TALLINN UNIVERSITY OF TECHNOLOGY

Faculty of Information Technology

Thomas Johann Seebeck Department of Electronics

Hip Kõiv

132707 IVEM

Developing tissue phantom materials with required electric conductivities

Master thesis

Supervisor: Senior research scientist Rauno Gordon

Tallinn 2015

Author's declaration

I declare that I carried out this master thesis independently, and only with the cited sources, literature and other professional sources. I certify that this research thesis or any part of it has not been previously submitted for a degree or any other qualification at the Tallinn University of Technology or any other institution.

Date: 04.06.2015

Author: Hip Kõiv …….

/signature/

Thesis Assignment

Name of topic:

Developing tissue phantom materials with required electric conductivities

Kudedele sarnaste elektrijuhtivustega materjalide disainimine ja valmistamine

Origin of topic: Tallinn University of Technology, Thomas Johann Seebeck Department of Electronics

Thesis goals: Give an overview of bioimpedance, phantoms used to mimic tissues, and van der Pauw resistivity measurement method. Develop a fixed recipe for gelatine phantoms with desired electric conductivities. Manipulate the conductivities of the phantoms with sodium chloride and accurately measure and calculate resistivity with van der Pauw method.

Expected results: Design tissue phantom material and manipulate its conductivities easily with NaCl. Use van der Pauw method and prove its reliability measuring resistivity of developed phantoms. To gain knowledge of bioimpedance, tissue phantoms and van der Pauw method.

Solved problems: It is proposed how to prepare low-cost and simple tissue similar gelatine phantoms and how to measure their resistivity with van der Pauw method.

Date: ……

Student: …… ……..……..

/given name(s) and surname/ /signature/

Supervisor: ...……. …..……..

/given name(s) and surname/ /signature/

Hip Kõiv

Developing tissue phantom materials with required electric conductivities

Master thesis

Abstract

This thesis is the final work for fulfilment of the Master of Science degree at the Thomas Johann Seebeck Department of electronics in Tallinn University of Technology.

The overall aim of this research is to develop inexpensive tissue similar phantoms with required electric conductivities and find a reliable measurement technique for said phantoms. The master project includes two main parts: review of the state of art and phantom construction and testing. First part introduces different phantoms used in biomedical fields and potentially suitable materials for mimicking tissues. It also reviews the main concepts of bioimpedance and van der Pauw method for determining conductivity. This section is mainly based on scientific publications analysed to give theoretical basis for assumptions made in the study. Second part includes development process of phantoms and their measurement results with implemented measurement technique. After analysing subject matter literature and considering possibilities, gelatine material is used to make homogeneous phantoms with tissue similar conductivities because other discussed materials were either too expensive or too problematic to prepare. Van der Pauw method is applied for conductivity measurements due the interest to investigate its accuracy and reliability measuring thick biological samples. Biological tissues have different electric conductivities, which means it should be also possible to manipulate with phantom conductivities.

Gelatine phantoms developed in current study are manipulated with NaCl to increase resistivity of samples and get desirable electric properties. NaCl dependence of conductivity is confirmed to be linear. Van der Pauw method for gelatine phantoms between 100 Hz to 1 MHz was proved to be applicable and gave satisfying results. In addition, graphite powder was added to the gelatine mixture to analyse its influence to electrical conductivity on low frequencies. Although, the results did not show reliable trend in conductivities measured, it is worth developing and researching further in future studies.

Presented gelatine phantoms and van der Pauw measurement system used, proved to meet the goal of the thesis. Experiments showed relatively good agreement between theory and implementation part and phantoms with desired electric conductivities were prepared for development of bioimpedance technologies.

The thesis is in English and contains 66 pages of text, 6 chapters, 30 figures and 6 tables.

Abbreviations

vdP – van der Pauw

CT – computed tomography

EIT – electrical impedance tomography

PVA – polyvinyl alcohol

MRI – magnetic resonance imaging

AC – alternating current

BLM – bilayer lipid membrane

2D – two dimensional

CNC – computer numerical control

PCB – printed circuit board

Wt/vol% - weight/volume percentage

NaCl – sodium chloride

List of figures and tables

Table of Contents

Introduction

Bioimpedance is the biological material's opposition to the alternating electric current of various frequencies [22]. There are significant impedance differences between tissues, which help to determine the state of material under study. This is also why bioimpedance applications are mainly used in medical field - to differentiate cancerogenous tissues or monitor physiological changes in them. New instrumentations in this ground have growing tendency of being designed to image electrical properties of tissues.

Phantom materials are used to mimic and reproduce the characteristics of biological matters and these tissue mimicking phantoms have great importance in development, testing and evaluating of bioimpedance measurement applications. The main purpose of present study is to have realistic homogeneous phantom material, which can be prepared with desired electrical conductivities and used to develop bioimpedance technology. There are countless phantoms available commercially, but due to the need for application related specificity, it is easier and cheaper to find a method to develop our own phantoms. These phantoms should be easy to prepare and use, stable over time and inexpensive. Phantoms would be used in development of medical diagnostic systems, which use bioimpedance measurement with either electrodes or eddy currents to estimate the physiological state and processes in the patient.

This research is a review of the basic fundamentals of tissue phantom properties, impedance of material under study and conductivity measurement method. First part of the research introduces the purpose of tissue similar phantoms, different materials used for mimicking tissues, basic factors in bioimpedance and van der Pauw method. Scientific articles are reviewed to find suitable materials and techniques needed for development of tissue phantom samples. Second part describes the experimental part, which consists of finding the most effective and inexpensive way to form homogeneous phantoms and measure them. Gelatine phantom material preparation and manipulation with NaCl is discussed together with conductivity measurements. Finally, graphite powder influence to the phantom material is presented.

Van der Pauw method, typically utilised in semiconductor industry, is used to measure and calculate developed phantom conductivities. Currently exists a small number of scientific articles studying the electrical impedance properties of biological phantom samples and using van der Pauw method to determine conductivity of biological materials. Therefore, there is a need for phantom materials with different electric properties in different shapes to analyse new technologies. Present research is an attempt to make cheap tissue mimicking phantoms and prove the reliability of van der Pauw technique when implemented on designed phantoms.

Main goals of the research are:

1. Give an overview of bioimpedance, phantoms used to mimic tissues, and van der Pauw method;
2. Develop a fixed recipe for gelatine phantoms with desired electric conductivities;
3. Find a way to accurately measure and calculate conductivities of developed tissue phantoms;
4. Manipulate the conductivities of the phantoms with sodium chloride and other matters to get the required electric conductivities.

1. Review of the state of art

Development of tissue similar materials in current study relies on a series of previously published articles that are reviewed in next chapters. Also, brief analysis of phantom technologies, advantages of different phantom materials, basic formulas used, introduction to bioimpedance and van der Pauw method, is given.

1.1. Tissue phantoms and their purpose

Phantoms are made to mimic biological tissues and are mostly used in medicine related fields in order to analyse, test the performance, establish or evaluate different medical devices. [37] Many researchers need phantoms to develop some novel measurement technique or check theories, uncover potential weaknesses in imaging systems or run simulations. Scientific studies [21][22] propose that malignant tissues show different impedance compared to normal tissues. This means that electrical impedance scanning could detect tumours and cancerous tissues, which make phantoms with specific electrical properties important and useful for many researchers in biomedical field. Due to this, present study is directed to developing phantoms with needed electrical conductivities.

Phantoms used in medical field started soon after the discovery of X-rays in 1896. The harmful effects of high radiation doses urged physicists to develop materials to simulate patients. The earliest phantoms were mainly made of water and water is still a very good approximation of the human tissue, but nowadays phantoms have become more complex, more reliable over time and more accurate over wider range of energies. [36] Alderson et al. [44] developed in 1960s first more complex phantom for dosimetry with human skeleton inside the basis material. Phantom material typically must have properties that are equivalent to those one desires to study for tissue, and phantom used to evaluate an imaging device should respond in a similar manner to how human tissues and organs would act in that specific imaging modality. [31] In 1970s number of phantoms were developed for imaging systems, such as for computed tomography (CT) and mammography. [36] Griffiths et al. [39] introduced saline phantom for electrical impedance tomography (EIT) in 1989 and for these phantoms it is important to be able to change the electrical conductivity likewise to the goal of present work.

1.2. Materials used for phantoms

There are many phantom materials available for mimicking human tissues, but it is necessary to find or design material that represents its properties accurately as possible. These properties could be radiological, electrical, mathematical or optical. In many cases phantom materials are separated by the energy range in which they should be used [36]. In present work, conductivity is the main factor affecting the choice of a phantom material for studying the electrical properties of biological tissues.

Polyacrylamide, polyvinyl alcohol (PVA), agar, agarose and gelatine are popular mimicking materials to be used in biomedical field. [36][41] Moldability, easy preparing and the ability to manipulate conductivity are the main reasons for using mentioned substances. [9] Polyvinyl alcohol and polyacrylamide gel are synthetic materials and more used for mimicking blood vessels in magnetic resonance imaging (MRI) experiments. [40] Polyacrylamide gels suffer from cluster formation and limited shelf life, also they are toxic and require special handling. [15] Comparing research by Kandadai et al. [9] suggest agar, agarose, and gelatine gels usage for mimicking tissue electrical parameters because these materials are usually inexpensive, easy to prepare, readily accessible and stable over time [15]. Agar and agarose, both are derived from red algae, but agarose has undergone purification and does not have protein in it. Agarose is electrically neutral and of high purity. Agar, however, is relatively impure and possesses multiple charges. [9] Gelatine is made of collagen in pork skins and bones. Main advantages of agar and agarose in front of gelatine:

1) Body temperature is around 36°C and many experiments should also take place on that temperature. Agar and agarose sustain the shape and structure also on higher temperatures, including body temperature. [9]
2) Gelatine has very good structure at room temperature but on higher temperatures it starts to lose its ability to sustain shape unless gelatine powder concentration is increased. Rising the concentration changes the structure and it may not be suitable to accurately mimic needed biological organ. [15][42]

In spite of agar and agarose practical advantages, in present research gelatine for producing tissue-similar phantoms with needed electrical conductivities, is used. Gelatine is easily

available, robustly processable, cheap, and it melts at 35°C, when agar and agarose need more heating (85°C). Similar research by Marchal et al. [6], Elbohouty et al. [10], Kandadai et al. [9] and Pinto et al. [15] suggest using gelatine phantoms for an easy and cheap simulation of many human tissues. Tabel 1. shows some important parameters of gelatine powder used in phantom preparation.

Table 1. Gelatine powder characteristics. [15]

Gelatine's main characteristics	
Parameters	Results
Viscosity	36.7 mP
Humidity	9.05 %
pH	5.45
Bloom*	240 g/Bl
Color	Light brownish

*Strenght of the gelatine gel

Gelatine has cationic and anionic groups together with hydrophobic segments, i.e. segments, which have apparent repulsion of water. Due to these positively and negatively charged sites, van der Waals, i.e. electrostatic interactions appear. Salt in gelatine will shield these electrostatic interactions and conductivity increases. Addition of NaCl to the gelatine mixture concludes in more Na^+ and Cl^- ions and gel becomes more ionised and more electricity is carried. [15]

1.3. Developed phantoms' usage

Tissue mimicking materials have been extensively studied in imaging, radiology, optical spectroscopy, elastography and dosimetry field, but there are less researches made on the electric properties of gelatine phantoms. [15] Prospective usage of phantoms prepared in current work would be in developing bioimpedance based diagnostic systems.

One possible application, where proposed phantoms are needed, is in the early stages of testing eddy current sensor for tissue conductivity measurement. Non-invasive eddy current method is based on creating current, which is induced by an AC-driven coil in the tissue. That current induces a secondary magnetic field in and around the tissue opposite to primary magnetic field in direction. [35] The developed sensor should be movable on the skin of a patient, in order to detect areas where electrical impedance deviates from normal. [34] Sequin et al. [32], Riedel et al. [34] and M. Tanha [35] investigated and described eddy current measurement method for biological tissues. These researches show the proposed technique potential to examine tissues and to discover changes in them, which means that prepared gelatine phantoms could be also useful for eddy current sensor development.

2. Bioimpedance

2.1. Overview

As was mentioned above, current study of making gelatine phantoms is put together to help develop applications for bioimpedance measurements. In this section overview of bioimpedance and its importance is presented.

Bioimpedance is basically the ability to oppose electric current flow inside the biological tissue and it varies with frequency and different tissue types. [23] Thus, for example, fat has high resistivity and blood lower resistivity. [55] Biopimpedance measurements are usually noninvasive and the main idea of different applications is to analyse the electrical conductive properties of various tissues. Bioimpedance measurement methods have been used in several medical applications:

a. Cellular measurements;
b. Volume changes;
c. Fluid overflow;
d. Body composition;
e. Lung function monitoring;
f. Skin cancer detection;
g. Tissue classification;
h. Tissue monitoring;
i. Electrical impedance tomography (EIT). [24]

Method used in these applications is harmless for the patient and in many cases more affordable than the alternative techniques. [25][26] To give the proper overview, short description of electrical terminology needed in tissue impedance studies is given.

In bioimpedance the main basic properties are the resistance (R), conductance (σ), capacitance (C) and permittivity (ε). Resistance element opposes the flow of electrons or, as in living tissue, the flow of ions among its cells, and conductance is the inverse of resistance. Resistance analogous is reactance (X), which shows how current is travelling through a capacitance. Capacitance is a parameter, which shows how much the system stores and releases energy as the current and voltage fluctuate with each AC cycle. [26] It opposes a change in voltage across an object and acts to store energy [21]. Permittivity is a property of the dielectric material and it is a measure how an electric field affects dielectric medium [27]. There is a relation between capacitance and permittivity as follows:

$$C = \frac{\varepsilon A}{d} \ , \tag{2.1.1}$$

where ε is relative permittivity, A is the area of capacitor plates and d is the distance between them. [21] The impedance of an element at a certain frequency is defined as the relation between the input voltage and the input current for that frequency. It is measured in ohms and

$$Z = V/I \ , \tag{2.1.2}$$

where Z is impedance, V is voltage and I is current. Complex bioimpedance consists of two components, resistance as the real part, and reactance, the out-of-phase data. Resistance is the x axis and reactance is y axis and this is said to be 'complex' impedance. [24] Putting these components in series, we obtain impedance in complex form:

$$\boldsymbol{Z} = R + jX\,, \tag{2.1.3}$$

where $\boldsymbol{Z}$ is complex impedance, R is resistance, X is reactance and j is the square root of minus 1. [29] Following equation shows how reactance is formed:

$$X = \frac{1}{(2 \cdot \pi \cdot f \cdot C)}\,, \tag{2.1.4}$$

where reactance X is in Ohms, frequency f is in Hz and capacitance C is in Farads. [26] The representation of the impedance can also be in the form of polar coordinates:

$$\boldsymbol{Z} = Z/\theta\,, \tag{2.1.5}$$

where Z is the magnitude and θ is the phase angle of the impedance. Z is also the square root of $R^2 + X^2$ and $\theta = arctan(X/R)$ like is shown in Fig. 1. [21]

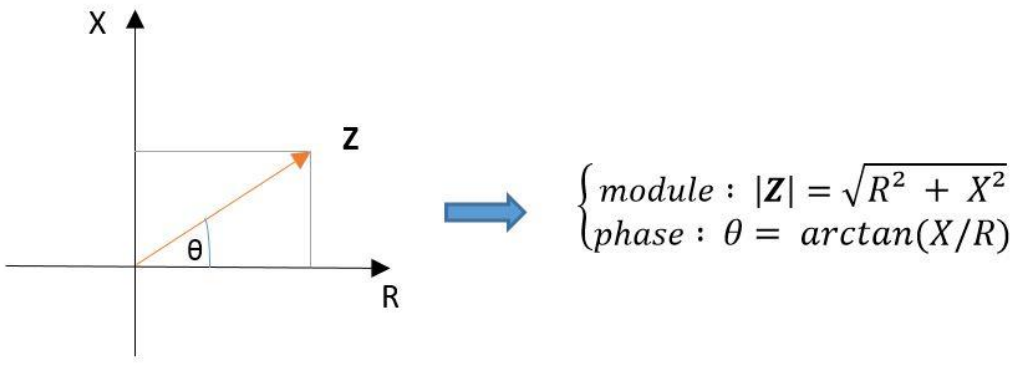

Figure 1. Complex plane

For example, complex impedance is written:

$$\boldsymbol{Z} = 660 + 340j$$

This means that resistance is $660\ \Omega$ and the reactance is $340\ \Omega$ and it can be displayed on 'complex plane' as is depicted on Fig. 1. Replacing real part R and imaginary part X to equations written before, we get the module and phase angle. So $660 + 340j$ converts to $742\ \Omega$ at 27 degrees. [26] These parameters help to give an understanding picture of different impedances of tissues on a graph.

