

École polytechnique de Louvain

Impedimetry and potentiometry for the design of paper-based biosensors

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Academic year 2021–2022

Master [120] in Electro-mechanical Engineering

Acknowledgements

Firstly, I wish to thank Margo Hauwaert and Grégoire Le Brun for their invaluable help and availability. Thank you for the enthusiasm, the support and the answers to my questionings. I wish you both a great continuation of your PhD.

I would like to thank Prof. Jean-Pierre Raskin, my supervisor, for having integrated me for a year in his team. It was an enriching experience.

I also acknowledge Prof. Marc Debliquy for having accepted to be part of my master thesis jury.

I wish to thank Prof. Ignace Adant for the time and enthusiasm devoted to my master thesis and the precious guidelines concerning the development of a more responsible approach in the science sector.

I acknowledge Pascal Simon, Souley Djadjandi and Benoît Hubert of the Welcome platform for their patience, advice and help. Thanks also to Fares Tounsi for the loan of his inductances and his answers to my questions even in the middle of his holidays. Finally, I would like to thank Oriane de Leuze for welcoming me to the Welcome laboratory at the beginning of this term.

Finally, I would like to express my gratitude to Véronique and Louis for their assiduous review of my master thesis and their constructive comments.

A special mention to Pierre for his Overleaf advice to the boomer that I am. Thanks also to Juliette for lending me her English skills for many hours.

I warmly thanks my family of course, my father and brother for their readings, my sister for her Procreate and Photoshop skills and my mother for her unfailing support.

Abstract

Monitoring water quality is of paramount importance, as demonstrated in the 2015 publication of the United Nations' 17 Sustainable Development Goals (SDGs). Access to safe drinking water for all was placed sixth among the 17 goals to be achieved by 2030 [1].

The project that led to this master thesis aims at designing a Point-of-Care paper-based biosensors for bacteria detection. The Point-of-Care aspect is important because it allows water quality assessment at the place where it is consumed. This biosensor is designed to meet the ASSURED criteria (Affordable, Sensitive, Specific, User friendly, Rapid and robust, Equipment free and Deliverable to end users).

This biosensor combines two main elements, the LFA and the detection method. Detection methods are colorimetric and electrochemical. Colorimetric detection is done by simple optical reading of the LFA. Within the framework of electrochemical detection, several options are available. This is where this master thesis comes in. Indeed, a comparison between two interesting electrochemical detections is carried out: the non-faradaic impedimetric detection and the potentiometric detection. These methods have the advantage of being cheap and easy to miniaturize. The comparison seeks to determine which detection should be encouraged in the future development of the biosensor. These detection methods are compared in three cases of variations of the sample characteristics. The sample may vary in conductivity, permittivity or permeability. The comparative analysis is also done in the case where the LFA has two kind of detection pad. One is constructed with a nitrocellulose membrane combined with a layer of polyester on one of its faces and the other without any polyester layer.

The analyses are carried out on simplified systems. The LFA is simply represented by a nitrocellulose membrane alone. The conductivity and permittivity variations are simulated by model fluids such as Phosphate-Buffered Saline (PBS) and deionized (DI) water. Permeability variation is simulated by a variable spiral planar inductance.

Simplified equivalent circuits are developed for each system. They are used to analyse the underlying physical characteristics behind each observations.

List of Acronyms

AgNP	Silver NanoParticles
AuNP	Gold NanoParticles
BNC	Bayonet Neill-Concelman
BP	Black Phosphor
CDs	Carbon Dots
CE	Counter Electrode
CI	Confidence Interval
CNB	Cellulose NanoBead
CNT	Carbon NanoTube
CPE	Constant Phase Element
CuONP	Copper Oxide NanoParticles
DI	Deionized
DUT	Device-Under-Test
ECS	Electrochemical Capacitance Spectroscopy
EIS	Electrochemical Impedance Spectroscopy
ELISA	Enzyme Linked Immunosorbent Assay
EPR	Extended Producer Responsibility
FRET	Fluorescence Resonance Energy Transfer
GO	Graphene Oxide
HDPE	High-Density Polyethylene
ISE	Ion-Selective Electrodes
LDPE	Low-Density Polyethylene
LCA	Life Cycle Assessment

LFA	Lateral Flow Assay
MNP	Magnetic NanoParticle
MST	Metal Shielding Tuned
NAT	Nucleic Acid Testing
NC	Nitrocellulose
NCs	Metal NanoClusters
NIR	Near-InfraRed
OD	Optical Density
PADs	Paper-based Analytical Devices
PBS	Phosphate Buffered Saline
PC	Polycarbonate
PCB	Printed Circuit Board
PET	Polyethylene Terephthalate
PoC	Point-of-Care
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl Chloride
PVD	Physical Vapor Deposition
QDs	Quantum Dots
RE	Reference Electrode
S	Sense electrode
SDGs	Sustainable Development Goals
SERS	Surface-Enhanced Raman Spectroscopy
SPR	Surface-Plasmon Resonance
SRF	Self-Resonant Frequency
UCNPs	Upconversion NanoParticles
VNA	Vector Network Analyzer
WE	Working Electrode

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Chapter 1

Introduction

The United Nations, in its action plan published in 2015 "The 2030 Agenda for Sustainable Development", placed the availability of drinking water for all and the sustainable management of this resource in 6th position among the seventeen Sustainable Development Goals (SDGs) for 2030 [1].

However, the development of industry and agriculture as well as the increase in world's population are putting de facto ever greater pressure on water [2, 3].

One aspect of the water supply security is the monitoring of its quality.

Conventional water quality analysis is expensive, time-consuming and requires trained personnel [3]. These characteristics do not facilitate the widespread implementation of water quality monitoring. Nowadays, as a direct consequence, the quality of the water consumed by nearly 2.5 billion people (31% of the world's population) is unknown [4, 5].

One way to promote access to clean water is to develop Point-of-Care (PoC) devices to assess water quality. PoC devices meet the ASSURED criteria: Affordable, Sensitive, Specific, User friendly, Rapid and robust, Equipment free and Deliverable to end users [6].

Such PoC devices can be paper-based biosensors that meet the ASSURED criteria. Among those, a certain type based on the Lateral Flow Assay (LFA) technique stands out. Therefore, this technique is the foundation of the paper-based biosensor developed by Jean-Pierre Raskin's research team at the Ecole Polytechnique de Louvain.

The design of this type of biosensor depends notably on three critical choices. Firstly, in order to determine the presence or absence of the target element (e.g. bacteria), it is important to determine what kind of variation is to be measured in the sample. The variations may be electrochemical. They concern the variation of the conductivity, permittivity or permeability of the sample. But the variation can also involve a variation in the colorimetry of the LFA at the test line. Secondly, the choice of the detection method used within the biosensor is significant. This choice of method is directly related to the chosen variation to be investigated. Finally, a third design consideration is the type of paper used in the sensor itself.

As biosensors cover a wide field of research, this master thesis will start with a *state of the art* regarding Lateral Flow Assays. Then a series of available detection methods for biosensors will be outlined. A large part of the state of the art is devoted to the concept of equivalent circuit. This equivalent circuit model serves then as a basis for interpreting the underlying physics of the studied systems.

The second chapter, entitled *Objectives*, exposes the objectives of this master thesis, i.e. the research question. This question deals with the three choices described earlier. The three types of sample variation and their impact on the impedimetric and potentiometric detection methods are studied. All of this will be evaluated based on the design choice for the LFA paper. That is the use of a nitrocellulose membrane either with or without a polyester layer bonded on one side.

The third chapter, *Materials and methods*, presents the different measuring instruments used during this work. It also includes the equivalent circuits developed to characterise the systems studied.

The fourth and fifth chapters, called *Results* and *Discussion* respectively, contain outputs associated with the research question. The *Discussion* Chapter begins with a summary of the information obtained in the *Results* Chapter. This summary will provide some guidelines concerning the next steps in the design of the paper-based biosensor developed by Jean-Pierre Raskin's research team. The limitations and strengths of this work are also discussed. Perspectives from the scientific literature for the future miniaturisation of detection systems are provided.

A final chapter, entitled *Responsible approach: Eco-design*, is devoted to the issue of eco-design and the recycling of the innovation studied in this master thesis.

As a *Conclusion*, the main results are summarized and perspectives for the future miniaturisation are outlined.

Chapter 2

State of the Art

This chapter provides an overview of the state of the art of Lateral Flow Assay (LFA), a paper-based analytical device technology. Colorimetric and electrochemical methods compatible with LFA technology are then reviewed. Two measurement devices capable of performing electrochemical detection are also presented. The equivalent circuit associated with the system measured by these devices is developed. Finally, the spiral planar inductor technology is explained.

2.1 Paper-based analytical devices

Current conventional diagnostic techniques include Enzyme Linked ImmunoSorbent Assay (ELISA) and Nucleic Acid Testing (NAT). These methods are used in a laboratory and it is therefore impossible to carry out on-site diagnostics. They also have other disadvantages. They are time-consuming and labour-consuming. In addition, they are expensive and rely heavily on expensive machinery [7].

In order to bring biosensors into the field and overcome the problem of traditional biosensors, Point-of-Care (PoC) devices have been developed. The term Point-of-Care devices comes from the fact that diagnostics are done in close proximity to the patient care. This innovation in the field of medical diagnostics has qualities such as an ability to perform diagnoses very quickly, a high specificity and sensitivity. In addition, these devices are inexpensive and highly efficient. The main characteristics of a Point-of-Care device are grouped under the acronym ASSURED. This means that a PoC device is Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-user [7, 8].

One type of PoC devices are the Paper-based Analytical Devices (PADs). The use of PADs offers some significant advantages. First of all, paper is cheap, abundant and its manufacture is simple. Moreover, it is stable over a wide temperature range and a long period of time. Paper has spontaneous microfluidic properties due to its capillarity. If the need arises for bioreceptors such as antibodies, they are easily attached to paper. However, there are also disadvantages to the use of paper. Optical detection sensitivity is low as only $10\mu\text{m}$ of paper depth is involved in the colorimetric signal. In addition, measurements are generally qualitative and at most quantitative [9, 10]. Paper-based Analytical Devices

(PADs) can be divided into three categories: dipsticks assays, microPaper-based Analytical Devices (μ PADs) and Lateral Flow Assays (LFAs) [11].

Lateral Flow Assays (LFAs) have reagents embedded in the paper prior to analysis. A flow of the sample along the paper is possible thanks to capillarity. A Lateral Flow Assay is made up of four parts and takes the form of a stick 4 to 6mm wide and 6 to 7cm long [9].

The four parts that make up this strip are [8, 9, 11]:

- The **sample pad**, made of cellulose.
- The **conjugation pad**, which is made of glass fibre.
- The **detection pad** is a membrane of nitrocellulose.
- The **absorbent pad** that is, like the sample pad, cellulose.

These parts are illustrated in Figure 2.1.

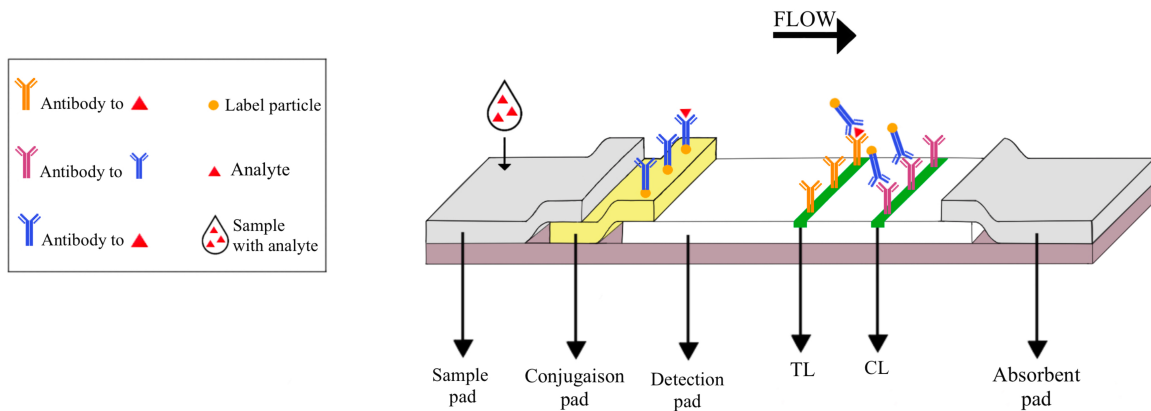


Figure 2.1: *Schematic of a positive lateral flow assay [12].*

Given the four main parts of the lateral flow assay, its operating principle can also be explained in four steps. First, the sample is added in liquid form to the sample pad. The cellulose in the sample pad filters the sample of any impurities. Afterwards, the sample arrives at the conjugate pad. This pad functions as a storage of label and bioreceptor previously combined. If the analyte is present in the sample, the bioreceptor and analyte bind. The combined product arrives at the test line (TL) and control line (CL) in the detection pad. The combination of bioreceptor and label is in all cases hooked by other bioreceptors to the control line, thus providing evidence that the Lateral Flow Assay is working. Only bioreceptors and labels linked to an analyte are attached to the bioreceptors in the test line. The appearance of two lines therefore means a positive test, the appearance of the control line means a negative test and the appearance of no lines or only the test line means that the assay has failed. The absorbent pad absorbs excess reagents and avoids backflow. It also increases the amount of sample that passes through the lateral flow assay and therefore increases the sensitivity of the assay [8, 9, 11, 12]. The operating

principle of LFA described above uses only colorimetric detection.

Table 2.1 lists the detection methods compatible with a Lateral Flow Assay and the advantages and disadvantages of this type of paper-based biosensor. Colorimetric, fluorescent and electrochemical detection are explained in the next Section.

Table 2.1: *Lists of different methods compatible with LFAs and advantages and disadvantages of LFAs.*

Detection method	
	Colorimetric
	Fluorescent
	Electrochemical
	Chemiluminescent
	Surface-Enhanced Raman Spectroscopy (SERS)
Advantages	
	User-friendly
	Quick-analysis (5-15min)
	Low-cost
	Multiplexed detection
	No sample pre-treatment required or washing step necessary
	Equipement-free
	Good stability
	Long lifetime
Disadvantages	
	Limited sensitivity
	Semi-quantitative detection
	Long fabrication
	Large sample volume ($100\mu L$)
	Detection time influenced by sample's viscosity

The different labels/tracers used depending on the detection method employed with the LFA are gathered in Figure 2.2 [8].

	Optical	Thermal	Magnetic	Electrochemical
Sensing Modality	Colorimetric Fluorescence Surface-enhanced Raman scattering Chemiluminescence	Thermal imaging Photoacoustic Speckle imaging	Giant magnetoresistance Tunneling magnetoresistance Magnetic particle quantification	Amperometry Cyclic voltammetry Impedimetry Electro-chemiluminescence
Tracer	 AuNP  AgNP  MNP  IrO ₂ NP  CNT  CNB  CuONP  Polymer  GO	 AuNP  AgNP  CNT  BP	 MNP	 AuNP  AuNP@Pt  MNP  GO

Figure 2.2: *Labels used depending on the detection method employed with the LFA. AuNP, gold nanoparticle (NP); AgNP, silver NP; CuONP, copper oxide NP; MNP, magnetic NP; IrO₂NP, iridium oxide (IV) NP; GO, graphene oxide; CNT, carbon nanotube; , cellulose nanobead; BP, black phosphor; Pt, platinum [8].*

2.2 Detection methods

In the design of diagnostic PoC systems, the choice of the detection method used is decisive for the efficiency, sensitivity and accuracy of the diagnosis. The following Section details the different types of detection methods that have so far been implemented in PoC device systems.

2.2.1 Electrochemical biosensors

The common feature of any electrochemical biosensor is that it relates a measurable electrical output signal to a variation in the structure of an electrode or the characteristics of the sample being measured in order to recognise the target analyte [13]. The advantages of electrochemical biosensors are that they have a fast detection rate, the detection instruments are simple to use and inexpensive, and the biosensors are easily transportable [7].

2.2.1.1 Voltammetric/Amperometric biosensors

There are different methods to implement a voltammetric biosensor: cyclic voltammetry, linear sweep voltammetry, square wave voltammetry and differential pulse voltammetry [7]. To determine the presence or absence of the target, voltammetric biosensors work by applying a potential to the indicator electrode relative to the reference electrode. This causes an electrochemical reduction or oxidation reaction at the working electrode. The electrolysis produced by this reaction creates a current that corresponds to the one measured in order to detect the target [7].

The working of an amperometric biosensor is similar to that of the voltammetric biosensor except that the applied potential, which increases gradually in the voltammetric case, is constant in the amperometric case [7]. It works under the application of a DC potential to the working electrode. It creates a current that is measured [8].

Amperometry and voltammetry implemented in a LFA uses redox tracers which transfer only a few electrons. Therefore sensitivity is low. Various chemical amplification strategies are developed to enhance the sensitivity [8]. Du et al. (2012) and Scida et al. (2014) present some of them [14, 15].

2.2.1.3 Impedimetric biosensors

Impedimetric detection is also called impedance spectroscopy in scientific literature. Impedance measurement is performed by applying an AC voltage of amplitude V_0 to one electrode of the system and reading the AC current generated at the other electrode. This generated current is called I_0 and is characterised by a phase ϕ and a fundamental frequency f . If V_0 is well below the thermal voltage ($U_t = k_B T/q \simeq 26mV$), the system is linear and the phasorial form of the impedance is given in Equation 2.1 [16, 17]. The capacitive and resistive elements of the equivalent Device-Under-Test circuit are then obtained from the impedance expression [7].

$$Z(\omega) = \frac{V_0 \exp j\omega t}{I_0(\omega) \exp j\omega t + \phi(\omega)} \quad (2.1)$$

where

- V_0 and I_0 are the maximum voltage and current signals,
- ω is the excitation frequency in $rad.s^{-1}$
- t is time,
- ϕ is the phase shift between the voltage-time and current-time functions

Since impedance is a complex number, it can be described by its magnitude ($|Z|$) and phase shift (ϕ) or by its real and imaginary part. Impedance spectroscopy has the advantages of being easy to miniaturize, able to perform *in situ* measurements, relatively cheap. Moreover it can perform label-free measurements with low disturbance amplitude and low power [7, 13, 16].

Two transduction mechanisms must be distinguished, the faraidic and the non-faraidic one [7, 17].

