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The effect of irregularity on the dielectric dispersion characteristics of spherical cellular suspension

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ABSTRACT

The dielectric dispersion characteristics of cellular suspensions are fundamentally determined based on the analogy to composite dielectric materials when periodically and discrete arrangement of cells is assumed. However, under native physiological conditions, when flocculation and clamping events usually occur, those assumptions are usually not valid. In the framework of this study, an examination of irregularity effect on the dispersion characteristics of spherical cellular suspensions is presented. Here, the permittivity spectra of the suspensions have been determined by both measurements of living K562 cell suspensions and finite numerical simulations. Based on the measured and simulated spectra, the dispersion characteristics of the suspensions, for several destinies and arrangements of cells, have been quantitatively analyzed using the Havriliak–Negami empirical formula. Generally, a strong correlation between the low dispersion characteristics was observed as the concentration and density of the cells was increased. In addition, all characteristics found to be significantly deviated in comparison to the characteristics of a periodically arrayed suspension. However, when low-dense arrangement was assumed, the correlation found to be much lower when all characteristics found to be less perturbated. Based on a simple model of interacting cells, it is suggested that those deviations are related to intercellular interactions between adjacent cells.

1. Introduction

Dielectric dispersion analysis of cellular suspensions is generally based on the analogy to composite dielectric materials. The dielectric characteristics of the suspensions are usually determined using analytical mixture formulas which are based on the effective medium theories or periodic arrangement of the inclusions [1–4]. The validity and applicability of those formulas have been confirmed for several regular structures of dilute mixtures [5,6]. For random mixtures, those are found to be quite effective at low concentration of inclusions but usually inefficient for high concentrations [5,6].

When a disordered arrangement of inclusions is considered, the probability of percolation and multipole interactions is usually getting higher [5,7,8]. Based on the packing density of the inclusions, percolating clusters which can influence the overall dielectric characteristics of the mixture can be formed. This behaviour is well characterized by a percolation threshold which depends on the geometry and the conduction of the mixture [9,10]. In the case of high volume fraction of inclusions, both the packing density and the structure of the composite become quite significant. Characteristics like morphology, distribution and cohesion force between adjacent inclusions can influence the filler content and isotropy of the mixture. In this case, when the percolation threshold is nearly achieved, most of the mixed dielectric formulas fail to simulate the true behaviour of the mixture. On the other hand, several theories have been elaborated by number of authors which can be used to predict the physical behaviour of certain mixtures [4,11].

As opposed to the intensive research done at the field of composite materials, the interpretations and expansions of the above realizations to the case of cellular suspensions were remaining quite limited. The dielectric characteristics of cellular suspensions are mainly characterized by β dispersion mechanism which is accounted for the Maxwell–Wagner effect (interfacial polarization) [12]. The suspension is considered as random composite when the complex permittivity can be calculated by assuming the analogy to equivalent periodic material with identical inclusions [13,14]. However, more precisely, when random distribution is considered within physiological suspensions, it is normally highly irregular. Under native physiological conditions, when flocculation and clamping events usually occur [15], the assumption that the cells are periodically arrayed (within the suspended medium) should be

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carefully considered. Since the cells are free to "move" within the medium, it is quite possible that under certain conditions (e.g. high cell concentration) the arrangement of the cells will not be uniform. For example, some cells can be arranged in clusters while other will remain isolated. Since the analogy to composite dielectric materials is generally based on periodically arrangement of inclusions, in the case of cellular suspensions this assumption is not quite trivial. This issue can introduce severe errors especially when attempting to probe the dielectric properties of cells under those conditions [16].

Giving these realizations, in this study, the effect of irregularity within spherical cellular suspensions was examined. The essence of the study is based on K562 cells (Human Erythroleukemia derived Cell line). These spherical like cells usually exhibit conservative structure when found in suspension, and therefore, can serve as an excellent model for dispersion analysis. Using dielectric spectroscopy (DS) technique, the permittivity spectra of K562 suspensions have been determined for several cell concentrations. Based on the dielectric properties of the cells, random arrays of double-shelled spheres were generated and used to imitate the disordered arrangement of the cells within the suspended medium. By applying a computational solution of a complex potential problem using the Boundary Element Method (BEM) [17], the permittivity spectra of the arrays were numerically calculated for several concentrations and different packing densities of inclusions. Based on the measured and simulated spectra, the corresponding dispersion characteristics of both the measured suspensions and the simulated arrays have been estimated using the Havriliak–Negami empirical formula [18]. The probed characteristics were than compared against the dispersion characteristics which have been extracted based on the permittivity spectra of a periodically arrayed suspension. This comparison allows quantitative and statistical examination regarding the deviations occurred in the dispersion characteristics of suspended cells in respect to their spatial arrangement within native physiological suspensions.