2.2. Electrical conductance of living tissue

Biological tissue is very heterogeneous material because cells are of uneven size and with very different functions. [23] Tissue consists of extracellular fluid and cells containing the intracellular fluid inside the cell membrane. [25] Extracellular medium consists of ionic solutions [21]. Cells in tissues may be modelled as a group of electronic components. Easiest way to depict it, consists of 3 components: the extracellular space as a resistor Re, intracellular space and the membrane is modelled as a resistor Ri, and C is the membrane capacitance (Fig. 2) [26]

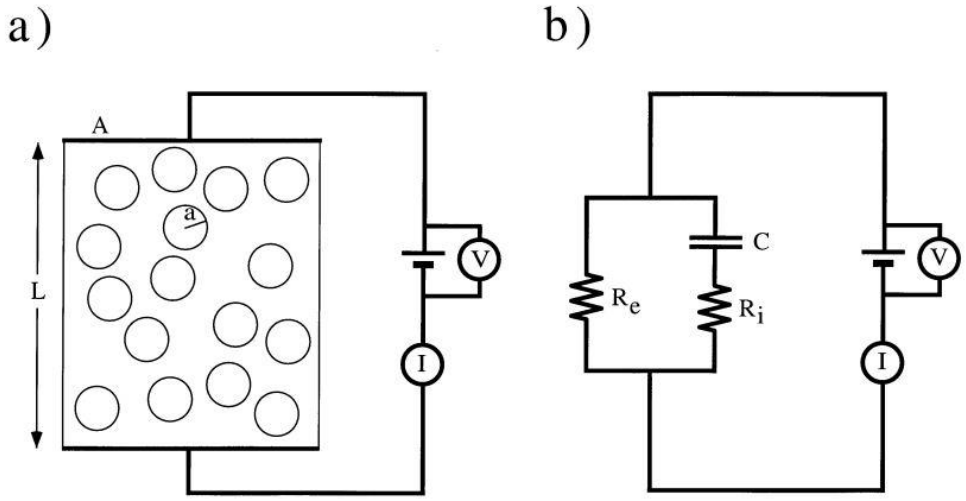

Figure 2. Suspension of spherical cells, each of radius a is showed (a). Electric circuit equivalent of the effective conductivity of the suspension. (b) [33]

Extracellular and intracellular space are highly conductive because they contain salt ions. Lipid membrane of cells is an insulator, which prevents current at low frequencies from entering the cells. So at lower frequencies current flows only through extracellular space. [26] At higher frequencies the membrane capacitance lets AC current pass (charges pass backwards and forwards more rapidly if the applied frequency is higher) [23][26]. The total ionic conductivity of a solution depends on the concentration, activity, charge and mobility of all free ions. The most important ions contributing to the ionic current in living tissue are K^+, Na^+ and Ca^{2+}. [25] As shown in Fig. 3, electric current passes through tissue in a frequency-dependent manner. Remarkable feature of live tissue is an extraordinarily high capacitance – 1000 times greater than inorganic materials. [26] This is because the passive part of the cell membrane is the bilayer lipid membrane (BLM). It allows lipid and water to pass through it, but not ions. Each cell membrane behaves as a dielectric, so the structure formed by the extracellular medium, the BLM and the intracellular medium together, make collection of closely positioned tiny capacitors. [24]

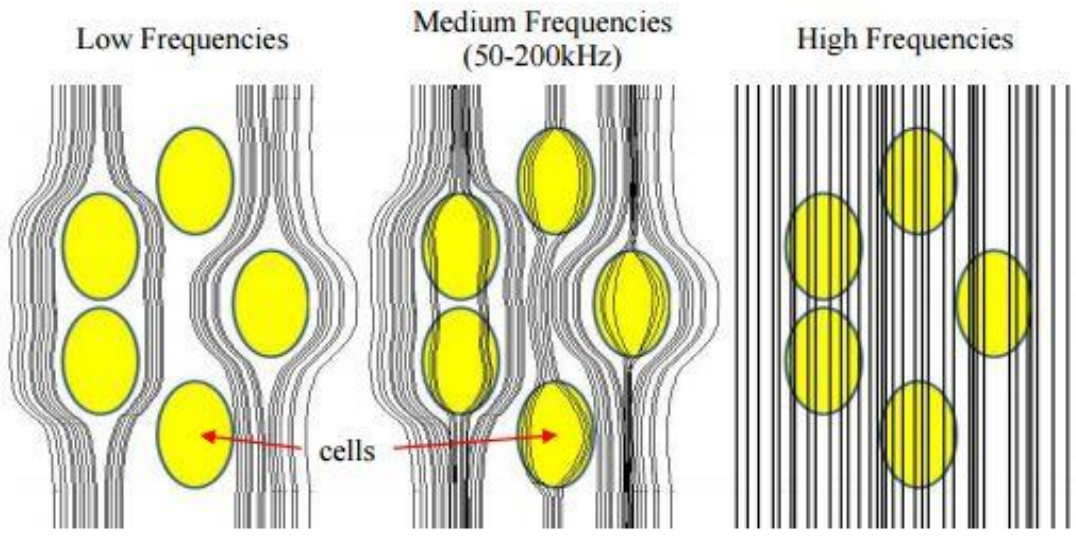

Figure 3. Overview of how alternating current passes through the cells in biological tissues on different frequencies.

Fig .3 shows how on lower frequencies current passes the cells without entering them. On medium frequencies (50 – 200 kHz) current goes through some cells because membrane lets current pass. On high frequencies current is able to flow through extracellular and intracellular space without any interruptions.

2.3. α-, β- and γ-dispersions

H.P. Schwan introduced already in 1957 that living tissue is a dispersive medium and there are three major dispersions: α-, β-, and γ-dispersions. [30] α-dispersions can be found down to below 1 Hz and up to 100 kHz but it is not so well defined. Schwan [30] proposed that it is due to surface admittance and diffusion processes, but the mechanisms behind dispersions are still unclear. β-dispersions are considered to be associated with the dielectric properties of the cell membranes and they appear in frequency range from 1 kHz to 100 MHz [24] The membrane only partially charges and current can flow through the lipid cell membranes, introducing a capacitive component. γ-dispersions occur due to the relaxation of water molecules and is centred at 10 GHz [26]. Grimnes and Martinsen [29] found that most of the alpha dispersions disappeared in the dead tissue after few hours and they raised a question whether alpha dispersion actually gives additional information for bioimpedance measurements.

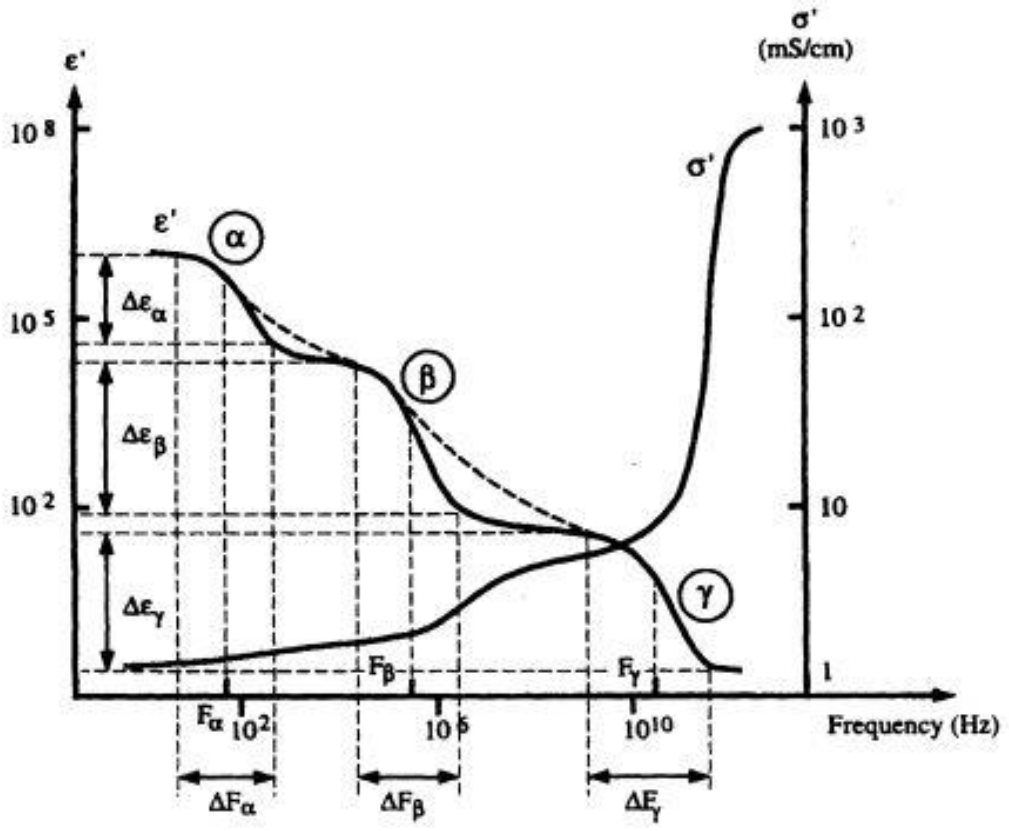

Figure 4. α-, β-, and γ-dispersions of permittivity (ε) and conductance (σ) showed in relation to frequency (f). [Bourne 1996, 24]

Current experiments on gelatine phantoms are made with 4 electrodes on each side of the sample and on low frequencies - until 1 MHz, which is in the range of alpha and in the beginning of the beta-dispersions. Despite the unclear information about alpha-dispersions, future development of current work will include trying to produce dispersions in alpha and beta region to make artificial tissue more similar to real tissue. Fig. 4. shows dispersion ranges which can be seen as regions of an increased increase of conductance in a plot of conductance against frequency. [24]

3. Van der Pauw method

Van der Pauw (vdP) method was used during experimental part of present study to measure and calculate sample resistivity. This chapter gives an overview of van der Pauw theory, limitations and field of application.

3.1. History and overview

Van der Pauw method was firstly mentioned in Philips Research Reports in February 1958 by Leo J. van der Pauw. His published paper was titled 'A method of measuring specific resistivity and Hall Effect of discs of arbitrary shape'. [43] L. J. van der Pauw described a new method of measuring resistivity. He took a flat lamella, completely free of holes and provided it with four small contacts (Fig. 5).

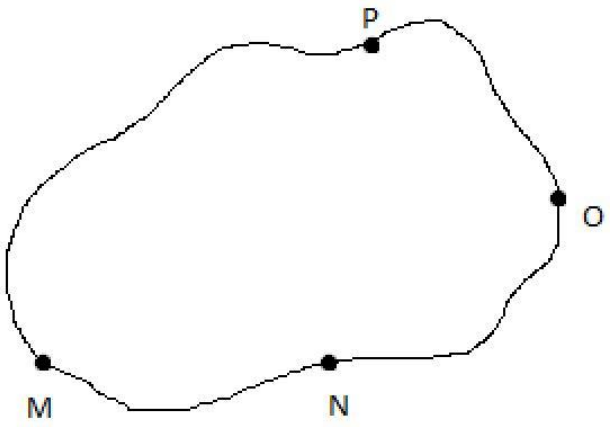

Figure 5. Flat lamella of arbitrary shape, with four small contacts M, N, O and P on the periphery. [43]

He measured the potential differences of the contacts $V_P - V_O$, when current i_{MN} was applied to contact M and taken off at contact N. As this new method is based on voltage and current ratio, resistance was defined:

$$R_{MN,OP} = \frac{V_P - V_o}{i_{MN}} \tag{3.1.1}$$

Analogously it was defined:

$$R_{NO,PM} = \frac{V_M - V_P}{i_{NO}} \tag{3.1.2}$$

L. J. van der Pauw presented that the new method of measurement is based on the theorem that between resistances $R_{MN,OP}$ and $R_{NO,PM}$ exists the simple relation:

$$\exp\left(-\frac{\pi d}{\varrho} R_{MN,OP}\right) + \exp\left(-\frac{\pi d}{\varrho} R_{NO,PM}\right) = 1\,, \tag{3.1.3}$$

where d is the thickness of the lamella and ϱ the resistivity of the material. [43] This relation is used very widely in evaluation of electrical properties in semiconductor materials and his contribution to electronics industry is well appreciated.

3.2. Method description

Van der Pauw method requires just two electrical resistance measurements to be made to get the resistivity, but it is reasonable to perform at least four combinations $R_{AB,DC}$, $R_{DC,AB}$, $R_{AD,BC}$, and $R_{BC,AD}$, (Fig 6.). [49] These combinations are based on voltage and current ratio showed in previous chapter (equation (3.1.1) and (3.1.2)). From these, 4 pairs of readings is extracted to place into (3.1.3) and solve for conductivity. This gives 4 (dependent) measurements of conductivity for each sample.

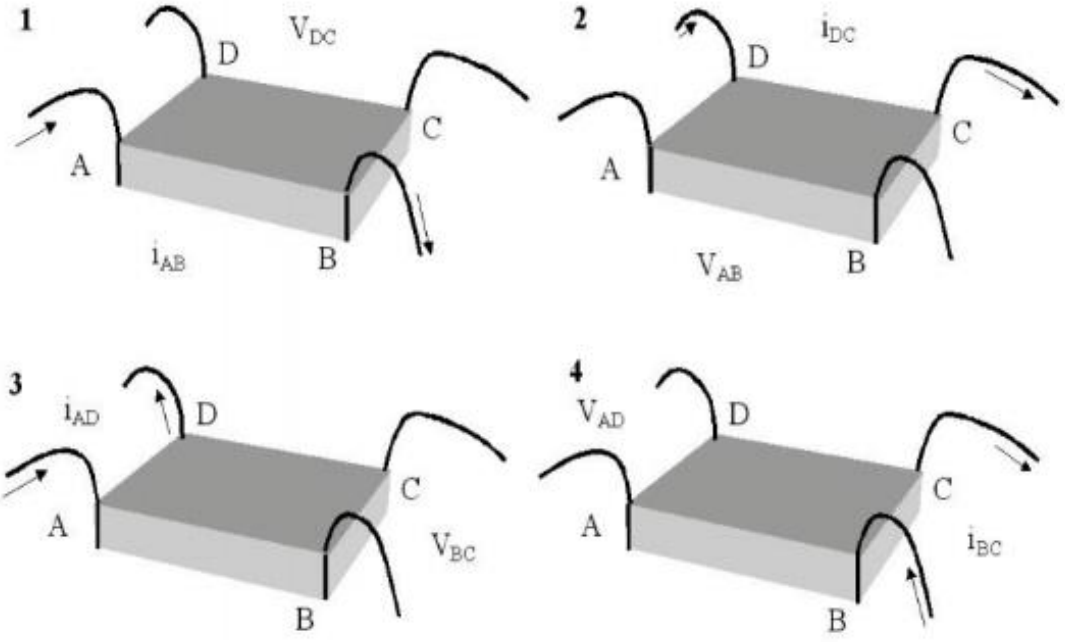

Figure 6. Schematic illustration of four contact configurations of Van der Pauw method [2].

In theory, when the sample is isotropic, all four conductivities should be identical. In practice, this does not always occur because the sample is anisotropic or there are poor connections between the sample and one of the electrodes. [49]

3.3. Main limitations

Several basic sample conditions must be satisfied to obtain accurate measurement results when using van der Pauw theory. Most semiconductors conform to these conditions easily, measurements are convenient and method is widely utilized in the field. [52]

The van der Pauw method requirements:

j. point contacts used for the resistance measurements must be placed at the edges of the sample;
k. must be thin samples of arbitrary shape , two-dimensional;
l. thickness must be constant;
m. resistivity must be uniform;
n. contacts should be sufficiently small;

o. there should be no isolated holes. [46][51][52]

These limitations lay strong restrictions on samples not used in semiconductor industry. However, since publishing van der Pauw method some researches are made to investigate the effects of sample inhomogeneity, electrode placement, sample shape, contact size and sample thickness. [51] Over the years, original ideas of L. J van der Pauw have been extended to use vdP theory in more applications.

