7. Relationship between the Cole-Cole  $\alpha$  and the peak width at half height of the cell size distribution in a batch culture of *S. cerevisiae*. Measurements were performed as described in the legends to Figs. 5 and 6.

As indicated above, the classical  $\beta$ -dispersion may be described by the equations:

$$\Delta\epsilon = 9PrC_m/4\epsilon_0$$

and

$$\tau = rC_m [(1/\sigma_i) + (1/2\sigma_o)]$$

If the value of  $C_m$  and the external (Fig. 1) and internal conductivities stay constant during a culture and are equal for all cells, we can say for notionally spherical yeast cell suspensions (with a volume given by  $\pi D^3/6$ ) that:

$$\Delta\epsilon' = K_1 ND^4 \quad (5)$$

$$\tau = K_2 D \quad (6)$$

where  $D$  = diameter of a yeast cell,  $N$  = number of cells per ml,

$$K_1 = 9\pi C_m/48\epsilon_0 \quad (7)$$

and

$$K_2 = C_m [(1/\sigma_i) + (1/2\sigma_o)]/2 \quad (8)$$

We may take for our purposes (for which normalisation is appropriate) that  $C_m = 1 \mu\text{F cm}^{-2}$  [4,7]. Any inaccuracies in this will show up as inaccuracies in the absolute values of the cell size (or number), but not of their distribution.

An ideal Debye dispersion of the complex permittivity  $\Delta\epsilon^*$  is described [3] by the equation:

$$\Delta\epsilon^*(\omega) = \epsilon_h + \Delta\epsilon/(1 + (i\omega\tau)) \quad (9)$$

so

$$\Delta\epsilon' = \Delta\epsilon/(1 + \omega^2\tau^2) \quad (10)$$

where  $\Delta\epsilon'$  is the real part of the dielectric increment and  $\omega$  the frequency in  $\text{rad s}^{-1}$ .

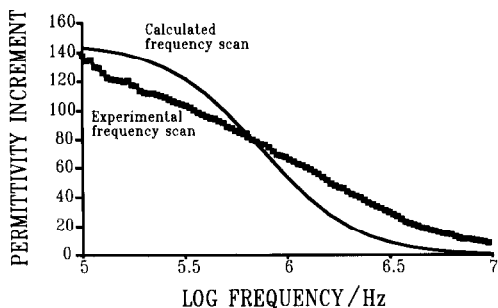


Fig. 8. Relationship between the dielectric dispersion found for a suspension of yeast cells (Fig. 3A) with the dielectric properties expected for a suspension of cells whose mean dielectric properties were as described in the text and whose cell size distribution was as in Fig. 5E. The permittivity increment is given relative to a normalised value for  $\epsilon_h$  of zero.

For the calculation of  $K_1$  and  $K_2$  we take (Fig. 5)  $D_{\text{peak}} = 5.02 \mu\text{m}$ .  $f_c$  was determined experimentally to be 1.1 MHz. From this we get  $\tau = 0.145 \mu\text{s}$ .  $\Delta\epsilon'$  was found to be 127. This information is sufficient for us to estimate  $K_1$  and  $K_2$ :  $K_1 = 6.55 \times 10^6 \text{ cm}^{-1}$ ,  $K_2 = 2.88 \times 10^{-4} \text{ s cm}^{-1}$ , and  $P = 0.020$ ,  $N = 3.05 \times 10^8 \text{ ml}^{-1}$  (see also ref. 17).

We can then calculate the  $\Delta\epsilon'$  for each number of cells of each cell size (corresponding to individual channel numbers in the original flow cytometer data file) in the cell size distribution plot obtained by flow cytometry using the Debye equation for different frequencies, using the data in Fig. 5E. These are then added to give a total  $\Delta\epsilon'$ . We thus get a frequency plot, which is given in Fig. 8. Also given in Fig. 8 is the actual dielectric spectrum obtained from the cell suspension at the same time, showing a large difference between the frequency scan of the suspension and the frequency scan calculated from the cell size distribution. The suspension had a Cole-Cole  $\alpha$  of 0.22, whilst the Cole-Cole  $\alpha$  fitted to the calculated frequency scan was only 0.008.

