Assessment of suspension medium conductivity by means of micro electrodes

Maximilian Westenthanner¹, Andreas Barthel¹, Ping He¹,², Dieter Beckmann¹, Arndt Steinke², Dr. Ingo Tobehn², Dr. Thomas Frank², Uwe Pliquett¹

¹Institut für Bioprozess- und Analysenmesstechnik, Heilbad Heiligenstadt, Germany
²CiS Forschungsinstitut für Mikrosensorik und Photovoltaik GmbH
³Southern Illinois University, Carbondale, IL, USA

Email: maximilian.westenthanner@iba-heiligenstadt.de

Abstract. Electrolytic conductivity of cell-based suspensions is an important parameter which can be easily and non-destructively measured as part of the electrical impedance. The low frequency conductivity of a cell suspension with very low cell density equals nearly the medium conductivity. However, a high cell density decreases the low frequency conductivity due to the insulating behaviour of the cell membranes. Here we use miniaturized electrode structures, smaller than the size of typical cells for impedometric conductivity measurement which allows an impedance measurement independent of the electrical properties of suspended cells or particles.

1. Introduction

There are many applications of impedometric monitoring, where not the cell membranes are the primary structure of interest, but the conductivity of the medium surrounding the cells. Typical examples are the monitoring of cellular growth in batch reactors, dialysis control [1], or diagnosis of dairy cattle mastitis. The last application uses the fact that the conductivity of milk from infected tips shows an elevated conductivity [2]. In case of low cell density, sufficiently low that the polarization of the cells (ß-dispersion) does not influence the measured impedance, the medium conductivity can be easily measured even with complicate electrode geometry. This approach needs only calibration with an electrolyte of known conductivity. This results in the bulk conductivity, which equals at low concentration the conductivity of the suspension medium. Since there is a functional dependence of the low frequency conductivity of a cell suspension, the density of cells and their passive electrical properties, it is theoretically possible to derive the medium conductivity from the spectrum within the ß-dispersion by using proper modelling. However, this approach depends on multiple factors and is critical if for instance the cell density of the cell size is poorly determined or simply unknown.

Another critical point is the homogeneity of the suspension. If the impedance is for instance measured in the presence of gas bubbles or additional particles, the selectivity in terms of medium conductivity vanishes. A way out is centrifugation and subsequent conductivity measurement using well calibrated equipment. This however, is not an option for inline measurement like for the detection of mastitis or monitoring of bioreactors.

However, even without excessive preparation, a conductivity measurement which is unaffected by cells can be achieved, if the electrode structures are smaller than the dimensions of the cells. Therefore the electrical field extension is limited to the bulk between the electrodes, a space free of cells. Moreover, due to the small dimension, a fast equilibration of the ion content between this region and the bulk electrolytes would be expected.
Figure 1 shows the principle of a microelectrode, where the main electric field extends into the suspension less than the size of a cell.

Figure 1A: scheme of a two-electrode microstructured electrode. The electrical field is concentrated between two electrodes (1) which results in a negligible sensitivity of the electrodes within the bulk electrolyte (2). This makes the electrodes essentially insensitive to cells (3). B: Microstructured gold-on-glass electrode chip with 24 individually contactable electrodes. The electrodes have a width and space of 5 µm.

The critical point of such a structure is a high sensitivity for the conductivity of electrolytes but a negligible sensitivity with respect to the presence of particles or cells.

1 Methods

Impedance spectra are recorded using different methods and devices. A phase sensitive multimeter (NumetriQ-PSM1735, Newtons4th Ltd, UK) in combination with a computer assessable multiplexer/frontend combination is used for frequency domain measurements. Other measurements are done in time domain by using a square wave generator (500 Hz, 2 ns rise time) for excitation and an oscilloscope (8GHz) for tracing current and voltage between the electrodes. An almost perfect match to the electrical behaviour of the electrodes was achieved using a custom built front end.