Faraidic mode

Faradaic transduction is also called Electrochemical Impedance Spectroscopy (EIS) [17]. Reduction and oxidation reactions take place among electroactive species. These reactions generate a current responsible for a physical transfer of electrons or ions from the electrode to the solution and vice versa [16]. This transfer is quantified by the charge transfer resistance. Faradaic sensors involve redox probes and the application of DC conditions to enhance the development of electrochemical reactions [7, 13]. Faradaic sensors offer better sensitivity than non-faradaic sensors [13].

Non-faraidic mode

Nanofaradaic transduction is also called Electrochemical Capacitance Spectroscopy (ECS) [17]. There is no electron transfer from the electrode to the electrolyte and vice versa. The impedance response of the sensor comes only from variation in the interfacial capacitance, called double layer capacitance. This is charged and discharged due to changes in dielectric properties, charge redistribution due to ion displacement, etc [7, 13, 16, 17]. The double layer capacitance is described by the Helmholtz model, given in Equation 2.2.

$$C = \frac{\epsilon_0 \epsilon_r A}{d} \quad (2.2)$$

where

- ϵ_0 is the free space permittivity,

- ϵ_r is the relative solution permittivity,
- d is the distance of the Helmholtz layer and
- A is the surface area of the electrode.

The advantage of non-faradaic detection is that it does not require redox probes, which makes it simple and therefore easily miniaturised in a PoC device. It also allows measurements at a single frequency [13, 16].

However, non-faradaic sensors lack specificity and therefore require the functionalization of the electrode (for example, but if the detection is used on an LFA, it is the LFA that is functionalized). Moreover, non-faradaic sensors are less sensitive than faradaic sensors [16].

2.2.1.4 Potentiometric biosensors

A potentiometric biosensor has the advantage of being small, fast responding, easy to use, cheap and not dependent on the size of the sample being measured [7].

A potentiometric biosensor uses an electrochemical cell with two reference electrodes. These electrodes are usually Ion-Selective Electrodes (ISE), i.e. they translate the activity of the target ion into an electrical potential. The final objective is to measure this electrical potential. These electrodes are operated by applying a current close to zero. To improve the quality of the signal produced in the case of biological samples, enzymes are used to catalyse chemical reactions and produce ions closer to the electrodes [7, 18].

2.2.2 Optical biosensors

An optical biosensor works through optical imaging or a spectroscopic system. It is usually equipped with a light source, which may be only ambient light, and lenses capable of collecting optical signals. Once collected, these optical signals are measured by photo-sensitive detectors [8].

2.2.2.1 Colorimetric biosensors

The advantages of colorimetric biosensors are their ease of preparation, comprehensible results, low cost and ability to be easily transported.

The principle of operation of these biosensors is based on the measurement of contrast or optical density (OD) modulation of images that result either from the oxidation of peroxidase or peroxidase-like nanomaterials or from the aggregation of nanomaterials at a specific location [7, 8]. A colorimetric biosensor is equipped with an external light source such as LEDs or simply uses ambient light. Once the contrast or optical density modulation image is obtained from the lights, it is processed by an image sensor. This sensor segments the test and control lines using various filters. It is then the pixels that are observed on the different lines that allow conclusions to be drawn about the sample analysed [8].

The nanomaterials used in colorimetric biosensors are usually gold nanoparticles (AuNPs), platinum nanoparticles, silver nanoparticles (AgNPs) or magnetic nanoparticles (MNPs). AuNPs are currently the most widely used nanoparticles due to their ease of preparation, the simplicity of their surface modification and their ability to provide a fast colorimetric signal following their aggregation [7].

Surface Plasmon Resonance (SPR)-based biosensors are used in some colorimetric biosensor. SPR-based biosensors have the advantage of providing highly reproducible results, good reuse performance and being non-invasive, label-free and cost-effective. These biosensors use a surface plasmon wave which causes a molecular interaction on the surface of a metal and this induces a variation in the refractive index. It is the measurement of this variation that allows detection of the presence or absence of the target. Since SPR-based biosensors require large sample volumes, have a high power consumption and their sensitivity and resolution is not yet optimal, improvements are being investigated. These include the use of nanomaterials, microfluidic devices and compact power-free pump systems [7].

2.2.2.2 Fluorescence biosensors

Fluorescence biosensors have the advantage of being highly sensitive, inexpensive, easy to use and provide rapid results. However, they have a strong background signal and are unable to perform multiplex detection [7].

The method used in fluorescence biosensors is Fluorescence Resonance Energy Transfer (FRET), i.e. the transfer of energy from a fluorophore donor to a fluorophore receptor. Fluorophores are either fluorescent dyes or fluorescent nanomaterials. Labelling these is fundamental to the design of a fluorescence biosensor. There are three types of labelling which are presented below [7].

- ***Direct fluorescence labeling biosensors***

Fluorescent dyes are labelled with a ligand capable of detecting in a very specific way the target element that is to be recognised. The fluorescence signals are therefore proportional to the amount of target in presence. Nanomaterials are widely used to act as fluorescent dyes. Four types of nanomaterials used in the design of fluorescence biosensors are presented below [7].

1. **Quantum dots (QDs)**

QDs are nanoparticles generally smaller than 10nm. The advantages of these nanoparticles are their high quantum yield, tunable emission spectrum and excellent photostability [7].

2. **Carbon dots (CDs)**

CDs are zero-dimensional carbon-based fluorescent nanoparticles of size less than 10nm. They present the advantage of having very good fluorescent properties and being biocompatible [7].

3. Upconversion nanoparticles (UCNPs)

UCNPs are inorganic fluorescent nanoparticles. They have the ability to absorb a minimum of two low-energy photons of light and convert them into higher energy photons. These are then responsible for the emission of a fluorescent signal. UCNPs have many advantages such as low toxicity, long luminescence lifetime, high photostability like CDs, high signal-to-noise ratio and high resistance to photobleaching [7].

4. Metal nanoclusters (NCs)

Metals NCs are very small nanoparticles. They consist of a few to a few dozen atoms. The metals used can be gold or silver to form AuNCs or AgNCs, respectively. The advantages of these nanoparticles are their biocompatibility and their high fluorescence emission [7].

- ***"Signal-on" fluorescence biosensors***

The intensity of the fluorescent signal is proportional to the amount of target present in the sample being measured. When the sample does not contain the target, a slight fluorescent signal can still be observed [7].

- ***"Signal-off" fluorescence biosensors***

The principle is the opposite of "signal-on" labelling. In this case, the fluorescent signal is inversely proportional to the amount of target element present in the sample. When the target is not present, the fluorescent signal is at its maximum intensity [7].

2.3 Electrochemical devices

Two measuring devices are detailed in this section. Firstly, the LCR meter, capable of performing impedance spectroscopy. It uses a frequency response analysis. The impedance is extracted by fitting the impedance spectrum to an equivalent interfacial circuit model. Secondly, the Autolab Potentiostat/Galvanostat is presented. Obtaining the impedance of the Device-Under-Test (DUT) with the potentiostat differs from the impedance spectroscopy because the impedance value is extracted from the estimated time constant via a complicated data fitting process [19].

2.3.1 LCR meter

An LCR METER is a measuring device used to determine the inductance, capacitance and resistance values of a Device Under Test (DUT). This is where its name comes from: L for inductance, C for capacitance and R for resistance [20].

Usually, an LCR METER determines the impedance of the DUT by comparing the magnitude and phase of the voltage across the DUT with the magnitude and phase of a current that is applied to the DUT. The impedance value is then obtained from the ratio

of the measured voltage to the applied current [20, 21].

In order to understand the principle of impedance, it is important to review the definitions of capacitance, inductance and resistance. Capacitance is the measure of the amount of charge that an electrical body can hold for a certain applied electrical potential. Inductance is the property of an electrical circuit responsible for the proportionality between the voltage generated and the rate of change of current in the circuit. That is, the more the current changes, the more voltage is generated. Resistance is the measure of the opposition that is made to the electric current in the circuit.

To derive these quantities in a circuit, the impedance of the circuit is usually measured. Impedance is expressed in ohms and is defined as the total opposition of a device or circuit to an alternating current (AC) flow at a certain frequency. When a circuit is subjected to a steady continuous current, the impedance is equal to the resistance. It is under the effect of alternating current that impedance differs from resistance and the principles of inductance and capacitance come into play. In this case, impedance is a vector quantity as shown in Figure 2.3(a) and expressed in Equation 2.3. The impedance vector has a real part equal to the resistance and an imaginary part equal to the reactance X . There are two forms of reactance, X_L and X_C , and, combined with R , they give the inductance and capacitance values of the circuit, respectively, as shown in Figure 2.3(b) [22, 23, 24].

$$Z = R \pm jX \quad (2.3)$$

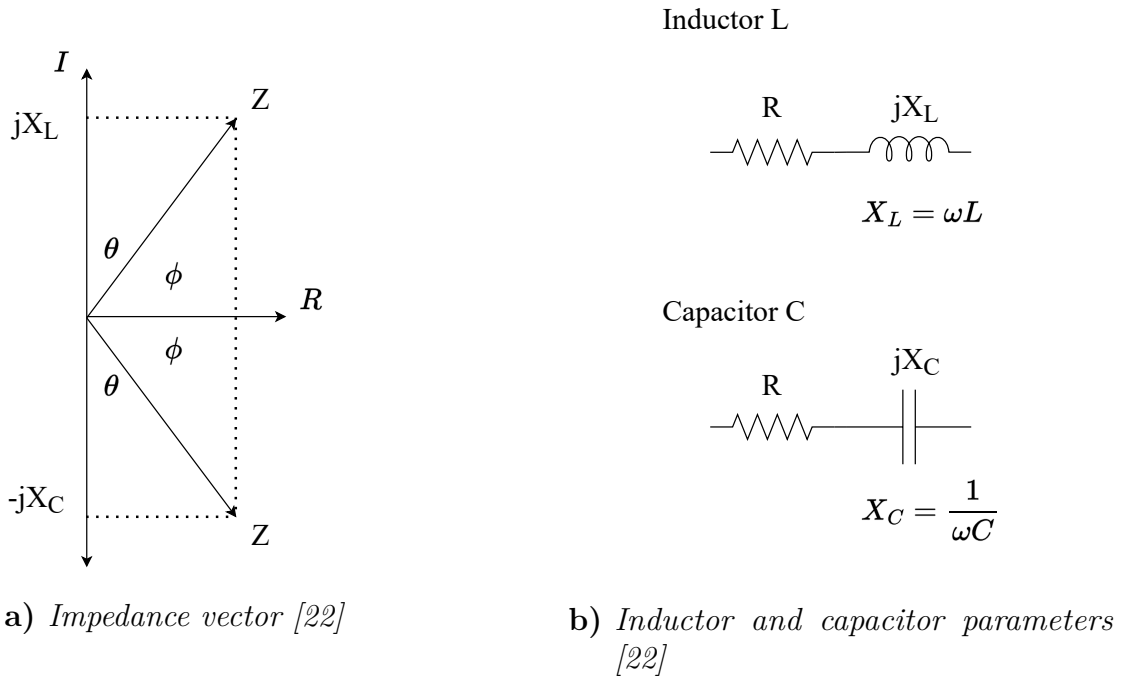


Figure 2.3: Vector representation of impedance and equivalent circuit of an inductor and capacitor.

Figure 2.4 shows the general block diagram of a typical LCR meter [25].

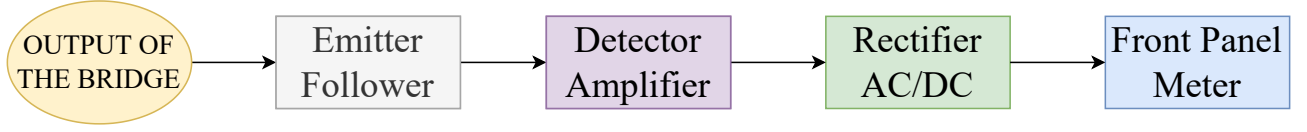
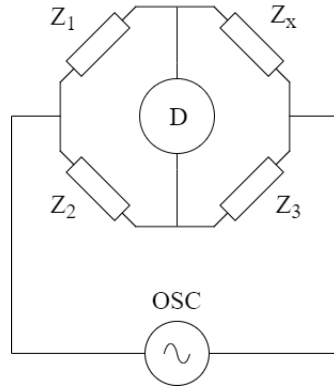


Figure 2.4: Block diagram of LCR meter [25].

In order to determine the impedance of a Device Under Test (DUT), most modern impedance measuring instruments measure the imaginary and real part of a vector impedance. These values are then converted to the desired values ($|Z|$, θ , $|Y|$, R , X , G , B). The defining element of an LCR METER is its bridge. There are different bridges used in the LCR METER. The oscillator presented in Figure 2.5 to 2.9 allows the bridge to be supplied with an AC signal when AC measurements are made [25].

1. Wheatstone bridge method [24]

When no current is flowing through detector D, the value of Z_x can be determined from the known values of the other impedances as shown in Expression 2.4. The Z blocks can take the form of a wide variety of combinations of L, C and R, depending on what is to be measured [24].



$$Z_x = \frac{Z_1}{Z_2} \cdot Z_3 \quad (2.4)$$

Figure 2.5: Simplified operation of the Wheatstone bridge method [24].

2. Resonant method [24]

Tuning capacitance C is adjusted to bring the circuit into resonance. The DUT is represented by the RL circuit composed of L_x and R_x in Figure 2.6. But it can be modified by a variety of combinations depending on what is to be measured. The quality factor Q of the circuit is obtained thanks to a voltmeter placed across the tuning capacitance. Q is a parameter used to describe the resonance performances of resonators. It is the ratio between energy stored in a component and dissipated by it. From a simplified point of view, it measures the reactance's purity, i.e. how far is it from being a resistance. Therefore a measure of Q allows to characterize how much of an inductance the DUT is [24].

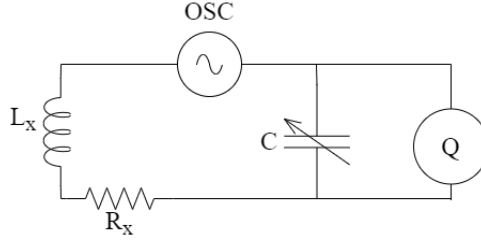
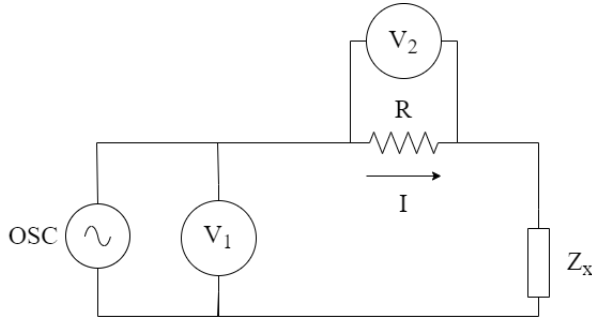


Figure 2.6: *Simplified operation of the Resonant method [24].*

3. I-V method [24]

The unknown impedance to be determined is represented by Z_x . As explained in Equation 2.5, this impedance can be determined by current and voltage measurements. The current is computed from the value of voltage V_2 measured across the low resistor R , whose value is known. R is sometimes replaced by a low-loss transformer, thus limiting the negative effects of a low value resistor in the circuit. However, the low-loss transformer does not allow to go as far down in low frequency measurements as with resistor R [24].



$$Z_x = \frac{V_1}{I} = \frac{V_1}{V_2} \cdot R \quad (2.5)$$

Figure 2.7: *Simplified operation of the I-V method [24].*

4. Network analysis method [24]

The Network Analysis method allows very high frequency measurements. The simplified operation is explained below and illustrated in Figure 2.8 [24].

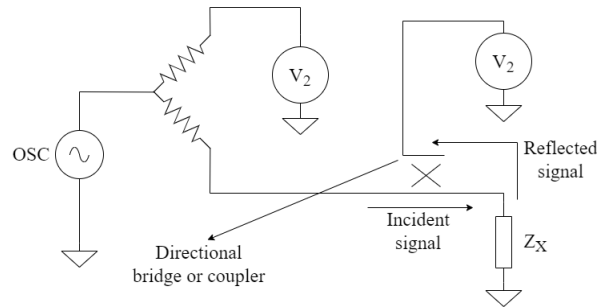


Figure 2.8: *Simplified operation of the Network analysis method [24].*

The network analyzer provides an incident signal which is reflected by the DUT. A bridge or directional coupler then detects the reflected signal which is measured

by the network analyzer. A reflection coefficient x is calculated by the ratio of the incident signal to the reflected signal. The impedance of the DUT is obtained via the reflection coefficient as explained in Equation 2.6 [24].

$$Coeffx = \frac{Z_x - Z_0}{Z_x + Z_0} \quad (2.6)$$

where

- Z_0 is the characteristic impedance of the measurement circuit (50Ω)
- Z_x is the impedance of the DUT in Ω .

5. Auto balancing bridge method [24]

A simplified diagram of an auto-balancing bridge is provided in Figure 2.9. The Device-Under-Test (DUT) is represented by Z_x . The voltage applied to the DUT is located at the High-Potential (Hp) terminal. This terminal is isolated from the High-Current (Hc) terminal to allow correct detection of the applied potential. The signal at the Hc terminal is the output signal [22].

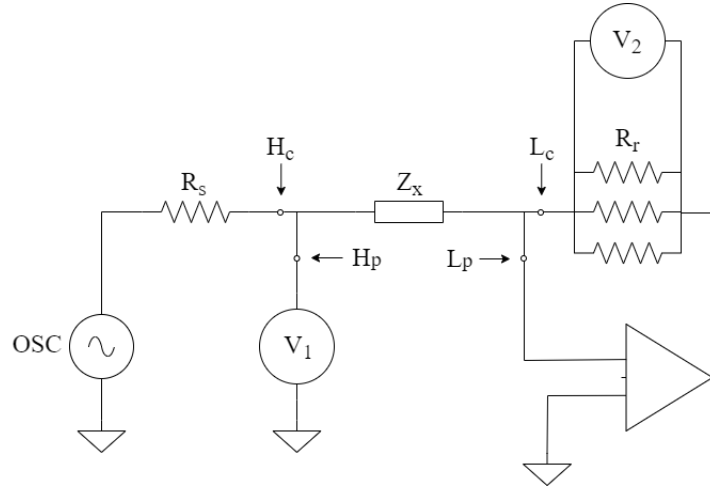


Figure 2.9: *Simplified diagram of the auto balancing bridge method [24].*

The Low-Current (Lc) terminal receives the current flowing through the DUT. At this terminal, a potential may exist that creates a stray capacitance between the terminal and ground. The current flowing through this terminal can then flow to earth. To avoid this, Lc terminal is kept at 0V. To do this, the current through R is balanced by the current through the DUT using the I-V converter amplifier and the feedback loop. In the simplified diagram in Figure 2.9, this I-V converter amplifier is simply represented by the operational amplifier. Node Lc is called a virtual ground because it is virtually maintained at 0V [22, 24].