The above analysis made two rather severe assumptions: that the internal conductivity and the membrane capacitance per unit area of all the cells was identical. It was therefore of interest to explore the consequences of relaxing that assumption. The strategy adopted for this was as follows.

We next calculated a mean value for the internal conductivity  $\sigma_i$ , from the measured values of the mean radius  $r_{\text{mean}}$ , the experimentally determined external conductivity  $\sigma_o$  ( $6.0 \text{ mS cm}^{-1}$ ) and the assumed value of the membrane capacitance per unit area  $C_m$ , mean, using eqn. (4). This gave a value for  $\sigma_i$  of  $2.05 \text{ mS cm}^{-1}$ , a value similar to those obtained in previous studies of the  $\beta$ -dispersion of yeast cells [46,47]. It is of interest to determine how sensitive  $\tau$  (or  $f_c$ ) is to  $\sigma_i$  in the range of values of  $\sigma_i$  centred on the mean value calculated. To this end, Fig. 9 shows a plot of eqn. (4) using the relevant values of  $r$ ,  $C_m$  and  $\sigma_o$  and a range of values for  $\sigma_i$ . It may be observed that halving or doubling the value of  $\sigma_i$  has an even less than

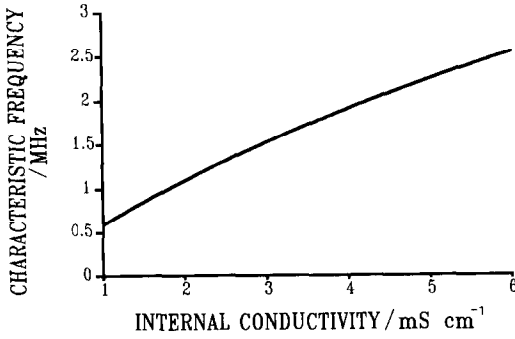


Fig. 9. Relationship between the characteristic frequency and the internal conductivity for a dielectric relaxation obeying that of the classical  $\beta$ -dispersion. Calculations were performed using eqn. (4) with the following values:  $r = 2.51 \mu\text{m}$ ,  $\sigma_0 = 6.0 \text{ mS cm}^{-1}$ ,  $C_m = 1 \mu\text{F cm}^{-2}$  and the values of  $\sigma_i$  indicated.

proportional effect upon the value of  $f_c$  (although the effect of this on the Cole–Cole  $\alpha$  is yet to be determined).

Since the distributions of cell sizes were fairly symmetrical (Fig. 5), we adopted the following “worst case” strategy which might be expected to increase the values of the Cole–Cole  $\alpha$ . We divided the cells into two halves, centred around the peak cell size; the smaller cells were given a value of  $\tau$  twice that of the mean whilst the larger half of the cells were given a value of  $\tau$  that was half that of the mean. This may be expected maximally to increase the breadth of the dispersion.

It is qualitatively easy to see, by inspection of eqn. (3), that variations in  $C_m$  per se should not be expected to have a substantive effect on the magnitude of the

