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DETECTION OF MICROBEADS IN SELF-DEVELOPED IN VITRO BIOIMPEDANCE ASSAY - A PROOF OF PRINCIPLE STUDY

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Abstract:
Research on micro- and nanoplastics is attracting greater interest. As current studies have shown, their presence is in environmental elements and living organisms, thus investigating their toxicity is of crucial concern. In previous work, the authors have developed a unique bioimpedance spectrum (BIS) measurement technology to study the biological processes of cell cultures in vitro. Exploiting the electrical properties of the material composition of plastics, it seems trivial to investigate how the BIS technique can detect them. Therefore, BIS data of plastic beads of 6 μm diameter were recorded at different concentrations. The correlation between microbead concentration and BIS data is established. The proof-of-principle measurements are successful and the technology is ready for testing with live cells.

Keywords: *Microplastics, In vitro assay, Bioimpedance spectrum, Sensitivity, Correlation*

Introduction

Microplastics (MPs) are ubiquitous in the environment and their widespread presence in water, air and soil has led to significant environmental problems nowadays [1,2]. MP accumulation has been shown to adversely impact fertility, development, metabolism, and immunity across various species [1-4]. Consequently, the accurate

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detection of microplastics in environmental samples is critical for assessing their ecological and human health risks. Conventional analytical methods, such as Raman spectroscopy, Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM), require expensive equipment and sample preprocessing, making them highly expensive, time-consuming and and limits their field application [5]. Therefore, there is still a need for techniques that can cost-effectively and rapidly quantify the presence of microplastics in environmental samples.

Biompedance spectroscopy (BIS) offers a promising method of analysing microplastics in liquid samples. [5-7]. BIS can detect the presence of microplastics by measuring changes in the electrical properties of the sample. The method does not require sample preparation, thus providing fast, cost-effective real-time field measurements.

Authors have introduced and validated a self-developed, four-electrode BIS system and associated measuring plates, which have previously been used in live cell assays [8,9]. The authors aim to be able to detect the presence of MP in the extracellular or even the intracellular space using the in vitro BIS system. Therefore, in this paper, the use of the self-developed BIS system for microplastic quantification in fluid samples containing different known concentrations of microplastics is demonstrated. Since the intend is to use BIS measurement procedure for a live cell assay in future studies, the MP concentrations were chosen to represent low, medium and high exposure levels in the human organs, for instance liver [10]. Experiment indicate that the proposed method has no physical limitations to make it suitable even for field measurement of environmental fluid samples or in vitro live cell assays.

1. The developed BIS measurement technique

The BIS measurements are performed using a self-developed, modified four-electrode technique introduced by Vizvari et al [9]. This technique suppresses errors from excitation electrodes and residual impedances by utilizing common-mode rejection. Common-mode rejection is based on the generator connected directly to one electrode, while the ground is linked to the other electrode through a resistor, causing the potential values shift by a constant value. Digital compensation and division

during impedance calculation eliminate errors in the symmetrical measuring circuit. The principle of the self-developed in vitro BIS technique is illustrated in Figure 1. (in the case of the experiments presented in this paper, microbeads are used instead of cells).

As shown in Figure 1. a series resistor (R_{ref}) shifts the voltage of the measuring cell by a constant value, causing the potential of the excitation electrodes (E1 and E4 in Figure 1a) to non-zero potentials, developing an electrical double layer on the both of the electrodes. The current flowing between the voltage generator (E1) and electrode E4 is the result of the potential difference between the generator and the electrode E4. In Figure 1b, these electrical double layers are represented by the simplified Randles circuit elements Z_{CPE1}, R_{E1}, Z_{CPE4}, R_{E4} and Z_S. The impedance Z_S symbolizes the impedance of the solution placed in the Petri dish, while the impedance of the Constant Phase Elements (CPEs, Z_{CPE1} and Z_{CPE4}) presents the capacitance arising at the electrodes. Since the double layer effect is strongest near the electrodes, the measuring electrodes (E2 and E3) are placed separately (Figure 1a).

