

# **A comparison of two-and four-electrode bio-impedance measurements in electrolytes of different conductivity for measurement of cell suspension condition**

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## **1 Introduction**

Dielectric studies of biological material have been of significant importance in revealing electrical properties of biological materials. Two-electrode and four-electrode techniques are both classic methods used in impedance measurement of biological systems. Two-electrode method is widely used in the measurement of dielectric properties of sample due to its simple system setup and ease of application [1]. However, electrode polarization, which is due to the form of charge at the interface between electrode and electrolyte [2, 3], is a remarkable problem in impedance and dielectric measurement of conductive materials, especially in the biological system. The contribution from  $\alpha$ -dispersion and  $\beta$ -dispersion of biological system is overshadowed by electrode polarization. To reduce the effect of electrode polarization on the measurement of conductive sample, especially at low frequency range, a great deal of work has been proposed to alleviate or reduce the electrode polarization influence on impedance measurement. Both four-electrode measurement and mathematical interpretation of electrode polarization turns out to be widely adopted methods [4-6]. Constant phase angle (CPA) has been applied to describe the frequency response of the electrode-electrolyte interface since it could represent the effect of electrode polarization quite well to the change of frequency [4, 6, 7]. Therefore, it is of significance to choose the suitable method for different researches. In this paper, two-electrode and four-electrode measurements were adopted and evaluated from the accuracy and frequency range of results. The purpose of this study is to review and compare these two traditional methods to choose the better method for further study on cell suspension based on current bioimpedance system.

## **2 Material and method**

### **2.1 Preparation of the electrolyte**

NaCl solutions with different conductivity were required for impedance measurement with their conductivity measured by conductivity meter.

### **2.2 System setup**

Solartron 1260 Impedance Analyser was adopted for impedance measurement in this study. A PC was connected with impedance analyser via a GPIB-USB cable to serve as the DAQ module and a LabVIEW programme was used for the interface controlling and functional parameter setting. An impedance measurement chamber was designed and adopted for the measurement of saline and cell suspension. The chamber is 4mm in diameter and 11 mm in

length. Two stainless steel plated disc electrodes are positioned at the ends of chambers which could be used to apply current and voltage as well. Another two stainless needle electrodes were placed 6mm apart on the bottom of chamber which could be used for the measurement of voltage. Two open holes (diameter around 1 mm) were left on the top and used for injecting saline. The sample was swept at 30 frequencies between 10 Hz and 10MkHz within a period of approximately 60 seconds. Amplitude of 10 mV was chosen for the impedance measurements. All the measurements were conducted at  $20 \pm 0.5$  °C.

### 2.3 Data analysis

In order to extract parameters involved in electrode polarization in two-electrode measurement, the raw data of resistance and reactance for saline solutions of different conductivities was fitted to the equations from the electrical model (错误! 未找到引用源。) by MATLAB (Mathworks Inc.). The best-fit parameters were estimated by automatically comparing the theoretical simulations to the real data. The Mean Squared Error (MSE), as given in equation 1, was employed to evaluate the quality of fitting. The less MSE is, the better the fitting quality is. In equation 1, N is the number of frequency points, D is the observed data and  $D_{cal}$  is the output data after function fitting. To fit measurement results into models,  $Z_{real}$  was used, instead of D in equation 1, to fit into the electrical circuit model and physical model, respectively.

$$MSE = \frac{\sum_{i=1}^N (D - D_{cal})^2}{N} \quad (1)$$

### 2.4 Equivalent circuit model

Equivalent circuit model was used for analysis to illuminate the electrode polarization effect on the measurement result (Figure ).The equivalent circuit model for electrode polarization adopted in this study comprised a constant phase angle (CPA) impedance  $Z_{CPA}$  in parallel with  $R_{ct}$ . The CPA impedance is a measure of the non-faradaic impedance arising from the interface polarization, and is given by empirical  $Z_{CPA} = K \times (j\omega)^{-\beta}$  (2).  $R_{ct}$  represents the charge transfer resistance. The resistance of NaCl solution was in series with the electrode polarization model.

