ON THE RELIABILITY AND REPEATABILITY OF BIOIMPEDANCE MEASUREMENTS

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Abstract: Wide and attractive, challenging in many aspects, bioimpedance measurements today reveal an important interest as one among the methods for noninvasive exploration of human body physiological properties or events, "in vivo" or on the species-"in vitro". Measurements are non-invasive, instrumentation is relatively simple and non-expensive, and require not highly skilled staff and medical environment. There is an extremely wide range of diagnostic bioimpedance measurements applications, such as: tissue or organ state and tumours detection, total body composition and fluid compartments, cardiovascular system, respiratory system, state of skeletal muscles, brain impedance, skin impedance, blood and blood flow, imaging techniques (EIT), etc., and whole range of "in vitro" techniques. In our research, we focused interest on measurements on the gymnasts and the patients with ischaemic syndromes in lower legs. We proved that a good "follow-up" of gymnast's training efficiency and an early diagnosis of one leg ischaemia can be achieved. However, it should be emphasized that the main problems concerning bioimpedance measurements are reliability and repeatability of results. Discussing these problems, in the paper some problems of choice between bipolar and tetrapolar techniques, constant current vs. constant voltage measurement techniques and elimination the effect of electrodes impedance are presented.

Introduction

Since the end of the nineteenth century, electrical impedance measurements have been used to study biologic systems, "in vitro" or "in vivo" [1-2]. It could be of interest to reveal that the concept of tetrapolar constant current impedance measurement was introduced in 1884 [3]. Between 1920 and 1950 an important series of fundamental papers was produced [4-7]. From this époque till nowadays, bioimpedance measurements reveal an important interest as a method for noninvasive, simple, and not expensive way of exploration of physiological state of human body (such as tissue or organ state and tumours detection, total body composition and fluid compartments, cardiovascular system, respiratory system, state of skeletal muscles, brain impedance, skin impedance, blood and blood flow, imaging techniques (EIT), etc., and wide range of "in vitro" techniques [8-14].
We concentrated our interest on measurements on the gymnasts and the patients with ischaemic syndromes in lower legs. We proved that a good "follow-up" of gymnast's training efficiency and an early diagnosis of one leg ischaemia can be achieved [15-18]. However, it should be emphasized that the main problems concerning bioimpedance measurements are reliability and repeatability of results. The choice between bipolar vs. tetrapolar & constant current vs. constant voltage measurement techniques seems to be essential as far as reliability, repeatability and reproducibility of results are concerned.

Materials and Methods

In this work, we have concentrated our interest on the comparison of skeletal muscle bioimpedance properties, using two and four electrodes measurement techniques.

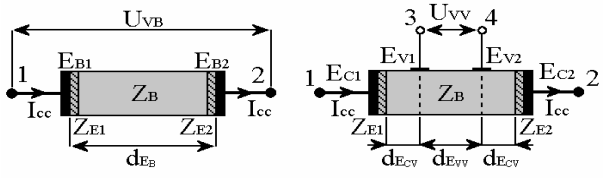

Figure 1: Bipolar (two electrodes) and tetrapolar (four electrodes) technique

Main problems, when bioimpedance of living subjects is concerned, are caused due to the two facts:

$$\mathbf{Z_M} = \mathbf{Z_E} + \mathbf{Z_B} \qquad (1)$$

and

$$\mathbf{Z_M} = f_1 [A, \mathbf{d}] \times f_2 [\rho (\mathbf{J}, \omega), \varepsilon_R (\mathbf{J}, \omega)] \qquad (2)$$

where

- $\mathbf{Z_M}$ = measured impedance (Ω)
- $\mathbf{Z_E}$ = electrode impedance (Ω)
- $\mathbf{Z_B}$ = biological (tissue) impedance (Ω)
- A = cross-sectional area of specimen (cm^2)
- ω = radian frequency (s^{-1})

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J = *current density* (A/cm^2)
d = *distance* between (*voltage, if tetrapolar!*) electrodes (cm)
ρ = specific resistivity of the specimen (Ω cm)
ε_R = relative permittivity of the specimen

