JOURNAL OF BIOELECTRICITY, 5(1), 139-144 (1986)

SHORT COMMUNICATION

A NOMOGRAPH FOR CALCULATING THE OPTIMAL FREQUENCY FOR DIELECTROPHORESIS AND THE CHARACTERISTIC FREQUENCY OF THE β-DISPERSION OF CELL MEMBRANE VESICLES

Douglas B. Kell
Department of Botany and Microbiology
Unversity College of Wales
Aberystwyth, Dyfed SY23 3DA, U.K.

Alan Burns
Postgraduate School of Studies in Computing
University of Bradford
Bradford, West Yorkshire BD7 1DP, U.K.

ABSTRACT

The first stage of the two-stage cell electrofusion technique involves the dielectrophoretic apposition, in an AC field, of protoplasts suspended in a medium of relatively low specific conductivity. A frequency at which the maximum dielectrophoretic force is exerted is given by the characteristic frequency for the dielectric relaxation by a Maxwell-Wagner type of mechanism. We provide a nomograph for the rapid calculation of this frequency.

INTRODUCTION

When a strong electric field is applied to a suspension of cells or protoplasts in a medium of a low electrical conductivity, a phenomenon of (mutual) dielectrophoresis occurs (1,2). The cells line up to form, depending upon the cell density of the suspension, pairs or pearl chains, and they may subsequently by fused by the imposition of a short electrical pulse of high field-strength (3,4). The physical basis of this dielectrophoretic effect resides in the difference in complex permittivity between the vesicles and the

0730-823X/86/0501-0139$3.50/0

medium in which they are suspended (1,2,5), and thus the dependence of the dielectrophoretic force upon the frequency of the exciting field is reflected in the frequency-dependent dielectric properties of the suspension. In other words, for a given field strength, the dielectrophoretic efficiency is greatest at the characteristic frequencies f_c of the dielectric relaxations exhibited by the vesicles.

In the frequency range up to around 100 MHz, it is generally observed that (charged) biological cells or vesicles exhibit two major dielectric dispersions, usually referred to as the α- and β-dispersions (2,6-8). Our attention is here focussed on the more rapidly relaxing β-dispersion, which is dominated by capacitive charging of the cell membrane, and we treat only spherical shell protoplasts, which are of the greatest interest so far as practical exploitation of the technique is concerned (9,10).

For such a case, we assume:

(a) that the protoplasts are characterised by a uniform radius r μm;

(b) that, in contrast to, say, skeletal muscle (11), the electrical conductivity of the cell interior is isotropic, and of magnitude σ_i mS/cm;

(c) that the protoplasts are immersed in a medium of bulk conductivity σ_o mS/cm, and

(d) that the protoplasts are possessed, in the frequency range of interest, of a membrane with a conductance sufficiently small to be neglected, and a static capacitance C_m = 1 μF/cm^2.

The last assumption deserves comment. The static membrane capacitance as measured with transmembrane electrodes is often as low as 0.5-0.6 μF/cm^2 (8,12). Both the dielectrophoretic induction phase of the electrofusion process itself (3,4,10,13), and dielectric measurements made directly (8,14-16) indicate that (probably partially restricted) motions of membrane and double-layer components contribute to the dielectric properties in the frequency range usually associated with the β-dispersion, when measurements are made with extravesicular electrodes. This complication, however, is not important here.

In this case, then, the relaxation time for the charging of the membrane capacitance ($f_c = 1/2\pi\tau$, where f_c = the characteristic frequency), is given by (6):

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

If r is in μm, σ_i and σ_o are in mS/cm, f_c is in kHz and $C_m = 1\ \mu F/cm^2$, equation (1) may be rewritten:

$$f_c = 1591.5/r[(1/\sigma_i) + (1/2\sigma_o)] \tag{2}$$

$$\text{or } f_c = 1591.5B/r \tag{3}$$

$$\text{where } B = 2\sigma_i\sigma_o/(\sigma_i + 2\sigma_o) \tag{4}$$

The units of B are therefore mS/cm.