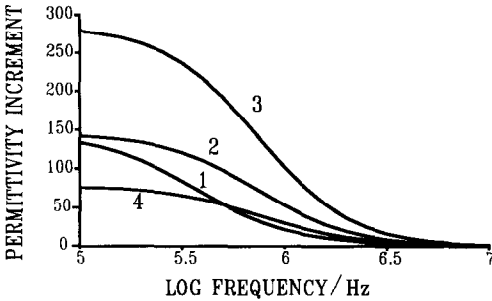


Fig. 10. Effect of varying the individual dielectric parameters of cohorts of cells on the dielectric dispersion to be expected. Simulations were carried out as described in the legend to Fig. 8. Curve 1 represents a simulation in which the smaller half of the cells were given a value of  $\tau$  equal to twice the mean and the larger half were given a value of  $\tau$  equal to one-half of the mean. Curve 2 shows the simulation of Fig. 8. Curve 3 shows the simulation of Fig. 8 but in which the value of  $C_m$  for the larger half cells is twice the mean and for the smaller half of the cells is one half of the mean. Curve 4 is the same except that the smaller half of the cells are given a value of  $C_m$  equal to twice the mean whilst that of the larger half of the cells is one half the mean.

Cole–Cole  $\alpha$  obtained: if the large cells are given a value of  $C_m$  twice the mean they will tend to dominate the response, decreasing the Cole–Cole  $\alpha$ , whilst if they are given a value of  $C_m$  of half the mean there will be a “bunching effect” as they look more like the smaller cells.

Figure 10 therefore displays plots similar to that of Fig. 8 in which the simulated data are plotted for the different cases ( $2 \times$  and  $0.5 \times \tau$ , with or without doubling and halving the  $C_m$  values in each direction). The following remarks may be made. It is evident, as expected, that increasing or decreasing the value of  $C_m$  for a fraction of the cells, whilst maintaining the overall  $C_m$  at a value of  $1 \mu\text{F cm}^{-2}$ , distorts the dispersion very greatly. Doubling the value of  $\tau$  for the smaller half of the cells and halving that of the larger cells makes the overall  $\tau$  noticeably greater (and the  $f_c$  noticeably smaller, at 0.37 MHz), whilst, of course, having no effect upon  $\Delta\epsilon'$ . However, the value of the Cole–Cole  $\alpha$  remains very low, at 0.043. Thus realistic (and even unfavourable) variations in the value of the internal conductivity of the cells can not be used to account for the value (0.22) of the Cole–Cole  $\alpha$  observed.

## DISCUSSION AND CONCLUSIONS

In the study of biological dielectrics, it has been common to find that the dielectric dispersions observed have a breadth substantially greater than that of a single Debye dispersion. Since the dielectric dispersions observed seem invariably to be symmetrical, most workers have found it convenient to describe this increase in breadth over the Debye type of relaxation by the use of the Cole–Cole equation. The physical significance of the extra term of the Cole–Cole equation, the Cole–Cole  $\alpha$ , is usually taken to imply a distribution of relaxation times [28] in the system under study, and it was stressed by Schwan [22] that whilst the distribution of relaxation times underpinning the Cole–Cole equation was rather complex, a variety of relaxation-time distributions gave behaviour indistinguishable from “true” Cole–Cole behaviour.

Following the pioneering and historically important studies of Fricke [48], it was established that biological cell suspensions exhibit a substantial dielectric dispersion (known as the  $\beta$ -dispersion [22]) in the radio-frequency range, which could be ascribed to their possession of more-or-less insulating cell membranes separating two conductive aqueous phases, with dielectric increments and relaxation times defined by eqns. (3) and (4) (above). Many data, reviewed e.g. in refs. 3, 4, 26, 49, 50, are broadly consistent with this explanation of the factor dominating the  $\beta$ -dispersion. In particular, in a recent, most elegant experiment [51], Asami and Irimajiri showed that a single, macroscopic spherical bilayer gave the behaviour expected, with a calculated value of  $C_m$  of  $0.54 \mu\text{F cm}^{-2}$  and (as expected, since only a single membrane was studied at any one time) a Cole–Cole  $\alpha$  indistinguishable from zero.

In biological tissues or cell suspensions, however, the values of the Cole–Cole  $\alpha$  observed are much greater, typically in the range 0.1 to 0.35 [13,50,52–65]. Figure 11 provides a compilation of some relevant data.