Obviously, if we intend to maintain constant and repeatable *current density* (currents up to 1 mA_{RMS}, if sinusoidal), we are limited to the two possible methods: *bipolar* or *tetrapolar constant current techniques*.
Although a lot of books and papers treat problems of $\mathbf{Z_E}$ influence on the *reliability* and *repeatability* of bioimpedance measurements results, when *bipolar technique* is used, only a few serious papers discuss *reliability* and *repeatability problem***,** when *tetrapolar* ("theoretically" more accurate) *technique* is used [19].
The main problem is that, using tetrapolar technique, we can "eliminate" electrode impedance influence, but the results of tetrapolar measurements *depend* on electrode shape, positioning, and primarily of the *distance* between voltage-voltage and voltage-current electrodes. Using bipolar technique, we have a problem of electrode impedance influence, but using *M*ulti*F*requency *BI*oimpedance *A*nalysis (*MFBIA*), or some kind of similar techniques, it can be eliminated (see *Appendix*) in rather satisfactory manner [18, 20].
Measurements were performed with self-adhesive surface electrodes on lower leg (m. gastrocnemius) in healthy males, measuring repetitively three times/week, during 30 days (student's population; using constant current technique 100 μA_{RMS} in the range 0.1-500 kHz, with HP LCR Meter 4284A). Measurements were performed by changing distances (d_{Eb} or d_{Evv}) between E_B (bipolar) or E_V (tetrapolar) electrodes (Fig. 1.) i.e. measuring Z_{m1} and Z_{m2}, bipolarly and tetrapolarly (Fig.2), *and* by simulation on model (U_{m1} and U_{m2} - Fig.4), respectively.

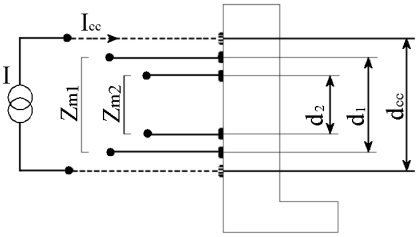

Figure 2: Electrode's position for lower leg measurements

Results were compared for student population between themselves, and with results obtained by modeling.
For modeling, we used EMAS™ (***E**lectro**MA**gnetic Field **S**imulation)* software, using Finite Element Analysis (Fig. 3.), and model shown in Figure 4.
Due to the symmetry of electromagnetic fields, only one quadrant of specimen was simulated with interpolation of data for specific resistivity and relative permittivity following equations, adapted from [9],
$\rho\,(f) = 590{,}67\, e^{-0{,}0012\, f}$ (Ω cm)
$\varepsilon_R\,(f) = 70{,}718\, e^{-0{,}0036\, f}$

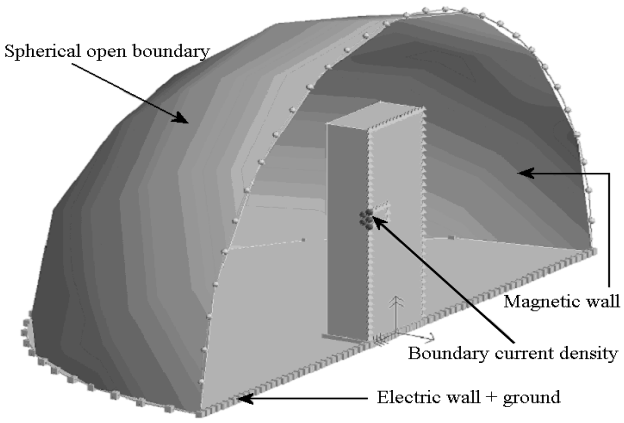

Figure 3: EMAS formal model

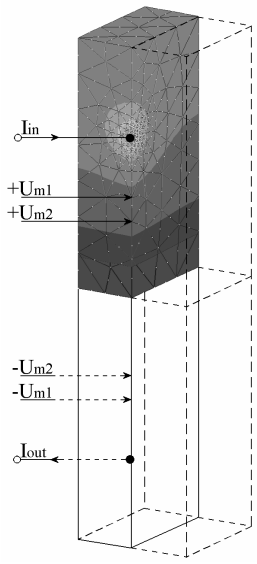

Figure 4: EMAS simulation for bipolar and tetrapolar methods of measurements (calculations were performed only for one fourth of the simulated object, and results were obtained using boundary symmetry)