The current flowing through the DUT then passes through one of the available resistors. Measuring the voltage across the resistor allows the current flowing through

the DUT to be determined [22, 24]. The multiple resistors allow a high resolution for various current measurements. Once the current flowing through the DUT is known, its impedance can be calculated since the value of the applied potential is known.

An auto-balancing bridge uses a simple operational amplifier to act as an I-V converter at low frequencies ($< 100kHz$). Above $100kHz$, the operational amplifier provides performance that is too limited to meet measurement accuracy requirements. For measurements between $100kHz$ and $110MHz$, the I-V converter is composed of a null detector, a phase detector, an integrator and a vector modulator [24].

Table 2.2 lists the advantages, disadvantages and frequency range of the different bridges presented.

Table 2.2: *Comparison of the advantages, disadvantages and frequency range of the different bridges shown in Figure 2.5 to 2.9 [22, 24].*

Method	Advantages	Disadvantages	Frequency range
Wheatstone bridge	High accuracy	Manually balanced	DC to 300 MHz
	Wide frequency coverage (if combined with different type of bridge)	Narrow frequency coverage if one type of bridge is used.	
	Low cost		
Resonant method	Good Q accuracy	Need tuning to resonance	10 kHz to 70 MHz
	Low Z measurement accuracy		
I-V method	Grounded device measurements	Limited frequency range	10kHz to 100MHz
	Probing measurements	Limited impedance range	
Network analysis method	Broad frequency range	Z_x range limited to value close to Z_0	$> 300kHz$
Auto balancing bridge method	Good accuracy (if Z_x close to Z_0)	Recalibration needed when frequency changed	5Hz to 40 MHz [22]
	Broad frequency range (LF to HF).	Higher frequency ranges not available.	
	Highly accurate over a large impedance measurement range.		20Hz to 110MHz [24]
	Grounded device measurement.		

2.3.2 Potentiostat

The potentiostat described here as an example is the Autolab potentiostat/galvanostat from Metrohm (PGSTAT302N, Metrohm Belgium, Antwerp, Belgium). This potentiostat

is equipped with four electrodes, whose distinct roles are described below:

- Counter electrode (CE)

The counter electrode closes the current circuit of the DUT by collecting the AC current applied to WE but does not usually participate in the electrochemical reaction. The CE is made of an inert material such as Pt, Au, graphite, glassy carbon, etc [26].

- Reference electrode (RE)

The reference electrode is a reference in the sense that it has a stable and well known potential. It is used as a reference point for measurements and for voltage control. The current through the reference electrode is kept close to zero [26].

- Working electrode (WE)

AC current is applied to WE [27]. As with the counter electrode, the working electrode is made of inert materials [26].

- Sense electrode (S)

The sense electrode provides accurate impedance measurement by minimizing the noise level [27].

Figure 2.10 provides a schematic representation of the basic operating principle of the Autolab potentiostat/galvanostat in a four electrode system. The operating principle is described hereafter.

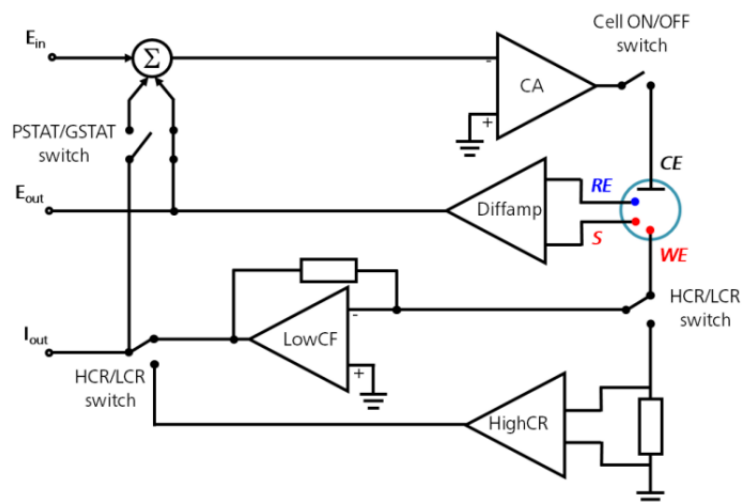


Figure 2.10: *Schematic representation of the basic working principle of the Autolab potentiostat/galvanostat in a four electrode system [28].*

1. E_{in} signal is generated by a digital-to-analog converter.
2. The PGSTAT/GSTAT switch allows the potentiostat or galvanostat mode to be selected.

3. If potentiostat mode is selected, E_{in} and the output of the differential amplifier Diffamp are summed at the summation point Σ . If galvanostat mode is selected, E_{in} and the output of the current follower (LowCF or HighCR) are summed.
4. The HCR/LCR switch before the summation point allows to choose between the output of the LowCF if the current is low or the output of the HighCR if the current is high.
5. The summation of the two signals is then sent to the Control Amplifier CA. First, the CA amplifies the output of the summation point. Then, it controls the output voltage of the counter electrode CE relative to the working electrode WE to maintain a potential difference equal to the value imposed by the user between WE and S (Sense electrode) and between RE (Reference Electrode) and WE. In addition, CA connected to CE allows the current to be forced through the DUT.
6. The measured electrochemical cell is represented by the electrodes CE, RE, S, WE. After this, the HCR/LCR switch allows the signal to be sent to the LowCF current follower if the current is low or to the HighCR current follower if the current is high.
7. The Current Follower CF measures the current between WE and CE. Its output I_{out} is sent to the summation point if the galvanostat mode is selected. I_{out} is also sent to the digital-to-analog converter which generates E_{in} .
8. The differential amplifier Diffamp measures the potential difference between RE and S. Its output E_{out} is sent to the summation point if the potentiostat mode is chosen. E_{out} is also sent to the digital-to-analogue converter which generates E_{in} .

2.3.3 Electrochemical system equivalent circuit

An electrochemical system exhibits such complexity that its electrical parameters are not directly calculated on the basis of the area of the electrode and the measured voltage and current. Indeed, the current distribution through the electrode is non-uniform and this makes the determination of the current flow path more complex. To remedy this, a simplified equivalent circuit is used. This allows to curve fit the experimental data and to extract information about the electrical parameters of the system. Once this information has been collected, the influence of each electrical parameter on the impedance variation of the measurements can be analysed. A simplified equivalent circuit is usually composed of resistances, capacitances and inductances. These elements represent independent physico-chemical processes that occur in the electrochemical system. Although simple, the main disadvantage of the equivalent circuit technique is that several quite different circuits may fit the experimental data. The choice of circuit should be made based on the one that best explains the physical behaviour of the Device Under Test (DUT) [29, 30].

The equivalent circuit of an electrochemical system can be developed using a case of solid electrode immersed in an electrolyte solution. In this situation, the charged surface

of the electrode, whose potential Ψ_0 is known, attracts oppositely charged ions in the solution through strong electrostatic interactions. A layer of diameter a containing an excess of one type of ion is absorbed on the surface of the electrode as shown in Figure 2.11(a). This layer is called the Stern layer or the compact layer. It is framed on the one hand by the electrode and on the other by the diffuse layer or Gouy-Chapman layer, which also contains an excess of one type of ion, as shown in Figure 2.11(a). The accumulation of charge prevents the electric field from the electrode to penetrate too far into the electrolyte solution. The potential at the interface between the Stern layer and the Gouy-Chapman layer is called the zeta potential ζ . It is slightly lower than the electrode potential Ψ_0 as shown in Figure 2.11(a).

In the diffuse layer the potential drops exponentially as one moves away from the electrode and reaches zero equilibrium in the bulk electrolyte. The diameter of this layer is called the Debye length λ_D or Double layer thickness. This diameter covers the range in which the voltage applied by the electrode can have an impact on the charge distribution of the electrolyte solution [17, 30, 31]. The Debye length can be expressed by means of Equation 2.7 [17]:

$$\lambda_D \cong \sqrt{\frac{\epsilon_{sol} U_t}{2 F C_{ions} \cdot 10^3}} [m], \quad (2.7)$$

where

- ϵ_{sol} is the electrolyte permittivity [$F.m^{-1}$],
- U_t the thermal voltage [V],
- F the Faraday constant [$C.mol^{-1}$] and
- C_{ions} the ionic strength [$mol.L^{-1}$].

This formula has the advantage of being simple to use as it only depends on the characteristics of the electrolyte. However, it is only valid if the local potentials are always lower than the thermal voltage. This condition ensures that the simplification of the Poisson-Boltzmann equations, from which Equation 2.7 is derived, remains valid. This double layer at the electrode interface can be assimilated as a capacitance, from which the principle of double layer capacitance is derived. The spectral behaviour of the double layer is modelled by the Randles circuit in Figure 2.11(b) [17, 30, 31].

The equivalent circuit model of an electrochemical system usually includes a solution resistance, a double layer capacitance, a dielectric capacitance, a bias resistance, a charge transfer or faraidic electron-transfer resistance, a Warburg impedance, an interfacial impedance, a coating capacitance and virtual inductances. A randle circuit generally consists of four of these elements, as shown in Figure 2.11(b), which is explained below [17, 29, 30, 32].

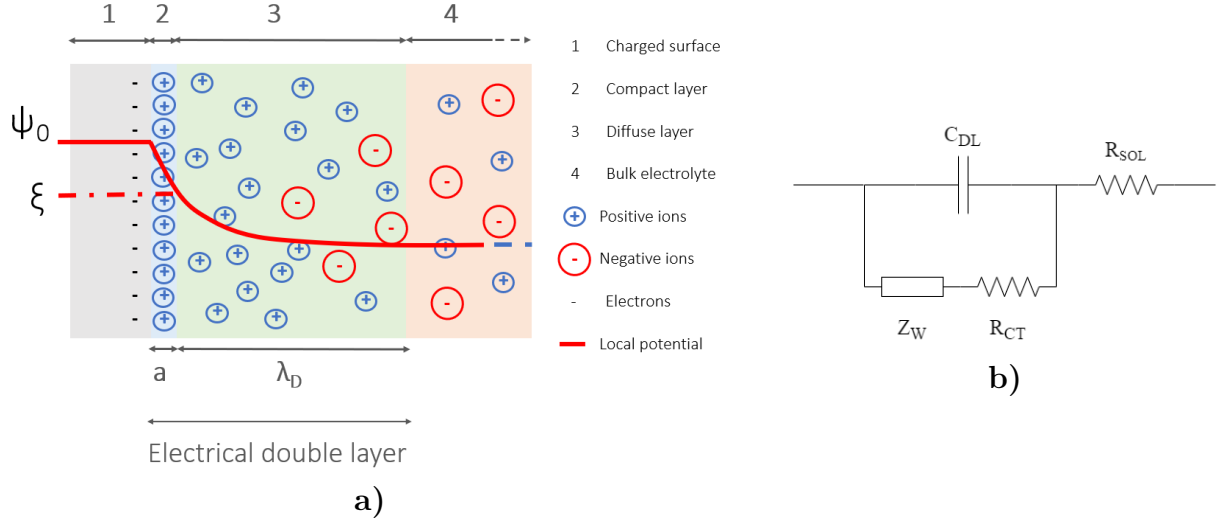


Figure 2.11: (a) Schematic representation of the electrode-electrolyte interface and dependence of the local potential with the distance. Ψ_0 is the surface potential, ζ is the zeta potential [17, 31]. (b) Representation of the equivalent circuit model to analyze impedance named Randles model [30].

- **Charge transfer resistance R_{CT}**

The resistance R_{CT} is found at the electrode/solution interface when a redox probe is present. This resistance represents the current flow generated by the electrochemical reactions. More precisely, this resistance is equivalent to the opposition to the access of the redox probe to the electrode surface during a faradaic process [17, 30, 32].

- **Warburg impedance Z_W**

Z_W characterises the ionic diffusion through the double layer, that is from the bulk electrolyte to the electrode surface [17, 30].

- **Capacitance C_{DL}**

C_{DL} is therefore the series combination of the capacitance representing the compact layer and the capacitance representing the diffuse layer. C_{DL} is expressed in Equation 2.8 [17].

$$C_{DL} \triangleq (C_{compact}^{-1} + C_{diffuse}^{-1})^{-1} \quad (2.8)$$

The value of C_{DL} depends on variables such as electrode potential, temperature, ionic concentration, types of ion present in the electrolyte solution, electrode roughness and impurity absorption. In particular, electrode roughness means that the actual electrical double layer rarely exhibits purely capacitive behaviour and a constant phase element (CPE) is usually used to replace C_{DL} [30].

- **Solution resistance R_{SOL}**

R_{SOL} represents the conductivity variation of the bulk medium and the charge transport through the bulk solution [32].

R_{CT} and Z_W are connected in parallel to C_{DL} because the total current flowing through the electrode is the sum of the contributions of the faradaic processes. These processes are characterised by R_{CT} and Z_W . The interfacial charging is characterised by C_{DL} . R_{SOL} is in series with the combination of the other elements because all the current flow passes through this resistance [30].

In addition to the four elements listed below, the equivalent circuit of a dielectric material subjected to an electric field can be characterised by a capacitance and a resistance at any frequency. If the material under consideration suffers dielectric losses, the capacitance and resistance used to characterise it are set in parallel [33]. A capacitance of the solution C_{SOL} is therefore included in addition to R_{SOL} to characterise the electrolyte solution. The total equivalent circuit of an electrochemical system is therefore based on the Randles circuit shown in Figure 2.11(b) with the addition of the dielectric capacitance of the C_{SOL} solution, in parallel with R_{SOL} [32]. It is illustrated in Figure 2.12.

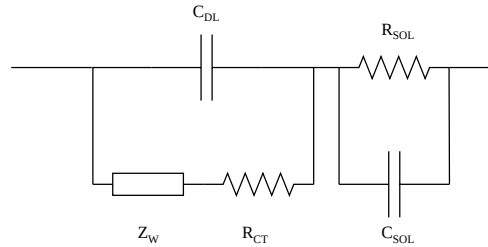
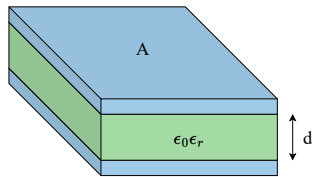


Figure 2.12: Representation of the equivalent circuit Randles model with the additional dielectric capacitance of the solution C_{SOL} .

Determining all the parameters of an equivalent circuit such as the one shown in Figure 2.12 requires clarification of the concept of polarisation and its link with dielectric permittivity. Indeed, the value of a capacitor depends on the permittivity of its dielectric as shown in Figure 2.13 and Equation 2.9, in the simple case of a parallel plate capacitor.



$$C = \frac{\epsilon_0 \epsilon_r A}{d} \quad (2.9)$$

Figure 2.13: Parallel-plate capacitor.

When an alternating electric field is applied to a dielectric, the electric charges within it rearrange in space. That is, the positive charges in the dielectric move in the same direction as the applied electric field and the negative charges move in the opposite direction. This charge shift is responsible for the conductive properties of a dielectric. Indeed, a dielectric does not have free electrons, which are responsible for conduction in other types of materials. This phenomenon of electrical charges reorganisation in space is called polarisation. It

can be classified into four main groups: ionic polarisation, dipolar polarisation, interfacial polarisation and distortion polarisation. These phenomena are illustrated in Figure 2.14. Polarisation distortion can itself be characterised by two sub-types of polarisation: atomic and electronic polarisation [33, 34].

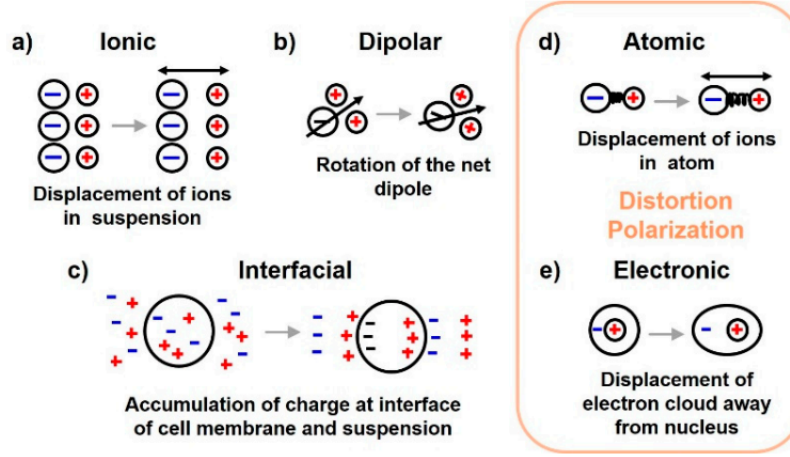


Figure 2.14: The five different types of polarisation phenomenon. The distortion polarisation is represented in (d) and (e) by the atomic and electronic polarisation respectively [34].

- **Ionic polarisation**

Ionic polarisation is the displacement, due to the application of an alternating electric field, of cations and anions in an aqueous and ionic medium from their equilibrium position, as illustrated in Figure 2.14(a). In this type of polarisation, as well as in interfacial and dipolar polarisation, no direct mechanical force is attempted to move the dipoles back into their random orientation [33, 34].

- **Dipolar polarisation**

Dipolar polarisation occurs when polar molecules are present within a material. These have a net permanent dipole oriented randomly and under the application of an electric field orient themselves in the direction of this field. This phenomenon is illustrated in Figure 2.14(b). The result is a net polarisation in the direction of the field [33, 34].

- **Interfacial polarisation**

While distortion and dipolar polarisation occur as a result of the presence of atoms and molecules, interfacial polarisation requires the presence of charge carriers capable of migrating a certain distance by diffusion or jumping under the application of a low frequency electric field. This causes charges to accumulate at the interfaces of the material, as depicted in Figure 2.14(c) [34].