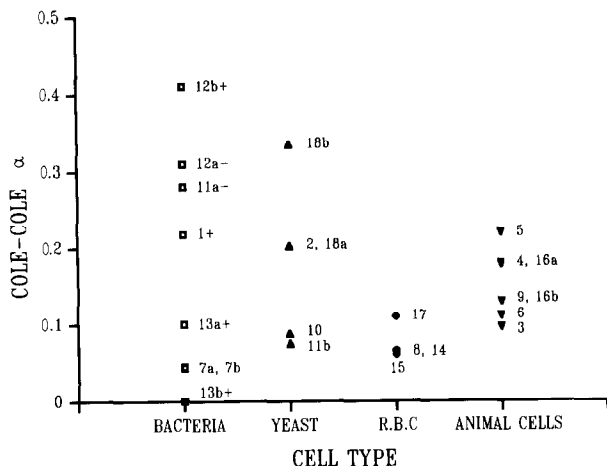


Fig. 11. Some values of the Cole-Cole  $\alpha$  determined in biological cell suspensions. Data were taken from experiments on the following systems in the references indicated: (1) *Staphylococcus aureus* [66]; (2) *Saccharomyces cerevisiae* [47]; (3) murine lymphoblasts [57]; (4) murine lymphocytes [67]; (5) murine lymphocytes [68]; (6) rat basophil leukaemia cells [64]; (7a, b) *Halobacterium halobium* and *H. marismortui* [69]; (8) human red blood cells [70]; (9) LS-L929 mouse fibroblasts [13]; (10) *Saccharomyces cerevisiae* [46]; (11a, b) *Escherichia coli* and *Saccharomyces cerevisiae* [26]; (12a, b) *Methylophilus methylotrophus* and *Bacillus subtilis* [62]; (13a, b) *Micrococcus lysodeikticus* cells and protoplasts [54]; (14) human erythrocytes [71]; (15) carp (*Cyprinus carpio*) red blood cells (22°C) [72]; (16a, b) human T and B lymphocytes [63]; (17) human red blood cells [10]; (18a, b) *Saccharomyces cerevisiae* stationary and exponential phases (this work).

If one is to explain this large value of the Cole-cole  $\alpha$  on the basis of a distribution of relaxation times of cell properties conforming to eqns. (3) and (4), the free variables that one may consider are the size (and shape), the membrane capacitance per unit area  $C_m$  and the internal conductivity. Since cells of the budding yeast *S. cerevisiae* are very good approximations to spheres we need not here consider shape, although it is worth remarking that *significant* changes in shape towards bacillary [54,62] or invaginated morphologies [13,64,65] are certainly accompanied by increases in the Cole-Cole  $\alpha$ . In previous work with washed cell suspensions of *S. cerevisiae* that had proceeded well into the stationary phase before harvest (and had thus completed the possible rounds of replication and division), the Cole-Cole  $\alpha$  was found to be less than 0.1 [46]. By contrast, in the present work with *growing* cells the values of the Cole-Cole  $\alpha$  were in the range 0.2 to 0.3, and did not change enormously during the course of batch culture. Thus one must look towards a distribution in the cell radii or the internal conductivities if one is to explain the fact that the Cole-Cole  $\alpha$  is normally significantly greater than zero.

Flow cytometry provides a convenient and powerful means for obtaining accurate and detailed data on the distribution of cells sizes, via the extent of their low-angle light-scattering. Although other workers measuring the  $\beta$ -dispersion have also studied cell size distributions, this has been only in the form of histograms

based on electron microscopic measurements [13,56,60,63–65]. We found (Fig. 7) that there was little correlation between the *breadth* of the cell size distribution and the magnitude of the Cole–Cole  $\alpha$ . More quantitatively, for a suspension of cells whose Cole–Cole  $\alpha$  was 0.22 (fig. 3B), the distribution of cell sizes was far too low alone to account for the the magnitude of the Cole–Cole  $\alpha$  (Fig. 8).