Results

Sensitivities to electrodes placing (distance&position) changes were estimated using relations

$$s_Z = |Z_m - Z_n|\,(d_m + d_n)\,|\,/\,|d_m - d_n|\,(Z_m + Z_n) \qquad (3)$$

$$s_\varphi = |\varphi_m - \varphi_n|\,(d_m + d_n)\,|\,/\,|d_m - d_n|\,(\varphi_m + \varphi_n) \qquad (4)$$

where $Z_{m\&n}$ are impedances magnitudes, $\varphi_{m\&n}$ phase angles, and $d_{m\&n}$ (d_{Eb} or d_{Evv} - Fig. 1.) distances between voltage (voltage & current, when bipolar technique is used) electrodes positions, respectively. At the same time, interrelation and correlation of student population results [768 data = 16 students x 12 (3x4weeks) measurements x 4 data (Z_{m1} & Z_{m2} x bip. + tet.) /measurement] were examined and analyzed.

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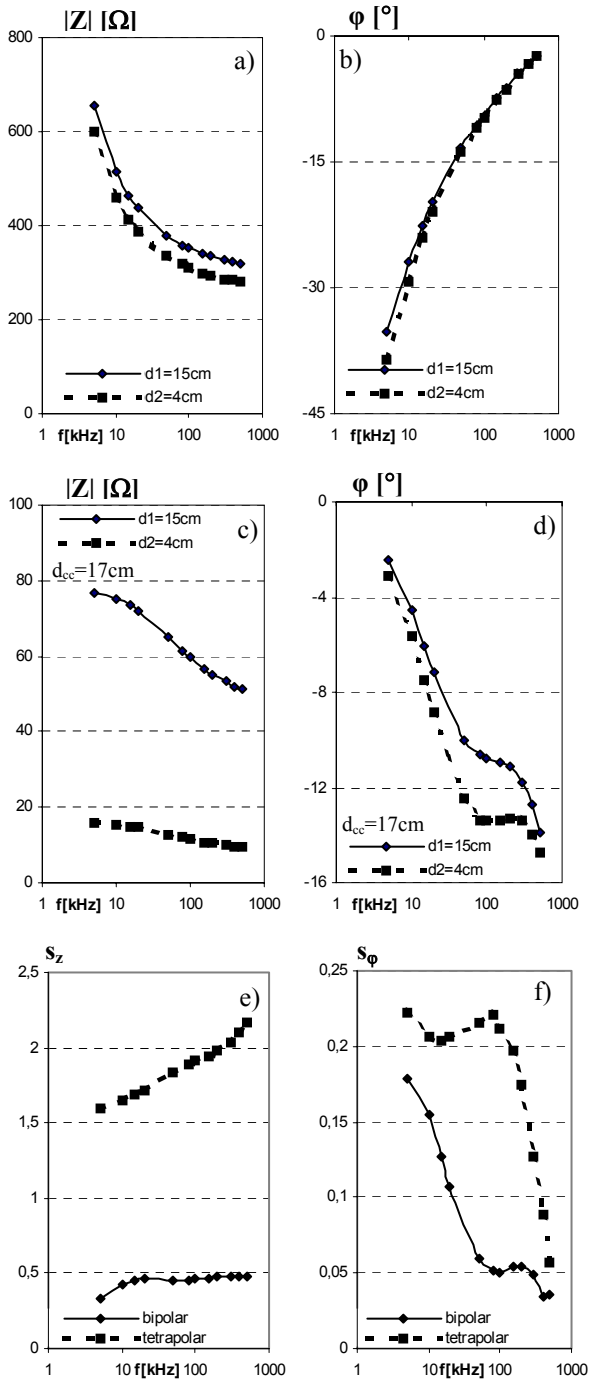

Figure 5: *Averaged* results (16 students) of measurements as shown in Figure 2.

