A New Method for the Investigation of Cellular Dielectrophoresis

Jerzy J. Zieliński, Piotr Marszałek

Electrotechnical Research Institute, Department of Fundamental Research in Electrotechnics, 28 Pożaryskiego st., 04-703 Warsaw, Poland

and

Magdalena Fikus

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 36 Rakowiecka st., 02-532 Warsaw, Poland

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A new method and a new type of measuring-microchamber for the investigation of cellular dielectrophoresis is presented. The method is based on the observation of the trajectory of a single cell in gravitational and electric fields being crossed and orientated perpendicularly to the observation direction. By means of this method the whole dielectrophoretic spectrum ranging from negative to positive dielectrophoresis may be easily and quickly obtained. The experiments carried out on different cell types showed that the dielectrophoretic spectra, as well as the dependence of the critical frequency upon medium conductivity and cell size agree well with the predictions of a new model of the dielectrophoretic mechanism proposed by Sauer.

Introduction

Dielectrophoresis is the motion of a neutral or charged particle caused by the action of a non-uniform DC or AC electric field [1]. Qualitative and quantitative dielectrophoretic investigations of living organisms were originally initiated in the 60 s [2, 3]. Their purpose was to gain insight into the physical mechanism of this phenomenon [4–7] (and especially into the dependence of the dielectrophoretic force on frequency) as well as to assess its practical value, *i.e.* in the technique of cell separation [8]. Theoretical works analyzed the effect of an electric field upon a single cell [4, 6, 7] whereas experimental methods measured the size of chains appearing on an electrode surface, but only gave a rough estimate of the force acting upon the cell population. Strong cell interactions precluded explicit verification of theoretical assumptions referring to a single particle model.

New dielectrophoresis models were announced from 1983 [9–11], showing that the previously applied method for calculating the force from the potential electric energy [4, 6, 7] was correct only for particles having negligible losses, and could not be applied in cases of cell dielectrophoresis. Significant discrepancies in forecasts made by the old and new theories are expected especially at low frequencies *i.e.* in the frequency range where the electric force acting on the medium may exceeds that of the force acting on a cell (negative dielectrophoresis). However, no experimental work has yet been made to verify these discrepancies, because the method used so far [1, 12–15] did not permit measurements in the region of negative dielectrophoresis.

It is the purpose of this paper to propose a novel method for observing cell dielectrophoresis and a novel measuring set-up to implement this method. The new method enables all necessary measurements to be made on a single, isolated cell, to obtain a full dielectrophoretic spectrum ranging from negative to positive dielectrophoresis.

Principles of the method

In a majority of the methods used until now, the gravitational settling of cells during dielectrophoresis significantly hindered measurement and analysis of the cell trajectory. The method developed in our laboratory [16] is based on the observation of the trajectory of a single cell travelling in a gravitational field and in a horizontal non-uniform electric field (AC). Motion of the cell is a combination of vertical sedimentation at constant speed and horizontal dielectrophoresis and is observed under a microscope with a horizontal optical axis coupled to a TV

Reprint requests to P. Marszałek.

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camera, VDU and video recorder. The direction of the horizontal path component defines the type of dielectrophoresis: travel in the direction of the $\nabla|\vec{E}|$ vector is positive dielectrophoresis, and travel in the opposite direction – negative dielectrophoresis. The electric field has a specific frequency, called the critical frequency (f_0) [17], at which no horizontal component of the cell motion is present. At f_0 the effective polarizability (the polarizability of the cell less the polarizability of the medium) is equal to zero. The time required by the cell to travel a known distance is measured. The reciprocal of that time at a given electric field frequency is proportional to the effective polarizability of the cell at that frequency, *i.e.* is a measure of the dielectrophoretic force [1, 7, 15]*. Implementation of this method requires the construction a new type of measuring microchamber (Fig. 1), the electrodes of which have to fulfil the following conditions. They must:

- be parallel to each other and to the gravitational field
- be of adequate length to permit a large number of measurements to be made during the gravitational sedimentation of the cell,
- produce a strong, non-uniform electric field with monotonously changing intensity, to ensure the constant direction of the $\nabla|\vec{E}|$ vector over the whole inter-electrode space.