- **Distortion polarisation**

The principle of distortion polarisation is as follows: the applied electric field attempts to vary the distance between the charges in the material. In response, a restoring force opposes the action of the field. The appearance of this force is what distinguishes

distortion polarisation from other types of polarisation. Like dipolar polarisation, distortion polarisation occurs when molecules or atoms are present in the material [34].

– **Atomic polarisation**

As with ionic polarisation, atomic polarisation arises from the movement of negative and positive ions in opposite directions under the application of an alternating electric field. Figure 2.14(d) shows that ion displacement takes place within the atom. [33, 34].

– **Electronic polarisation**

Electronic polarisation is illustrated in Figure 2.14(e). It acts on every atom or molecule present. Electrons surrounding an atom move away from the atom's nucleus as a result of the application of an alternating electric field. Since an electron is very light, this displacement is particularly fast and this allows the electron polarisation to be observed at so-called optical frequencies. These include the visible electromagnetic spectrum and the infrared ($>3\text{THz}$) [33, 34].

Debye's equations provide a link between frequency and permittivity. This link is polarisation. At low frequencies all types of polarisation participate in the total polarisation because all dipoles have time to follow the variations of the electric field. As shown in Figure 2.15(a), permittivity is therefore maximum. When frequency is increased, polarisations that are no longer able to align the dipoles according to the directional field reversals, because they are accelerated, are dropped. These dipoles therefore no longer contribute to the total polarisation of the material. The frequencies where polarisation losses occur are called relaxation frequencies. These frequencies coincide with a drop in real permittivity ϵ' and a peak in imaginary permittivity ϵ'' . These are shown in Figure 2.15(a) and are referred to as f_{r1} , f_{r2} and f_{r3} for the relaxations associated with, respectively, ionic polarisation, which is therefore dropped first, interfacial polarisation and dipolar polarisation. Frequency dependence of permittivity is called dispersion. Figure 2.15(b) illustrates dispersion as a function of frequency variation.

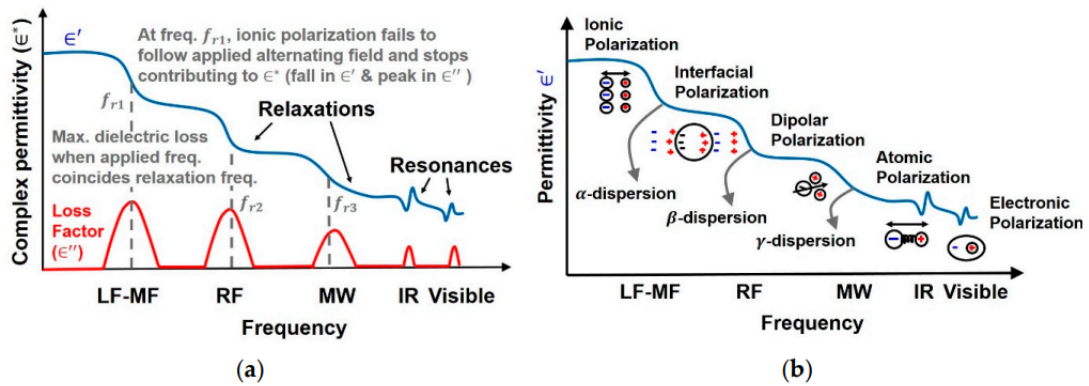


Figure 2.15: (a) Evolution of the complex permittivity of a dielectric as a function of frequency when an alternating field is applied. f_{r1} , f_{r2} and f_{r3} are the relaxation frequencies. (b) The order in which polarisation phenomena disappear as the frequency increases [34].

The first dispersion occurs in kHz region and is called α -dispersion. It is the result of ionic relaxation. The second dispersion, β -dispersion, occurs in MHz range and is associated with interfacial relaxation. The third, γ -dispersion, takes place in GHz range and is the result of dipolar relaxation [33, 34].

2.4 Spiral planar inductor

To perform inductive detection, two types of coils can be used. The first type is called planar inductor. Spiral planar inductors are the most commonly used. There are several types of geometry. Those shown in Figure 2.16(a) are square, octagonal, hexagonal and circular geometries. Each geometry has its advantages and disadvantages and should be chosen according to the application. Hexagonal and octagonal geometries, for example, require more chip area than square and circular geometries and are therefore rarely used [35, 36].

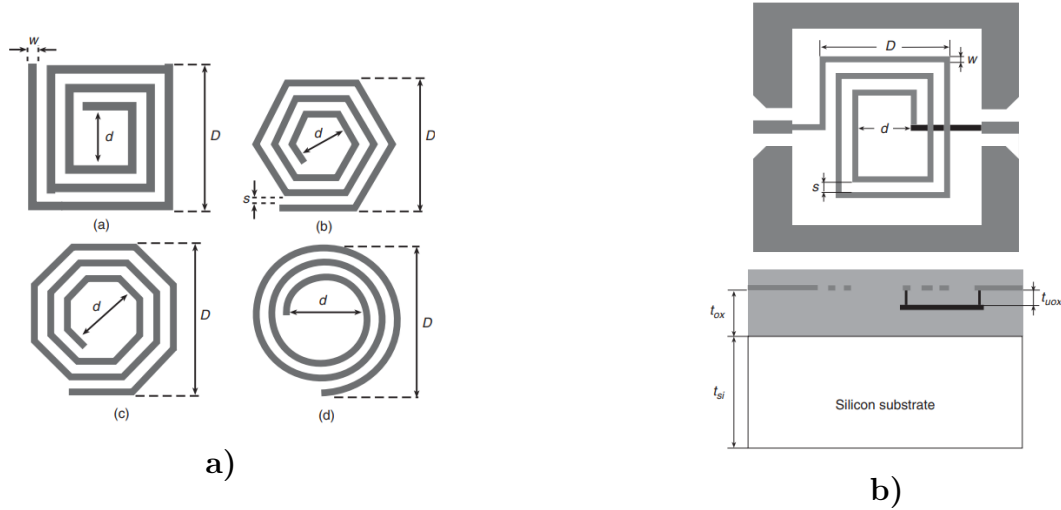


Figure 2.16: (a) Examples of possible geometries for the metal trace of a spiral planar inductor. (b) Cross section of a planar inductor [36].

An on-chip planar spiral inductor consists of a low resistivity trace metal such as aluminium, copper, gold or silver. This trace is usually supported on a silicon dioxide SiO_2 layer of thickness t_{ox} , which in turn is supported on a silicon (Si), gallium arsenide ($GaAs$) or silicon germanium ($SiGe$) substrate, as shown in Figure 2.16(b). The thickness of this layer varies between 500 and 700 μm . The SiO_2 layer isolates the trace metals from the substrate and is typically 0.4 μm to 3 μm thick. The outer end of the metal trace is directly connected to a port. The inner end is connected to the port via an underpass of another metal layer or via an air bridge. The underpass is located at distance t_{uox} from the spiral, as shown in Figure 2.16(b). The whole thing is connected to pads and is surrounded by ground plane. The most commonly used spiral planar inductor is the square inductor because it allows high inductance values per unit of wire length [36, 37, 38].

The second type of coil is called 3D solenoid. It consists of a superposition of several planar inductors, as shown in Figure 2.17. The inductances are superimposed in such a way that the current always has the same direction [39].

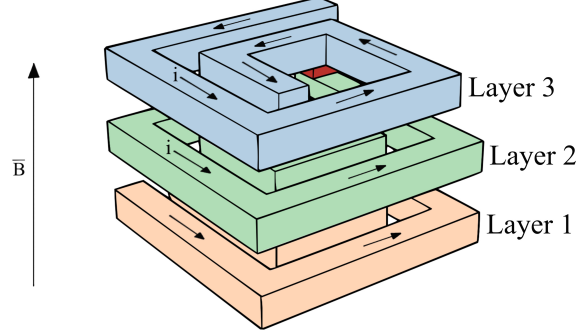


Figure 2.17: Concentric flow of current in a 3D solenoid inductor showing the direction of the associated magnetic field [39].

The equivalent circuit that models single layer planar spiral inductors is the two-port π -model shown in Figure 2.18(a). To be precise, this model is that of a planar inductor on a Si substrate. This lumped element model is composed of the inductor and the parasitic elements of the system. Indeed, since the purpose of an inductor is to store energy only, the resistors and capacitances present in a real inductor prevent this storage phenomenon, therefore being parasites. This model takes into account the frequency-dependent phenomena inherent to the system such as the eddy current effect in the conductor or the proximity effect. In general, the higher the frequency, the greater the influence of these parasitic phenomena. This model is valid up to the self-resonant frequency of the inductor, a concept that will be discussed shortly. L_s is the inductance of the metal trace and the underpass. R_s is the resistance of the spiral and the underpass. It dissipates energy by ohmic loss. C_s is the capacitive coupling due to the crosstalk between the adjacent turns C_{tt} and the overlap between the spiral and the underpass C_{su} , as illustrated in Figure 2.18(b) [36, 38, 39, 40, 41].

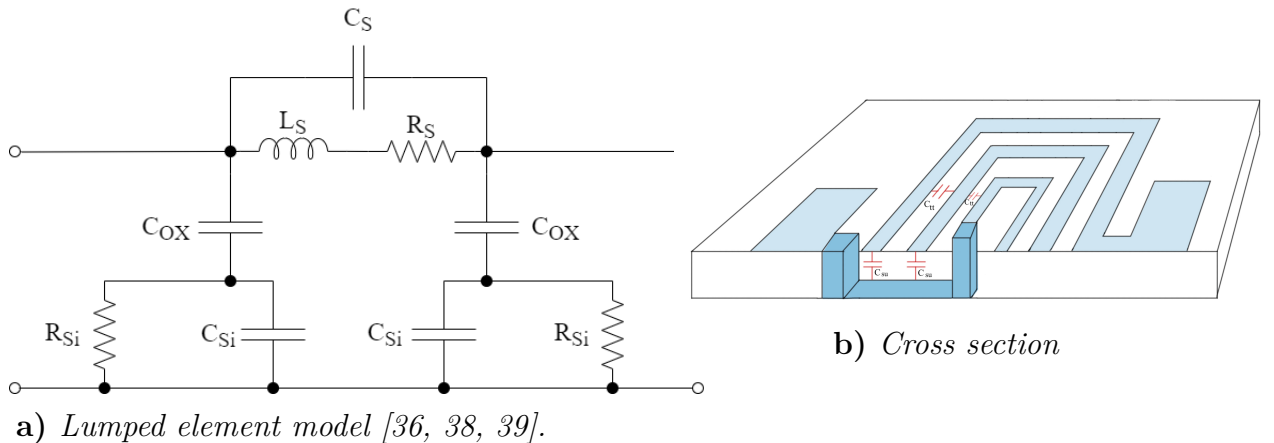


Figure 2.18: Planar spiral inductor on a silicon substrate [40].

This capacitive coupling allows the current to flow from the input to the output of the port without passing through the inductance. The electrical energy stored in the turn-to-turn C_{tt} capacitances is proportional to the square of the voltage. The displacement current across the turn-to-turn capacitances $i_C = C_{tt} \frac{dv}{dt} \cong C_{tt} \frac{\Delta v}{\delta t}$ is very small. Consequently, the effect of the turn-to-turn capacitances is negligible. The effect of the overlap capacitance on the other hand is not negligible since the potential difference between the spiral and the underpass is large. C_{ox} models the oxide capacitance between the metal traces and the silicon substrate. C_{si} and R_{si} model the capacitance and resistance of the silicon substrate, respectively [36, 37, 38, 39, 40].

The parameters R_S , L_S and C_S are explored in more detail below. Information about C_{si} , R_{si} and C_{ox} can be found in documents [36], [38], [39] and [40].

Series resistance R_s

Before being able to give the expression for the series resistance R_s , it is important to clarify the concepts of eddy current, skin effect and proximity effect.

Eddy currents take place in any conductor that is subject to a time-varying magnetic field. The physical manifestations of eddy currents within the conductor are skin and proximity effects. In order to understand the concept of eddy current, consider the diagram in Figure 2.19.

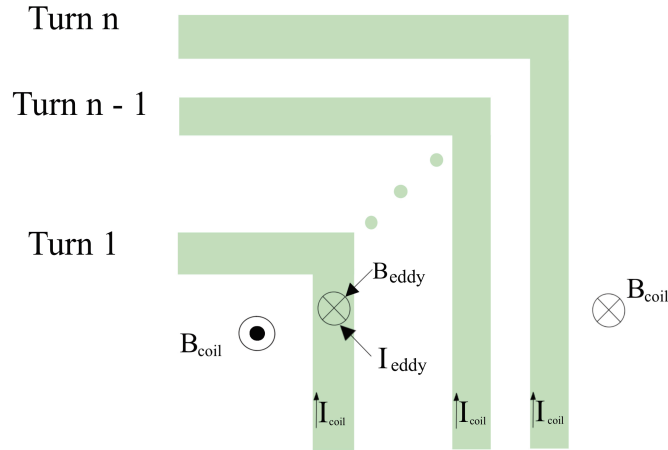


Figure 2.19: Section of an n -turn square-shaped inductor with the fields and the eddy currents [38].

It represents an n -turn section of an inductor. The current in the metal traces is called I_{coil} and its associated magnetic flux B_{coil} . The magnetic flux lines that enter the page next to the n -turn emerge from the page in the centre of the inductor. If the inner diameter of the inductor is very small, the magnetic flux lines generated by the current flowing in the outer conductor segments pass through the inner segments [38]. Faraday's and

Lenz's laws predict that a current is then induced within these inner turns. These induced currents are called eddy currents. They then produce their own magnetic field, B_{eddy} , which opposes the basic magnetic field B_{coil} . At high frequencies, the skin effect is the tendency of the current to flow mainly at the surface of the conductor. Indeed, a close look at turn 1 in Figure 2.19 allows to see that I_{eddy} has the same direction as I_{coil} when looking at the inner edge of turn 1, i.e. the edge on the left. I_{coil} and I_{eddy} therefore add up on this edge. On the other hand, on the outer edge of turn 1, the edge on the right of Figure 2.19, I_{eddy} has a direction opposite to that of I_{coil} . The two currents are therefore subtracted at the right-hand edge. This results in a higher current density on the inner side of turn 1 and a current non-uniformity in the metal traces [36, 38, 40]. As for the proximity effect, the explanation concerns adjacent conductors. When a conductor is subjected to an alternating current, an alternating magnetic field is created around it. This magnetic field induces eddy currents in the conductors near this first conductor. The distribution of the current in the neighbouring conductors is then altered. The current seeks to concentrate in the areas furthest away from the other conductors whose current flows in the same direction [40, 42].

Spiral planar inductors can be affected by the skin effect and the proximity effect since they are composed of several conductors close to each other. Indeed, the current through each of these conductors can induce eddy currents in the other segments. Proximity effect can be neglected frequencies are below 1 GHz and each segment of the inductance is in the same plane [40].

At low frequencies, the series resistance R_s is given in Equation 2.10 because the current density is uniform in the wire [36, 38, 39, 40].

$$R_s = \frac{\rho l}{wt} \quad (2.10)$$

where

- ρ is the copper resistivity in $[\Omega.m]$,
- l is the overall length of the spiral inductor $[m]$,
- w is the width of the copper conductor trace in $[m]$ and
- t is the thickness of the copper conductor trace in $[m]$.

At high frequencies, the formation of eddy currents within the conductor leads to a non-uniformity of the current density called skin effect. The series resistance R_s becomes frequency-dependent and increases as the frequency increases. The skin effect results in a decrease in the effective thickness t_{eff} of the metal. This thickness is given in Equation 2.11 [38, 39, 40].

$$t_{eff} = \delta(1 - e^{-\frac{t}{\delta}}), \quad (2.11)$$

where δ is the skin depth of the metal in m. This parameter is given in Equation 2.12 [43].

$$\delta = \sqrt{\frac{2\rho}{\omega\mu}} \sqrt{\sqrt{1 + (\rho\omega\epsilon)^2} + \rho\omega\epsilon} \quad (2.12)$$

where

- ω is the angular frequency in $[rad.s^{-1}]$,
- μ is the permeability of the conductor in $[H.m^{-1}]$ and equals $\mu_r.\mu_0$,
- μ_r is the relative permeability of the conductor,
- μ_0 is the vacuum permeability in $[H.m^{-1}]$,
- ϵ is the permittivity of the conductor in $[F.m^{-1}]$ and equals $\epsilon_r.\epsilon_0$,
- ϵ_r is the relative permittivity of the conductor and
- ϵ_0 is the permittivity of free space in $[F.m^{-1}]$.

Equation 2.12 can be simplified to become Equation 2.13 when frequencies are much below $\frac{1}{\rho\epsilon}$. Indeed, in this case the second square root of Equation 2.12 approaches unity [43].

$$\delta = \sqrt{\frac{2\rho}{\omega\mu}} = \sqrt{\frac{2\rho}{2\pi f \mu_0 \mu_r}} \quad (2.13)$$

The skin depth can also be called the depth of penetration. It describes the extent to which the electric current and magnetic flux can penetrate the conductor at high frequency. The effect of eddy current can be quantified by comparing the skin depth to the thickness of the conductor. If the skin depth is much greater than the conductor thickness, the effect of eddy current is negligible [40].

By replacing the value of t in Equation 2.10 with the effective thickness t_{eff} of the metal, defined in Equation 2.11, R_s at high frequency is derive in Equation 2.14 [36, 37, 38, 40]:

$$R_s = \frac{\rho l}{w\delta(1 - e^{-\frac{t}{\delta}})} \quad (2.14)$$

Parasitic capacitance C_s

The parasitic capacitance has two components as already explained. A component C_{tt} which characterises the crosstalk capacitance between adjacent turns and a component C_{su} which characterises the overlap capacitance between the spiral and the underpass. However, since the adjacent turns are almost equipotential, the effect of crosstalk capacitance is

assumed to be negligible. The impact of C_{su} on the other hand is much more significant given the large potential difference between the metal traces and the underpass. C_s can therefore be modelled as the sum of all overlap capacitances [36, 38, 40].

$$C_s = \epsilon_x \frac{mw^2}{t_x} \quad (2.15)$$

where

- m is the number of overlap,
- w is the metal traces width in $[m]$,
- ϵ_x is the permittivity of the layer between the traces and the underpass in $[F.m^{-1}]$ and
- t_x is the thickness of this layer in $[m]$.