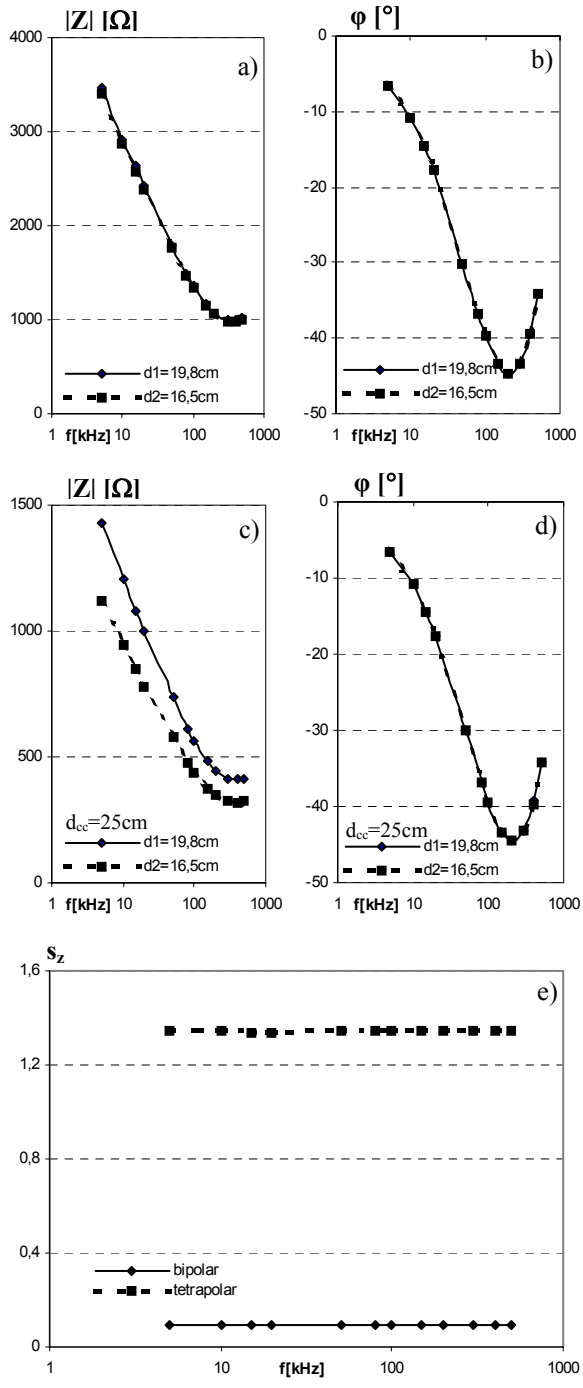

Figure 6: Results using simulation model and EMAS software

Obtained results can be elucidated in a number of ways. Figures 5. and 6. show some results of measurements and calculated sensitivities. The frequency dependences of a)-$|Z|$ bipolar; b)-φ bipolar; c)-$|Z|$ tetrapolar; d)-φ tetrapolar; e)-s_Z, and f)-s_φ are shown, respectively. Results of bipolar measurements (Figure 5.) are obtained and calculated using *MFBIA* technique (see *Appendix*). An analysis of performed results shows that sensitivity, as defined, could be a good measure of differences in results due to the unavoidable changes or variations in electrode's position and/or spacing in repeated measurements. Tetrapolar technique of measurements is more sensitive to the electrodes placing and spacing [see e) and f) in Figures 5. and 6.]. However, this is correct and factual only at "short" distances between electrodes (small d_{Evv} and $d_{Ecv} \approx d_{Evv}$)! Measured values of impedance magnitude are more sensitive to the electrode's placement variations (differences, in order of 12-85 Ω, not visible due to the scale, exist in Figure 6. a), too) than those of phase. This could be easily explained due to the fact that phase angle is relatively insensitive to *A* and *d* (2) changes.