2. Methods

2.1. Experimental procedures

2.1.1. Cell cultures

K562 cells were cultured in Dulbecco's Modified Essential Medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS), 1% glutamine and 1% antibiotics, and maintained in 5% CO_2 at 37 °C. Microscopy observations were carried out using inverted fluorescent microscope (Zeiss, Axiovert 200M). Images were acquired with an Axiocam MRm monochrome CCD camera. The CCD camera drive and color acquisition were controlled by Axiovision 4.4 Imaging System.

2.1.2. Permittivity measurements

Permittivity measurements of K562 cell suspensions were carried out within a frequency range of 1 kHz up to 100 MHz using 4294A Precision Impedance Analyzer (Agilent technologies) at voltage amplitude of 100 mV. All measurements were carried out using Teflon cell, total volume of 750 μl using oblong parallel-plate Pt black coated electrodes. In order to minimize fringing effects, all measurements were based on the three-terminal method [19]. Electrodes were tight to the condenser walls using Teflon screws in order to avoid gap capacitances. The measured air capacitance was 0.26 pF.

The Teflon cell was cleaned before each measurement using Piranha (1:3, 30% H_2O_2, 96% H_2SO_4) during 20 min. Cleaning of electrodes was performed in 1 M H_2SO_4 by passing cathodic current, 10 mA/cm^2 during 1 min. The durability of electrodes was tested before each measurement in order to avoid degradation of the platinum black which deteriorates with sequential use. The polarization capacitance of unused electrode in 1 M H_2SO_4 was found to be 24 mF/cm^2 (using C–V method in double layer region, V = 50 mV/s); the roughness factor (surface increase coefficient) was approximately 500. When the polarization capacitance was found to be significantly lower (approximately 5 times), the electrodes were replaced.

At the beginning of each set of measurement, total cell counts were performed using hemocytometer cell counting chamber. The cells were transferred into a sterile centrifuge tube and harvested at 1500 rpm at 4 °C for 7 min. The harvested cells have been resuspended in 6 different eppendorf tubes (total volume of 750 μl) in specific volumetric concentrations of P = 0.01, 0.05, 0.1, 0.15, 0.2 and 0.3 respectively. Each cell suspension has been transferred into the measuring cell immediately before the measurement. All measurements were performed at room temperature (25 °C); the measured data was corrected for leads/cell inductance and polarization impedance according to a method reported previously [20]. In addition, non-linearity of current was checked to avoid errors in correction of polarization effects which can have large impact on the measured data [21]. The measured permittivity spectrum was extracted from the corrected impedance data based on an equivalent circuit of the condenser. The extraction procedure can be found in previous work of the authors [20].

2.2. Modeling and computing procedures

2.2.1. Cell and array models

A double-shell spherical structure was used as a model for K562 cell (Fig. 1). The outer and inner shells stand for the cell membrane and nuclear envelop while the intermediate and inner cores represent the cytoplasm and nucleoplasm spaces. The outer radii of the shells have been determined using phase contrast microscopy based on the average dimensions of 20 cells and taken to be 6.8 (SD = 0.7) and 5.8 (SD = 0.5) μm. The shell thicknesses have been evaluated based on the average dimensions of several cell types and taken to be 10 and 50 nm respectively [22].

Cell arrays were constructed using cubic unit-cells which assembled to form a matrix of order $5 \times 5 \times 5$. Three configurations of arrays were designed which are accounted for different packing densities of inclusions (Fig. 2); those are based on different dimensions of the unit cells. The arrays were randomly inhabited by double-shelled spherical objects up to specific volume fraction of inclusions P. The number of spherical objects as function of the volume fraction for each of the arrays is given by Table 1. The design and construction of all arrays has been carried out using the graph-

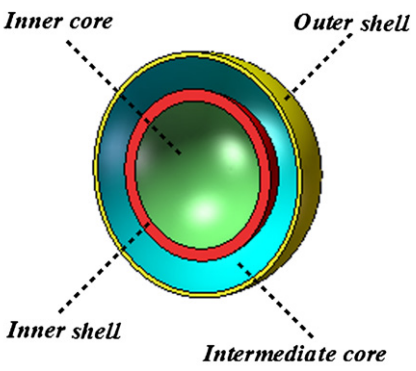

Fig. 1. Spherical double-shell model used to approximate the morphology of K562 cell.