Inductance L_s

Inductance L_s can be computed by means of the Greenhouse method. That is, it can be expressed as the algebraic sum of the self-inductance of each wire and the mutual inductance between all possible wire pairs. The mutual inductance is sometimes positive, when two parallel segments have the same current direction, sometimes negative, when the currents in the two parallel wires flow in opposite directions. The mutual inductance of two perpendicular conductors is zero since their magnetic flux is not linked [36, 38, 39, 40]. The Greenhouse method has sufficient accuracy and adequate speed. However, it is not possible to design an inductance based on specifications with this method. To do so, there are many simple approximations to L_s , many of which are listed in [44].

Chapter 3

Objectives

This work is part of the development of the biosensor developed by Jean-Pierre Raskin's team at the Ecole Polytechnique de Louvain. As a reminder, this one aims to provide a water quality assessment methods in remote areas. This shall be implemented by an electrochemical and colorimetric reading of a Lateral Flow Assay (LFA).

Therefore, this master thesis focuses on electrochemical detection. In particular, the main objective of this master thesis is to define the ideal detection method in order to read the LFA.

After analysis of the state of the art, potentiometric and non-faradaic impedimetric detection seem to be two electrochemical detection methods well suited to meet ASSURED criteria. However, some gaps emerged in the comparison between those detection procedure in the design of paper-based devices based on the LFA method. In particular, the scientific literature does not provide research studies on non-faradaic impedimetric detection on paper-based biosensors to my knowledge.

The various aspects of this research work are illustrated in Figure 3.1.

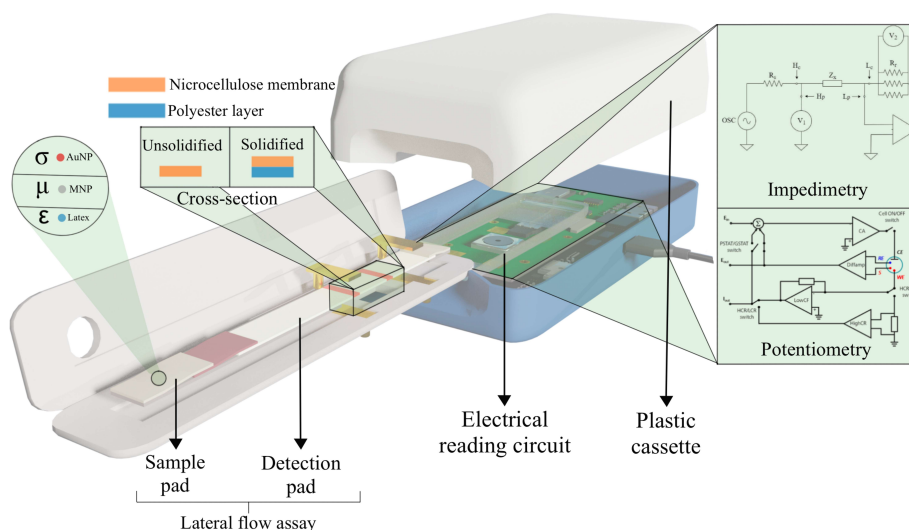


Figure 3.1: Research question related to the biosensor prototype (illustration by Nicolas Moreau).

Three areas of the biosensor are highlighted. These are the areas that the research question of this master thesis will focus on. On the right of Figure 3.1, the choice between impedimetric and potentiometric detection is illustrated. This choice of the best possible detection is constrained by two types of physical constraint on the Lateral Flow Assay.

1. The first constraint is the choice of the nitrocellulose NC membrane in the detection pad, as illustrated in the centre of Figure 3.1. This membrane can be solidified by a thin layer of polyester glued to one side of the membrane. Solidification of the membrane leads to easier handling. However, an additional polyester layer limits the possible conduction paths. The final question is to understand which technique is to be preferred in the case of a solidified membrane and how this polyester layer affects the detection.
2. A second constraint is the sample pad, as shown on the left of Figure 3.1. The Lateral Flow Assay benefits from the specific characteristics of the nanoparticles (NPs) that can compose LFAs (e.g. conductivity, permittivity or permeability for gold, latex or magnetic nanoparticles, respectively). These NPs concentrate at the test line and enhance electromagnetic transduction. The different types of NPs are not yet available for use in LFA in our lab. Therefore, in this work, we investigate these different types of transduction by using model samples that simulate the electromagnetic behaviour of the different NPs. In particular, to study the response of the sensor to changes in conductivity, permittivity and permeability, we used conductive biological fluids, permittive fluids and variable spiral planar inductance, respectively.

In short, the detection methods are compared to each other by comparing their detection behaviour in three main cases:

- In the case of **conductivity variation** of the measured sample.
- In the case of **permittivity variation** of the measured sample.
- In the case of **permeability variation** of the measured sample.

Cases of conductivity and permittivity variations are studied on simple system consisting of a nitrocellulose membrane soaked with different model liquids chosen for their known permittivity or conductivity.

The sensitivity of the methods to permeability variation is analysed via the impedance variation of the spiral planar inductors induced by the variation of their magnetic flux, itself induced by the deposition of an iron alloy foil on the inductor's spiral.

Three figures of merit are used to compare the two detection methods for the three cases of variation considered.

- Study of the system's **sensitivity**

The sensitivity of the two detection methods is compared in order to investigate which of the two methods is more suitable for the three types of variation.

- Study of the system **consumption**

The power consumption of the biosensor depends, among other things, on the voltage that needs to be applied to enable it to operate at its maximum capacity. The two detection methods are compared here to study their behaviour when faced with a voltage variation. A set of measurements with a low voltage is compared to a set of measurements with a voltage ten times higher. The question is whether the results obtained are the same, so the biosensor can operate with a low voltage and consume less. Or on the contrary, is a high voltage level necessary and if so, is it necessary for both detection methods.

- Study of the system's measurement **repeatability**

The two detection methods will be compared in terms of their measurement repeatability. Indeed, this figure of merit is important for the reliability of the measurements obtained.

In conclusion, the research should answer the question: « *Which **detection method** should be preferred, between **potentiometry** and **non-faraidic impedimetry**, in the design of a paper-based biosensor, depending on the **sample variation** that is to be analysed and the type of **paper** used in the Lateral Flow Assay **detection pad**? ».*

Chapter 4

Materials and methods

This chapter starts by presenting the different measurement devices used in this master thesis and the associated measurement procedures. The samples used in the measurements are then listed. A third section develops the sensitivity and repeatability formulae that are used to study the behaviour of the measurement devices in the various measurement cases. Finally, the equivalent circuits associated with the three types of sample variation studied are developed.

4.1 Instruments and measurements procedure

In order to study impedimetric and potentiometric detection, two main devices will be used: an Agilent LCR meter and the Methrom Autolab potentiostat. These two devices, their technical specifications and setup are detailed in the following section. In addition, two further devices are used in the validation of results and are therefore presented in this section: the novocontrol and a Vector Network Analyzer (VNA).

4.1.1 LCR meter

The nitrocellulose membranes were held between the two parallel-plates of the dielectric test fixture (16451B, Agilent, Santa Clara, CA, USA), as illustrated in Figure 4.1.

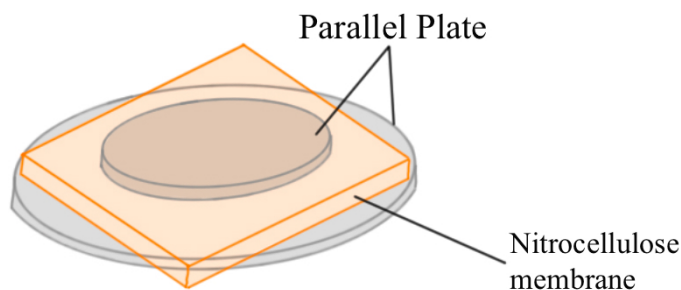


Figure 4.1: *Diagram of a nitrocellulose membrane deposited between the parallel-plate test-fixture electrodes [10].*

The test-fixture was connected to an impedance analyzer (LCR 4284A, Agilent, Santa Clara, CA, USA) through BNC connectors. The LCR meter was remotely controlled through LabVIEW software (Labview National Instrument, Austin, TX, USA) to perform an automatic sweep from 20 Hz to 1 MHz, at voltage amplitude of 20mV. The LCR meter was used to perform impedance spectroscopy measurements. From Labview software, the norm and phase values of the measured impedances were extracted. The measurement setup is presented in Figure 4.2.

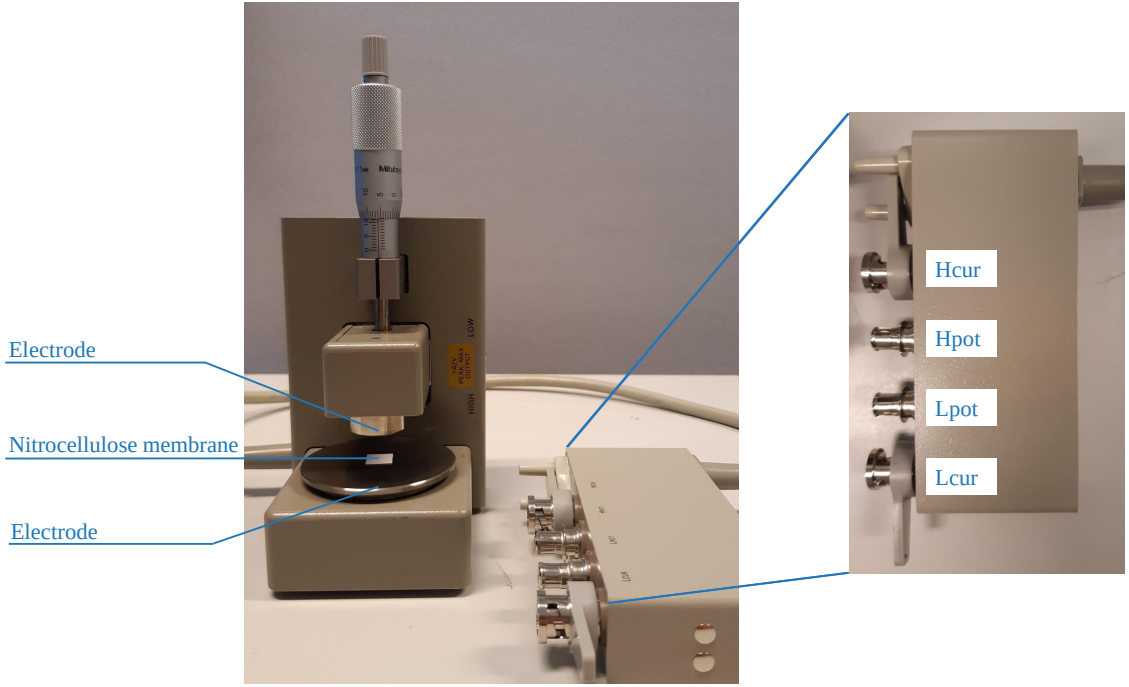


Figure 4.2: Setup enabling impedimetric measurement of nitrocellulose (NC) membrane saturated with specific liquid samples. The setup includes an Agilent LCR meter connected via BNC connectors to a parallel-plate test fixture.

Measurement conditions are based on the technical specifications of the LCR meter, given in Table 4.1. Furthermore, the applied voltage is constrained to be lower than the thermal voltage U_t ($=25mV$). Indeed, the development of equivalent circuits for the electrochemical systems studied introduces the concept of an electrical double layer. The approximation of this double layer at the level of an equivalent circuit requires simplifications that are only valid if the applied potential is lower than the thermal voltage. This is explained in more detail in Section 4.4. Measurements are performed at a voltage of $20mV$, to be lower than the thermal voltage but high enough to maximise the readability of the measurements.

Table 4.1: LCR 4284A Technical Specifications, Agilent [45].

Device references	Frequency range	Voltage range	Measured impedance range
LCR 4284A, Agilent	$20Hz \rightarrow 1MHz$	$5mV \rightarrow 2V$	$0.01m\Omega \rightarrow 99.99M\Omega$

Measurements carried out by the LCR meter necessarily contain some errors. These errors can be caused by instrument inaccuracies, residuals in the test fixture and cables or noise. The correction of the LCR meter is done in two steps. First, a calibration is performed, followed by a compensation. The calibration defines the calibration plane, at which the specified measurement accuracy can be obtained. In the case of 4TP configured instruments, the calibration plane is at the unknown BNC connectors, as illustrated in Figure 4.3 [24].

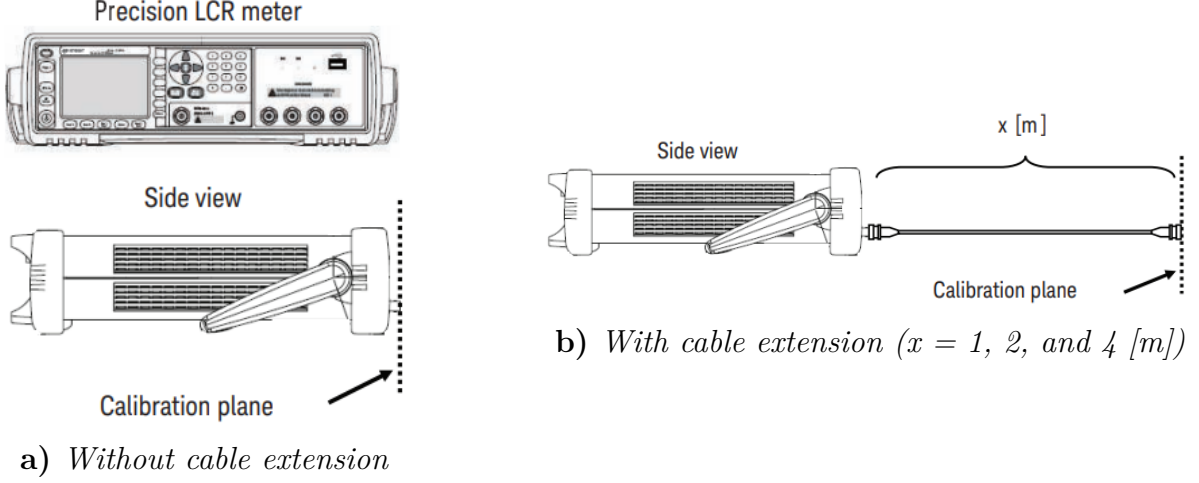


Figure 4.3: Calibration plane of auto-balancing bridge instruments [24].

When measuring, once the test fixture was connected to the LCR meter, an open and short compensation was performed. The compensation reduces the effects of error sources between the Device Under Test (DUT) and the calibration plane of the instrument. This compensation technique assumes that the residual test-fixture errors can be represented in an L/R/C/G circuit (see Figure 4.4) [24].

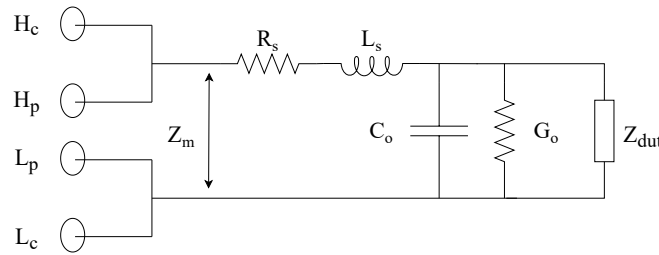


Figure 4.4: Test fixture residuals [24]. H_p , L_p , H_c , L_c are the input ports of the LCR meter and stand for high potential, low potential, high current and low current respectively.

These residual errors are called residual impedance $Z_s = R_s + j\omega L_s$ and stray capacitance $Y_o = G_o + j\omega C_o$. The open and short compensation allows to know their value. The open compensation was performed by keeping the parallel plate electrode of the test fixture at a maximum distance from each other. In this situation, the stray admittance Y_o is measured since the residual impedance Z_s is negligible (see Figure 4.5(a)). The short compensation

was performed by short-circuiting the test-fixture electrodes. The measured impedance is then equal to the residual impedance Z_s because Y_o is bypassed (see Figure 4.5(b)). Once Z_s and Y_o are computed, Equation 4.1, called compensation formula, allows to know Z_{dut} [24].

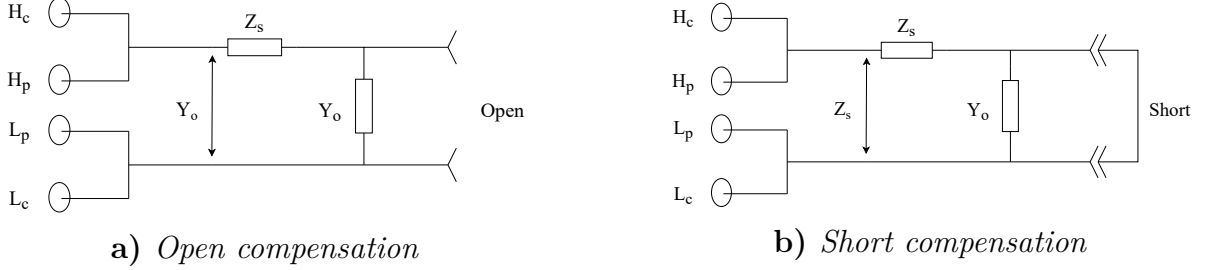


Figure 4.5: *Equivalent circuit illustrating the open and short compensation operations in an auto-balancing bridge device [24].*

$$Z_{dut} = \frac{Z_m - Z_s}{1 - (Z_m - Z_s)Y_o} \quad (4.1)$$

The LCR METER 4284A used for the measurements presented in the next sections has an auto-balancing bridge [24].

4.1.2 Autolab potentiostat

The Autolab (PGSTAT302N, Metrohm Belgium, Antwerp, Belgium) is a potentiostat/galvanostat designed for electrochemical impedance spectroscopy. Only the potentiostat mode is used to obtain the results presented in Chapter 5. Applied potential is therefore kept constant throughout all measurements.

The four electrodes required for measurements with potentiostats are *4mm* male banana connectors, as shown in Figure 4.6(a) [28]. There are three connection modes for the four electrodes. The mode used in the measurements in Chapter 5 is called "two electrode mode". In this configuration as shown in Figure 4.6(b), the counter electrode CE and the reference electrode RE are connected together to the Device-Under-Test (DUT) electrode. The working electrode WE and the sense electrode S are also connected together to the second electrode of the DUT. This type of connection results in ohmic losses. The two electrode mode is particularly suitable for high frequency measurements (>100 kHz) [26]. When the impedimetric measurements are non-faraidic, it is not necessary to control the solution potential and the two-electrode system is sufficient [17]. The fifth electrode in Figure 4.6(a), called the ground connection, allows a connection to a Faraday cage, but is not used in this case [28].