3.4. Effects of sample thickness

L.J. van der Pauw investigated the effects of contact size and placement himself after publishing the novel resistivity method. He showed error calculation for thin disk samples:

$$\Delta\rho/\rho \sim (t/D)^2 \,, \tag{3.4.1}$$

where t measures the contact size or distance from the edge and D the disk diameter. If special sample shape is selected, then it is possible to improve accuracy. [43] Also, Koon et al. [53] have made researches of the effects of sample shape and contact placement. His research includes also finite contact size and sample inhomogeneity. [51]

Effects of thickness is especially important in current research because for developing gelatine phantoms, measurements were made on samples, which had dimensions of $50\ mm\ x\ 50\ mm\ x\ 50\ mm$. Effects of thickness of the sample overall is not so well investigated, but Kasl and Hoch [51] and Weiss and Kaplar et al. [54] show how van der Pauw formula is also compatible and valid when sample thickness is non-zero.

Kasl and Hoch [51] examined the thickness correction experimentally by increasing the thickness range of the method. On Fig. 7 two sample geometries are shown and their conductivities are compared by using van der Pauw method.

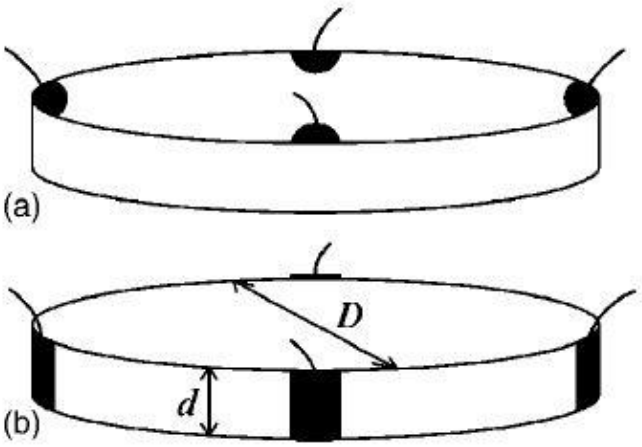

Figure 7. Very thin sample geometry. Contacts are at the perimeter (a). The contacts are placed across the entire edge to produce uniform current distribution (b). D is the diameter and d is the thickness. [51]

Experiments done by Kasl and Hoch showed that, the limiting thickness in case of arrangement (a), is about half the diameter of the disks. When sample thickness is considerably bigger than the diameter, arrangement (b) should be used because contacts along the edges enhance the effective 2D nature of the current distribution. Furthermore, using this approach gives improved accuracy and the reliability when the resistance of the electrodes is sufficiently low compared to the sample resistance. It gives good estimation over the whole range of thickness. [51] The effects of non-zero thickness and non-zero contact area is examined by Weiss and Kaplar et al. [54] as well. Research analysis are in basic agreement and analytic results can be compared to the experimental results of Kasl and Hoch. [51] Although, there has been relatively little research on sample thickness effect on van der Pauw theory, it is still possible to conclude that phantoms used in present work with non-zero thickness, are suitable and measurements give valid results.

3.5. Different fields using van der Pauw method

As was mentioned before, van der Pauw measurement is very common resistivity method used in the semiconductor industry. Semiconductors have high resistivity and special methods are required to measure it. [9] But in present paper given technique is used to measure the resistivity of gelatine phantoms. Van der Pauw method is typically used to avoid contact and isolation resistances, leakage currents as well as voltage, temperature and bandwidth effects and it is particularly useful for measuring very small samples because the

dimensions of the sample and the spacing of the contacts are unimportant. [56][38] This chapter will give some examples of untraditional van der Pauw method fields of application.

For instance, in 2012 M. Tokarska [46] from Lodz University made a new approach to determine electroconductive properties of a textile products which based on the Van der Pauw method. Two electroconductive woven fabrics were selected and examined if the fabrics have characteristics typical for van der Pauw structure. [48] The results showed that in case of selected range of the electrodes diameters, the contact diameters do not affect resistance when constant force is applied. It is successfully used to determine two characteristic resistances associated with four electrodes. The method is suitable for assessing electrical properties of textiles characterized by good conductivity. [46][48]

The electrical conductivity of mouse cortex has been measured at 10 kHz by Elbohouty et al. [49] with van der Pauw method. This research was one of the few papers that I found to be using van der Pauw four electrode method to measure electric conductivity of biological tissues. Brain slices from mice were prepared under seizing and non-seizing conditions and resistivity was calculated. Elbohouty et al. bring out why they used van der Pauw: there is no restriction on the shape of the slice and the positioning of the electrodes is arbitrary, so long as they are at the perimeter.

The errors due to electrodes being of finite width and not applied exactly at the perimeter have been investigated previously by Chwang et al. [50]. Even though researchers decided to use a rectangular sample to minimize the error, some geometrical effects are still affecting the result. Summary of errors is given in Table 2.

Table 2. Errors in the conductivity measurements [50]

Source	Contribution
Electrodes not at perimeter	+3%
Electrodes have finite width	+2%
Slice is not of infinitesimal width	≈0%
Correction to 25°C	±1%
Combined	+5%

Despite the 5% error rate, Elbohouty et al. conducted research to find conductivity of mice brain slices by using van der Pauw method, was successful.

Moroń and Grysiński in 2009 [45] proposed that van der Pauw four-point method can be applied also to measure electrical conductivity of electrolyte solutions. Aim of the research was to determine if it is possible to find electrolytic conductivity by and absolute method, using a conductance cell, which constants can be calculated from its geometrical dimensions. [45]

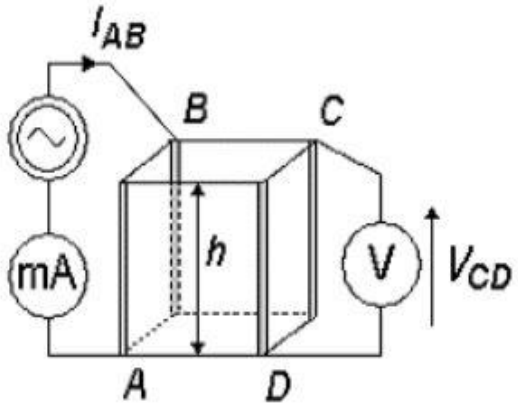

Figure 8. Designed conductance cell for four-electrode measurement, where h is the height of the cell. Electrodes are named A, B, C, D and voltage and current is applied. [45]

Fig. 8. shows the setup of the measurement system, which is similar to the arrangement described before (chapter 3.2). The results confirmed that the cell constant can be determined from its height only and van der Pauw method is thereby more than useful.

Basing on the researches gathered and analysed, it is safe to say, there is a growing literature on van der Pauw usage in other fields than semiconductor industry, and given four-electrode technique is very versatile.

4. Phantom construction and testing

4.1. Phantom development

Fundamental purpose of this work is to provide a phantom material with similar electrical conductivities to human tissues that can be used in biomedical field with different applications. While *in vivo* measurements are more representative of a biological tissue, *in vitro* experiments are more controllable and easier to carry out in a laboratory. Next chapter describes experiments made on how phantom preparation was performed and what kind of methods were applied. Tissue simulating phantoms have been studied in fields such as ultrasound, optical spectroscopy, imaging and dosimetry [1], but there are only few studies on electric properties of gelatine phantoms. [15]

4.1.1. Plexiglas enclosure with electrodes and planar coils

Samples with regular and reproducible shape were needed to easily measure the properties of phantom material. Moulds used in the experiments are in the shape of cube and made of plexiglas. Enclosure was designed in AutoCAD by Ph.D. student Ksenija Pesti, with whom we have worked together since 2013. The cubic is CNC-milled from 10 mm plexiglas. The dimensions of the inner cube are 50 mm x 50 mm x 50 mm to simplify the model when calculating phantom's electrical conductivity. The purpose of the box is to store gelatine mixture and hold electrodes against the phantom while measuring.

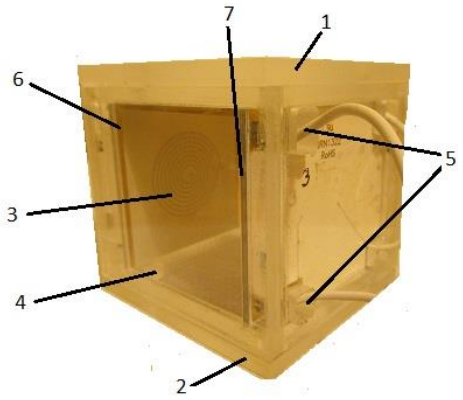

Figure 9. Plexiglas enclosure with PCB-s inside it.

The caps inside the plexiglas box hold the double-sided printed circuit board (PCB) with gold-plated contacts in place. Two gold-plated electrodes on two vertical sides are for van der Pauw measurements described in chapter 3. Electrodes in the inside are connected to the pads on the outer side where wire-leads are soldered. In addition, a planar coil was designed onto the PCB with corresponding pads on the outer side for connections. PCBs with different planar coils were made to test an eddy-current detection method. Fig. 9 shows the Plexiglas cube and planar coils inside it.

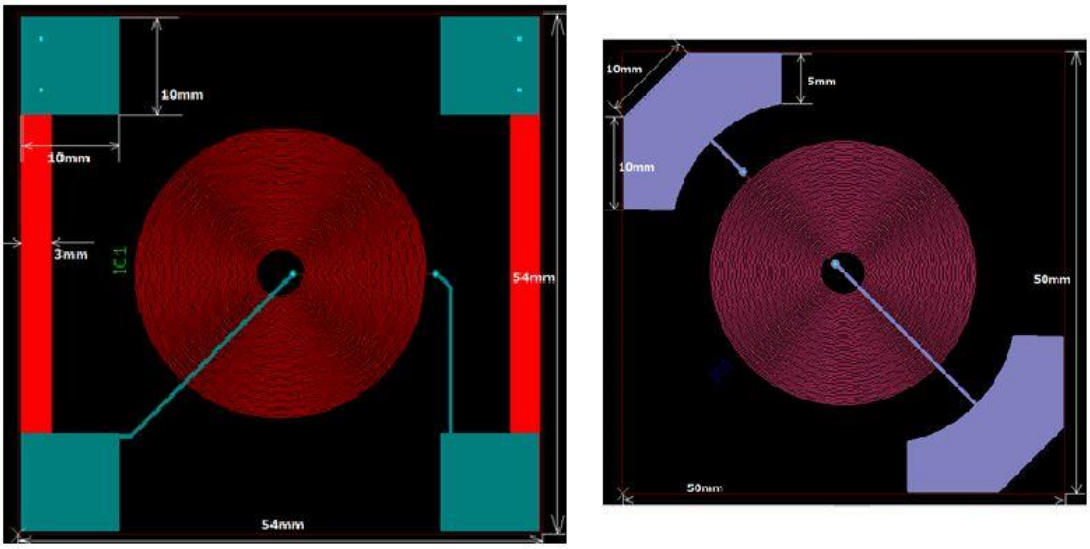

Figure 10. Examples of planar coils with electrodes. On the left there is a side PCB with two electrodes for van der Pauw measurement and coil with 19 turns, diameter of 2 cm, and trace width of 0.1 mm. On the right there is a planar coil that goes on top of the gelatine cube with the same coil parameters.

4.1.2. Recipe of phantom material

Gelatine phantom preparation started with analysing articles on tissue mimicking materials and finding suitable prime recipe for development. Main desired characteristics of the phantom material were: 1) inexpensiveness, 2) stability for long period of time, and 3) ease to manipulate electrical conductivity. Centre for Biorobotics in Tallinn University of Technology made low cost renal biopsy phantoms for interventional radiology trainees. They used gelatine gel as the tissue-mimicking material because it is self-supportive solid with proper range of achievable elasticity and relatively simple manufacturing process [5]. In current work gelatine and two other ingredients (Ethyl 4-hydroxybenzoate, Formaldehyde

solution) were employed from Biorobotics Centre's radiology phantom recipe to develop tissue phantoms with required electric parameters. Many other literature sources [6][15] suggest using gelatine as the main component for tissue mimicking as well. Biorobotic Centre scientists were making phantoms for radiology applications and graphite flakes or glass-beads were added to increase the ultrasound attenuation and backscatter. [5] Phantoms designed in present research are slightly different because ultrasound requirements of soft tissues do not have to be satisfied, but rather their electrical properties. In chapter 1.2 gelatine and other possible materials used for tissue mimicking phantoms were described more precisely.

Based on various research articles [5][9] and considering the possibilities, phantom based on gelatine material is used due to availability, simple production, and the ability to manipulate with its electrical conductivity without difficulty.

Table 3. Phantom mixture for 3 different phantom samples used in the research.

Ingredient	Amount	Purpose
Distilled water	250 ml for dilation 333 ml added later	Solvent
Sodium chloride (NaCl, 99+%, Sigma-Aldrich)	A: 0 g – 0 wt/vol% B: 0.5 g – 0.08 wt/vol% C: 5 g – 0.8 wt/vol%	Modifying electrical conductivity
Ethyl 4-hydroxybenzoate (99+%, Sigma Aldrich)	3 g	Antifungal and antibacterial preservative
Formaldehyde solution (37 wt.%, Sigma Aldrich)	2 ml	Rising melting temperature of gelatine and stabilizing the phantom over time
Gelatine powder (240TM, Poland)	40 g	Giving mechanical strength

In table 3. recipe for one phantom in plexiglas mould is given. A, B and C represent possible NaCl weights used in one phantom sample expressed as a percentage of the total solution volume. [16] When solute (NaCl) is added to the gelatine solution the percentage solution is calculated from equation:

$$\frac{weight}{volume}\% = \frac{weight\ of\ solute}{volume\ of\ solution} * 100 \qquad (4.1.2.1)$$

For example, 0.5 g of NaCl is added to 583 ml of gelatine mixture. Volume of solution is then 583 ml and weight of solute is 0.5 g. Replacing these figures into the equation (4.1.2.1) gives the percentage of concentration of NaCl in gelatine mixture, which is 0.082 wt/vol%.

4.1.3. Preparing of the gelatine phantoms

Preparation method was fairly simple and easily manageable when recipe was developed and worked out. Due to the toxic and carcinogenic components of the mixture (formaldehyde) protective equipment was used: safety goggles, latex gloves and all the mixing was performed in fume hood. For various experiments different sizes and shapes of beakers, flasks, test tubes, and pipettes were needed (ordered from Sigma Aldrich and Labochema).

Procedure for preparing the gelatine phantom material is as follows:

- First, distilled water is added to the unflavoured gelatine powder for dilation for approximately 10 minutes.

- After that, it is heated in a hot water bath (~60°C) and stirred constantly till the solution of gelatine and water liquefies and becomes homogeneous. Fig. 11 shows how gelatine mixture is being melted on hot water bath.

- Then, other ingredients are added: ethyl 4-hydroxybenzoate, formaldehyde solution and sodium chloride.

Melting point of gelatine gel is 35°C and formaldehyde solution is used to rise the melting temperature to over 65°C and stabilize the phantom. Formaldehyde increases the crosslinking of the molecules on the gelatine matrix. Ethyl 4-hydroxybenzoate is used as preservative and it has antifungal and antibacterial effect. [1] Different electrical conductivities of phantoms are achieved by adjusting the amount of sodium chloride. The solution is cooled to 40°C and distilled water is added to regulate phantom's gelatine concentration. Distilled water is used because its electrical conductivity is zero and it does not influence mixture overall conductivity. Stirring during supplementing extra components is crucial to achieve uniform solution. The mixture is placed into the containers described before, in chapter 4.1.1, and rotation machine is used during solidifying to get more homogeneous and even mixture.

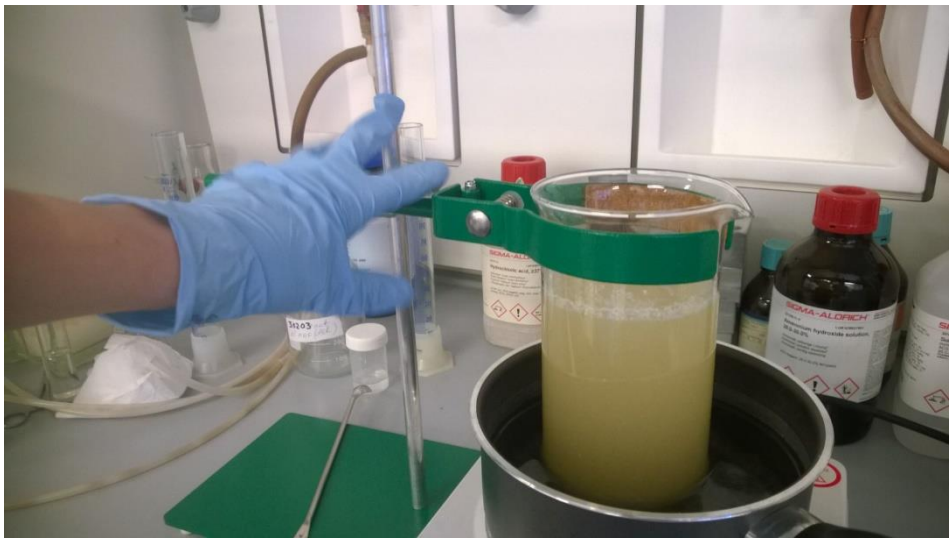

Figure 11. Heating the gelatine mixture on water bath in the fume hood.