Cole himself [73] has also expressed disquiet with the view that the breadth of the  $\beta$ -dielectric dispersion can be accounted for solely in terms of a distribution of cell sizes. We therefore simulated the effects of increasing the distribution of relaxation times by increasing the range of internal conductivities of the cells around the mean value estimated from the dielectric spectrum (Fig. 10). As discussed in more detail elsewhere [13,26], our present measuring system and fitting procedure provide an extremely accurate reflection of the dielectric properties of the system under study. However, changing the internal conductivities over a factor of 4 increased the Cole–Cole  $\alpha$  to only 0.04, whilst varying the value of  $C_m$  gave simulated data that were very different from those actually found (Figs. 8, 10). Thus, even substantial variations in  $\sigma_i$  or  $(r)C_m$  cannot account for the magnitude of the Cole–Cole  $\alpha$  of the  $\beta$ -dispersion of biological cell suspensions.

Of the other mechanisms, additional to the “classical” charging of a static membrane capacitance, which may be expected to contribute to the  $\beta$ -dispersion, that involving a lateral mobility of membranous components [4,7,10,11,50,62,74] seems to be the most plausible, since its has the combined merits of explaining (i) the positive temperature coefficient observed for the dielectric increment, (ii) the fact that the value of  $C_m$  calculated for *biological* membranes is typically twice as great as that observed with pure lipid membranes, and (iii) the sensitivity of the  $\beta$ -dispersion to chemical cross-linking reagents, as well as the excessive magnitude of the Cole–Cole  $\alpha$  observed herein.

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#### REFERENCES

- 1 E.H. Grant, R.J. Sheppard and G.P. South, *Dielectric Properties of Biological Molecules in Solution*, Oxford University Press, Oxford, 1978.
- 2 O.F. Schanne and E.R.P. Ceretti, *Impedance Measurements in Biological Cells*, Plenum Press, New York, 1978.
- 3 R. Pethig, *Dielectric and Electronic Properties of Biological Materials*, Wiley, Chichester, 1979.
- 4 D.B. Kell and C.M. Harris, *J. Bioelectr.*, 4 (1985) 317.
- 5 K.R. Foster and H.P. Schwan In C. Polk and E. Postow (Eds.), *CRC Handbook of Biological Effects of Electromagnetic Fields*, CRC Press, Boca Raton, 1986, p. 27.
- 6 D.B. Kell in A.P.F. Turner, I. Karube and G.S. Wilson (Eds.), *Biosensors: Fundamentals and Applications*, Oxford University Press, Oxford, 1987, p. 428.
- 7 R. Pethig and D.B. Kell, *Phys. Med. Biol.*, 32 (1987) 933.
- 8 K.R. Foster and H.P. Schwan, *CRC Crit. Rev. Biomed. Eng.*, 17 (1989) 25.