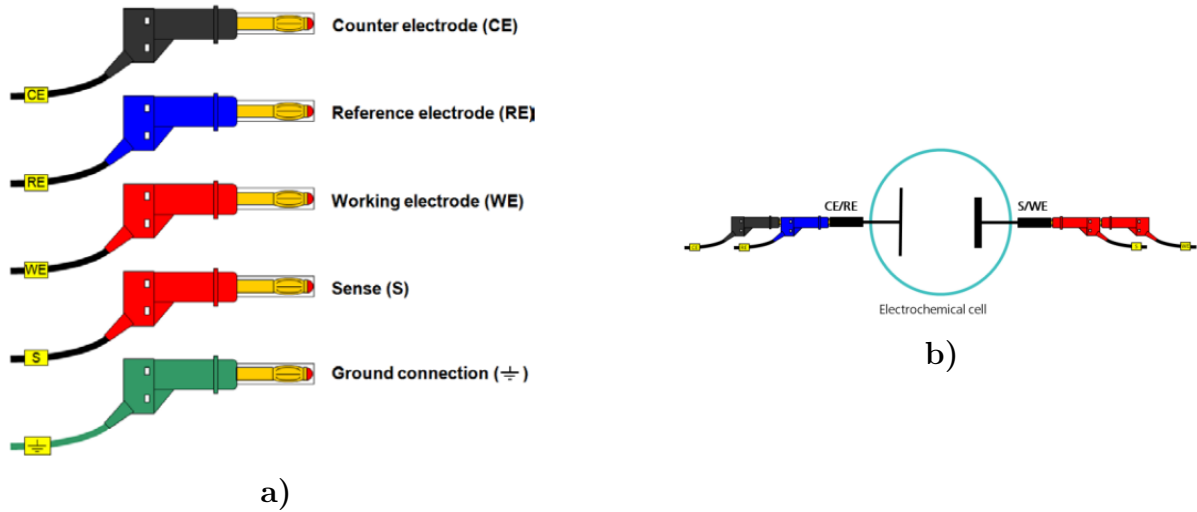


Figure 4.6: (a) Connections available on the cell cables of the Autolab PGSTAT [26]. (b) Schematic of the two electrode mode setup [26].

3.1.2.1 Limits of the Autolab potentiostat

Two types of limitations encountered by the Autolab potentiostat are particularly important in the measurement choices in Chapter 5. They are presented below.

- **Limited resolution**

The resolution of the measurements made with the autolab potentiostat is limited because the recording of the measurements is digital. This limited resolution can have negative impacts when low currents are measured over a wide possible current range. Indeed, the potentiostat automatically chooses the most suitable current range for the measurement but if during the measurement it has to change range, it takes 0.5ms to 2ms. An error may be observed during this switch time [28].

- **Bandwidth settings**

The control amplifier CA is equipped with three bandwidth settings: high stability, high speed and ultra-high speed. The frequency range covered by the measurements presented in Chapter 5 extends to 1 MHz. The bandwidth setting automatically chosen by the Frequency Range FRA mode of the NOVA software is therefore ultra-high speed which allows a frequency range of up to 1.25 MHz. However, if the DUT has a high capacitance, which is the case for measurements on solidified nitrocellulose membranes, there is a high risk of oscillations in the measurements. In addition, the noise in the measurements is higher than for a bandwidth setting high stability. In this case, it is advisable to perform these measurements with adequate shielding of the DUT and the electrodes, e.g. by means of a Faraday cage [28].

The differential amplifier has a low capacitive load. If the capacitive part of the impedance between CE and RE has a large capacitance compared to the capacitive

load, phase shifts occur and inject instability into the potentiostatic measurements when the bandwidth setting is other than high stability. [28].

Under an ultra-high speed mode bandwidth setting it is advisable to measure a maximum impedance of $35\text{ k}\Omega$ between CE and RE. Measurements on solidified nitrocellulose membrane give impedance values up to $10^8\ \Omega$ as shown in Figure 4.7(a), which presents measurements on LCR meter. Figure 4.7(b) shows a measurement on Autolab with solidified nitrocellulose membrane where the instability of the measurements is noticeable in impedance phase measurements [28].

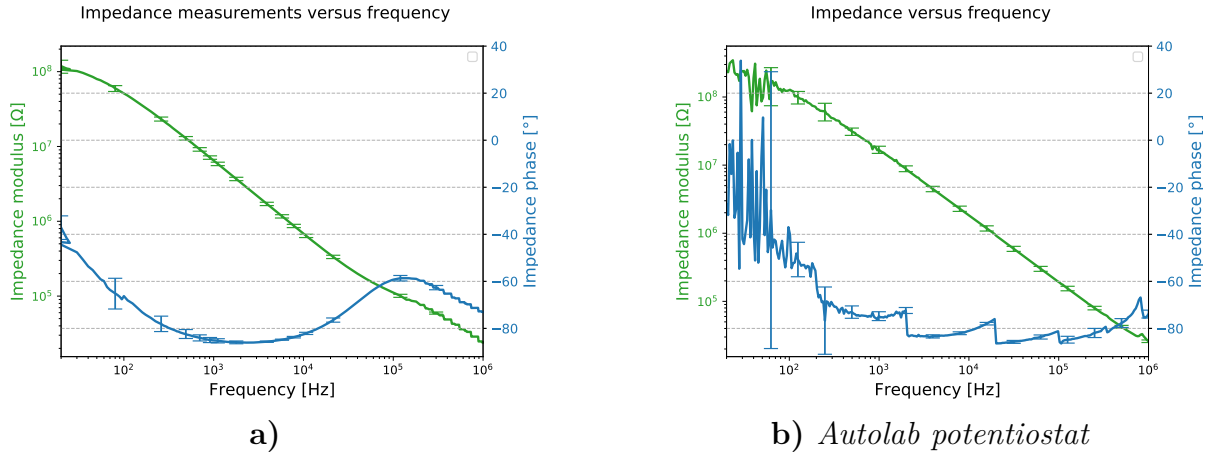


Figure 4.7: *Measurements carried out with the LCR meter on a solidified nitrocellulose membrane saturated with $1\ \mu\text{M}$ PBS. (b) Measurements carried out with the Autolab potentiostat on a solidified nitrocellulose membrane saturated with $1\ \mu\text{M}$ PBS.*

Ideally, measurements should be performed with the bandwidth setting high stability. In this case the control amplifier uses the slowest possible feedback to guarantee a stable control loop and a minimum of noise in the measurements. However, to work in this setting, the frequency range should not exceed 10kHz [28].

3.1.2.2 Measurements set-up

The set-up for the Autolab measurements is schematically illustrated in Figure 4.8. It consists of a clamp with two gold electrodes at the ends. The gold electrodes are covered at the end with a silver paste to ensure a good grip of the crocodile clips which are connected to the males banana connectors of the Autolab. The end of the clamp can be rotated, thanks to a joint, to ensure that the gold electrodes are as parallel as possible to each other.

The gold electrodes were fabricated by deposition of 1 micrometer-thick gold layer on PET substrates, with a Physical Vapor Deposition (PVD) e-gun evaporation technique. The electrodes were then obtained by simply cutting the plastic supports to the desired shape. They were cut into squares of 0.5 cm by 0.5 cm , which is the diameter of the

parallel-plate electrodes used in the LCR meter measurements setup.

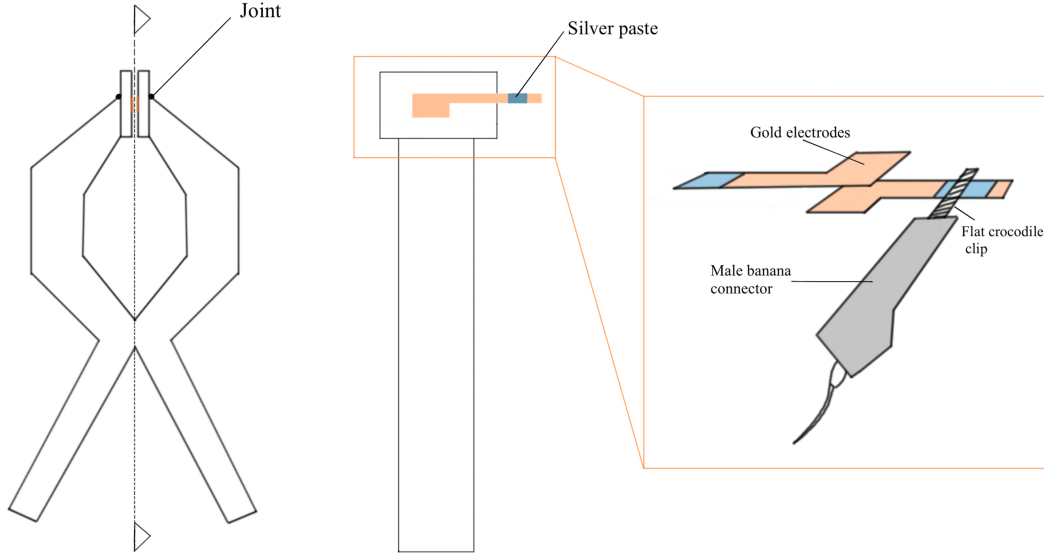


Figure 4.8: *Schematic of the setup for Autolab potentiostat measurements*

From the squares extend 1 "gold rod" of 0.2cm wide by 2cm long. At the end of these rods, a layer of silver paste (Silver nanoparticles ink for screen printing, Sigma-Aldrich, Burlington, MA, United States) is deposited [46]. The whole is then placed in an oven at 100°C for 24 hours.

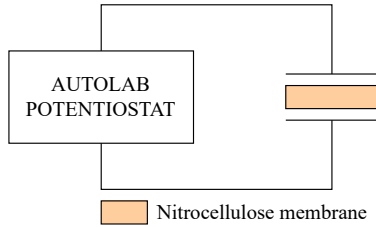


Figure 4.9: *Simplified equivalent circuit of the autolab set-up.*

The set-up is assumed to be equivalent to that of the LCR meter as shown in Figure 4.9. The parasitic elements introduced are studied in *Discussion* Chapter.

4.2 Sample presentation

In the course of the measurements, various samples are used. A first set of five liquid samples with significantly different conductivities is used to analyse the sensitivity of the LCR meter and the Autolab potentiostat to a variation in conductivity. A second set of three liquid samples with different permittivities was used to study the sensitivity of the same devices to a variation in permittivity. These liquid samples are deposited on squares of nitrocellulose membrane of 1 cm side. Finally, in order to study the sensitivity of the machines to a variation in permeability, four different inductors are used and their impedance is varied by means of two samples of iron alloy with different permeabilities.

4.2.1 Liquid samples

4.2.1.1 Phosphate-Buffered Saline (PBS)

For the conductivity variation measurements, five samples of Phosphate-Buffered Saline (PBS) (Phosphate buffered saline, Sigma-Aldrich, Burlington, MA, United States) are used [47]. Each sample is diluted by a factor of ten with deionised water. Thus the five samples have conductivities varying by a factor of ten. The samples and their respective conductivities are shown in Table 4.2.

Table 4.2: *Samples of conductivity variation measurements*

Solution	Concentration [μM]	Conductivity σ [S.m^{-1}]
PBS	1	1.587×10^{-4}
PBS	10	16.57×10^{-4}
PBS	100	181.5×10^{-4}
PBS	10×10^3	1.709×10^{-1}
PBS	10×10^4	17.49×10^{-1}

The conductivity of the samples was measured using a conductivity meter (SEVEN-COMPACT S230, Mettler Toledo, Greifensee, Switzerland) equipped with a conductivity microprobe (INLAB 452-6mm, Mettler Toledo, Grefensee, Switzerland) with a conductivity range of 0.01..112 mS/cm [48]. Table 4.2 shows that the conductivity measured for the PBS sample with a conductivity of $1\mu\text{M}$ is below 0.01 mS.cm^{-1} . However, the value given is a factor of ten lower than the conductivity given for the $10\mu\text{M}$ PBS sample, which is consistent. It is therefore assumed that this measured conductivity value is correct.

4.2.1.2 Deionized (DI) water

A sample of deionized DI water (Deionized water, S.Faniel, WINFAB Uclouvain, Belgique) is chosen as a model liquid to simulate a change in permittivity [49]. It is chosen for its low conductivity and variable permittivity relative to ethanol and isopropyl alcohol permittivity, the other model chosen. In addition, DI water is non-destructive to nitrocellulose.

$$\sigma_{DI} = 5.49 \times 10^{-6} \text{ [S.m}^{-1}] \quad \text{and} \quad \epsilon_{DI} = 80$$

4.2.1.2 Ethanol

A sample of ethanol (Ethanol, Welcome Uclouvain, Belgique) is chosen as a model liquid to simulate a change in permittivity. It is chosen for its low conductivity and variable permittivity relative to DI water and isopropyl alcohol permittivity. In addition, ethanol is non-destructive to nitrocellulose.

$$\sigma_{ET} = 1.119 \times 10^{-4} \text{ [S.m}^{-1}] \quad \text{and} \quad \epsilon_{ET} = 24.5$$

4.2.1.2 Isopropyl alcohol

A sample of isopropyl alcohol (Isopropyl alcohol, Welcome Uclouvain, Belgique) is chosen as a model liquid to simulate a change in permittivity. It is chosen for its low conductivity and variable permittivity relative to ethanol and DI water permittivity. In addition, isopropyl alcohol is non-destructive to nitrocellulose.

$$\sigma_{IS} = 3.8 \times 10^{-6} [S.m^{-1}] \quad \text{and} \quad \epsilon_{IS} = 17.9$$

4.2.2 Nitrocellulose membrane

The support for measurements of conductivity and permittivity variation is a nitrocellulose NC membrane (UniSart CN140 Membrane, Sartorius, Goettingen, Germany) cut into a 1cmx1cm square. These nitrocellulose membranes have a polyester layer glued to one side of the square or not. A nitrocellulose membrane combined with a polyester layer is called a solidified nitrocellulose membrane. The addition of a polyester layer prevents conduction paths between the two electrodes of the setup. The nitrocellulose membrane has a thickness of 225 - 255 μm , so a value of 240 μm is assumed hereafter. The polyester layer has a thickness of 100 μm .

4.2.3 Spiral planar inductances

Measurements of permeability variation are made using four squared spiral inductors built on Printed Circuit Board (PCB), as shown in Figure 4.10 [37, 39]. The permeability of these inductors is altered by depositing Fe-based alloy foils, constructed from nanocrystalline soft ferromagnetic Fe-based ribbons or amorphous soft ferromagnetic Fe-based ribbons, on the coils of the inductor [50].

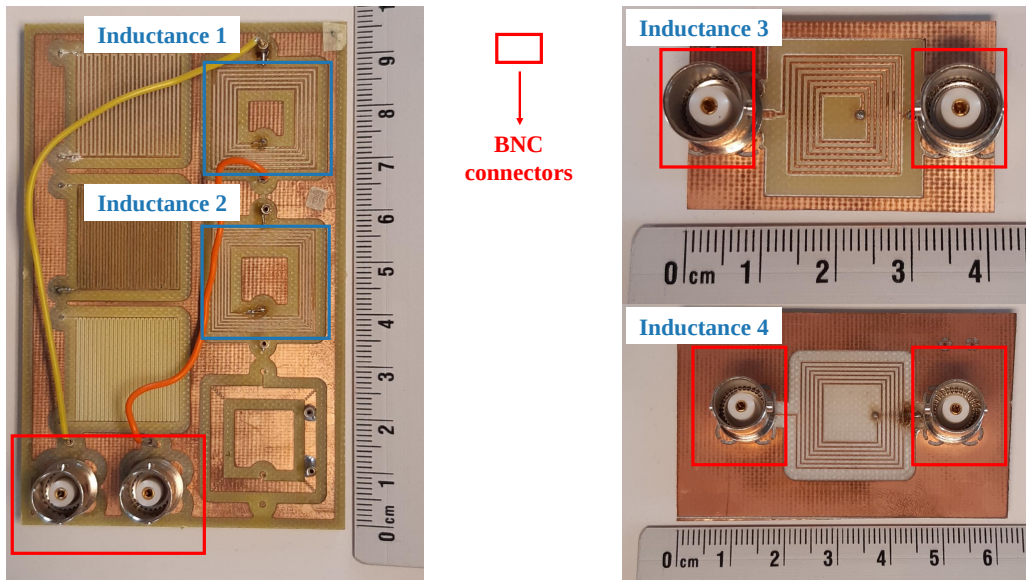


Figure 4.10: Photos of the four spiral planar inductors used to measure a change in permeability and their BNC connectors. Inductance L_1 and inductance L_2 share the same substrate and have the centre of their spiral filled with a copper square.

This type of inductor has the advantage of being low cost, easy to mass produce, durable and can be made on a wide variety of substrates. The coil is constructed on an FR4-type board, which acts as a substrate, as illustrated in Figure 4.11(a) [36, 37, 38, 39].

The characterisation of an inductor is done by means of its number of turns n , its spiral track width w , the spacing between two consecutive turns s , the inner diameter d_{in} , the outer diameter D_{out} , the thickness of the conductor t and the total conductor length l [36, 37, 38, 39]. These geometrical parameters are illustrated in Figure 4.11(b).