Gelatine phantom is later placed in the refrigerator for storage. Due to the fact that temperature influences impedance measurement results (described in chapter 4.2), the gelatine samples were removed from the refrigerator at least 24 hours prior to measurements, insuring these were carried out at room temperature (23.5°C). Gelatine gives mechanical strength to the phantom, salt and water control the conductivity and permittivity. Gelatine concentration in present research is approximately 6.8 wt/vol%. If the concentration of the gel mixture is over 13 wt/vol% it is proven to be difficult to handle due to the fact that gel viscosity and solidification time is too big. [3]

If we have reliable homogeneous phantoms with known conductivities, we can start to develop more tissue-like heterogeneous phantoms by adding different substances or making layers of various materials.

4.2. Temperature effect on phantom impedance measurement

On logical ground, there is no compelling reason to argue that temperature influences the impedance measurements. Resistivity decreases as the sample temperature rises. The main theoretical premise behind the rise of conductivity is that ions in phantom material have greater mobility. As the phantom is made of biological material the current is carried by cations and anions. With temperature increase the heat energy breaks the electrostatic forces between ions and there is more mobility which results in higher conductivity. [4]

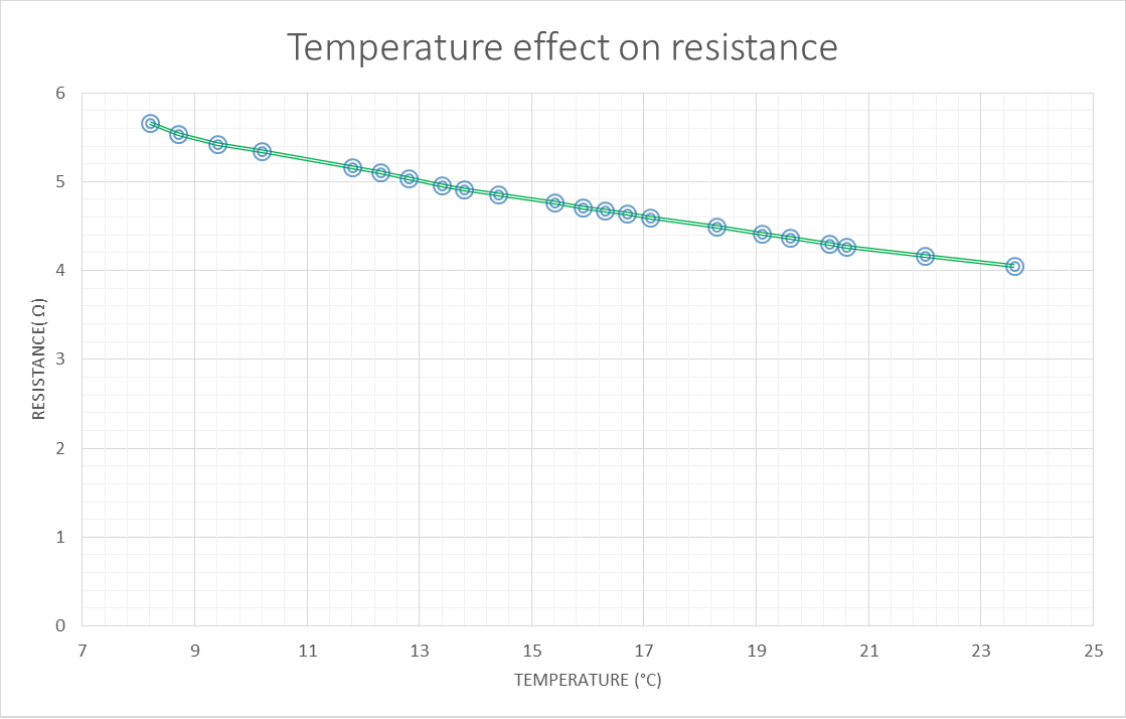

Figure 12. When temperature rises resistance decreases and the relation to temperature is fairly linear.

In metals, conductivity appears due to free electrons and electrical conductivity decreases with increasing temperature. There are plenty of mobile carriers (travel through a lattice of fixed atoms) and when the temperature rises the atoms that are in lattice start to move out of their lattice sites and interfere the electrons travelling through the channels. This means that

when temperature increases, conductivity in metal decreases on contrary to biological tissues.[7] To check the theory, subsequent experiments conducted on gelatine samples were formed and it showed the electrical resistivity to be a linear function of temperature as is depicted in Fig. 12. Fig. 12, shows the influence of temperature to the gelatine phantom described in this study. When sample was removed from the refrigerator, the gelatine temperature was around 5°C. Sample was connected to the impedance analyser measuring impedance and phase angle of the material over 5 hour time. From the graph it is possible to conclude that 5°C in temperature rise responds to approximately 0.4 Ω decrease in impedance. Also, it can be estimated that with 2 hours temperature increased with 10 degrees and slowed its pace when reaching near the room temperature. [7]

4.3. Measurement of phantoms

Electrical conductivity (σ) is defined by how easily current flows through a material under the influence of an external electric field and it is the inverse of electrical resistivity (ρ). [8][15] One interest and goal of current work is to find conductivities of gelatine samples prepared by the recipe described in chapter 4.1.2.

For desired measurements, edges of the plexiglas box, where the electrodes are placed, were labelled as *A, B, C* and *D*. If the contacts are not placed exactly in the periphery of the test object it will cause inaccuracies in the results [17]. The sample is symmetrical and in the shape of a square, which reduces measurement fault. The side length (L) is 50 mm and the width (w) of contacts is 1 mm. The measuring error due to the size of the contacts is under 10% because $s/L<0.1$ [17] The van der Pauw method as described in chapter 3 [2, 3] is used in this research to measure and calculate the electrical conductivity of the designed gelatine samples. Four thin electrode stripes on two PCB's on either side have direct contact to the gelatine phantom, which is inside the plexiglas box. When viewed top down as 2D, the point-like electrodes are exactly in the corner of the sample which makes the vdP method compatible. Also, thickness of the sample must be constant and homogeneous [52]. Usually, vdP method is used on samples where the sample thickness is comparable to the surface dimensions. In this study given technique is used to measure resistance of gelatine cube. Research made by Kasl and Hoch [51] shows that the limiting thickness is about half the diameter of the samples when contacts are placed on the edge of the surface. But the technique

is valid also for arbitrarily thick samples when low resistance electrodes are placed across the entire edge, perpendicular to the surface, in order to maintain an effective two-dimensional current distribution. Kasl and Hoch research [51] results are analysed more precisely in chapter 3.4.

For current and voltage measurements, gelatine sample electrodes were connected to the Wayne Kerr 6500B precision impedance analyser [28]. It provides precise and fast testing at frequencies up to 120 MHz, but due to cable resistance and capacitance effects, it is possible to make measurements until 1 MHz with low conductivity phantom samples. Instrument has an anomalous behaviour at high frequencies - phase shift occurs. Studies [24][29] suggest that these results may be due to stray capacitances of the connecting cables. A 1V AC voltage was applied across the sample's one side and current was measured on two other electrodes. AC measurements were used, to avoid effects of polarisation across the electrodes [9].

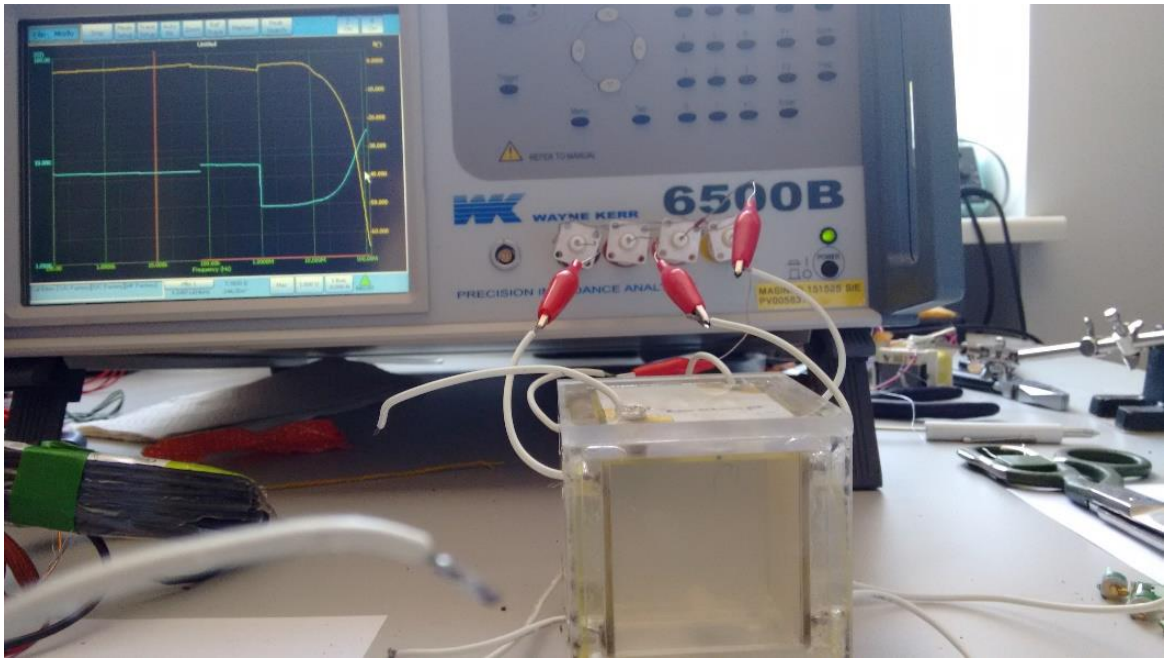

Figure 13. Wayne Kerr impedance analyser connected to gelatine sample with four electrodes.

When doing van der Pauw measurements four switching configurations was used to get the optimal result. Fig. 13 shows the placement of the electrodes which are labelled as *A, B, C* and *D* counter clockwise. Four different electrode placements were used to get two resistances from one sample:

1) Measurement 1: current through the electrodes A and D, measuring voltage from B and C (Fig. 14.1);
2) Measurement 2: current through A and B, measuring voltage from C and D (Fig. 14.2);
3) Measurement 3: current through B and C, measuring voltage from A and D (Fig. 14.3);
4) Measurement 4: current through C and D, measuring voltage from A and B (Fig. 14.4).

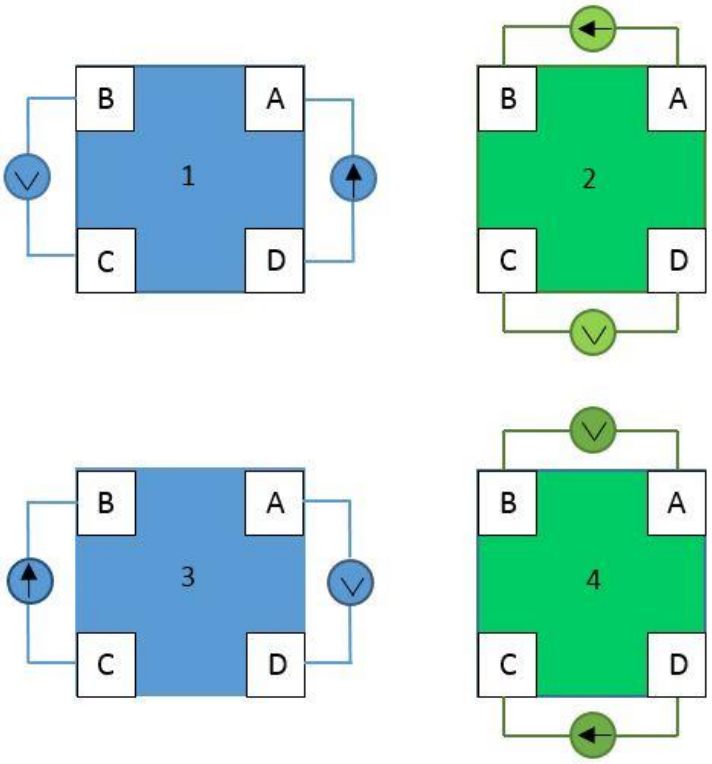

Figure 14. Van der Pauw measurement method set up used in the research. Blue colour indicates one pair of measurement (1 and 3) and green colour other pair (2 and 4).

Resistivity (ρ) and conductivity (σ) are calculated from the measured values and the thickness d, of the measured sample. Two parameters are displayed on impedance analyser screen at the same time: impedance and phase in relation to frequency logarithmically-spaced from 100 Hz to 120 MHz with 800 points.

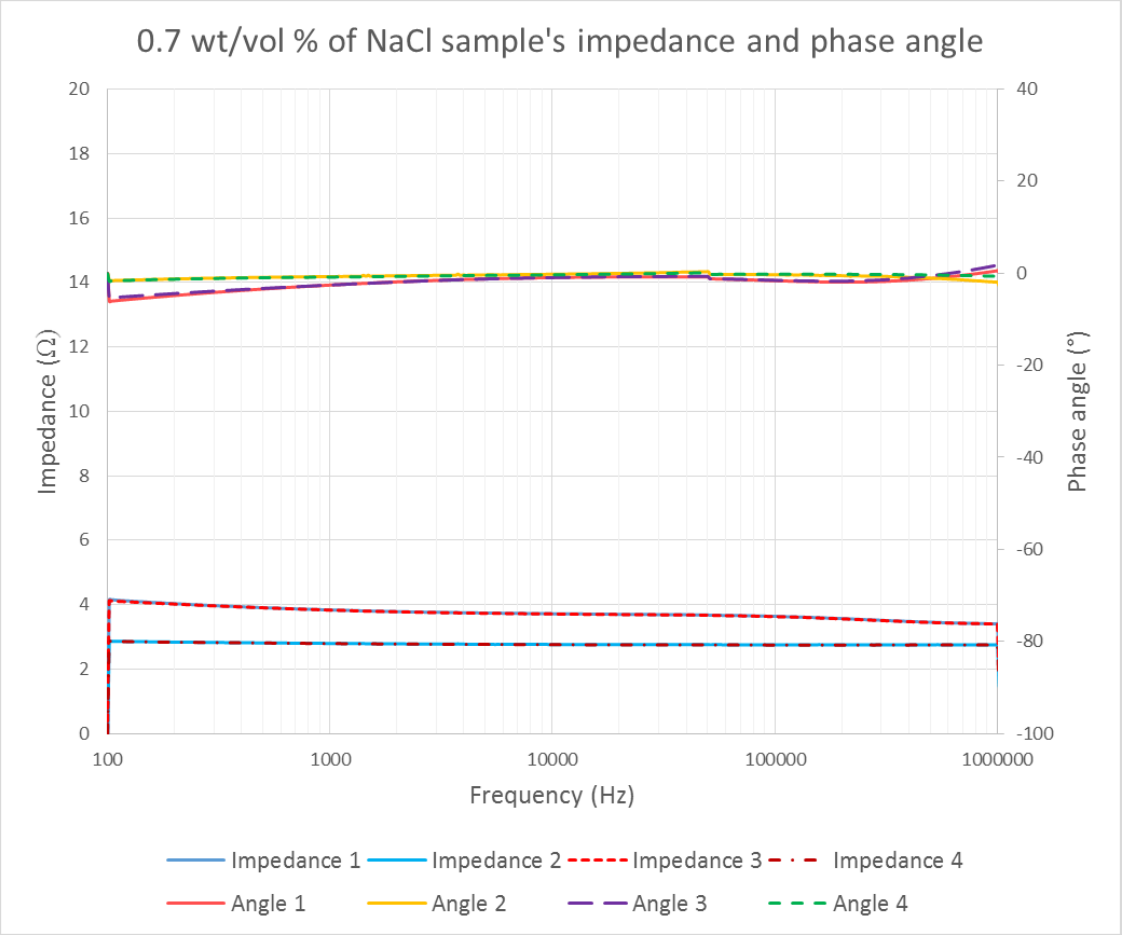

Figure 15. 0.7 wt/vol% of salt is added is added to the gelatine mixture and four measurements are made.