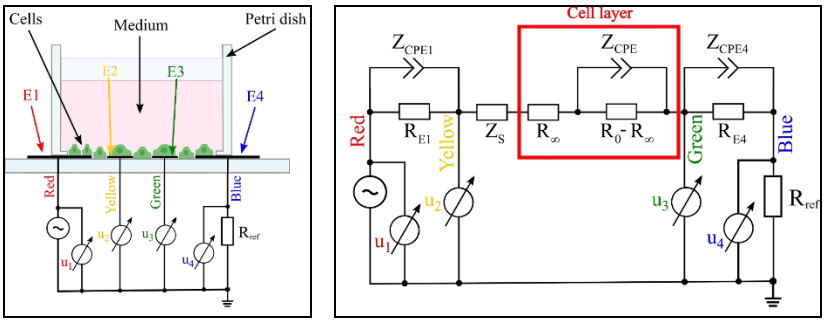


Figure 1. *Schematic drawings of the self-developed in vitro BIS technique application in case of (a) the self-produced graphene plates ; (b) the equivalent circuit model [9]*

The spectrum of the measured sample (Z) at each frequency point is calculated using the potential values u_2, u_3 and u_4 using the following formula [9]:

$$Z = R_{ref} \frac{u_2 - u_3}{u_4} \tag{1}$$

Model-based evaluation is preferred to evaluate the measured spectra, allowing the study of the dielectric properties of biological structures. For this purpose, the most commonly used model is the Cole-Cole model, which describes the frequency domain behaviour of biological structures. The Cole-Cole equation describing an equivalent circuit can be expressed as follows [9]:

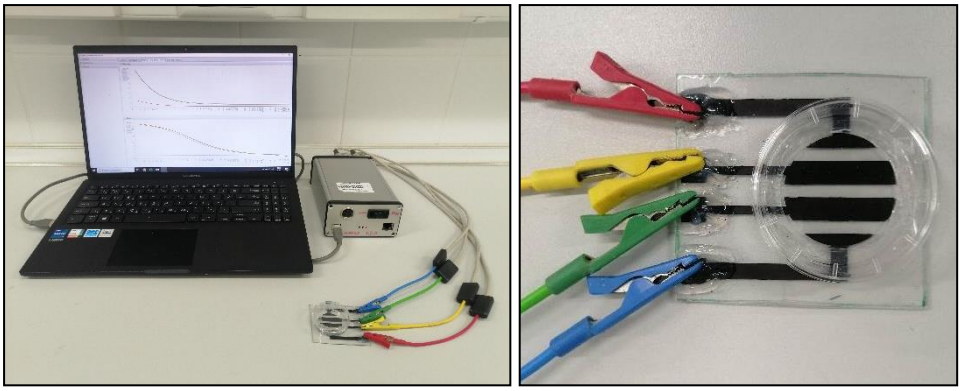
$$Z(j\omega) = R_{\infty} \frac{R_0 - R_{\infty}}{1 + (j\omega\tau)^{\alpha}} \tag{2}$$

where Z($j\omega$) is the complex impedance, R_{∞} is the resistance corresponding to the ∞ frequency, R_0 is the resistance corresponding to 0 Hz frequency, τ is the time constant, α is the exponent parameter ($0 < \alpha \leq 1$), ω is the angular frequency, and $j=\sqrt{(-1)}$.

2. Experiments and Results

MPs of around 10 μm are able to enter the human circulatory system and are transported to various organs where they accumulate [10]. Considering this, polystyrene (PS) microbeads with an average particle size of 6 μm were used as MP samples in the experimental work. Working solutions with varying MP concentrations were prepared in physiological saline solution with final concentration 0, 20, 40, 60, 80 and 100 μg/ml PS microbeads. The density of PS microbead is 1.05 g/cm^3, therefore the MP concentrations in the solutions are 19, 38, 57, 76 and 95 ppm.

The self-developed BIS measuring plates consist of graphene electrodes painted on 1 mm thick polycarbonate sheets. Graphene was chosen as the material of the electrodes mainly due to its advantageous properties of measurement and biological point of view. 0.5 mm silver wire was applied to the graphene painted surface, allowing easy attachment of the cables to the electrodes during the measurement. Bottom-open Petri dishes were fixed to the polycarbonate sheets as sample containers. The assembled measuring system with the self-made measuring plate is shown in Figure 2.