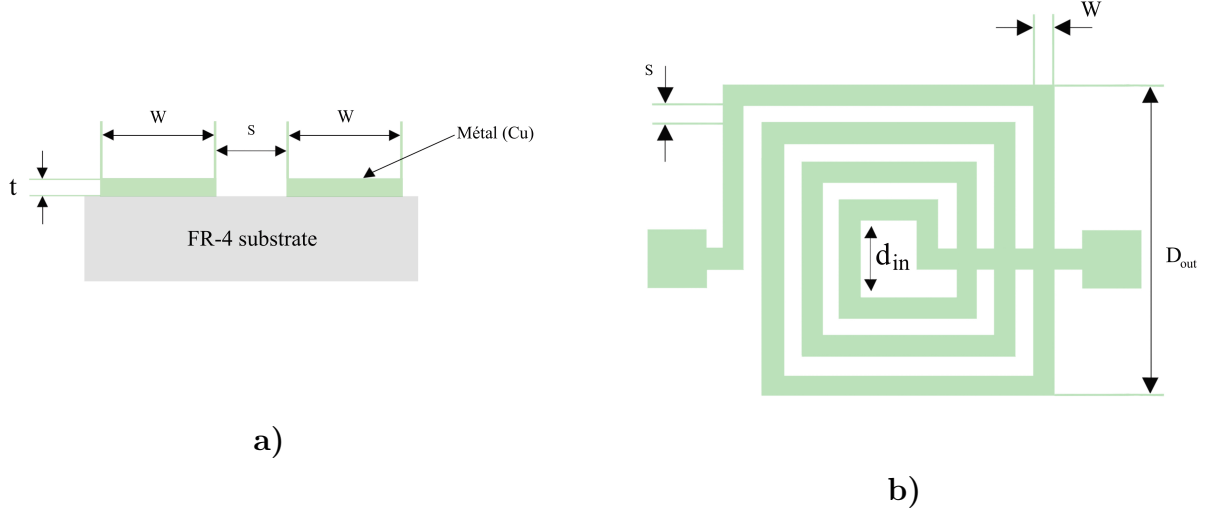


Figure 4.11: (a) Side-view of a square-shaped PCB inductor [39]. (b) Geometrical parameters of the same inductor [37, 39].

Total length of the conductor l is obtained using Equation 4.2.

$$l = 4nD_{out} - 4nw - (2n + 1)^2(s + w) \quad (4.2)$$

The geometric parameters of each inductor and their respective DC resistances measured with a multimeter are given in Table 4.3.

Table 4.3: Geometrical parameters and DC resistance R_{DC} of the four PCB inductors used in Chapter 5 [37].

Inductance	n	w [mm]	s [mm]	D_{out} [mm]	d_{in} [mm]	l [mm]	R_{DC} [Ω]
1	6.5	0.4	0.4	20.4	10.7	363.2	0.9
2	6.5	0.4	0.4	20.1	13.4	355.4	1.5
3	6.5	0.3	0.4	15.6	5.67	260.6	0.8
4	6.5	0.4	0.4	20	10.4	384	2.1

The parameters of the three samples used, i.e. air, amorphous iron alloy and nanocrystalline iron alloy, are given in Table 4.4.

Table 4.4: *Samples of permeability variation measurements [50, 51].*

Sample	Conductivity σ [$\mu S.cm^{-1}$]	Initial Relative Permeability μ_i [/]	Max Relative Permeability μ_m [/]
Air	2.95×10^{-11}	1	1
	to		
	7.83×10^{-11}		
Fe-based amorphous	7.692×10^{-3}	5000	50000
Fe-based Nanocrystalline	8.696×10^{-3}	80000	400000

4.3 Formulae to study figures of merit

In order to study the behaviour of impedimetric and potentiometric detection and to compare these behaviours, three figures of merit are studied: sensitivity, consumption and repeatability. To study the sensitivity and repeatability, two formulas have to be developed.

4.3.1 Sensitivity equation

LCR meter and Autolab sensitivity is evaluated as a function of the variation in modulus impedance. It is measured following a variation in the input signal introduced by the sample. The impedance modulus and input signal variations are compared to a reference impedance modulus and input signal. For measurements concerning the change in conductivity and permittivity, the reference sample is a DI water sample. For measurements of permeability variations, the reference is the impedance modulus measurements of the inductances without the addition of Fe-based foil.

The sensitivity S_i is computed using Equation 4.3.

$$S_i = \frac{|Z_{ref,meas} - Z_{mod,meas}|}{|x_{ref} - x_{mod}|} \cdot \left| \frac{ref}{Z_{ref,meas}} \right| \times 100 \quad [\%], \quad (4.3)$$

where

- $Z_{ref,meas}$ is the impedance modulus measured of the reference sample chosen in $[\Omega]$,
- $Z_{mod,meas}$ is the impedance modulus measured of the model chosen to simulate the target variation in $[\Omega]$ ($\Delta\sigma$, $\Delta\epsilon$, $\Delta\mu$),
- x_{ref} is the sample variation studied of the reference sample (σ [$S.m^{-1}$], ϵ [$F.m^{-1}$] or μ [$H.m^{-1}$]),
- x_{mod} is the sample variation studied of the model (σ [$S.m^{-1}$], ϵ [$F.m^{-1}$] or μ [$H.m^{-1}$]).

4.3.2 Repeatability equation

Repeatability of measurements is crucial to judge of their reliability. Repeatability is calculated by performing the same experiment several times, under the same conditions, and by performing a statistical analysis of these measurements. Measurements are here repeated 12 times for each case. Repeatability accounts for the statistical dispersion of measurements. It is associated with the standard deviation. In order to evaluate the repeatability of the measurements, the repeatability factor R is introduced in Equation 4.4. This factor oscillates between 0 and 1. It is equal to 1 when there is no dispersion with respect to the calculated mean. This means that the measurements are perfectly repeatable.

$$R = \frac{\bar{x}}{\bar{x} + \frac{c \cdot STD}{\sqrt{n}}} \quad [/], \quad (4.4)$$

where

- $\bar{x}$ is the **average** of a set of measurements obtained under identical conditions.
- STD is the **standard deviation** which describes the dispersion of the set of measurements.
- c is the **critical value**. The dispersion of the data covers a range called confidence interval (CI). This interval is limited by a confidence level. The confidence level is the percentage of times you would expect to reproduce an estimate between the upper and lower limits of the confidence interval. The critical value c indicates how many standard deviations from the mean are required to reach the desired confidence level [52].
- n is the **number of tests** done under identical conditions.

Standard deviation STD is computed using Equation 4.5 [52].

$$STD = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2} \quad (4.5)$$

where

- $x_1, x_2, \dots, x_n$ are the measured values of the sample items.

To obtain the critical value c , a confidence level must be chosen. A confidence level of 95% is chosen. Since the measurements have few samples ($n = 12 < 30$) and the data are assumed to be approximately normally distributed, the t-distribution is used to find the critical value [52]. Table B.1 (see Appendix B) gives the value of 2.201 for c , with df , the degree of freedom, being equal to $n - 1$, so 11.

4.4 Equivalent circuit

The equivalent circuit before simplification chosen to represent the electrochemical phenomena in Chapter 5 is shown in Figure 4.12(a). Detailed developments on how to obtain this circuit are available in Section 2.3.3 of the state of the art.

Since the experiments considered in Chapter 5 involve non-faradaic interfaces, no DC current passes through the solid-electrolyte interface and R_{CT} and Z_W are neglected. Indeed the charge transfer into the electrolyte solution after applying a potential via the electrode occurs without a redox probe. The total impedance measured is therefore only influenced by interfacial charging, represented by C_{DL} , in series with C_{SOL} and R_{SOL} , which are parallel to each other. The simplified equivalent circuit is shown in Figure 4.12(b) [17, 30].

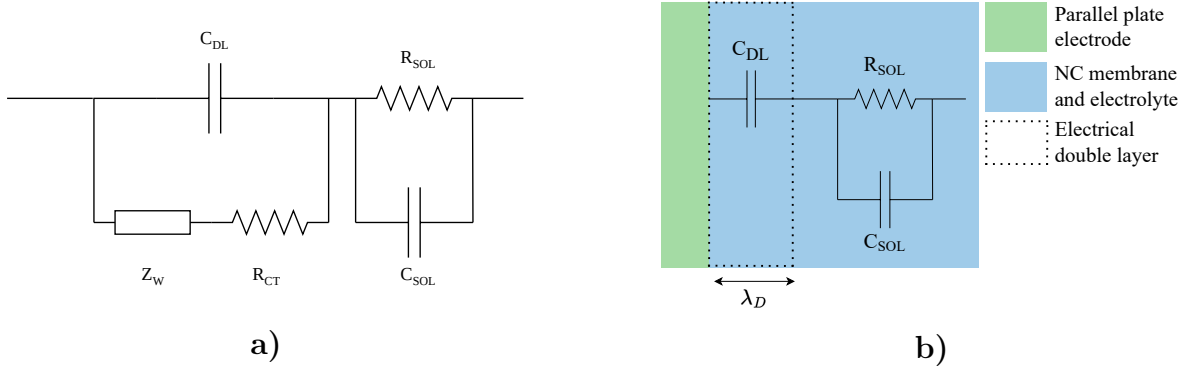


Figure 4.12: (a) Representation of the equivalent circuit Randles model with the additional dielectric capacitance of the solution C_{SOL} . (b) Representation of the simplified equivalent circuit Randles model.

Equivalent circuit capacitances are computed by assuming each to be equivalent to two parallel plate conductors. The derived expressions for C_{SOL} and C_{DL} are therefore given in Equations 4.6 and 4.7 [17, 30].

$$C_{DL} = \frac{\epsilon_0 \epsilon_{r,sol}(f) A}{\lambda_D(f)} \quad [F], \quad (4.6)$$

$$C_{SOL} = \frac{\epsilon_0 \epsilon_{r,sol}(f) A}{d} \quad [F], \quad (4.7)$$

where

- ϵ_0 is the permittivity of vacuum [$F.m^{-1}$],
- $\epsilon_{r,sol}$ is the relative permittivity of the electrolyte solution combined with the NC membrane,
- A is the area of the electrode [m^2],
- d is the thickness of the nitrocellulose membrane [m] and

- λ_D is the debye length [m].

The expression for the debye length is given in Equation 4.8. This expression is only valid if the local potential applied is lower than the thermal voltage U_t . Indeed, the expression is obtained through the linearisation of the Poisson-Boltzmann equation, which is only valid if the voltage is lower than U_t [17].

$$\lambda_D \cong \sqrt{\frac{\epsilon_0 \epsilon_{r,sol} U_t}{2 F C_{ions} \cdot 10^3}} [m], \quad (4.8)$$

where

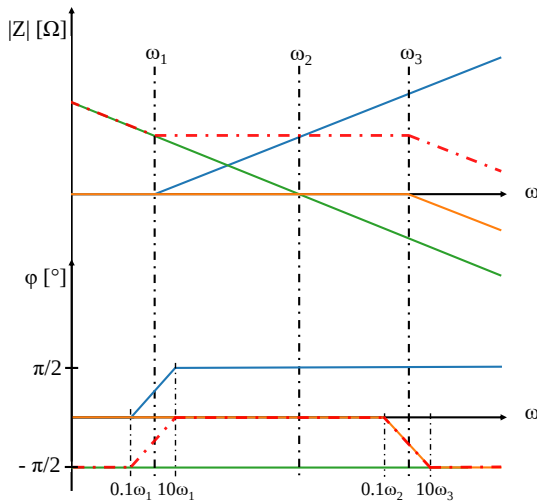
- U_t is the thermal voltage [V],
- F is the Faraday constant [$C.mol^{-1}$] and
- C_{ions} is the ionic strength [$mol.L^{-1}$].

Solution resistance R_{SOL} is obtained from Equation 4.9.

$$R_{sol} = \frac{d}{A \sigma(f)} [\Omega], \quad (4.9)$$

where $\sigma(f)$ is the electrolyte conductivity in [$S.m^{-1}$].

In the analysis of an equivalent circuit, the determination of its cut-off frequencies is a particularly important step. Figure 4.13 shows the impedance phase and modulus of an equivalent circuit such as the one in Figure 4.12(b). Each cut-off frequency is shown with the knowledge that $\omega = 2\pi f$. These cut-off frequencies are obtained via the impedance expression as explained in Equation 4.10.



$$Z = \frac{1 + \frac{j\omega}{\omega_1}}{\frac{j\omega}{\omega_2} \left(1 + \frac{j\omega}{\omega_3}\right)} \quad (4.10)$$

Figure 4.13: Qualitative representation of the Bode plot of simplified equivalent circuit, when $\omega_1 < \omega_2 < \omega_3$ [49].

For the equivalent circuit in Figure 4.12(b), the impedance expression is given in Equation 4.11 and the corresponding cut-off frequencies in Equation 4.12. The expressions for the impedances and cut-off frequencies of the equivalent circuits developed in the following three Sections are given in Appendix F.

$$Z = \frac{1 + j\omega R_{SOL} (C_{DL} + C_{SOL})}{j\omega C_{DL} (1 + j\omega C_{SOL} R_{SOL})} \quad (4.11)$$

$$\omega_1 = \frac{1}{R_{SOL}(C_{DL} + C_{SOL})} ; \quad \omega_2 = \frac{1}{C_{DL}} ; \quad \omega_3 = \frac{1}{C_{SOL} R_{SOL}} \quad (4.12)$$

In the data analyses to follow, it is expected that C_{DL} and R_{SOL} have a dominant contribution to C_{SOL} at low frequency, i.e. below 1MHz. However, at high frequencies, beyond 1MHz, the contribution of C_{SOL} is expected to dominate the total measured impedance [29].

4.4.1 Conductivity variation

Conductivity variation measurements are carried out on nitrocellulose NC membrane. These NC membranes are saturated with PBS of various concentrations. The membrane may or may not be solidified by a layer of polyester, called backing. This backing is only represented by a capacitance $C_{backing}$ because the dielectric losses in the polyester layer can be considered negligible in the frequency range studied in Chapter 5 [10]. Therefore $C_{backing}$ is added in addition to the model developed in Figure 4.12(b). The analytical model for the conductivity variation measurements is shown in Figure 4.14. Figure 4.14(a) is therefore the model for a solidified NC membrane measurement and Figure 4.14(b) for a unsolidified membrane measurement.

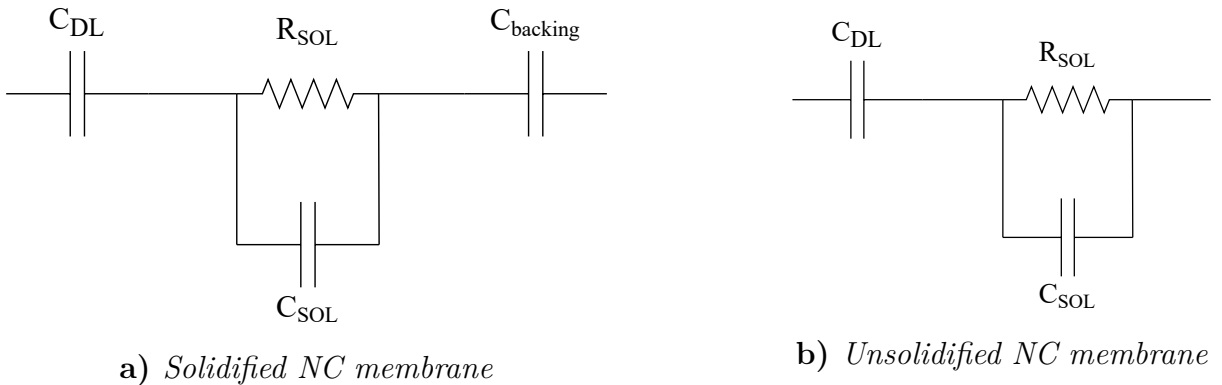


Figure 4.14: (a) Representation of the simplified Randles equivalent circuit model with an additional backing capacitance $C_{backing}$. It is used when measurements are carried out with solidified NC membrane. (b) Representation of the simplified Randles equivalent circuit model. It is used when measurements are carried out with unsolidified NC membrane.

A LCR meter measurement of a polyester layer, without nitrocellulose, between the two electrodes of the parallel-plates has made it possible to determine the value of the capacitance $C_{backing}$. It has a value of about $100pF$ [10].

In order to compute the other equivalent elements of the circuit in Figure 4.14, relative permittivity and conductivity of the electrolyte solution combined with the NC membrane, $\epsilon_{r,sol}$ and σ_{sol} , respectively, and C_{ions} , the ionic strength, must be approximated. To do this, relative permittivity and conductivity of the liquid sample, $\epsilon_{r,PBS}$ and σ_{PBS} , respectively, alone must be determined.

1. Relative permittivity $\epsilon_{r,PBS}$

Permittivity of a perfect dielectric material is a real number. However, due to electromagnetic losses caused by polarisation (see Section 2.3.3) and current conduction, which arise from charge displacement, an imaginary component is added to the concept of dielectric permittivity. Therefore, the absolute complex permittivity ϵ^* of a dielectric material can be expressed as [33, 34],

$$\epsilon^* = \epsilon' - j\epsilon'' \quad (4.13)$$

The imaginary part of the absolute complex permittivity is also called the loss factor since it is associated with the energy dissipation in the material. The real part of the absolute complex permittivity is associated with the energy stored in the material. In order to simplify operations, the relative complex permittivity ϵ_r is used instead of the absolute complex permittivity. Indeed, ϵ_r is dimensionless and can be determined with simple numerical values. The relative complex permittivity is obtained from Equation 4.14 [33, 34].

$$\epsilon_r = \frac{\epsilon^*}{\epsilon_0} = \epsilon'_r - j\epsilon''_r \quad (4.14)$$

ϵ'_r is therefore the relative real permittivity and ϵ''_r the relative imaginary permittivity. The latter is always a positive quantity and much lower than ϵ'_r [33, 34].

Relative permittivity of a Phosphate-Buffered Saline (PBS) sample is approximated as being equal to the relative permittivity of deionized (DI) water [17]. This assumption becomes less valid as the PBS concentration of the samples increases.

Figure 4.15 provides an evolution from 100Hz to 1MHz, of the relative real permittivity of the deionized water and of the evolution of the dielectric loss tangent $\tan \delta$, the ratio of emitted energy to stored energy over a period of time. As the curve at 32 °C is the closest to the real conditions of the measurements carried out in Chapter 5, this is the one chosen to approximate the relative permittivity of deionized water [33, 53].