RESULTS AND DISCUSSION

In dielectrophoretic or cell electrofusion experiments, it would be convenient to be able rapidly to calculate f_c from known or assumed values of r, σ_i and σ_o. Thus, our aim here is to provide, for the benefit of workers using the dielectrophoretic technique, a nomograph relating f_c to r, σ_i and σ_o.

From the theory of nomographs (17-20), we can construct a nomograph (Figure 1) to solve equation (3), using the following principles: (a) the B and r axes are parallel, and perpendicular to the σ_o axis; (b) the f_c axis joins the zero points on the B and r axes; (c) the angle between the σ_i and B

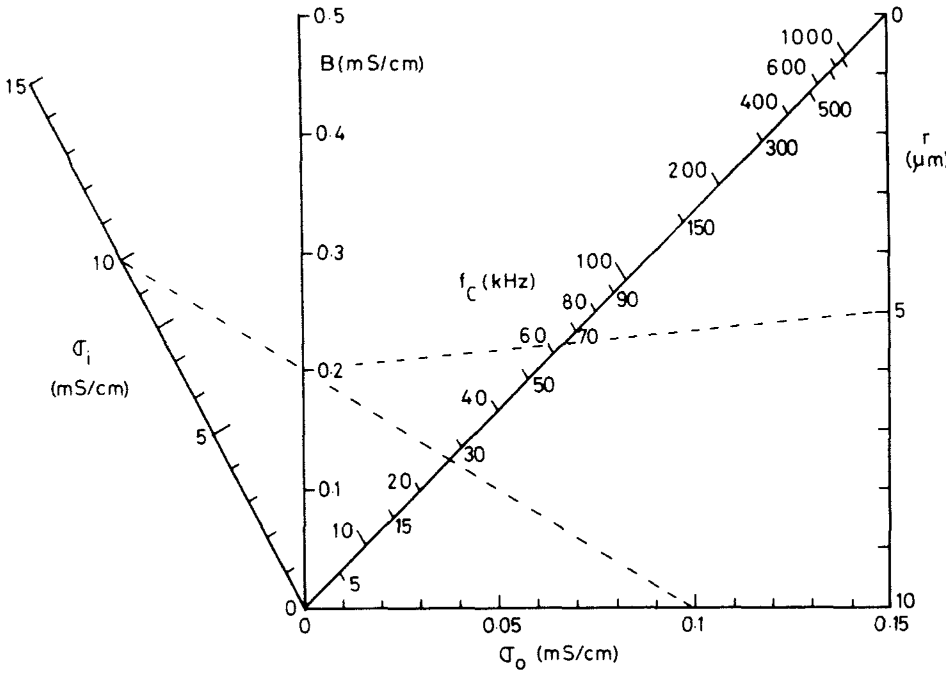

FIGURE 1

A nomograph to calculate the optimal frequency for dielectrophoresis and pearl chain formation in protoplast (or cell) suspensions. Note that B may be in arbitrary units for the present purposes, but must have the units of mS/cm. The value of B is obtained by joining the values for σ_i and σ_o, and f_c is obtained by joining the value for B and r. In the example shown, σ_i = 10 ,S/cm, σ_o = 0.1 mS/cm and r = 5 μm. It may be calculated in this case that, as observed, B = 0.196 mS/cm and f_c = 63.4 kHz.

axes is arbitrary (although it affects the scale on the f_c axis); (d) all scales except that for f_c are linear. We have used for Figure 1 a range of 0-10 μm for r; if one wishes to consider larger cells, the scale may be redrawn to read, say, 0-100 μm, in which case f_c values are decreased by a factor of 10. Similarly, the value of C_m (1 $\mu F/cm^2$) is subsumed in r; if one wished to use a value for C_m of n $\mu F/cm^2$, then f_c values should be decreased by a factor n. However, if one wishes to consider different scales for σ_i and

σ_o, the nomograph must be redrawn, and scales of B (if of interest) and f_c amended accordingly.