- 9 S. Takashima, *Electrical Properties of Biopolymers and Membranes*, Adam Hilger, Bristol, 1989.
- 10 C.L. Davey and D.B. Kell in M. O'Connor, R.H.C. Bentall and J.S. Monahan (Eds.), *Emerging Electromagnetic Medicine*, Springer, Berlin, 1990, p. 19.
- 11 D.B. Kell and C.L. Davey in A.E.G. Cass (Ed.), *Biosensors; a Practical Approach*, Oxford University Press, Oxford, 1990, p. 125.
- 12 C.M. Harris, R.W. Todd, S.J. Bungard, R.W. Lovitt, J.G. Morris and D.B. Kell, *Enz. Micr. Technol.*, 9 (1987) 181.
- 13 C.L. Davey, D.B. Kell, R.B. Kemp and R.W.J. Meredith, *Bioelectrochem. Bioenerg.*, 20 (1988) 83.
- 14 C.A. Boulton, P.S. Maryan and D. Loveridge, *Proc. 22nd European Brewing Convention*, Zürich, 1989, p. 653.
- 15 D.B. Kell and R.W. Todd, U.S. Patent 4, 810, 650, 1989.
- 16 N.G. Stoicheva, C.L. Davey, G.H. Markx and D.B. Kell, *Biocatalysis*, 2 (1989) 245.
- 17 D.B. Kell, G.H. Markx, C.L. Davey and R.W. Todd, *Trends Anal. Chem.*, 9 (1990) 190.
- 18 D.B. Kell and C.L. Davey in ref. 11, p. 125.
- 19 G.H. Markx and D.B. Kell, *Biofouling*, 2 (1990) 211.
- 20 G.H. Markx, C.L. Davey and D.B. Kell, *J. Gen. Microbiol.*, (1990)
- 21 A.M. Woodward and D.B. Kell, *Bioelectrochem. Bioenerg.*, 24 (1990) 83.
- 22 H.P. Schwan, *Adv. Biol. Med. Phys.*, 5 (1957) 147.
- 23 H.P. Schwan, in W.L. Nastuk (Ed.), *Physical Techniques in Biological Research*, Vol. 6, Academic Press, New York, 1963, p. 323.
- 24 H.P. Schwan and C.D. Ferris, *Rev. Sci. Instr.* 39 (1968) 481.
- 25 C.L. Davey, G.H. Markx and D.B. Kell, *Eur. Biophys. J.*, 18 (1990) 255.
- 26 L.E. Ferris, C.L. Davey and D.B. Kell, *Eur. Biophys. J.*, 18 (1990) 267.
- 27 P. Debye, *Polar Molecules*, Dover, New York, 1929.
- 28 K.S. Cole and R.H. Cole, *J. Chem. Phys.*, 9 (1941) 341.
- 29 M.R. Melamed, P.F. Mullaney and M.L. Mendelsohn (Eds.), *Flow Cytometry and Sorting*, Wiley, New York, 1979.
- 30 H.M. Shapiro, *Practical Flow Cytometry*, Alan Liss, New York, 1988.
- 31 J.E. Bailey, J. Fazel-Madjlessi, D.N. McQuitty, L.Y. Lee and J.A. Oro, *AI Ch E J.*, 24 (1978) 570.
- 32 L. Alberghina, L. Mariani, E. Martegani and M. Vanoni, *Biotechnol. Bioeng.*, 25 (1983) 1295.
- 33 T.K. Sharpless, M. Bartholdi and M.R. Melamed, *J. Histochem. Cytochem.*, 25 (1977) 845.
- 34 G.C. Salzman, P.F. Mullaney and B.J. Price in ref. 29, p. 105.
- 35 P. Latimer, *Annu. Rev. Biophys. Bioeng.*, 11 (1982) 129.
- 36 G.C. Salzman in N. Catsimpoalas (Ed.), *Cell Analysis*, Vol. 1, Plenum Press, New York, 1982, p. 111.
- 37 M. Kerker, *Cytometry*, 4 (1983) 1.
- 38 A.L. Koch, *Am. Soc. Microbiol. New*, 50 (1984) 473.
- 39 S.H. Harding, *Biotechnol. Appl. Biochem.*, 8 (1986) 489.
- 40 A.L. Koch, *J. Microbiol. Meth.*, 5 (1986) 221.
- 41 H.B. Steen, *Cytometry*, 11 (1990) 223.
- 42 C.L. Davey, N.M. Dixon and D.B. Kell, *Binary*, 2 (1990) 119.
- 43 C.L. Davey, D.B. Kell and N.M. Dixon, *Binary*, 2 (1990) 127.
- 44 R. Firstenberg-Eden and G. Eden, *Impedance Microbiology*, Research Studies Press, Letchworth, 1984.
- 45 B.M. Ranzi, C. Compagno and E. Martegani, *Biotechnol. Bioeng.*, 28 (1986) 185.
- 46 C.M. Harris and D.B. Kell, *Bioelectrochem. Bioenerg.*, 15 (1983) 11.
- 47 K. Asami, T. Hanai and N. Koizumi, *J. Membr. Biol.*, 28 (1976) 169.
- 48 H. Fricke, *J. Gen. Physiol.*, 9 (1925) 137.
- 49 K.S. Cole, *Membranes, Ions and Impulses*, University of California Press, Berkeley, 1972.
- 50 D.B. Kell and C.M. Harris, *Eur. Biophys. J.*, 12 (1985) 181.
- 51 K. Asami and A. Irimajiri, *Biochim. Biophys. Acta*, 769 (1984) 370.
- 52 H. Fricke, H.P. Schwan, K. Li and V. Bryson, *Nature (London)*, 177 (1956) 134.
- 53 H. Pauly, L. Packerand, H.P. Schwan, *J. Biophys. Biochem. Cytol.*, 7 (1960) 589.