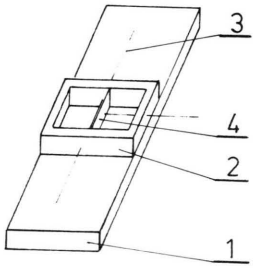

Fig. 1. The measuring microchamber. (1) – microscopic slide; (2) – vessel made of plexiglas (capacity ca. 0.5 cm^3); (3) – wire electrode (Pt), length – 15 mm, $\varnothing = 0.2$ mm; (4) – plate electrode (Pt), $15 \times 3 \times 0.1$ mm. Distance between electrodes – 0.2 mm.

Fulfilment of the last condition permits easy assessment of whether the given motion is equivalent to positive or negative dielectrophoresis. All these conditions are fulfilled by the plate and cylinder system of electrodes shown in Fig. 1. The plate electrode is $15 \times 3 \times 0.1$ mm while the cylindrical electrode is 15 mm long at $\varnothing$ 0.2 mm (distance between electrodes $d = 0.2$ mm). Thus, the mutual sizes and spacing of the electrodes allow the plate electrode to be treated as an infinite conducting plane. The electric field formed here will therefore be identical in distribution with a field formed by two parallel cylinders having a gap of 2 d (mirror symmetry). The intensity, $\vec{E}$, and the gradient, $\nabla|\vec{E}|$, in the cylinder-

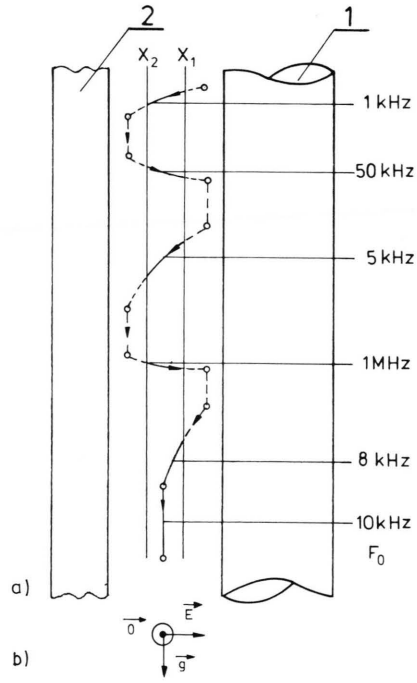

Fig. 2. (a) The principles for determining complete dielectrophoretic spectrum and critical frequency f_0, for a single cell. (1) – wire electrode; (2) – plate electrode. The axes X_1 and X_2 on the VDU screen limit the path in direction $\vec{E}$, over which duration of cell motion is measured. At frequencies corresponding to negative dielectrophoresis (*e.g.* 1 kHz), the sedimenting cell moves towards the plate electrode. A change in frequency to *e.g.* 50 kHz causes movement of cell towards the wire electrode (positive dielectrophoresis). At the critical frequency f_0, cell trajectory is reduced to a vertical line (in Fig. 2 $f_0 = 10$ kHz). (b) Orientation of three vectors: $\vec{g}$ – gravitation; $\vec{E}$ electric field; $\vec{0}$ – observation. $\odot$ – vector perpendicular to the drawing plane.

* Cell effective polarizability in this work was calculated using Sauer's theory [9, 10] using effective electric parameters for one-shell (cytoplasm + membrane) cell model [17, 19]. Electric conductivity and permittivity of the cell interior and its cytoplasmatic membrane were determined earlier [17].

cylinder system are a relatively simple function of the distance from the electrode [18] (when observation of the cell motion is restricted to the symmetry plane of the c-c system; *cf.* Fig. 2). In contrast to the cylinder-cylinder system, where $\nabla|\vec{E}|$ reverses direction at mid-distance between the electrodes, the plate-cylinder system maintains the same $\nabla|\vec{E}|$ vector direction over the whole space between the electrodes.

The course of a typical experiment carried out using the new measuring chamber and new observation method was as follows:

- filling of the microchamber with a suspension containing a low number of cells and placing it on the microscope table in such a way that the electrodes fall in the same direction as the gravitational field,
- selection of one cell in the suspension, preferably found in the upper part of the microchamber
- an a.c. voltage (10^3–10^7 Hz, 4 V_{p-p}), is switched on,
- the whole dielectrophoretic spectrum of the investigated cell is determined by measuring the time required to complete travel between axes X_1 and X_2 at frequencies relating to positive and negative dielectrophoresis (see Fig. 2),
- determination of the critical frequency, f_0.