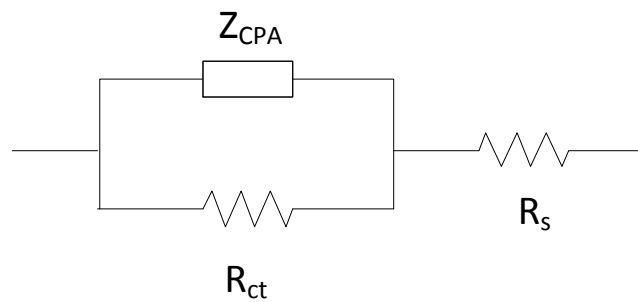


Figure 1. Equivalent circuit model for electrode polarization.

$$Z_{CPA} = K \times (j\omega)^{-\beta} \quad (2)$$

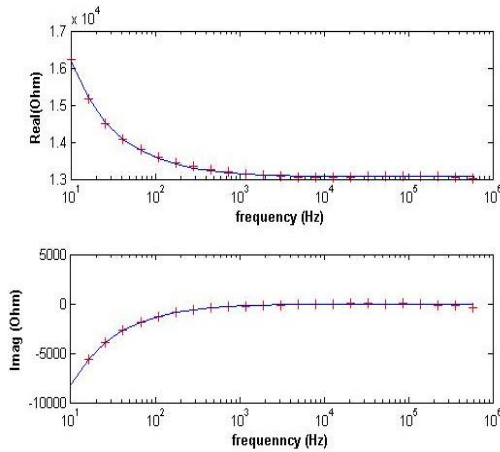
Where  $0 < \beta < 1$  and  $R_{ct}$  is the resistance with that due to faradaic charge transfer and  $R_s$  represents the resistance of the electrolyte.

### 3 Result

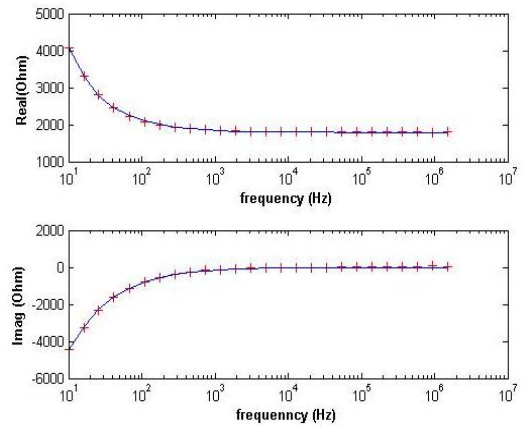
#### 3.1 Electrical polarization

It was observed that there is apparent electrode polarization effect in the frequency range from 10 Hz to several kHz. Moreover, the ratio of max real value to the average value of saline solution is increasing as the conductivity of saline solution increases, which verified that the effect of electrode polarization became more potent as the ion concentration of saline solution increases. Parts of the fitting result of the saline in different conductivity were shown in Figure and the four parameters in the model were extracted and summarized in

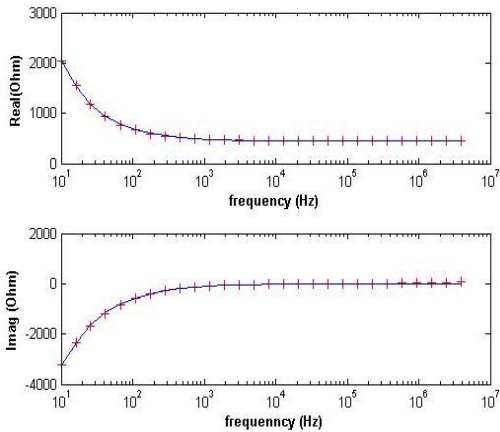
Table . It could be seen from the fitting result that the model with  $Z_{CPA}$  parallel with  $R_{ct}$  turned out to fit the data well.



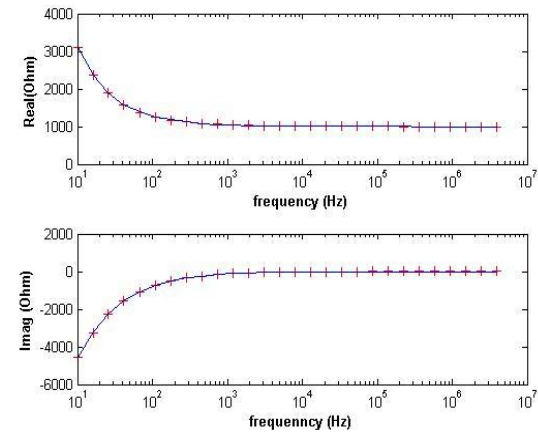
(A) 0.650 mS/cm



(B) 4.70 mS/cm



(C) 8.79 mS/cm



(D) 20.01 mS/cm

Figure 2. Part of fitting results of the electrolyte solution with electrical circuit model. The conductivity is 0.650mS/cm, 4.70 mS/cm, 8.79 mS/cm and 20.01mS/cm respectively for (A), (B), (C) and (D).