- 54 H. Pauly, *IRE Trans. Biomed. Electron.*, 9 (1962) 93.
- 55 C.W. Einolf and E.L. Carstensen, *Biophys. J.*, 9 (1969) 634.
- 56 K. Asami, T. Hanai and N. Koizumi, *Biophys. J.*, 31 (1980) 215.
- 57 A. Irimajiri, Y. Doida, T. Hanai and A. Inouye, *J. Membr. Biol.*, 38 (1978) 209.
- 58 H.P. Schwan and K.R. Foster, *Proc. IEEE*, 68 (1980) 104.
- 59 R.D. Stoy, K.R. Foster and H.P. Schwan, *Phys. Med. Biol.*, 27 (1982) 501.
- 60 K. Asami, A. Irimajiri, T. Hanai, N. Shiraishi and K. Utsumi, *Biochim. Biophys. Acta*, 778 (1984) 559.
- 61 C.M. Harris, G.D. Hitchens and D.B. Kell in M.J. Allen and P.N.R. Usherwood (Eds.), *Charge and Field Effects in Biosystems*, Abacus Press, Tunbridge, 1984, p. 179.
- 62 C.M. Harris and D.B. Kell, *Eur. Biophys. J.*, 13 (1985) 11.
- 63 A. Surowiec, S.S. Stuchly and C. Izaguirre, *Phys. Med. Biol.*, 31 (1986) 43.
- 64 A. Irimajiri, K. Asami, T. Ichiniwatari and Y. Kinoshita, *Biochim. Biophys. Acta*, 896 (1987) 203.
- 65 A. Irimajiri, K. Asami, T. Ichiniwatari and Y. Kinoshita, *Biochim. Biophys. Acta*, 896 (1987) 214.
- 66 A.A. Laogun, *J. Bioelectr.*, 5 (1986) 129.
- 67 W. Jaroszynski, J. Terlecki, A. Misliwski, J. Misliwska and J. Witkowski, *Fol. Histochem. Cytobiol.*, 21 (1983) 161.
- 68 W. Jaroszynski, J. Terlecki and J. Sulocki, *Studia Biophys.*, 107 (1985) 117.
- 69 H. Morgan, M. Ginzburg and B.Z. Ginzburg, *Biochim. Biophys. Acta*, 924 (1987) 54.
- 70 C.L. Davey and D.B. Kell in R. Paris (Ed.), *Electric Field Phenomena in Biological Systems*, IOP Short Meetings Series, Vol. 21, Institute of Physics, London, 1989, p. 51.
- 71 M. Bautembach and J. Terlecki, *Post. Fiz. Med.*, 15 (1980) 87.
- 72 I. Bielinska and J. Terlecki, *Folia Histochem. Cytobiol.*, 23 (1985) 33.
- 73 K.S. Cole in F. Snell, J. Wolken, G. Iverson and J. Lam (Eds.), *Physical Principles of Biological Membranes*, Gordon and Breach, New York, 1970, p. 1.
- 74 D.B. Kell, *Bioelectrochem. Bioenerg.*, 15 (1983) 405.