In the model, we explored also the influence of distance between electrodes on the sensitivity. At the frequency of 50 kHz, we changed d_{cc}, preserving distance d_{cc} - d_{vv} (I_{in} - $+U_{m1}$ and I_{out} - $-U_{m2}$ in Figure 4.) at 2,5 cm [≈same

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conditions as in Figure 6. c) and d)]. Obtained results show that, increasing electrode's distance, sensitivity s_Z [Figure 6. e)] for tetrapolar configuration decrease nearly exponentially, approaching to the bipolar (difference less than 0.2 at $d_{cc} \approx 65$ cm).
At the same time, results of measurements performed on student's population, as described (3 times/week/30 days), were treated statistically, in order to estimate repeatability and/or reproducibility of repeated ("follow-up") measurements. We will present only averaged correlations for Z_{m1} (see Figure 2.) among results for bipolar and tetrapolar measurements at the frequencies of 20 kHz and 50 kHz, i.e. $|Z_{Bb20}|$, $|Z_{Bb50}|$, $|Z_{T20}|$ and $|Z_{T50}|$, respectively (index $_{Bb20}$ means: bipolar measurement at the frequency of 20 kHz and $|Z_B|$ calculated following procedure explained in *Appendix*).
Averaged correlations "horizontally", i.e. "interrelations" for 1 to 16 students for 12 measurements

$$\begin{aligned} |Z_{Bb20}| &= 0.908 \\ |Z_{Bb50}| &= 0.912 \\ |Z_{T20}| &= 0.949 \\ |Z_{T50}| &= 0.952 \end{aligned} \qquad (5)$$

Averaged correlations "vertically", i.e. "per" student (1-16) for 12 measurements

$$\begin{aligned} |Z_{Bb20}| &= 0.922 \\ |Z_{Bb50}| &= 0.931 \\ |Z_{T20}| &= 0.879 \\ |Z_{T50}| &= 0.891 \end{aligned} \qquad (6)$$

As evident, from figures and results of statistical analysis, when accuracy and reliability are considered (e.g. in physiological measurements: muscle or tissue state, body composition, comparison of lower legs in ischaemic or compartmental syndrome, etc.) tetrapolar technique is more accurate (although requires four, instead of two electrodes, but does not require MFBIA and calculation of electrode's impedance). But, as far as repeatability and/or reproducibility are concerned (in repeated measurements; e.g.: "follow-up" of patients treated by vascular surgery, "follow-up"of the efficiency of sportsman training, etc.), bipolar technique should be recommended. Measurements at the frequency of 50 kHz give slightly superior results than those at the frequency of 20 kHz (variance of results is lowest in this range).

Conclusion

For bioimpedance measurements, constant current, bipolar or tetrapolar measuring techniques, should be favored. We have compared accuracy, reliability, repeatability and reproducibility of these two techniques. As could be expected, tetrapolar technique is more accurate and reliable, and sensitivity, as defined, could be a good indicator of repeatability and reproducibility of measurements. But, as far as reliability, in relationship with reproducibility *and* repeatability of results, is concerned (i.e., for repeated measurements), bipolar technique should be recommended when distance between voltage electrodes is smaller than ~ 30 cm, although MFBIA calculation is required to eliminate electrode impedance. Main reason is that results, at small electrodes distances, using tetrapolar technique, depends greatly (among other factors) on voltage electrodes placing and spacing.

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APPENDIX:

The idea of *M*ulti*F*requency *BI*oimpedance *A*nalysis (*MFBIA*) is to measure bioimpedance in larger frequency range than needed (100 Hz – 500 kHz), using "low" frequency range (100 Hz-1000 Hz) for calculation of R_e and C_e [15, 16, 20]. The inter-electrode impedance, $\mathbf{Z_M}$, can be generally represented as serial connection of two parallel RC impedances, $\mathbf{Z_E}$ and "true" $\mathbf{Z_B}$ (Figure A.1.).