Table 1. The fitting results of parameters involved in equivalent circuit model

Conductivity (mS/cm)	Two-electrode measurement				Four-electrode measurement
	K ( $\Omega/s$ )	$\beta$	$R_{ct}$ ( $\Omega$ )	$R_s$ ( $\Omega$ )	$R_s$ ( $\Omega$ )
0.650	2.148e+05	0.773	9.401e+05	1.307e+04	7120.87
1.31	1.090e+05	0.732	6.258e+05	6.701e+03	3591.5
2.64	1.315e+05	0.741	9.401e+05	3.199e+03	1717.4
4.70	1.310e+05	0.773	4.064e+04	1.791e+03	979.7
5.41	1.157e+05	0.712	1.684e+05	1.315e+03	883.7
8.79	1.419e+05	0.795	4.261e+04	999.42	555.7
15.8	8.644e+04	0.767	4.687e+04	578.63	312.2

As observed from

Table , with the increase of conductivity of saline solution,  $\beta$  didn't change with the conductivity and it fluctuated around 0.76 as the conductivity of NaCl changes, which is consistent with the study by Bordi that  $\alpha$ , counterpart of  $\beta$  in this study, was approximately 0.75 to univalent ion while as to the divalent ion, the value amounts to 0.68 when stainless steel electrode was adopted for the measurement [4]. The value of  $\beta$  is completely dependent on the properties of electrode and the saline solution used in the measurement system.

While it can be seen from the result, K changed significantly for electrolytes of different conductivities. The result is consistent with the study by Stoneman *et al* [6]. Since parameter K is actually representing the magnitude of capacitance of the electrical double layer existing at the interface of electrode and electrolyte which is dependent on property of electrode and sample, ion concentrations of buffer according to Gouy-Chapman-Stern model (GCS) [8]. Therefore the value of K varied from sample to samples under the same system.

### 3.2 Evaluation of accuracy of two methods

Evaluation of the fitting result with the real value could be found from the comparison between the resistivity derived from fitted  $R_s$  value and the conductivity as shown in 错误！未找到引用源。 . The theoretical resistivity  $\rho$  was deduced from the measured conductivity  $\sigma$  and the fitting resistivity from fitting result of  $R_s$  due to the relationship between in conductivity and resistivity shown in  $\rho = \frac{1}{\sigma}$  (3).