From graph (Fig. 15), the impedance measurement 1 and impedance measurement 3 have very similar values like impedance 2 and impedance 4. Also, phase angle pairs are matching. How measured impedances are put into van der Pauw equation, is described in the next chapter (chapter 4.4).

Van der Pauw method is very convenient for samples that are not cut into perfect geometric shape and the position of the electrodes is arbitrary, so long as they are at the perimeter. In practice, it is problematic to attach electrodes exactly at the perimeter and therefore, inevitable errors come forward. [10] Because of this reason, in present experiments cubic samples are used, so that electrodes would be placed as close as possible to the corners and could reduce the number of faulty measures. In practice, not all the measurements were considered true because number of assumptions were made. Firstly, it was considered that good electrical contact is maintained by the electrodes to the phantom sample. Secondly, it

was figured that conductivity of homogeneous sample is not dependent on frequency. Few experiments showed change in the frequency range and these results were rejected. Lastly, temperature was not controlled in the laboratory. Room temperature fluctuation was quite significant, varying from 23.4 to 25 °C. In this analysis, these errors are not investigated further and faulty measurements are rejected if two measurements differ at least 20% from each other.

4.4. Conductivity calculations

With the impedance analyser the relation between impedance and frequency was given. There was a negligible dependence of impedance on frequency, as also seen in other work [9] and because of that we used only one frequency (10 kHz) to depict the conductivity of sample. Figure 18. in chapter 5 shows how impedance did not change with the frequency and the graph is flat (literature suggests the same at 10 kHz, [10]) until the wire errors appear. Conductivity was calculated using van der Pauw formula from different measurements of impedance on 10 kHz. Van der Pauw theorem and its disposition for measuring was described before. (chapter 3, Fig. 6) To improve precision of the result, four measurements were made and labelled: $R_{AD,BC}, R_{AB,CD}, R_{BC,AD}, R_{CD,AB}$, where 2 measurements are similar to each other because 4 electrodes' configuration is symmetrical. To find resistivity of the sample, firstly, 2 resistances ($R_{AD,BC}, R_{AB,CD}$) are placed into the equation (4.4.1) and then 2 other resistances ($R_{BC,AD}, R_{CD,AB}$). After resistivity calculations reciprocal of these results give 2 similar conductivities. Later, average of these two calculated conductivities are used to describe the conductivity of particular sample. Calculations were made in Microsoft Excel software using modified van der Pauw formula:

$$=(\mathrm{PI}()*d/\mathrm{LN}(2)*(R_{AD,BC}+R_{AB,CD})/2)\ , \qquad (4.4.1)$$

where d is sample thickness in meters (0.05 m), $R_{AD,BC}$ is measured impedance 1 and $R_{AB,CD}$ is measured impedance 2. [11]

4.5. Accuracy of van der Pauw method

To check the correctness of experiments and to get the reliable results, checking the accuracy of van der Pauw conductivity calculations was needed. For that, plastic mould, a syringe, was used and two tin coated disk shaped copper electrodes in the both ends of the cylinder were added. Also, one hole was made to eliminate the air bubbles, which emerge during addition of gelatine mixture. On figure 16. syringe with gelatine gel inside it, is showed. Electrodes that are on both sides of the sample are marked as electrode 1 and electrode 2.

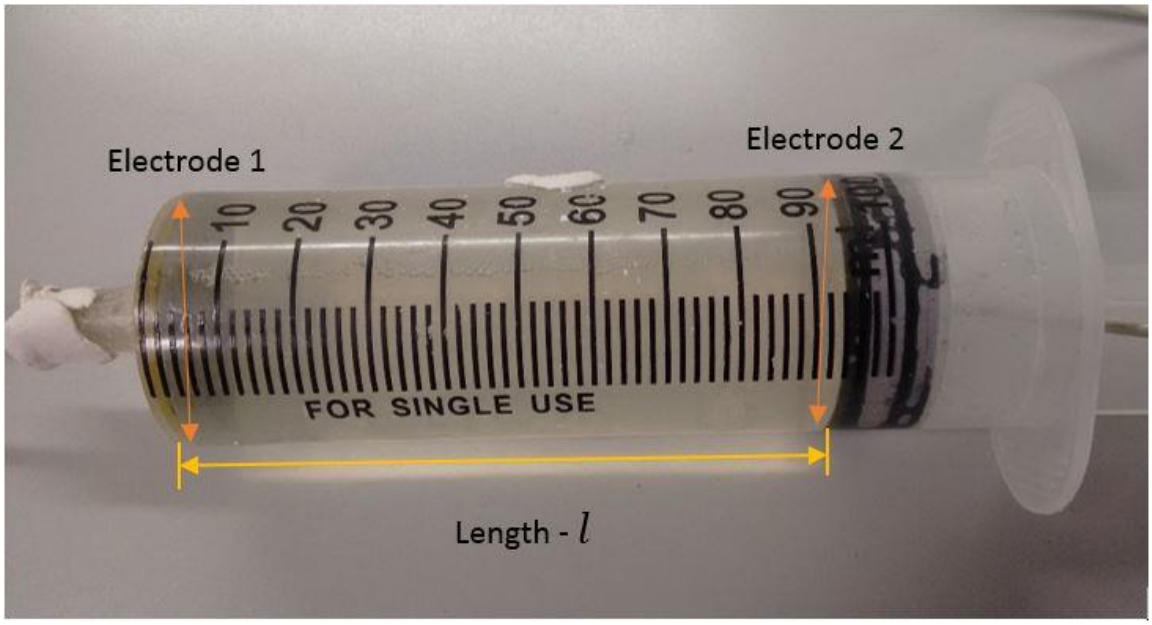

Figure 16. Syringe with gelatine mixture for resistivity measurement and for checking the correctness of our interpretation of van der Pauw method.

Tabel 4. represents three measurement results made with syringe. Syringe parameters are also depicted. To find the conductivity, similarly, AC voltage was applied across the gel in the syringe and the conductivity was calculated from the impedance using following equation (4.5.1):

$$\sigma = \frac{1}{\rho} = \frac{1}{\frac{RA}{l}} \tag{4.5.1}$$

where, σ is the electrical conductivity in Siemens per meter, ρ is the electrical resistivity (Ohms-m), R is the electrical resistance (Ohms), A is the cross sectional area of the gel sample in square meters and l is the length of the gelatine sample in the syringe in meters. Calculated conductivities together with vdP method results are shown in the table 5. In present work finding the conductivity with the equation (4.5.1) is named *syringe method*.

Table 4. 3 phantom samples with different NaCl concentrations and their conductivities calculated with syringe method.

	NaCl (wt/vol%)	Sample length (m)	Area (m²)	Conductivity on 10 kHz (S/m)
Syringe 1	0.51	0.082	0.00101	**0.75**
Syringe 2	0.20	0.072	0.00101	**0.41**
Syringe 3	0.29	0.092	0.00101	**0.53**

Table 5. 3 phantom samples and their conductivities calculated with vdP method.

	vdP 1	**vdP 2**	**vdP 3**
NaCl (wt/vol%)	0.51	0.20	0.29
Sample thickness (m)	0.05	0.05	0.05
Resistance 1 (Ω)	5.69	10.59	8.45
Resistance 2 (Ω)	5.16	9.71	7.35
Resistance 3 (Ω)	5.67	10.60	8.47
Resistance 4 (Ω)	5.16	9.72	7.36
Resistivity (Ω-m)	1.23	2.30	1.79
Conductivity on 10 kHz (S/m)	**0.80**	**0.43**	**0.55**

Conductivity on 10 kHz found with syringe method is slightly smaller when using vdP technique. Estimated possible error is 4.8% and standard deviation of conductivity results is 0.03, which appear due to measuring errors. Graph in figure 18. represents 3 samples with 0.51 wt/vol%, 0.20 wt/vol% and 0.29 wt/vol% concentration of salt. Comparison of syringe method and vdP method is depicted. As was already seen from the table 4 and 5. vdP results were acceptably similar to syringe method results on frequency 10 kHz, which proves the

correctness of vdP method interpretation in present study considering the possible measurement errors.

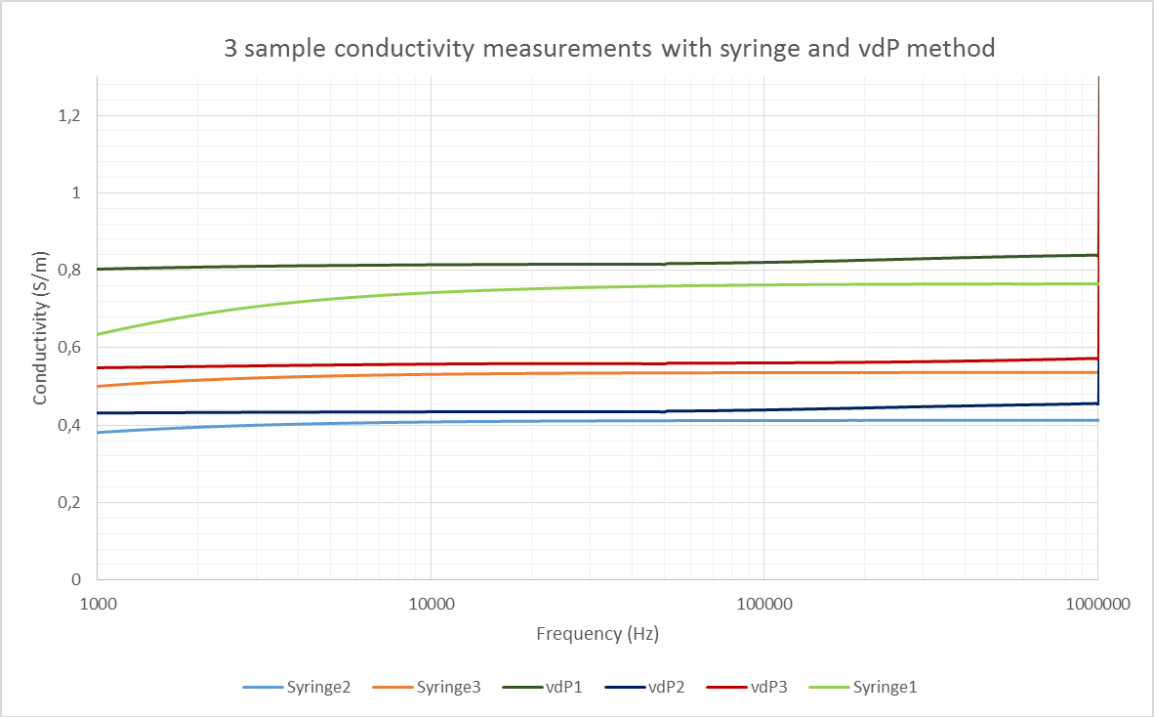

Figure 17. 3 samples with different salt concentrations were measured and conductivities were calculated with syringe and vdP method. Light blue, light red and light green lines represent conductivities found with syringe method and blue, red and green lines are conductivities calculated with vdP method.

The data yielded by this experiment provides convincing evidence that van der Pauw method for the measurement of electrical conductivity of materials – is reliable for measurement and calculation of the electrical conductivity of tissue mimicking phantom non-zero thickness materials.

5. Conductivity measurements

This chapter introduces the main conductivity measurements made and results obtained. Conductivity (σ) is one of the main factors affecting the choice of a phantom material for studying the electrical properties and effect of electric fields on different biological tissues. [9]. Human tissue is heterogeneous and electrical conductivity changes over the frequency range. Dispersions (described in chapter 2.3) are evident in human soft tissues, which means that conductivity changes over frequency range. Phantoms developed in present study are homogeneous as is seen from the Fig. 18, which shows frequency and conductivity dependence.

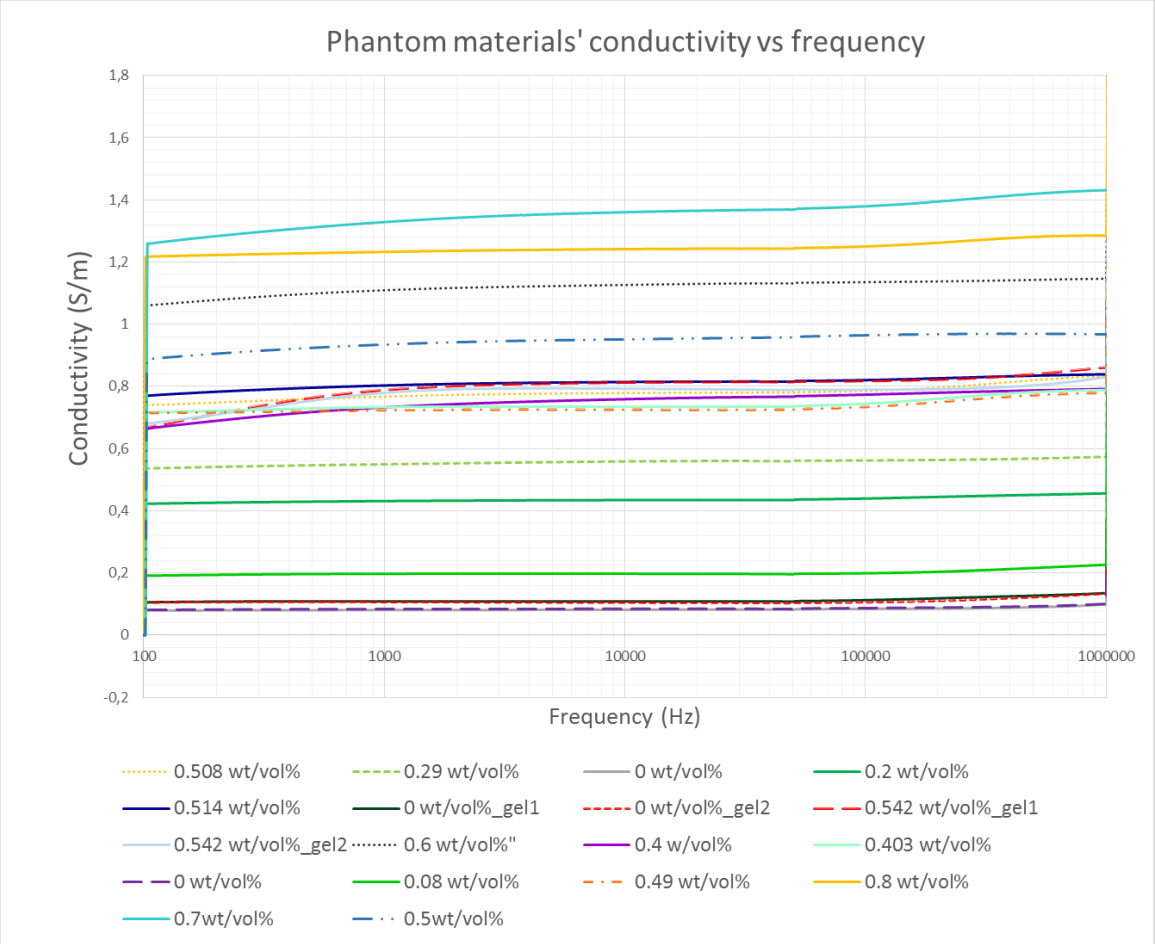

Figure 18. Conductivity of 18 different gelatine samples calculated.

18 different samples are showed in the graph. It can be seen from the figure that conductivity is same throughout the frequency range from 100 Hz to 1 MHz, which means that electrical

conductivity is nearly independent of frequency. Gelatine phantoms are usable and suitable for one frequency testing. No dispersions are shown like in biological tissues.

