SHORT COMMUNICATION

A NOMOGRAPH FOR CALCULATING THE OPTIMAL FREQUENCY FOR
DIELECTROPHORESIS AND THE CHARACTERISTIC FREQUENCY OF
THE \( \beta \)-DISPERSION OF CELL MEMBRANE VESICLES

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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 be 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...
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 \(\alpha\)- and \(\beta\)-dispersions \((2,6-8)\). Our
attention is here focussed on the more rapidly relaxing \(\beta\)-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 \(r\ \mu\text{m}\);
(b) that, in contrast to, say, skeletal muscle \((11)\), the electrical
conductivity of the cell interior is isotropic, and of magnitude \(\sigma_i\ \text{mS/cm}\);
(c) that the protoplasts are immersed in a medium of bulk conductivity
\(\sigma_0\ \text{mS/cm}\), 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 \(C_m = 1 \ \mu\text{F/cm}^2\).

The last assumption deserves comment. The static membrane capacitance as
measured with transmembrane electrodes is often as low as 0.5-0.6 \(\mu\text{F/cm}^2\)
\((8,12)\). 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 \(\beta\)-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 \((f_c = 1/2 \pi r, \text{ where } f_c = \text{the characteristic frequency})\), is given by (6):

\[
r = rC_m[(1/\sigma_1) + (1/2\sigma_0)]
\]

If \(r\) is in \(\mu\)m, \(\sigma_1\) and \(\sigma_0\) are in \(\text{mS/cm}\), \(f_c\) is in kHz and \(C_m = 1 \mu\text{F/cm}^2\), equation (1) may be rewritten:

\[
f_c = 1591.5/(r[(1/\sigma_1) + (1/2\sigma_0)])
\]

or \(f_c = 1591.5B/r\)

where \(B = 2\sigma_1\sigma_0/(\sigma_1 + 2\sigma_0)\)

The units of \(B\) are therefore \(\text{mS/cm}\).

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 \(r, \sigma_1\) and \(\sigma_0\). Thus, our aim here is to provide, for the benefit of workers using the dielectrophoretic technique, a nomograph relating \(f_c\) to \(r, \sigma_1\) and \(\sigma_0\).

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 \(\sigma_0\) axis; (b) the \(f_c\) axis joins the zero points on the \(B\) and \(r\) axes; (c) the angle between the \(\sigma_1\) and \(B\)
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 $\sigma_0$ and $f_c$ is obtained by joining the value for $B$ and $r$. In the example shown, $\sigma_1 = 10$ mS/cm, $\sigma_0 = 0.1$ mS/cm and $r = 5 \mu m$. 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 $\mu$m for $r$; if one wishes to consider larger cells, the scale may be redrawn to read, say, 0-100 $\mu$m, 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 $\sigma_1$ and
$\sigma_0$, 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 $\sigma_1$ and $\sigma_0$, 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 of $r$ crosses the $f_c$ axis gives the characteristic frequency. Thus, for example (Figure 1), if we choose values for $\sigma_1$, $\sigma_0$ and $r$ of respectively 10 mS/cm, 0.1 mS/cm and 5 $\mu$m, 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.

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REFERENCES