$$\rho = \frac{1}{\sigma} \quad (3)$$

The

result

from

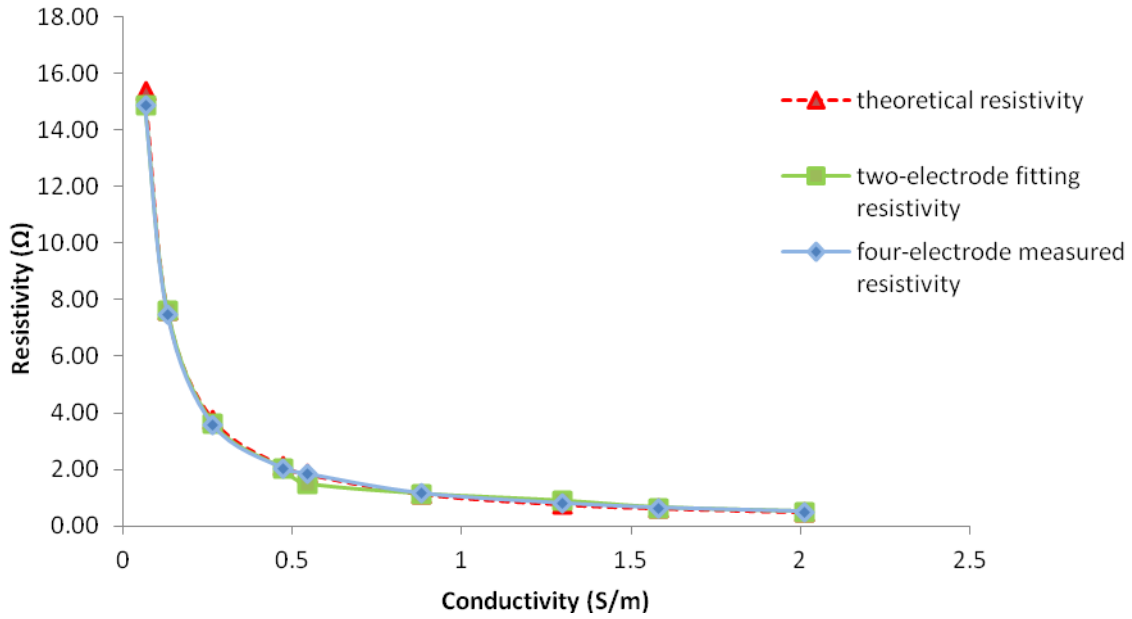


Figure 3 verified the good fitting result which indicated that the both two-electrode measurement together with equivalent circuit adopted and four-electrode measurement could reflect the accurate impedance of samples.

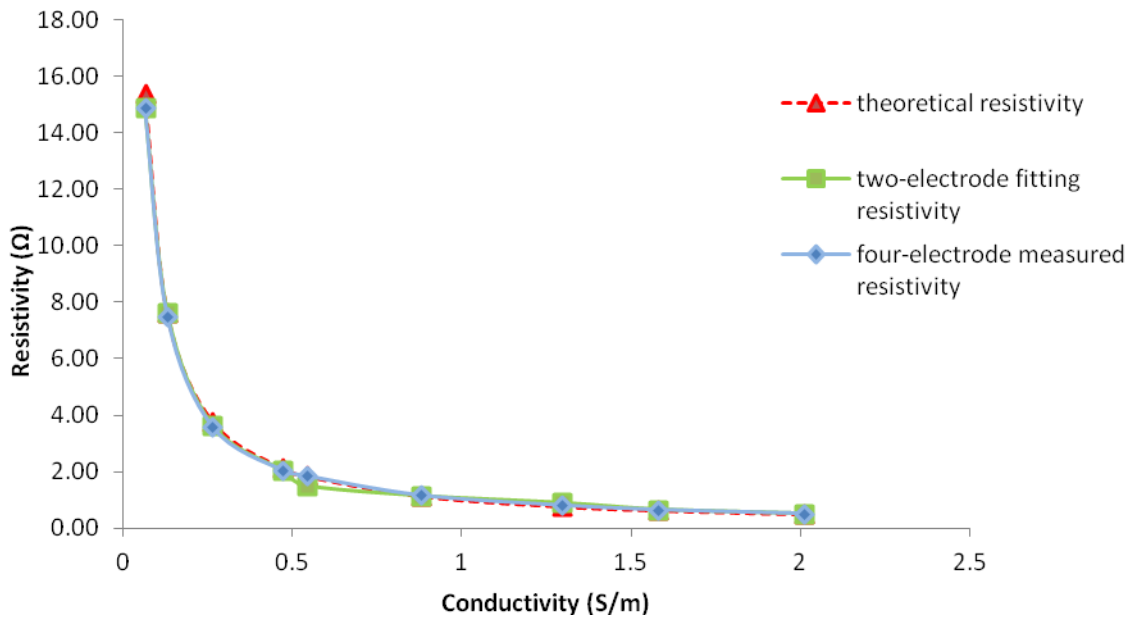


Figure 3. Comparison between the resistivity derived from fitted  $R_s$  value and the conductivity in two-electrode and four-electrode measurement.

### 3.3 Comparison of two-electrode and four-electrode measurement

In two-electrode method, both the current and the voltage are measured by the same two electrode while as to four-electrode method, current is passed through the sample via 2 outer

current electrodes, the potential drop across the sample is measured by the inner voltage electrodes thus the impedance by four-electrode measurement is lower when compared to the two-electrode measurement as to the same sample. The current flows through voltmeter are negligible due to the high impedance of voltmeter. As a result, it is possible to measure the impedance of the biological sample without the contribution of the electrode polarization by four-electrode technique [9].

The frequency range comparison of two methods indicated the bandwidth changes with different loading. The higher loading is, and the narrower the bandwidth to achieve. It was observed that the bandwidth to two-electrode measurement was higher when compared to four-electrode measurement (Figure 4), which is due to the typical “stray capacitance” effect in the measurement system, i.e., cable connected the voltage measurement electrode and voltmeter in four-electrode measurement exhibits the stray capacitance thus leads to the decreasing the impedance and finally results in the narrower bandwidth when compared to the two-electrode measurement.