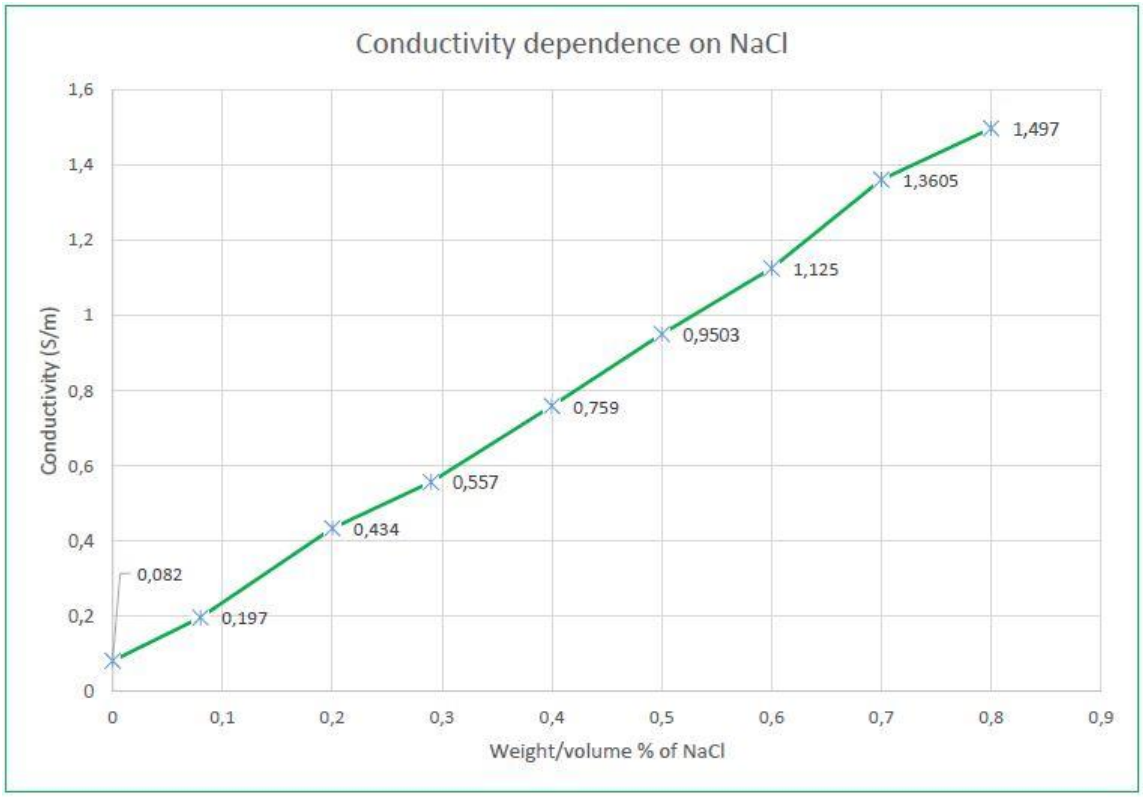

Figure 19. Conductivity dependence on NaCl.

Gelatine has cations and anions in its segments, thus there are positively and negatively charged sites. The addition of salt leads to an increase of the ionisation of the gelatine, due to the formation of ions [18]. Consequently, there is an increase in the conductivity due to the rise of salt content in the gelatine, thus lowering the impedance value. [15] Already a small amount can change the material less resistive to electrical current. Salt is proved [6][15][19] to be very reliable ingredient to manipulate with the gelatine sample conductivity. Fig. 19 describes conductivity dependence on amount of NaCl on 10 kHz. 9 samples with varying NaCl concentrations are depicted together with their conductivities. Rising the NaCl amount increases the electric conductivity. Relation between percentage of salt in the mixture and conductivity is strongly linear.

Table 6. Human tissue conductivities on different frequencies.

Tissue	***1/ρ* at 10 kHz, *S/m***	***1/ρ* at 100 kHz, *S/m***	***1/ρ* at 1MHz, *S/m***
Muscle	0.34083	0.36185	0.61683
Blood	0.7000	0.7029	1.0967
Dry skin	0.00045	0.00045	0.19732
Heart	0.15421	0.21511	0.50137
Body fluid	1.5	1.5	1.502

Various biological tissues have very small conductivity and in some cases there is no need to add any NaCl to the gelatine when mimicking these tissues. Table 6, introduces different human tissues and their conductivities on three frequencies. Lowest conductivity can be smaller than 0.0005 S/m. In current study, having 120g of gelatine powder added to 1300 ml of water gives the gelatine percentage of 9 wt/vol%. Using this concentration gives the minimum conductivity 0.07 S/m but to get lower conductivity more distilled water needs to be added. For example, adding 400 ml to the usual 9 wt/vol% gelatine concentration gives result of 0.06 S/m. There is a problem how to obtain smaller conductivities. It is not reasonable to add a lot of distilled water because the mechanical strength of phantom needs to stay at level where the sample can be used without the mould.

5.1. Adding substances and variations of recipes

In this section, the discussion will point to dispersions in tissue-mimicking phantoms. Biological tissues do not have the same electrical conductivity in different frequency levels as was described in the chapter 2. about bioimpedance. Desired dispersion would be from 100 Hz to 1 MHz which means from alpha region to beta region. Experiments were made by adding graphite in various ways to the gelatine mixture. Graphite's electrical conductivity is very high, it can reach to 25000 S/cm [12]. Graphite is a crystalline solid of black-grey colour. It is added to make tissue mimicking materials for ultrasound applications because its speed of sound properties mimic the human soft tissue and have proper range of achievable elasticity characteristics. [13][14] Making dispersions was not the intentional goal to achieve with this thesis work, but some experiments were still made to get a quick overview how heterogeneous samples' conductivity changes with frequency.

Firstly, graphite powder was added to the gelatine mixture to see how it influences the phantom electrical parameters. Results can be seen on Fig. 20.

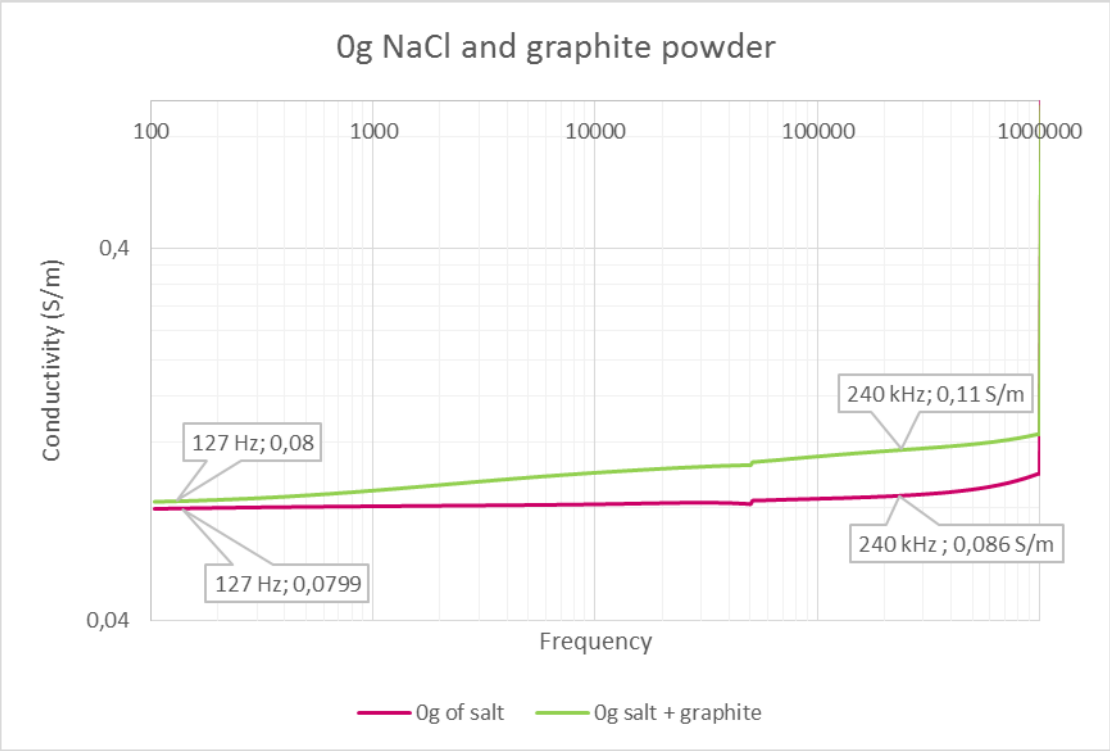

Figure 20. Graphite powder added to the gelatine phantom.

Graphite powder has been found to be useful in manipulating conductivity and permittivity mostly at microwave frequencies, but research made by T.J. Kao et al. [20] shows the increase in conductivity with the addition of graphite at frequencies from a few kilohertz to 1 MHz.. The results in current work, shown in Fig. 20, suggest that graphite does not influence gelatine sample's conductivity as much as was expected. Compared to the phantom, which had 0g of salt and sample with 0g salt and graphite powder, at around 100 Hz conductivity is basically the same – 0.079 S/m and 0.08 S/m. On higher frequencies, at 240 kHz, graphite powder sample conductivity increases to 0.11 S/m. Experiment did not support the hypothesis that with addition of graphite, conductivity increases significantly at all frequencies. Although, it could be that conductivity will increase to a greater extent at higher frequencies, but more experiments needs to be done.

Secondly, experiment with three contrasting samples was made, which has the following main components:

Sample 1 → Layer 1: 1.6 wt/vol% NaCl

Layer 2: 0 wt/vol% NaCl

Sample 2 → Layer 1: 1 wt/vol% NaCl

Layer 2: 0 wt/vol% NaCl + 44g of graphite powder

Sample 3 → Layer 1: 4 wt/vol% NaCl

Layer 2: 0 wt/vol% + 30g of graphite powder

Layer 3: 0 wt/vol% NaCl

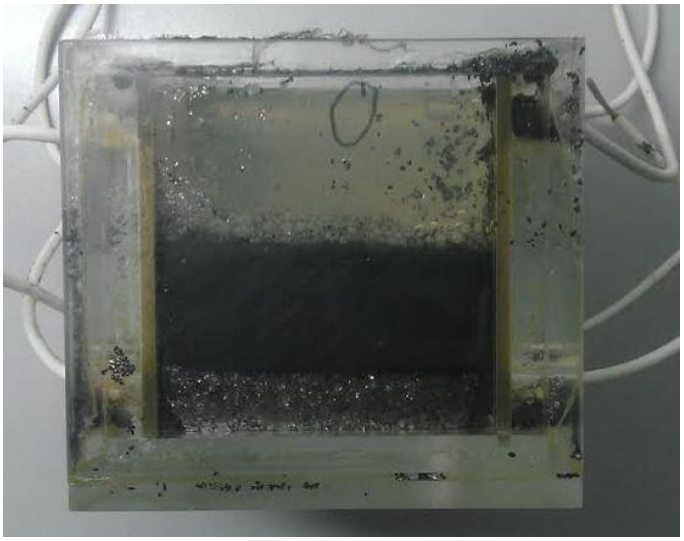

Figure 21. Sample 2 - 1 wt/vol% of NaCl and 44 g of graphite powder. Graphite flakes have settled in the bottom.

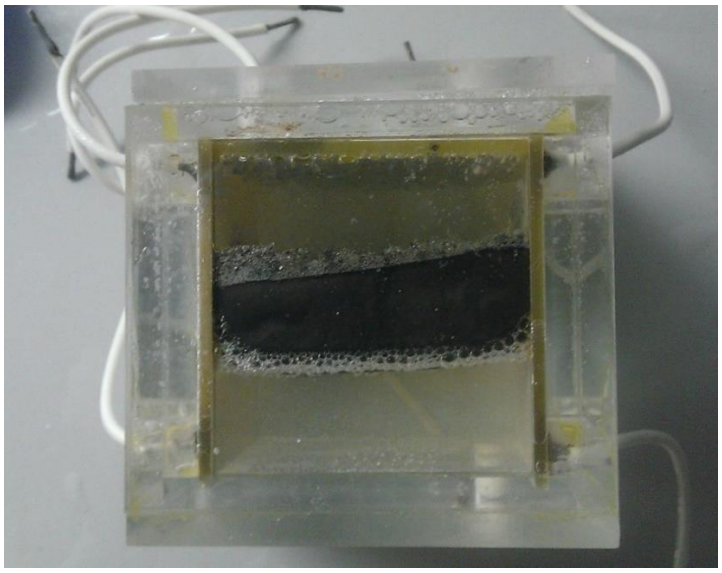

Figure 22. Sample 3 – 4 wt/vol% NaCl, 0 wt/vol% NaCl + graphite, 0 wt/vol% NaCl.

Main purpose was to see some changes in conductivity in given frequency range, which is by bioimpedance definition alpha- dispersion and the beginning of beta dispersion region. From Fig. 23, first two samples seemed to be quite similar to each other and first phantom's conductivity did not depend on frequency as was expected due to only changing salt percentage. Second sample had similarly flat graph until 800 kHz then, conductivity started to rise. Third phantom differs most from the other two. Conductivity starts to increase already from 500 kHz and continues to rise until 1 MHz. This could mean that graph follows beta dispersion characteristic, but closer look at the graph indicates that more likely the graphite powder in the mixture starts to influence the conductivity.

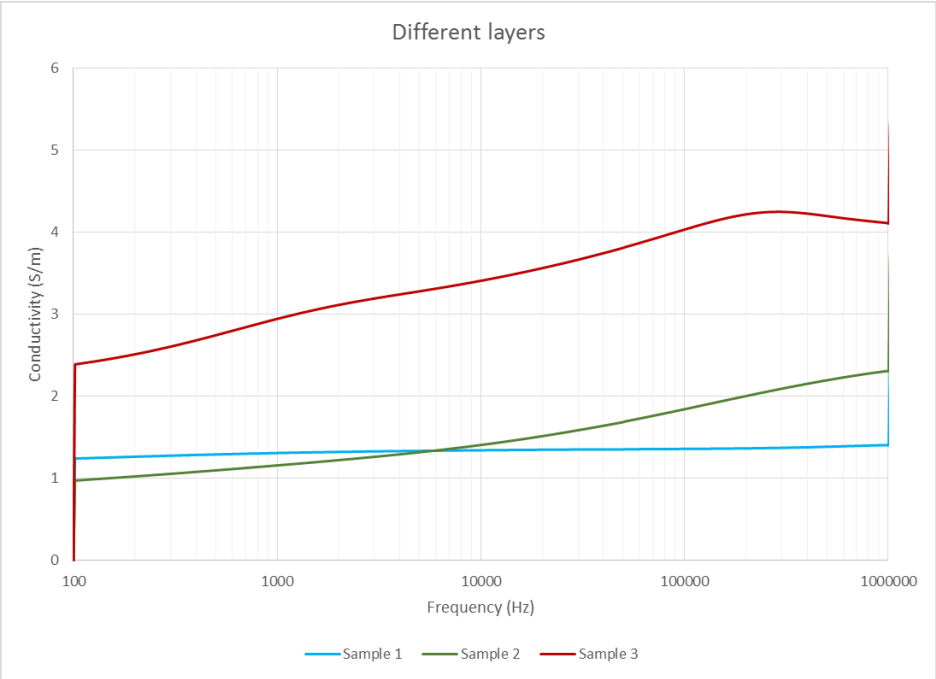

Figure 23. 3 different samples with different layers constructed.

Figure 23. shows how different layers with different amount of salt and graphite powder added draws the conductivity graph. Sample 3 conductivity rises from 2.5 S/m to 4.2 S/m in the frequency range of 100 Hz to 200 kHz. Sample 2 shows also signs of growing conductivity – from 1 S/m to 2.2 S/m. These two samples had graphite powder added to them and they differ slightly form the Sample 1, which had only salt added to change the conductivity.

From these two experiments it is not possible to make straightforward conclusions and these results do not provide confirmatory evidence that graphite powder forms any dispersions or any significant changes in the conductivity at all. But further research in this area may include analysing graphite powder influence to the gelatine phantom electrical conductivity and dielectric permittivity.

5.2. Comsol Multiphysics

COMSOL Multiphysics is a computer simulation program, which translates real-world physical laws into their virtual form [47]. Next figures (Fig. 24, 25, 26) show gelatine phantoms simulated in Comsol software with 4 copper electrodes on the edges. Materials

are defined in the program in terms of permittivity and conductivity. Gelatine phantom conductivity was chosen to be similar to blood at 10 kHz - 0.700 S/m and relative permittivity 90, because animal and human tissue permittivity varies from 95 to 180. [6] Copper electrodes were part of Comsol's integrated material library. Purpose of the Comsol calculated simulations in present study are mainly illustrative. Fig. 24 shows current density streamlines, which describe how current is going through the phantom sample when voltage is applied.