In use, one first obtains the value of B by joining the points representing the chosen (known or assumed) values of σ_i and σ_o, the value of B being where this line crosses the B axis. The point where the line joining this value of B and the chosen value or r crosses the f_c axis gives the characteristic frequency. Thus, for example (Figure 1), if we choose values for σ_i, σ_o and r of respectively 10 mS/cm, 0.1 mS/cm and 5 μm, we obtain values for B and f_c respectively of ca. 0.2 mS/cm and 63 kHz. By calculation from equation (1), values of 0.196 and 63.4 may be obtained for B and f_c; thus the nomograph accurately reflects the equation it was designed to solve.

ACKNOWLEDGEMENT

DBK thanks the Science and Engineering Research Council, U.K., for financial support. We thank Sian Evans for typing the manuscript, Jean Patrick for drawing the figure and Anthony Pugh for photographic assistance.

REFERENCES

(1) Pohl, H.A.: Dielectrophoresis. Cambridge University Press, 1978.

(2) Pethig, R.: Dielectric and Electronic Properties of Biological Materials, Wiley, Chichester, 1979.

(3) Zimmermann, U.: Electric Field-Mediated Fusion and Related Electrical Phenomena, Biochim. Biophys. Acta 694, 227-277, 1982.

(4) Zimmermann, U. and Vienken, J.: Electric Field-Induced Cell-to-Cell Fusion, J. Membr. Biol. 67, 165-182.

(5) Schwan, H.P. and Sher, L.D.: Alternating-Current Field-Induced Forces and their Biological Implications, J. Electrochem. Soc. 116, 22C-26C.

(6) Schwan, H.P.: Electrical Properties of Tissue and Cell Suspensions, in Advances in Biological and Medical Physics, J.H. Lawrence and C.A. Tobias, eds., Academic Press, Vol. 5, 147-209, 1957.

(7) Schanne, O.F. and Ceretti, E.R.P.: Impedance Measurements in Biological Cells, Wiley, Chichester, 1978.

(8) Kell, D.B. and Harris, C.M.: Dielectric Spectroscopy and Membrane Organisation, J. Bioelectricity 4, 317-348, 1985.

(9) Zimmermann, U.: Electrofusion of Cells: Principles and Industrial Potential, Trends in Biotechnol. 1, 149-155.

(10) Zimmermann, U., Vienken, J., Pilwat, G. and Arnold, A.M.: Electrofusion of Cells: Principles and Potential for the Future, Ciba Foundation Symp. 103, 60-85, 1984.

(11) Epstein, B.R. and Foster, K.R.: Anisotropy in the Dielectric Properties of Skeletal Muscle, Med. Biol. Eng. Comput. 21, 51-55, 1983.

(12) Almers, W.: Gating Currents and Charge Movements in Excitable Membranes, Rev. Physiol. Biochem. Pharmacol. 82, 96-190, 1978.

(13) Zimmermann, U., Buchner, K-H. and Arnold, W.M.: Electrofusion of Cells: Recent Developments and Relevance for Evolution, in Charge and Field Effects in Biosystems, M.J. Allen and P.N.R. Usherwood, eds., Abacus Press, Tunbridge Wells, 293-318, 1984.

(14) Kell, D.B.: Dielectric Properties of Bacterial Chromatophores, Bielectrochem. Bioenerg. 11, 405-415, 1983.

(15) Kell, D.B. and Harris, C.M.: On the Dielectrically Observable Consequences of the Diffusional Motions of Lipids and Proteins in Membranes. 1. Theory and Overview, Eur. Biophys. J. 11, 181-197, 1985.

(16) Harris, C.M. and Kell, D.B.: On the Dielectrically Observable Consequences of the Diffusional Motions of Lipids and Proteins in Membranes. 2. Experiments with Microbial Cells, Protoplasts and Membrane Vesicles, Eur. Biophys. J. 12, 11-24, 1985.

(17) Levens, A.S. Nomography, Wiley, New York, 1948.

(18) Johnson, L.H.: Nomography and Empirical Equations, Wiley, New York, 1952.

(19) Allcock, H.J., Reginal Jones, J. and Michel, J.G.L.: The Nomograph: The Theory and Practical Construction of Computation Charts, Isaac Pitman, London, 1963.

(20) Kuong, J.F.: Applied Nomography, Gulf Publishing Company, Houston, 1965.