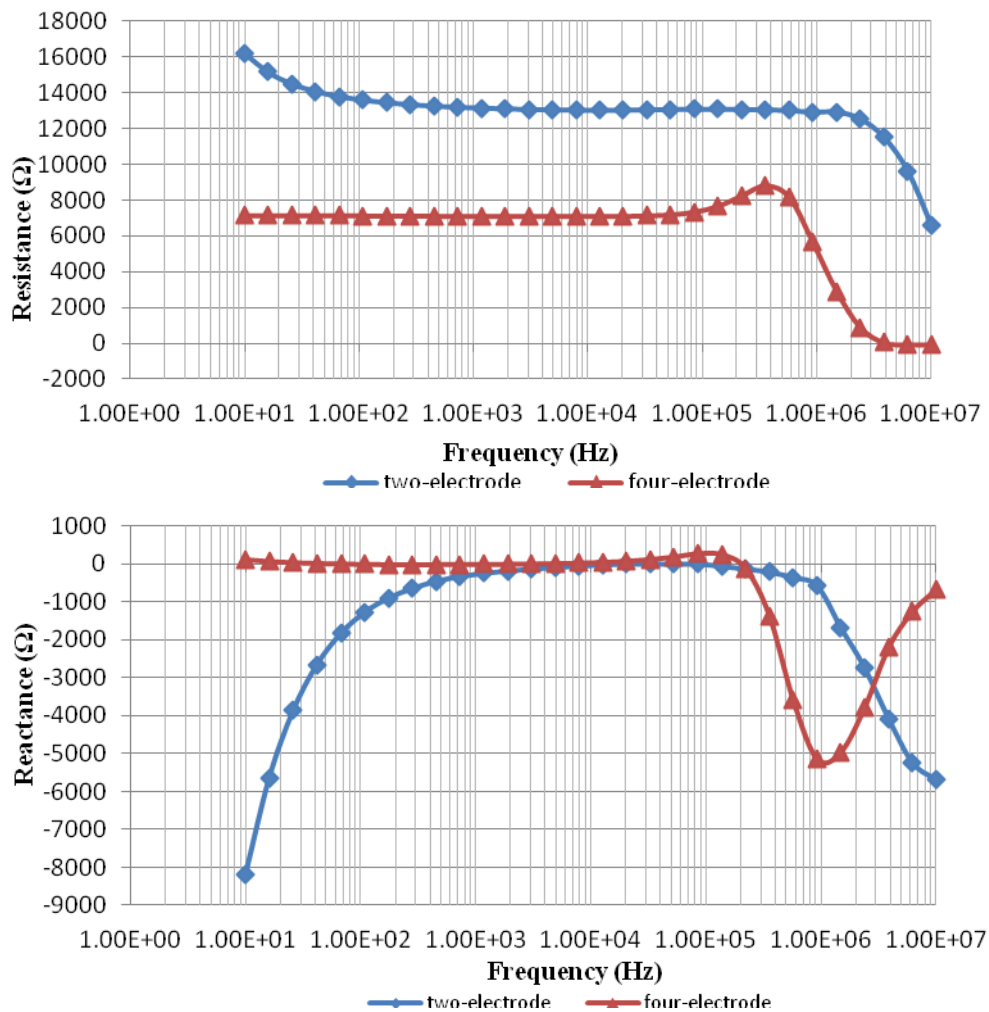


Figure 4. Comparison of frequency ranges by two-electrode and four-electrode measurement of electrolyte of conductivity of 0.650 mS/cm.

## 4 Discussions

Based on the comparison provided above, it was found that both the two methods stand out at different aspects. As to two-electrode measurement, it showed the advantages on the wider bandwidth and relatively simple setup for the system. Our analysis on the electrode polarization showed that the influence of electrode polarization could be reduced to some extent by modelling its effect with CPA. However, the parameters involved were related to many factors which lead to inaccuracy interpretation of the result. Therefore, more studies on effects of different factors to CPA are required before yielding to accurate result. As to four-electrode measurement, the bandwidth is comparatively narrow, which is dominated by stray capacitance in the measurement system. This needs further improvement in the near future. However, four-electrode measurement has demonstrated great advantage in the reduction of electrode polarization thus the complicated interpretation of derived data is not required. The investigation of this study provide a useful reference for studies of cell biological system based on bioimpedance technique, and also provide an reference in choosing the suitable method for cell biology measurement in future.

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