(*a*) **(*b*)**

Figure 2. *BIS experimental setup: (a) assembled measuring system; (b) connected BIS measuring plates*

During the BIS measurements, a 2,5 ml of solution from each of the six concentrations is placed into the actual Petri dish before the BIS measurements are started and repeated five times in succession. The measurements were carried out in the frequency range 0.1-10kHz repeated on six different plates.

To evaluate the results of the BIS analysis, a standard single-dispersion Cole–Cole model is used, and by extracting the R_0, R_∞, τ, α model parameters, the possibility is gained to compare the solutions with different MP concentration based on the model. A Z-test was used to identify outliers, after which the outliers were excluded from further analysis. Correlation values are calculated using the Pearson correlation coefficient. Comparison of R_0, R_∞, τ, α values for each solution shown in Figure 3.

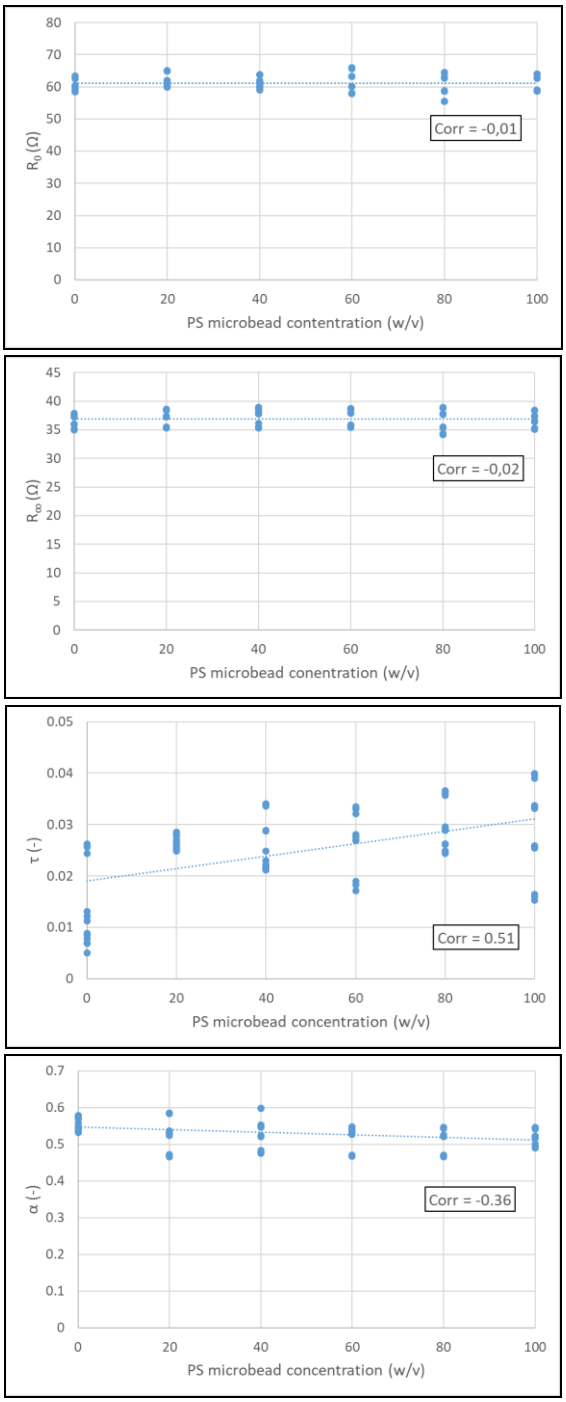


Figure 3. *Comparison of R_0, R_∞, τ, α values for each measured solution*

The diagrams grouped by parameters represent BIS measurements as individual points. Additionally, each diagram includes a linearly fitted trendline and the value of the Pearson correlation coefficient.