Unfortunately, the multiplexer made from solid state switches (ADG333) caused non-acceptable signal distortion. Since new multiplexer is under development, we undertook several measurements without multiplexing, by directly contacting the electrodes. This increased the sensitivity of the measurement but fortunately, did not change the overall outcome. To get the impedance spectra, the input (excitation) and the output (response) were transformed by the fast Fourier Transform algorithm (FFT) into frequency domain. The quotient FFT(U) / FFT(I) yielded the impedance spectrum [4].

Fig. 2: Impedance spectra of a KCl dilution series, (left) magnitude, (right) phase angle

To evaluate the sensitivity of the system with respect to electrolytic conductivity, the smallest available electrode distance of 5 µm was selected to assess the impedance spectrum of varying concentrations of KCl from 0.25 mM to 300 mM.

The sensitivity for cell based suspensions is evaluated using model suspensions in PBS dilutions. Yeast (Saccharomyce Cervisiae) suspended in 20 mM PBS from $10^7$ to $10^9$ cells/ml and pig erythrocytes from $4 \times 10^4$ to
2.5x10^9 cells/ml are used. The iso-osmolarity between the cytosol and the electrolyte was adjusted by adding sucrose.

2 Results

Two adjacent electrodes with a distance of 5 µm were directly contacted and the impedance spectra was taken using a time domain approach. As shown in Fig. 2, the impedance spectra of a miniaturized electrode pair is influenced by electrode polarization over the full accessible frequency spectrum. The sensitivity of the electrode to the electrolytic conductivity is evident for the whole frequency range. As seen in Fig.3, the electrode polarization yields a non-linear characteristic, especially for the low frequency range.

![Fig. 3: Conductance with respect to the electrolytic conductivity at different frequencies revealing the sensitivity of the 5 µm structured micro electrodes.](image)

The measurements are corrected for the electrode resistance.

To test the hypothesis of dramatically decreased sensitivity to cells and particles, model suspensions of different density were used. Besides polystyrene spheres (12 and 25 µm diameter) and glass spheres (5 – 150 µm), yeast and erythrocytes are used in 20 mM PBS and 140 mM PBS. Fig.4 shows the sensitivity of the 5 µm structured micro electrodes to yeast cells.

![Figure 4: Conductance of cell suspensions with different cell density in PBS at different frequencies. A: 20 mM PBS; B: 140 mM PBS](image)

By visual inspection, 140 mM are nearly insensitive to yeast cells, ranging between zero and 10^9 cells/ml. 20 mM PBS shows still some effect of the suspended cells on the conductance. Using the same setup, pig erythrocytes in osmotically balanced PBS (20 mM and 140 mM) were investigated (Fig.5).
While cells in 140 mM PBS influence the impedance of the suspension only negligibly, a more pronounced effect was found for the cells suspended in 20 mM PBS. Although the medium conductivity was determined after centrifugation we cannot completely rule out the influence of dying cells during the measurement as well as the a kind of shadow effect arising from very small cells (< 5 µm) covering the electrode partially.

3 Results and Discussion

Impedance spectroscopy with electrodes having a dimension below this of the suspended cells or particles yields predominantly information about the medium conductivity. Here we showed the example of 5 µm electrodes in the presence of Yeast and Erythrocytes, both slightly bigger than the electrode structure. These electrodes are sensitive for conductivity, even in a range where the electrode polarization dominates but they are essentially insensitive to the presence of cells and particles.

4 References

[1] Di Filippo S., Pozzoni P., Manzoni C., Andrulli S., Pontoriero G., Locatelli F., Relationship between urea clearance and ionic dialysance determined using a single-step conductivity profile; Kidney Int.; 68(5), 2389-95, 2005
[2] E. Norberg, Electrical conductivity of milk as a phenotypic and genetic indicator of bovine mastitis: A review, Livestock Production Science, 96(2-3):129-139, 2005
[3] R. D. Fomekong, U. Pliquett, F. Pliquett, Passive electrical properties of RBC suspensions: changes due to distribution of relaxation times in dependence on the cell volume fraction and medium conductivity, Bioelectrochemistry and Bioenergetics, 47-1, 81-88, 1998
[4] U. Pliquett, E. Gersing, F. Pliquett, Evaluation of Fast Time-domain Based Impedance Measurements on Biological Tissue, Biomed. Technik, 45(2000), 6-13, 2000