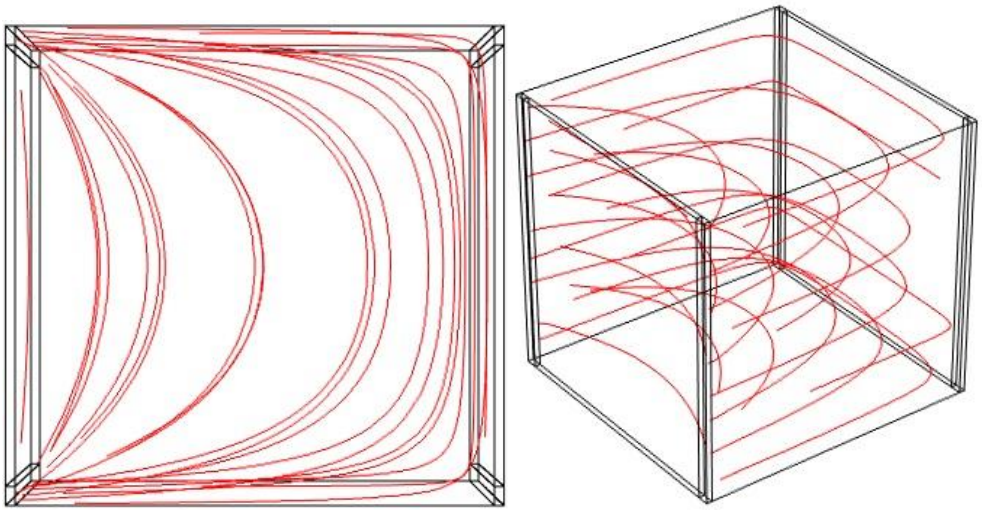

Figure 24. Current density streamlines of gelatine phantom

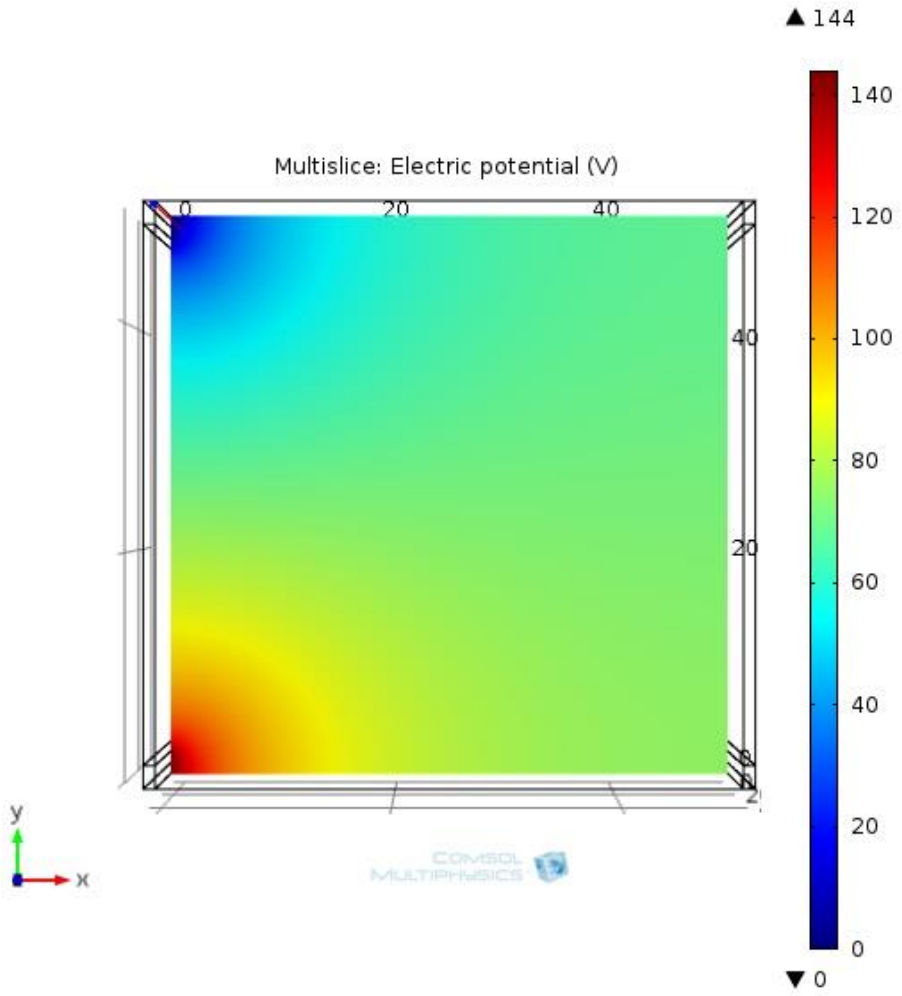

Figure 25. Electric potential on simulated phantom.

Fig. 25 illustrates electric potential between two nodes from which one node is ground and other has 1 V applied. Electric potential shows steady and continuous electric current in conductive media.

Fig. 26 shows resulting plot of current density of the vector. Electrode contacts in two corners have higher density than rest of the phantom and plot is almost uniform due to the low density in material.

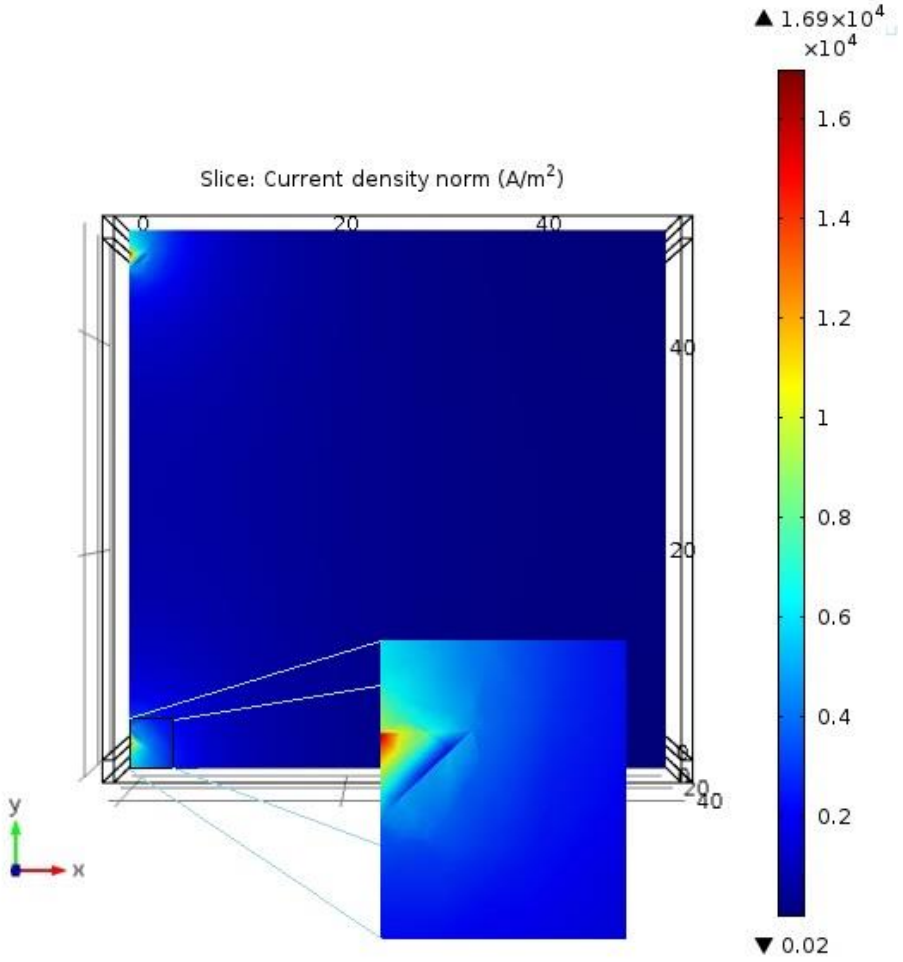

Figure 26. Absolute value of the current density vector.

6. Results and discussion

Recipe for gelatine phantom was developed and used to make different samples with various electrical conductivities.

Van der Pauw conductivity measurement method, needed mostly in semiconductor industry, was used to measure gelatine phantom materials. Electrodes placed in four edges of the sample gave impedance results on frequency range until 1 MHz. Van der Pauw technique proved to be reliable and convenient method to measure and calculate electrical conductivities with improved equation shown below:

$$=(\mathrm{PI}()*d/\mathrm{LN}(2)*(R_{AD,BC}+R_{AB,CD})/2)\ , \qquad (4.4.1)$$

Figure 27. shows the NaCl concentration effect on the electrical conductivity of gelatine phantom at room temperature ($23 \pm 1.5°C$). Rising the NaCl percentage in the solution increased the conductivity. Conductivity changed from 0.082 S/m to 1.497 S/m and NaCl concentration changed from 0 to 0.8 wt/vol% accordingly.

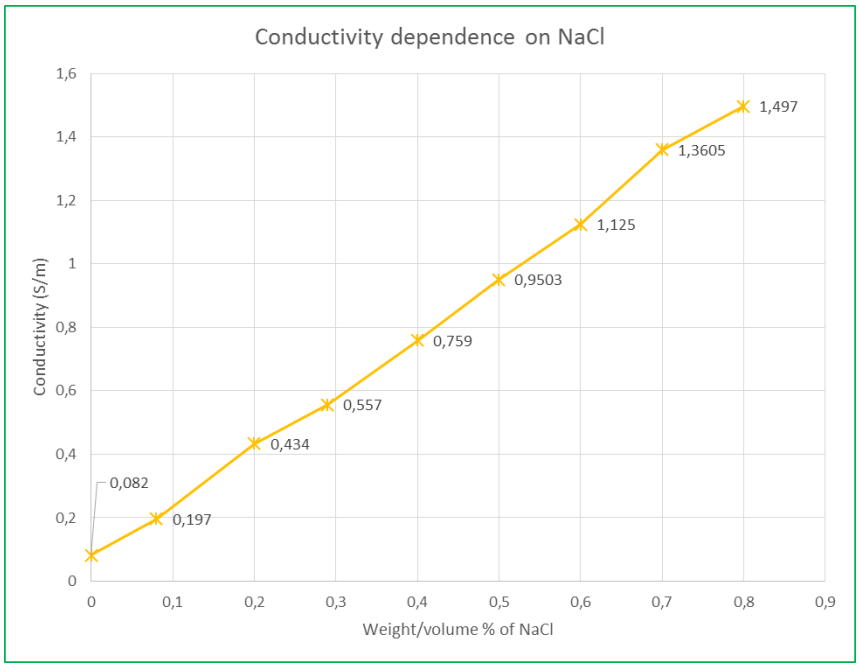

Figure 27. Conductivity change with NaCl concentration.

Conductivity of gelatine was constant across the frequency range (100 Hz – 1 MHz). (Fig. 28 shows sample conductivity dependence on NaCl concentration of 0 wt/vol% to 0.7 wt/vol%). Increasing the gelatine concentration increased the conductivity and adding more distilled water decreased the conductivity.

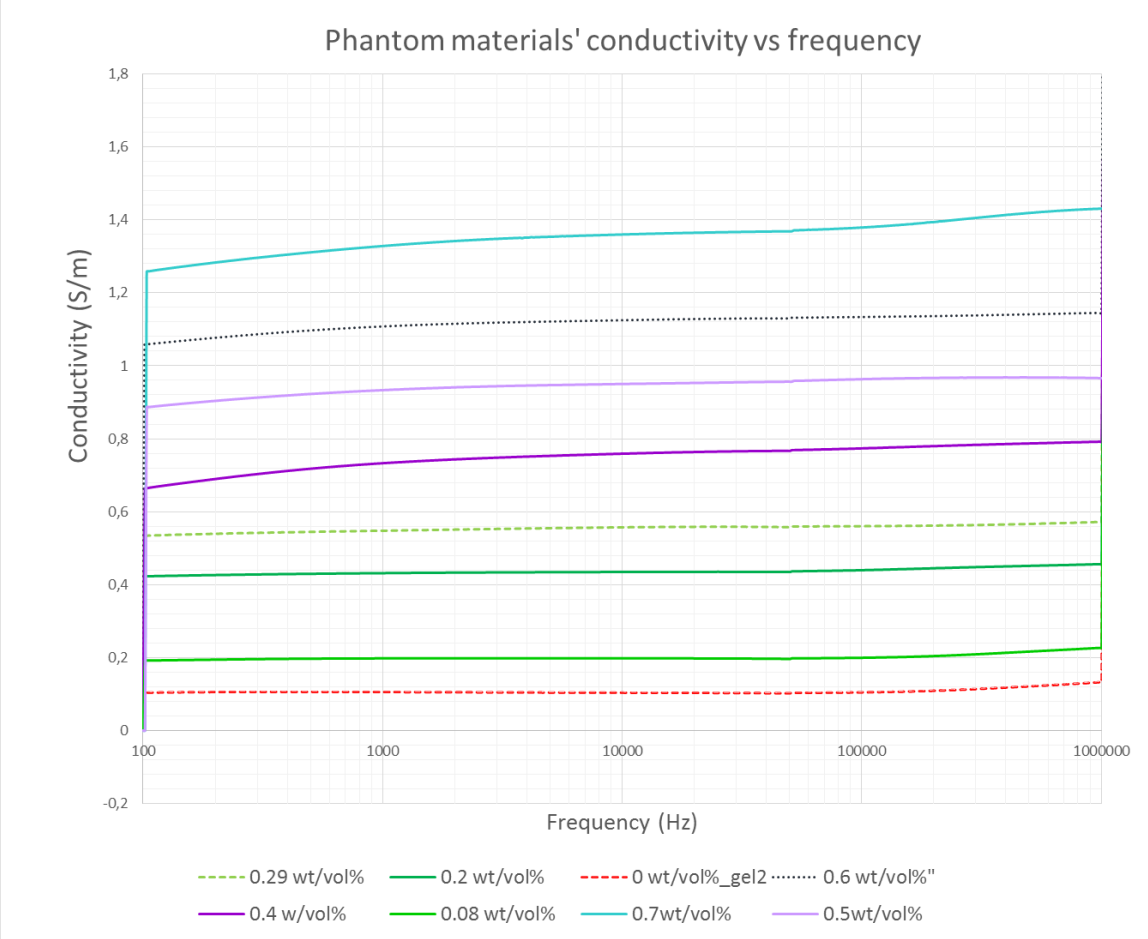

Figure 28. 8 different samples with varying NaCl concentration are shown. 270 frequencies are distributed evenly on a log scale from 100 Hz to 1 MHz. Conductivity change is negligible in this frequency range.

Conductivity also increased with the rise of temperature due to the increased mobility of ions in the mixture. Fig. 29. depicts the linear graph of resistance dependence on temperature.

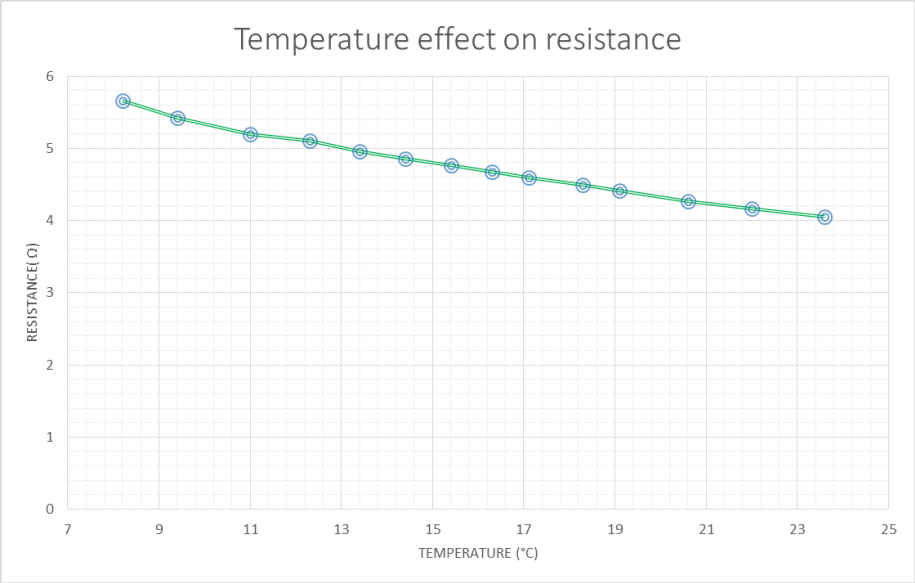

Figure 29. Gelatine phantom's resistance was measured during 4 hour time until sample temperature raised from 8°C to 23.5°C

Few experiments were made by adding graphite powder to gelatine mixture. There were only small, unsystematic variations in phantom's conductivity when graphite was added to gelatine alone. When different layers were constructed, graphite showed more of an impact to the mixture. Fig. 30, shows minor conductivity rise in frequency range from 100 Hz to 1 MHz.