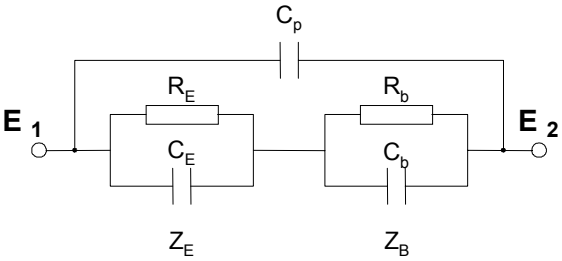

Figure A.1.: Simplified inter-electrode impedance, $\mathbf{Z_M}$, represented as serial connection of two parallel RC impedances, $\mathbf{Z_E}$ and "true" $\mathbf{Z_B}$

Neglecting the influence of inter-electrode parasitic capacitance (reason to avoid frequencies higher than 500 kHz), we tried to calculate, using an iterative procedure, $\mathbf{Z_E}$ and "true" $\mathbf{Z_B}$.
Starting (using MFBIA), with presumption that a value of bioimpedance magnitude at the frequency of 100 Hz ($|Z_{b\text{-}100Hz}|$) is 5 kΩ, and, the phase angle has initial value ($\varphi_{b\text{-}100Hz}$) of -40 °. The influence of parasitic capacitance C_p will be neglected. With this neglect, total measured impedance, referring Fig. A.1., is:

$$Z_m = Z_e + Z_b \tag{A1}$$

Real and imaginary parts of measured impedance can be calculated as

$$\mathrm{Re}\{Z\} = |Z| \cdot \cos\varphi \tag{A2}$$

$$\mathrm{Im}\{Z\} = |Z| \cdot \sin\varphi \tag{A3}$$

Than, presumed bioimpedance at 100 Hz is :

$$Z_{b-100Hz} = |Z_{b-100Hz}| \cdot \cos\varphi_{b-100Hz} + j \cdot |Z_{b-100Hz}| \cdot \sin\varphi_{b-100Hz} \tag{A4}$$

Impedance of electrodes can be calculated as follows :

$$Z_{e-100Hz} = Z_{m-100Hz} - Z_{b-100Hz}$$

$$= \mathrm{Re}\{Z_{m-100Hz}\} - \mathrm{Re}\{Z_{b-100Hz}\} + j \cdot (\mathrm{Im}\{Z_{m-100Hz}\} - \mathrm{Im}\{Z_{b-100Hz}\}) \tag{A5}$$

R_e and C_e can be calculated, using known values at the frequency of 100 Hz - $Z_{e-100Hz}$, from the formulas for parallel connection of capacitance and resistance :

$$\mathrm{Re}\{Z\} = \frac{R^2}{R^2 + \omega^2 R^2 C^2} \tag{A6}$$

$$\mathrm{Im}\{Z\} = \frac{\omega R^2 C}{R^2 + \omega^2 R^2 C^2} \tag{A7}$$

Solving equations (6) and (7), one can calculate electrode resistance, R_e as follows :

$$R_e = \mathrm{Re}\{Z_{e-100Hz}\} + \frac{\mathrm{Im}\{Z_{e-100Hz}\}^2}{\mathrm{Re}\{Z_{e-100Hz}\}^2} \tag{A8}$$

and then calculate electrode capacitance, C_e :

$$C_e = \left| \frac{\mathrm{Im}\{Z_{e-100Hz}\}}{\mathrm{Re}\{Z_{e-100Hz}\}} \right| \cdot \frac{1}{\omega_1 \cdot R_e} \tag{A9}$$

ω_1 is radial frequency at 100 Hz.
Knowing R_e, C_e, and using (A6) and (A7), electrode impedance value at 200 Hz - $Z_{b-200Hz}$, can be calculated:

$$Z_{b-200Hz} = Z_{m-200Hz} - Z_{e-200Hz}$$

$$= \mathrm{Re}\{Z_{m-200Hz}\} - \mathrm{Re}\{Z_{e-200Hz}\} + j \cdot (\mathrm{Im}\{Z_{m-200Hz}\} - \mathrm{Im}\{Z_{e-200Hz}\}) \tag{A10}$$

Also, using (8) and (9), one can calculate values of R_b and C_b. Knowing R_b and C_b, new value of bioimpedance, $Z'_{b-100Hz}$, using (6) and (7), can be calculated.
Now, one must compare calculated value of bioimpedance with the presumed one. Among several methods at the disposal, we choused a comparison of impedance real parts:

$$\left| 1 - \frac{\mathrm{Re}\{Z'_{b-100Hz}\}}{\mathrm{Re}\{Z_{b-100Hz}\}} \right| \le 10^{-N} \tag{A11}$$

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where N is real positive number, determining a number of iterations and accuracy of fitting (following our experience, $N = 5$ can be recommended giving acceptable accuracy without large number of iterations). If calculated bioimpedance, using (A11) is not acceptable, than iterative procedure can be repeated starting with the value of calculated biompedance instead of presumed one.

