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# Reply to comment on experimental methods and conclusions of impedance spectroscopy of solutions at physiological glucose concentrations by A. Caduff, M. S. Talary, Y. Feldman

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The comment to the manuscript [1] by Caduff et al. discusses some possible limitations of the study. The first criticism is related to the reported phase values. In science, experiments are carried on to validate theories, and not the opposite. Caduff et al. claim that the phase values are not convincing as they are not in agreement to what one would expect for a RC sensor. The experiments were performed with commercially available equipment of proven quality, especially the impedance analyzer. Thus, obvious reply is that the RC model may be oversimplified for complex systems such those investigated in our study, especially for blood. Furthermore, even in the case that phase values outside $\pm\pi/2$ were incorrect, it must be noted that such values were found only at the low and high ends of the studied frequency band. In our study the findings of major interest were observed in frequency intervals where the phase values were almost zero, thus certainly within what expected from the RC sensor theory (see Figs. 2, 3 and 4).

Second criticism to our study is that the measurement probe was not adequate for electro-impedance spectroscopy (EIS), because it was designed for conductivity measurements. It is right that the probe is usually sold coupled with a conductivity meter, but this is not enough to conclude that the probe cannot be used for EIS. Caduff et al. just claim that the probe and its electrodes are not adequate, but they fail to explain why. Moreover, it must be noted again that our major findings were related to differences in the impedance modulus at frequency values where the phase was small, or even almost zero. This is particularly verified in blood and sodium chloride samples (Figs. 3 and 4), but also for water (Fig. 2) in a still relatively wide frequency interval. Clearly, when the phase was zero the impedance reduced to conductance, and hence the probe was certainly used properly at those frequency values.

The third criticism is that our impedance measurements may be unreliable because of some electrode polarization phenomena. First, we performed four-electrode measurements: Caduff et al. should be aware that this technique strongly reduces the problem of electrode polarization [2,3]. Furthermore, it is well known that the electrode polarization is more relevant at low frequency values. In the study [4], the electrode polarization phenomena in the analysis of some biological tissues were considered relevant below 100 Hz and still present up to 1 kHz, but negligible at higher frequencies. In other studies electrode polarization was observed also for higher frequency values, but it is unlikely at frequencies above a hundred of kHz. Moreover, electrode polarization has effects more pronounced on the capacitance rather than the conductance of the investigated medium [4], and in our study the major findings were observed at frequencies where the capacitance was small or also negligible. Finally, we used platinum electrodes, which are less prone to electrode polarization phenomena compared to other materials independently from black platinum covering [2,5,6], though such covering may further reduce the amplitude of the electrode polarization phenomena by a factor of 4 or more [6].

Despite all the considerations above, let's suppose that we underwent some electrode polarization phenomena that affected our results. There are two possible cases:

1) Electrode polarization is independent by the glucose concentration of the samples. This is likely, since electrode polarization should occur only in the presence of ions within a solution, and glucose does not dissociate into ions in solutions (therefore, it does not add new ions to those already present in the solution). In this case, for each series of experiments in our study, i.e. water, blood and sodium chloride, the amplitude of the electrode polarization had to be the same for all the samples. Thus, we might have found

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inaccurate absolute values in the impedance of the samples at different glucose concentrations (due to the possible electrode polarization systematic error), but still the impedance differences between samples would be right. In fact, possible impedance differences between samples were of special interest in our study, and not the absolute impedance values.

2) Electrode polarization is dependent by the glucose concentration of the samples. In this case not only the absolute impedance values, but also the impedance differences between samples might be inaccurate. However, though the impedance differences might be overestimated they still need to be present to some extent. Otherwise, there is no reason for the glucose dependent electrode polarization. In any case, this possibility is unlikely for the reason explained at case 1).

Thus, even in the hypothesis that some of the criticisms by Caduff et al. were right, the main conclusions of our study would remain correct, i.e. glucose directly affects the impedance (at least the conductance) of the investigated solutions at relatively low frequencies, independently from other possible mechanisms triggered by glucose. It is true that the glucose effect may be small; for this reason, we are certainly extremely cautious to suggest that our findings can be used for clinical applications, as we already clearly claimed in the Discussion of our study.

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