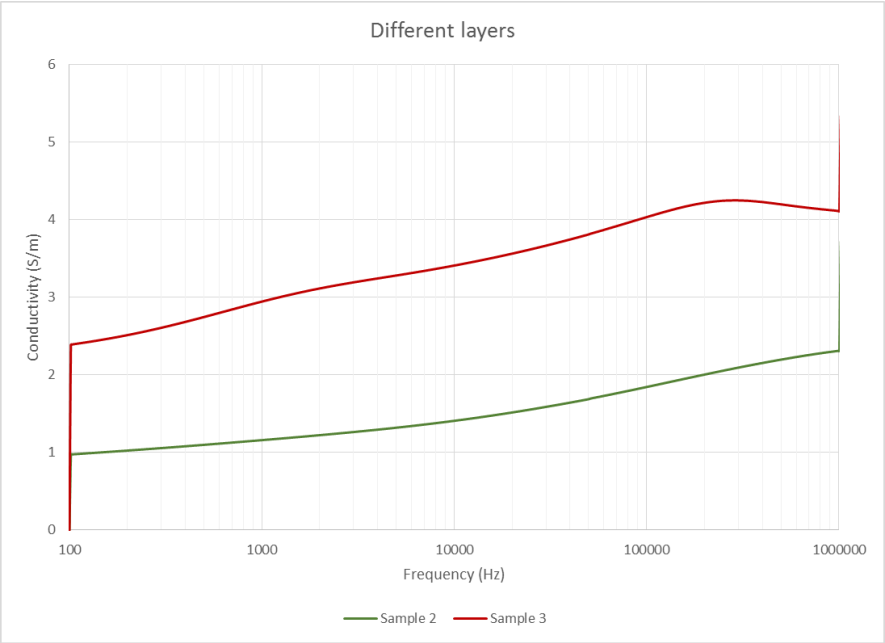

Figure 30. Graphite Sample 2 and Sample 3 (from chapter 5.1) show how conductivity increases slightly with frequency.

Using gelatine phantom to mimic tissue conductivities is inexpensive, stable over time and easy to prepare. Phantom preparation did not require special equipment and conductivity measurement method was convenient after some practice. Changing conductivity with NaCl was efficient and predictable due to the linear relation between conductivity and salt concentration. Additionally, van der Pauw technique proved to be effective way to determine conductivity of samples.

Given technology, technique to measure phantoms, sample making methods and preparations did not give overly accurate results because number of assumptions were made. It was assumed that samples were homogeneous without any isolated holes, recipe for phantoms was followed precisely and electrical contact between sample and electrodes was good. For these reasons it is possible to give only overall estimation to phantom samples. One other negative part of phantoms developed in current work is their homogeneous and isotropic nature in contrast to biological tissues that are anisotropic and heterogeneous. However, main purpose of this research was to prepare homogeneous phantom materials that match the average human tissue at room temperature. The analysis presented is intended to be a part of experiments of future research where main focus is towards understanding heterogeneous and more complex biological tissues. Preliminary results showed that gelatine

phantoms prepared are useful for research involving testing or development of applications using tissue phantoms.

Conclusion

Present research showed how homogeneous tissue equivalent phantoms that have similar electrical properties of a biological system, is designed and developed. Main goals of the thesis were to develop reliable recipe for gelatine phantoms with required electric conductivities for bioimpedance applications and find a method to accurately measure and calculate phantom conductivities.

The work was divided into two main parts: theoretical part, where overview of literature was done and experimental part, that introduced techniques and materials used. Theory part started with general description about the possible phantom materials, their usage, description of real human tissue measurement – bioimpedance and introduction to van der Pauw method. Experimental part described the gelatine mixture recipe developed, making of phantoms, how measurements were conducted, temperature, NaCl and graphite effect on electrical conductivity of gelatine phantom materials.

Gelatine sample phantoms with different NaCl concentrations were prepared and measured and fixed recipe was developed. Van der Pauw method was used to find numerical conductivity values. Measurements were held at frequency range from 100 Hz to 1 MHz and all the experiments with NaCl showed the change in conductivity which were nearly independent of frequency in the range. Conductivities increased with the greater amount of salt in the mixture and with the rise of temperature. Graphite powder added to the gelatine mixture did not change the conductivity as was expected, but conductivity increased in the 100 Hz to 1 MHz range when graphite was used only in one layer of sample.

In present study gelatine sample phantoms with needed electric conductivities were successfully designed. Manipulating with the amount of NaCl in the gelatine gel mixture, changed the conductivity as was expected and van der Pauw technique used in the research proved to be reliable method for measuring and calculating conductivities of developed phantom materials. Although, adding graphite powder to the gelatine mixture did not show any significant change in conductivity results after few experiments, it is studied further.

In the future, accuracy of measurements, methods and techniques must be improved to use developed phantoms in research about heterogeneous tissue mimicking materials for bioimpedance application measurements.

Acknowledgements

I would like to thank my supervisor, Rauno Gordon and Ph.D. student Ksenija Pesti for guidance in the field of bioimpedance, discussions, encouragement and support throughout this thesis.

Also, I would like to thank Raul Land for providing help with measurements and Jürgen Arund for friendly working environment.

This research was supported by the European Union through the European Regional Development Fund in the frames of the centre of excellence in research CEBE, competence centre ELIKO, of the Competence Centre program of Enterprise Estonia, National Development Plan for the Implementation of Structural Funds Measure 1.1 project 1.0101.01-0480 and also by Estonian Science Foundation Grant 9394.

References

1. S.Podtaev, D. Nikolaev, V. Samartsev, V. Gavrilov, K. Tsiberkin, “Frequency and temperature dependence of skin bioimpedance during a contralateral cold test”, Physiol. Meas. 36, Russia, pp. 561-577, February 2015.

2. D. Daghero “Resistivity measurements the conventional and van der Pauw techniques” extracted from the PhD thesis, Polytechnic University of Turin, 2002

3. R. Villenberg “Kirurgiliste ultrahelifantoomide materjalide arendamine”, extracted from the master thesis, Tallinn University of Technology, 2011.

4. Conductivity Theory and Practice. Website at: http://www.tau.ac.il/~chemlaba/Files/Theoryconductivity.pdf

5. A. Hunt, A. Ristolainen, P. Ross, R. Öpik, A. Krumme, M. Kruusmaa “Low cost anatomically realistic renal biopsy phantoms for interventional radiology trainees”, European Journal of Radiology 82 (2013) pp. 594-600, December 2012.

6. C. Marchal, M. Nadi, A. J. Tosser, C. Roussey, M. L. Gaulard “Dielectric properties of gelatine phantoms used for simulations of biological tissues between 10 and 50 MHz”, Int. Journal of hyperthermia, France, vol. 5, no. 6, pp. 725-732, November 1988.

7. P.V. Kozlov, G.I. Burdygina “The structure and properties of solid gelatin and the principles of their modification“, Polymer, Vol. 24. pp. 651-666, June 1983

8. F. T. Ulaby “Fundamentals of Applied Electromagnestics” Media Editioned ed. 2004, Upper Saddle River: Pearson Prentice Hall. 433, 2004

9. M. A. Kandadai, J. L. Raymond, G. J. Shawa “Comparison of electrical conductivities of various brain phantom gels: Developing a ‘Brain Gel Model’”, Mater Sci Eng C Mater Biol appl, 32(8): pp. 2664-2667, December 2012

10. M. T. Wilson, M. Elbohouty, L. J. Voss, D. A. Steyn-Ross “Electrical imoedance of mouse brain cortex in vitro from 4.7 kHz to 2.0 MHz”, NZ, Physiol. Meas. 35, pp. 267-281, January 2014

11. H. Kõiv, K. Pesti, R. Gordon “Electric impedance measurement of tissue phantom materials for development of medical diagnostic systems”, Tallinn, 2015

12. G. Juri, H. Wilhelm, J. L'Heureux "High-purity graphite powders for high performance ", Website at: http://www.thegraphite.com/Scopi/Group/Timcal/timcal.nsf/pagesref/MCOA-7S6H6L/$File/High_purity_graphite_powders_for_high_performance.pdf

13. N. Deprez, D. S. McLachlan "The analysis of the electrical conductivity of graphite conductivity of graphite powders during compaction", J. Phys. D: Appl. Phys. Vol. 21, 1988.

14. B. Marinho, M. Ghislandi, E. Tkalya, C. E. Koning, G. de With " Electrical conductivity of compacts of grapheme, multi-wall carbon nanotubes, carbon black, and graphite powder", Powder Technology, Vol. 221, pp. 351-358, May 2012.

15. A.M. R. Pinto, P. Bertemes-Filho, A. S. Paterno "Gelatin as a skin phantom for bioimpedance spectroscopy", VI Latin American Conference in Biomedical Engineering, Brazil, 2014.

16. Percent solutions calculator. Website at: http://www.physiologyweb.com/calculators/percent_solutions_calculator.html

17. K. D. Schroder, "Semiconductor Material and Device Characterization," 3rd ed. IEEE Press, Wiley Interscience, 2006

18. K. V. Thimann "The effect of salts on the ionisation of gelatin", J Gen Physiol. ; 14(2): 215–222, November 1930.

19. H. Ikehira, T. Sunaga, T. Obata, S. Furukawa, M. Tamura, E. Yoshitome, S. Tanada "Development of a dielectric equivalent gel for better impedance matching for human skin", Proc. Intl. Soc. Mag. Reson. Med. 11, 2003.

20. T-J. Kao, G. J. Saulnier, D. Isaacson, T. L. Szabo, J. C. Newell "A versatile high-permittivity phantom for EIT", IEEE Trans Biomed Eng.55(11): pp. 2601-2607, November 2008.

21. T. A. Hope, S. E. Iles "Technology review: The use of electrical impedance scanning in the detection of breast cancer", Breast Cancer Res 2004, 6:69-74, November 2003.

22. U. Birgersson “Electrical impedance of human skin and tissue alterations: mathematical modelling and measurements”, extracted from thesis, Karolinska Institutet Stockholm, Sweden, 2012.

23. S. Grimnes, O. G. Martinsen, “Bioimpedance and bioelectricity basics”, Elsevier Ltd., UK,USA, Third edition, 2015

24. A. Ivorra “Bioimpedance Monitoring for physicians: an overview”, extracted from thesis “Medicine for non physicians”, July 2002

25. F. S. Martinez “Electrical bioimpedance cerebral monitoring”, extracted from PhD thesis, Chalmers University of Technology, Göteborg, Sweden, 2007.

26. D. Holder Appendix A. “Brief introduction to bioimpedance”, Electrical Impedance Tomography: Methods, History and Applications , pp. 411-, 2010.

27. A. C. Buchholz, C. Bartok, D. A. Schoeller "The validity of bioelectrical impedance models in clinical populations", Nutr Clin Pract 19 (5): 433–46., 2011.

28. Wayne Kerr Electronics, Precision Impedance Analyzers 6500B Series. Wayne Kerr Electronics, Precision Impedance Analyzers 6500B Series. Website at: http://www.waynekerrtest.com/europe/ html/ products/impedanceanalysis

29. S. Grimnes, Ø. G. Martinsen “Alpha-dispersion in human tissue”, Journal of Physics: Conference Series 224, 012073, Norway, 2010.

30. H. P. Schwan, C. F. Kay “The conductivity of living tissues”, vol. 65, issue 6, 1975.

31. Biomedical optics research laboratory. Website at: http://www.ucl.ac.uk/medphys/research/borl/imaging/phantoms

32. E. K. Sequin, J. West, V. V. Subramaniam “Eddy current measurement for characterizing soft tissues”, ASME 2012 International Mechanical Engineering Congress & Exposition, November 2012.

33. B. J. Roth “The electrical conductivity of tissues”, CRC Press LLC, 2000. Website at: http://www.cmu.edu.cn/jcyxy/upl_files/20081122184243806.pdf

34. C.H. Riedel, M.-A. Golombeck, O. Dössel “Simulation of non-contact measurement of the electrical impedance using an anatomical modell”, Biomedizinische Technik, vol. 47-1, Sep. 2002.

35. M. Tanha “Eddy Current Sensor for Tissue Conductivity Measurement” master thesis, Umea University , Umeå [Online] http://umu.diva-portal.org/smash/record.jsf?pid=diva2:607825 (03.05.2015)

36. L. A. DeWerd, M. Kissick “The pahntoms of medical and health physics: Devices for research and development”, Springer Business Media, New York, Heidelberg, Dordrecht, London, 2014.

37. D. M. Peterson “Tissue equivalent phantom development for biomedical applications”, extracted from PhD thesis, University of Florida, 2009.

38. R. Siegela, Y. Bar-Cohenb, W. Grandiac “Noninvasive medical diagnostics & treatment using ultrasonics”, ASNT '98 Spring Conference, Joint with 9th Asia-Pacific Conference on NDT, 24-26, March 1998.

39. H. Griffiths, “Magnetic induction tomography,” Measurement Science and Technology, vol. 12, no. 8, pp. 1126–1131, 2001.

40. J. Fromageau, E. Brusseau, D. Vray, G. Gimenez, P. Delachartre “ Characterization of PVA Cryogel for Intravascular Ultrasound Elasticity Imaging”, IEEE transactions on ultrasonics, ferroelectrics, and frequency control, draft, 0885–3010

41. A. Hellerbach, V. Schuster, A. Jansen, J. Sommer “MRI Phantoms – Are There Alternatives to Agar?”, PLoS ONE 8(8): e70343 Germany, August 2013

42. P. Walker, R. A. Lerski, M.-D. Vre R, J. Binet, F. Yane ”Preparation of agarose gels as reference substances for NMR relaxation time measurement. EEC Concerted Action Program”, Magn Reson Imaging. 6(2): pp. 215-22, April 1988.

43. L.J. van der Pau “Method of measuring the resistivity, and Hall coefficient on l amellae of arbitrary shape”, Philips technical review, vol. 20, No. 8, 1958/59.

44. S. W. Alderson, L. H. Lanzl, M. Rollins, J. Spira “ An instrumented phantom system for analog computation of treatment plans”, Am J Roentgenol Radium Ther Nucl Med. ;87:185-95, January 1962.*

45. Z. Moroń, T. Grysiński " Experimental investigations of van der Pauw method applied for measuring electrical conductivity of liquids", XIX IMEKO World Congress Fundamental and Applied Metrology, Lisbon, Portugal, September 2009.

46. M. Tokarska "Measuring resistance of textile materials based on Van der Pauw method", Indian Journal of Fibre & Textile Research, vol. 38, pp. 198-201, June 2013

47. Introduction to Comsol Multiphysics Version 4.2a, October 2011.

Website at:http://fab.cba.mit.edu/classes/S62.12/docs/COMSOL_Multiphysics.pdf

48. M. Tokarska "Determination of fabric surface resistance by can der Pauw method in case of contact distant from the sample edge", AUTEX Research Journal, Vol. 14, No 2, June 2014

49. M. Elbohouty, M. Wilson, L. Voss, A. Steyn-Ross, "Methodology to measure the electrical conductivity of seizing and non-seizing mouse brain slices," Phys Med Biol. 58(11):3599-613, June 2013.

50. R. Chwang, B. J. Smith, C. R. Crowell "Contact size effects on the van der Pauw method for resistivity and hall coefficient measurement" Solid-State Electron, vol. 17, issue 12, pp. 1217-1227, December 1974

51 C. Kasl, M. J. R. Hoch, "Effects of sample thickness on the van der Pauw technique for resistivity measurements", Review of scientific instruments 76, 033907, South Africa, February, 2005.

52. T. Matsumura, Y. Sato " A theoretical study on Van der Pauw measurement values of inhomogeneous compound semiconductor thin films," J.Mod. Phys., Japan, pp. 340-347, November 2010.

53. D. W. Koon "Effect of contact size and placement, and of resistive inhomogeneities on van der Pauw measurements", Rev. Sci. Instrum. 60, 271, 1989.

54. J. D. Weiss, R. J. Kaplar, K. E. Kambour "A derivation of the van der Pauw formula from electrostatics", Solid-State Electronics, vol. 52, issue 1, pp. 91-98, January 2008.

55. D.Andreuccetti, R.Fossi and C.Petrucci: An Internet resource for the calculation of the dielectric properties of body tissues in the frequency range 10 Hz - 100 GHz. Website at: http://niremf.ifac.cnr.it/tissprop/. IFAC-CNR, Florence (Italy), 1997. Based on data published by C.Gabriel et al. in 1996

56. A. J. Majid "An innovative method of measuring semiconductors resistivity using van der Pauw technique", International Journal of Scientific & Engineering Research Volume 2, Issue 10, 1 ISSN 2229-5518, October 2011.