One example of results (one subject/eight measurements during one month) in figures A.2., A.3., and A.4., evidently show reliability of measurements and iterative procedure calculations [20].

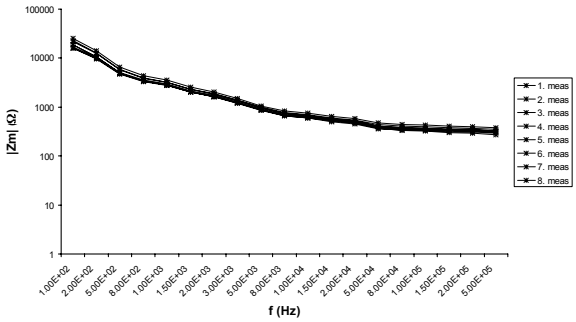

Figure A.2.: $\mathbf{Z_M}$ as a function of frequency

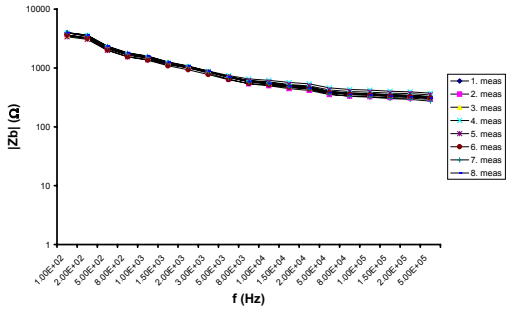

Figure A.3.: $\mathbf{Z_B}$ as a function of frequency

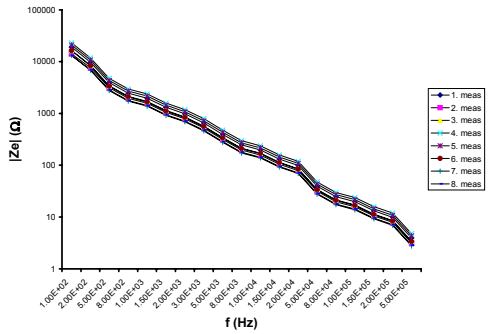

Figure A.4.: $\mathbf{Z_E}$ as a function of frequency

MFBIA allowed us to prove that the diagnostically useful frequency range, when measuring using cutaneous electrodes, is between 5 kHz and 200 kHz. Other measured values were used for iterative procedure and calculation of "true biological impedance", Z_B. The results, shown in Table I., reveal typical findings for one "normal" subject with calculated values of electrode resistance and capacitance, respectively.

			R_E (kΩ)	C_E (nF)		
			80.97	80.64	$Z_B=Z_M-Z_E$	
f (Hz)	Z_M (Ω)	φ_M (deg)	Z_E (Ω)	φ_E (deg)	Z_B (Ω)	φ_B (deg)
5000	981.84	-52.13	394.74	-89.72	711.10	-32.33
8000	756.66	-44.89	246.71	-89.83	607.53	-28.23
10000	679.86	-41.42	197.37	-89.86	568.46	-26.36
15000	574.99	-35.32	131.58	-89.91	510.15	-23.18
20000	520.08	-31.26	98.69	-89.93	476.28	-21.07
50000	411.98	-20.26	39.47	-89.97	400.01	-14.95
80000	379.96	-15.80	24.67	-89.98	373.99	-12.16
100000	368.35	-13.81	19.74	-89.99	364.14	-10.79
150000	351.40	-10.86	13.16	-89.99	349.16	-8.73
200000	342.28	-9.00	9.87	-89.99	340.88	-7.36

Table I.: Typical measured and calculated values for one "normal" subject

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