The results of the R_0 parameter extracted from the corresponding BIS data are introduced in the first diagram in Figure 2. The Pearson correlation coefficient between the resistance values measured at 0 Hz (R_0) and the concentration of PS microbead is -0,01, suggesting no correlation. The results of the R_∞ parameter also show no correlation with the concentration of MP (correlation coefficient -0,02). A medium strength correlation with a negative sign exists between τ values and increase in MP concentration, with a correlation coefficient value of -0.51. For the alpha parameter, the correlation coefficient is -0,36, which means that there is a medium correlation between the values of the alpha parameter and the concentration of the MPs.

3. Conclusions

This article presents a proof-of-principle study of correlation between BIS data and different concentrations of PS microbeads in physiological saline solutions. The aim of the study is to find out whether the developed BIS measurement method is suitable to detect and quantify microplastics even at low concentrations (>100 PPM). In order to demonstrate the beneficial properties of the proposed technology and the BIS data, basic outlier filtering method and Pearson correlation test are performed. The statistical results of collected BIS data in Figure 3 show that for the Cole-Cole parameters, τ and α values are moderately correlated negatively with the increase in PS MP concentration, while while there is no correlation find in R_0 and R_∞ parameters. These results suggest that, BIS measurements, by examining the τ and α Cole-Cole parameters, appear to be a promising method for quantifying microplastics in fluidic environmental samples and live cell assays. The results also encourage us to initiate the development of specific MP microsensors based on the BIS technique. Moreover, these results may contribute to understand the effects of microplastics on human health through in vitro studies.

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REFERENCES

[1] Lamichhane, G., Acharya, A., Marahatha, R., Modi, B., Paudel R., Adhikari, A., Raut, B. K., Aryal, S., Parajuli N. (2023). Microplastics in environment: global concern, challenges, and controlling measures. *Int. J. Environ. Sci. Technol.* 20, 4673–4694. https://doi.org/10.1007/s13762-022-04261-1

[2] Loganathan, Y., Kizhakedathil, M. P. J. (2023). A Review on Microplastics – An Idebible Ubiquitous Pollutant. *Biointerface Research in Applied Chemistry* Vol 13 (2), 126. https://doi.org/10.33263/briac132.126

[3] Vethaak, A. D., & Legler, J. (2021). Microplastics and human health. Science, 371(6530), 672–674. https://doi.org /10.1126/science.abe5041

[4] Domenech J., Annangi B., Marcos R., Hernández A., Catalán J., (2023). Insights into the potential carcinogenicity of micro- and nano-plastics. *Mutation Research/Reviews in Mutation Research* 791, article 108453, https://doi.org/10.1016/J.MRREV.2023.108453, 36739075

[5] Sarmiento, J., Anaya, M., Tibaduiza., D. (2024). Microplastic identification using impedance spectroscopy and machine learning algorithms. *International Journal of Distributed Sensor Networks* 2024 (1), article 5298635, https://doi.org/10.1155/2024/5298635

[6] Colson, B. C., Michel P. M. A. (2021). Flow-Through Quantification of Microplastics Using Impedance Spectroscopy. ACS Sensors 2021 6 (1), 238-244. https://doi.org/10.1021/acssensors.0c02223

[7] Gomes, D., Magalhaes, S., Rasteiro, M. G., Faia, P., (2024). Measuring mircoplastic concentrations in water by electrical impedanca spectroscopy. *Water* 16 (22) article 16223228. https://doi.org/10.3390/w16223228

[8] Vizvari, Z., Gyorfi, N., Odry, A. et al. (2020). Physical Validation of a Residual Impedance Rejection Method during Ultra-Low Frequency Bio-Impedance Spectral Measurements. *Sensors* 20(17), 4686, https://doi.org/10.3390/s20174686

[9] Vizvari, Z., Gyorfi, N., Maczko, G. et al. (2024). Reproducibility analysis of bioimpedance-based self-developed live cell assays. Sci Rep 14, 16380, https://doi.org/10.1038/s41598-024-67061-2

[10] Bristow, P. T. (2024). Microplastic exposure induces hepatotoxicity through oxidative stress: an investigation into the molecular mechanism (Doctoral dissertation, Stellenbosch University)