The method used for determining the full dielectrophoretic spectrum and critical frequency, f_0, for the same cell is illustrated in Fig. 2. The space between axes X_1 and X_2 is situated centrally in the chamber to minimize effects of electrode interference [13]. The ability to induce both positive and (for the first time) negative dielectrophoresis makes reversal of the horizontal motion component possible, and enables multiple measurements of one cell to be made under the same electric field conditions at different frequencies. This, in turn, enables the whole dielectrophoretic spectrum of the cell and its critical frequency f_0, to be established.

The low sedimentation speed (a few μm/s) and considerable length of the electrodes permit up to 20 measurements for one cell to be recorded, this being enough to scan the whole range of frequencies and assess the reproducibility of the measurements at selected frequencies (usually 1 kHz and 1 MHz).

Some Results and Conclusions

The new type of measuring microchamber equipped with the plate and cylinder electrode system and new method of observation were used to investigate the dielectrophoretic parameters of cells of different types (*Neurospora crassa* (slime), cucumber protoplast – cucumis anguria varlongipes). Both positive as well as negative dielectrophoresis was investigated. As motion of the sedimenting cell could be observed over a very long path (limited only by the length of the electrodes), detection of subtle effects of horizontal dielectrophoresis was possible. These effects were of prime importance in precisely determining f_0 because at frequencies close to the critical frequency, polarizability of the cell differs little from that of the medium and the resultant dielectrophoretic force is small.

It was observed that the dependence of f_0 on medium conductivity and cell size agrees well with Sauer's model [9, 10]. Fig. 3 shows an example of the dielectrophoretic spectrum of a *Neurospora crassa* (slime) cell. Culturing and preparation procedures have been described in detail earlier [17, 20]. Fig. 3 also shows the existence of a good correlation between the experiment and the theoretical approach

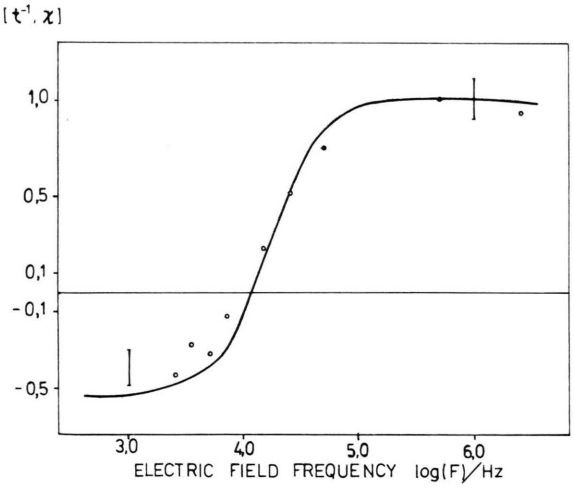

Fig. 3. Experimental (circles) and theoretical (continuous line) dielectrophoretic spectra of *N. crassa* (slime) cell. The effective polarizability (χ) of cell is calculated according to Sauer's model [9, 10] upon application of the electric effective parameters of cell [17, 19]. Experimental points are expressed as cell movement time reciprocals which are proportional to χ. They were assigned a sign consistent with that of χ. For comparison both values were normalized to unity. All experimental points, including those used for evaluation of reproducibility of the results (vertical bars at 1 kHz and 1 MHz represent the ranges of 4 results) were obtained by observation of the same, single cell. Cell radius – 10 μm, medium conductivity – 4.7 mS/m.

of Sauer over the whole range of frequencies investigated, including those of positive and negative dielectrophoresis.

The results obtained confirm the wide applicability of this method in the dielectrophoretic research of living cells. The method is simple and permits a large volume of results to be obtained rapidly (a complete dielectrophoretic spectrum is obtained within about 20 min). As the method ensures measuring conditions very close to the theoretical model, we feel that its application will not only verify the theory of dielectrophoretic mechanism, but will also lead to the refinement of the electric model of cells.

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