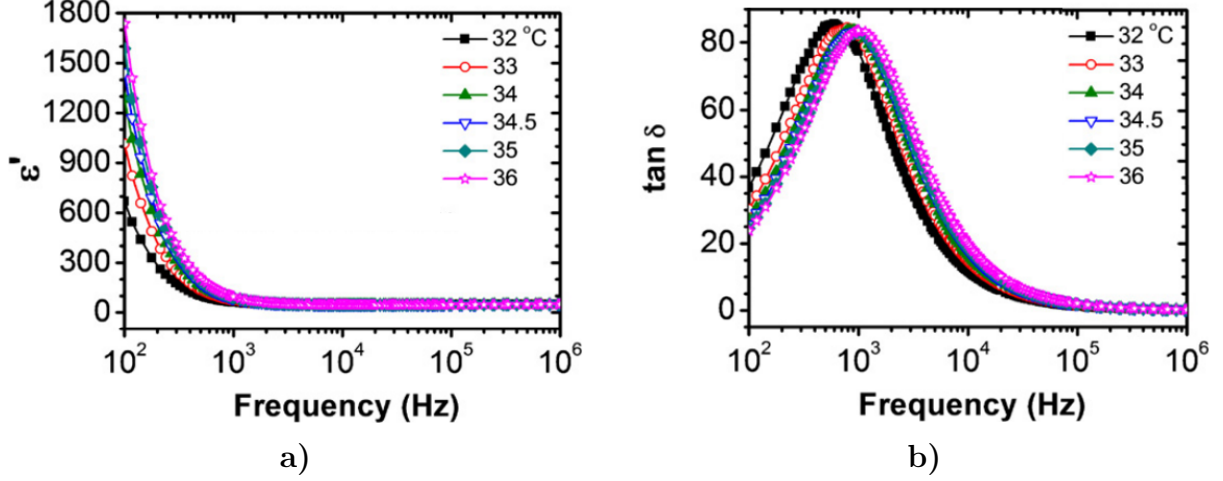


Figure 4.15: (a) Real part of relative permittivity of a sample of deionized (DI) water as a function of frequency. The frequency range goes from 100Hz to 1MHz. (b) Dielectric loss tangent of DI water as a function of frequency [53].

The dielectric loss tangent is defined in Equation 4.15.

$$\tan \delta = \frac{\epsilon_r''}{\epsilon_r'} \quad (4.15)$$

An hypothesis is made by assuming the complex permittivity equal to the real permittivity if ϵ'' is assumed to be sufficiently small compared to ϵ' . In order to support this hypothesis, the graphs in Figure 4.15 are studied at certain critical points, which are presented in Table 4.5.

Table 4.5: Coordinates of critical points of the graphs in Figure 4.15 and the value of the imaginary permittivity corresponding to the frequency.

x-axis: Frequency [Hz]	y-axis (1): ϵ_r'	y-axis (2): $\tan \delta$	$\epsilon_r'' = \tan \delta \cdot \epsilon_r'$
20	700	35	$35 \times 700 = 245 \times 10^2$
10 ³	80	85	$85 \times 80 = 68 \times 10^2$

Table 4.5 shows that from 20Hz to 1kHz, the imaginary permittivity decreases from 245×10^2 to 68×10^2 . $\tan \delta$ increases over this frequency range because ϵ_r'' decreases faster than ϵ_r' decreases. After 1kHz, Figure 4.15(a) shows that ϵ_r' is

almost constant but that $\tan \delta$ starts to decline towards zero from $100kHz$ onwards. ϵ_r'' therefore continues to decrease after $1kHz$ and tends to zero.

The assumption allows us to assume the value of the relative permittivity of DI water, and therefore of PBS, to be constant and equal to 80. This assumption loses validity for frequencies below $1kHz$.

$$\epsilon_{r,PBS} = 80$$

2. Conductivity σ_{PBS}

PBS samples conductivity is measured by a conductivity probe as explained in Section 4.2.1. Conductivity values are recalled in Table 4.6. Assuming that the conductivity of PBS is constant is a large approximation. Indeed, the conductivity of PBS varies by a factor of 100 over the frequency spectrum studied [54]. A constant mean value therefore always leads to being a factor of 10 larger or smaller than the real value. However, this approximation gives sufficient results for the purpose of this master thesis.

Table 4.6: Conductivity in $S.m^{-1}$ of the five Phosphate-Buffered Saline (PBS) samples.

Sample	Conductivity σ_{PBS}	Unit
PBS $1\mu M$	1.587×10^{-4}	$S.m^{-1}$
PBS $10\mu M$	16.57×10^{-4}	$S.m^{-1}$
PBS $100\mu M$	181.5×10^{-4}	$S.m^{-1}$
PBS $1mM$	1.709×10^{-1}	$S.m^{-1}$
PBS $10mM$	17.49×10^{-1}	$S.m^{-1}$

3. Ionic strength C_{ions}

Ionic strength of the different sample C_{ions} must be determined to compute the debye length λ_D . Equation 4.16 is used to compute it [55].

$$I = \frac{1}{2} \sum_{i=1}^n c_i z_i^2 \quad (4.16)$$

where

- c_i is the molar concentration of ion i in $[M]$ ($= mol.L^{-1}$) and
- z_i is its charge.

A 10mM PBS preparation consists of 137mM NaCl, 2.7mM KCl and 10mM Na_2HPO_4 [47]. Ionic strengths of the five PBS samples are computed using Equation 4.16 and gathered in Table 4.7.

Table 4.7: *Ionic strength in mol/L (=M) of the five Phosphate-Buffered Saline (PBS) samples.*

Sample	Ionic strength C_{ions}	Unit
PBS 1 μ M	16.97	μ M
PBS 10 μ M	169.7	μ M
PBS 100 μ M	1.697	mM
PBS 1mM	16.97	mM
PBS 10mM	169.7	mM

Once these three elements are obtained, the focus can be on computing the relative permittivities and conductivities of the nitrocellulose membrane saturated with PBS. Relative permittivity as well as conductivity of nitrocellulose membrane saturated with one of the five liquid samples are obtained using Bruggeman's formula (see Equation 4.17). Indeed, permittivity ϵ in Equation 4.17 is simply interchanged with conductivity σ [56].

$$\epsilon = \frac{H_b + \sqrt{H_b^2 + 8\epsilon_{r,m}\epsilon_{r,d}}}{4}, \text{ with} \quad (4.17)$$

$$H_b = (3c_d - 1)\epsilon_{r,d} + (3c_m - 1)\epsilon_{r,m}$$

where

- ϵ_m is the absolute complex permittivity of nitrocellulose in $[F.m^{-1}]$,
- ϵ_d is the absolute complex permittivity of liquid sample in $[F.m^{-1}]$,
- c_m is the volume fraction of the membrane pores and
- c_d is the volume fraction occupied by liquid sample.

Since membrane porosity is approximately 0.75, c_m is 0.25 and c_d is 0.75. This value comes from Odile Crahay's master's thesis, carried out in 2019-2020 on these same membranes [57].

The relative permittivity of nitrocellulose is obtained through a LCR meter measurement of a nitrocellulose membrane without any polyester layer. The membrane is placed between the two electrodes of the parallel plates and a voltage of 20mV is applied. Measurements are made over the frequency range of 20Hz to 1MHz. Measurements are repeated 12 times under the same conditions. This system can be characterised by a simple capacitance. Based on the formula for a parallel-plate capacitance and the

impedance measurements obtained by the LCR meter, the relative permittivity of the nitrocellulose membrane can be obtained. It varies slightly along the frequency range, as shown in Figure 4.16, but an average value of 4.88 is chosen for the rest of the developments.

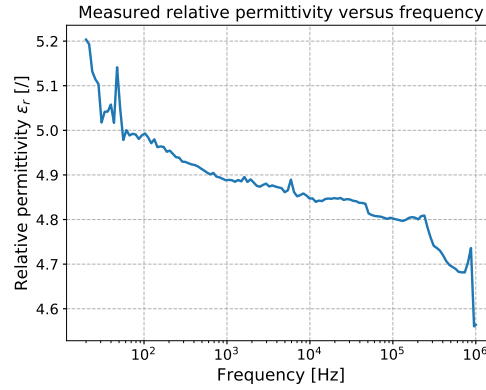


Figure 4.16: *Evolution of the mean relative permittivity measured of a nitrocellulose membrane in function of frequency. LCR meter measurements to obtain the data were performed 12 times under the same conditions.*

Finally, the nitrocellulose membrane conductivity varies between 1×10^{-9} and 1×10^{-11} $S.m^{-1}$, so a value of 1×10^{-10} $S.m^{-1}$ will be imposed [58]. Conductivities and permittivities of the NC membrane saturated with PBS are given in Table 4.8.

Table 4.8: *Conductivity of a nitrocellulose membrane saturated with Phosphate-Buffered Saline (PBS). Each conductivity corresponds to a different concentration of PBS.*

Sample	Conductivity σ_{sol}	Units	Permittivity $\epsilon_{r,sol}$
PBS $1\mu M$	99.19×10^{-6}	$S.m^{-1}$	53.07
PBS $10\mu M$	1.04×10^{-3}	$S.m^{-1}$	53.07
PBS $100\mu M$	11.34×10^{-3}	$S.m^{-1}$	53.07
PBS $1mM$	106.81×10^{-3}	$S.m^{-1}$	53.07
PBS $10mM$	1.09	$S.m^{-1}$	53.07

The frequency-independent parameters still needed to determine the equivalent circuit elements are listed in Table 4.9.

Figure 4.17 illustrates the comparison between the impedance measurements of the electrochemical system against the impedance curves that have been approximated by the equivalent circuit model. Only the cases of 1uM, 100uM and 10mM PBS are given as they are sufficient to allow an analysis of the theoretical model. All graphs (including the one with solidified nitrocellulose membrane) are provided in Appendix C.

Table 4.9: Physical parameters frequency-independent for the equivalent electrical model, in Figure 4.14, in the case of conductivity variations.

	Symbol	Description	Value	Units	Ref
Surroundings					
Nitrocellulose membrane	$\epsilon_{r,NC}$	NC relative permittivity	4.88	[/]	-
	σ_{NC}	NC conductivity	10^{-10}	$[S.m^{-1}]$	[58]
	d	NC thickness	240	$[\mu m]$	[59]
	t	Polyester thickness	100	$[\mu m]$	[59]
Electrodes	A	Electrode's air	1.96e-05	$[m]$	-
Constant	U_t	Thermal voltage	25.69e-3	$[V]$	[60]
	F	Faraday constant	96485.33	$[C.mol^{-1}]$	[61]

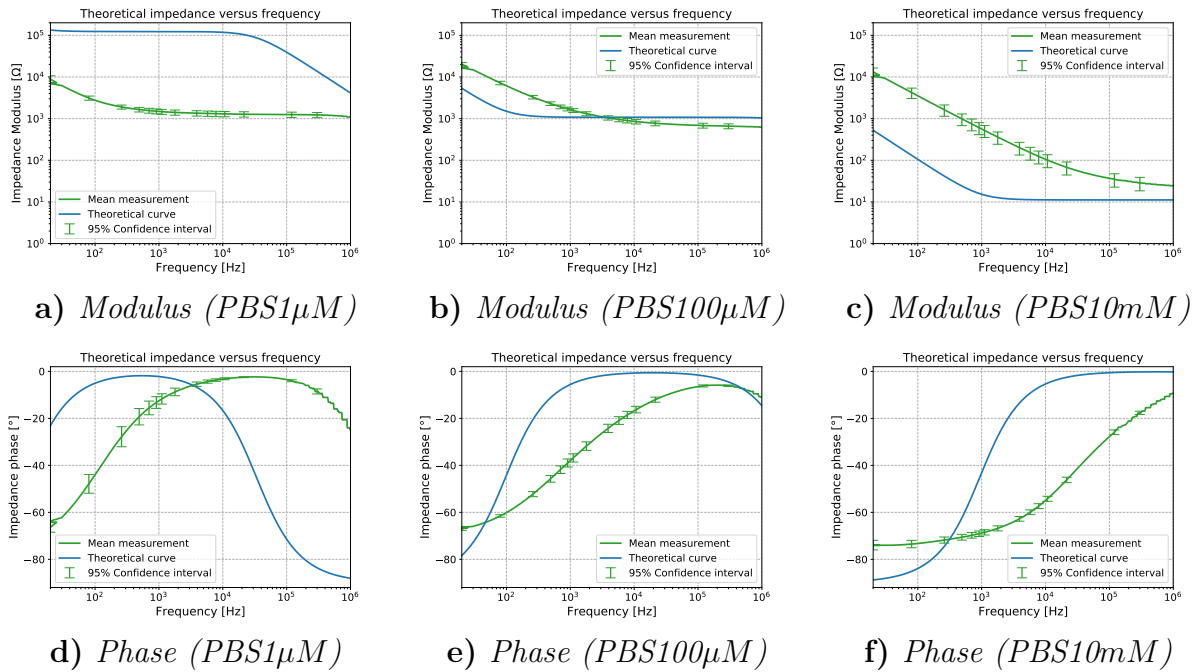


Figure 4.17: Comparison between the equivalent circuit impedance and the impedance measurements of the electrochemical system consisting of an unsolidified nitrocellulose membrane saturated with Phosphate-Buffered Saline, both as a function of frequency. The LCR meter measurements were repeated 12 times before averaging the impedance.

The fit between measurements and theory is not perfect. However, it provides a sufficient understanding of the electrochemical system to answer the questions addressed in this master thesis. This equivalent circuit will therefore not be explored further.

4.4.2 Permittivity variation

The equivalent circuit for permittivity change is the same as for conductivity change (see Figure 4.14). The backing capacitance $C_{backing}$ is found in the case where measurements are made on a solidified nitrocellulose NC membrane ($\pm 100 [pF]$). A double layer capacitance is present, as well as the resistance and capacitance of the solution, R_{SOL} and C_{SOL} ,

respectively. To determine these various parameters Equations 4.6, 4.7, 4.8 and 4.9 are used.

In order to compute the other equivalent elements of the circuit in Figure 4.14, relative permittivity and conductivity of the electrolyte solution combined with the NC membrane, $\epsilon_{r,sol}$ and σ_{sol} , respectively, and C_{ions} , the ionic strength, must be approximated. To do this, relative permittivity and conductivity of the liquid sample alone must be determined.

1. Relative permittivity ϵ_r

Relative permittivity of DI water has been approximated earlier in Section 1. It equals 80. Relative permittivities of ethanol and isopropyl alcohol are assumed to be constant as the frequency range studied is not sufficiently wide to observe a major permittivity variation [62, 63, 64]. The relative permittivities of ethanol ($\epsilon_{r,ET}$) and isopropyl alcohol ($\epsilon_{r,IS}$) are 24.3 and 17, respectively [65].

$$\epsilon_{r,DI} = 80 \quad ; \quad \epsilon_{r,ET} = 24.3 \quad ; \quad \epsilon_{r,IS} = 17$$

2. Conductivity σ

Conductivity σ also varies with frequency due to polarisation phenomena. However, since DI water, ethanol and isopropyl alcohol samples are weak electrolytes, it is assumed that the conductivity of the sample is constant over the frequency range considered. Conductivity values for each sample are recalled in Table 4.10 and were obtained thanks to a conductivity probe.

Table 4.10: *Conductivity in $S.m^{-1}$ of DI water sample, ethanol sample and isopropyl alcohol sample.*

Sample	Conductivity σ_{PBS}	Unit
DI water	5.49×10^{-6}	$S.m^{-1}$
Ethanol	1.119×10^{-4}	$S.m^{-1}$
Isopropyl alcohol	3.8×10^{-6}	$S.m^{-1}$

3. Ionic strength C_{ions}

Ionic strength of the different sample C_{ions} must be determined to compute the debye length λ_D . Ionic strength of DI water has been approximated to 0.001 M [66]. Finding the ionic strength of ethanol and isopropyl alcohol is not an easy task. A rough approximation is therefore necessary. These two liquids are even weaker electrolytes than DI water. Conductivity of a solution depends on the concentration of ions in the solution and their mobility. The higher the ion concentration, the higher the conductivity. The ionic strength is also proportional to the ion concentration as

explained in Equation 4.16 [55, 67].

It is therefore assumed that there is a linear relationship between the conductivity of the samples considered and their ionic strength [67]. The ionic strengths of ethanol and isopropyl alcohol samples is therefore obtained on the basis of the ionic strength of DI water. The latter is multiplied by the proportionality factor between each conductivity. A comparison between theory and measurements showed that the variation of the chosen value for the ionic strength does not have much influence on the fitting.

Figure 4.18 provides the theoretical curves associated with the designed equivalent circuit, as a function of the measured samples, DI water, ethanol and isopropyl alcohol. The impedance expression and cut-off frequency values are provided in Appendix F. It also shows where each element of the circuit is most influential as a function of frequency.

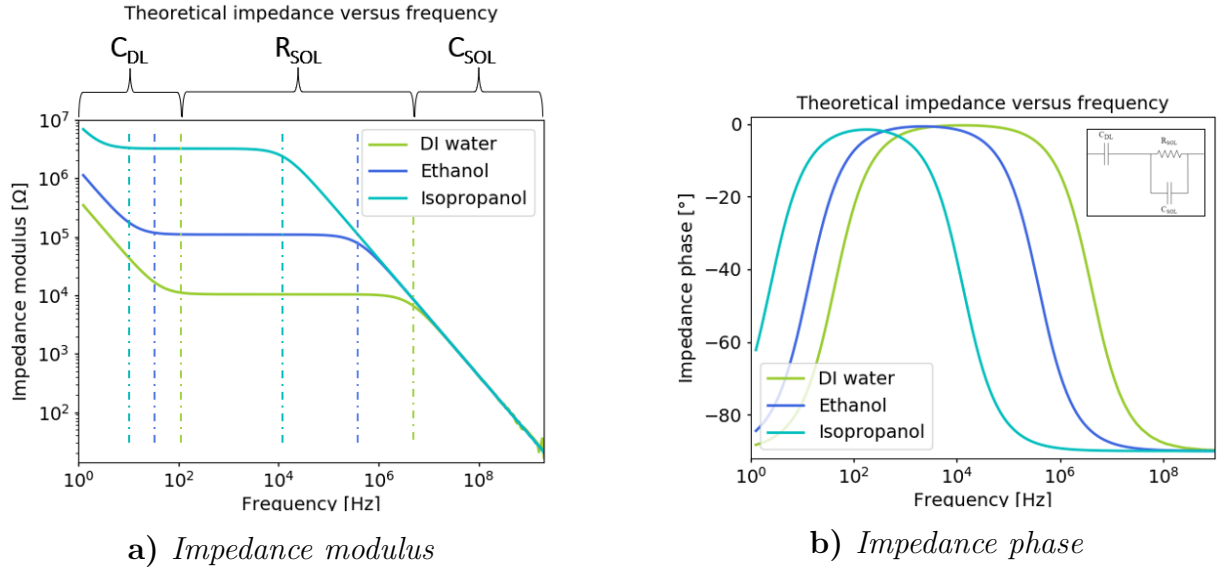


Figure 4.18: (a) Theoretical impedance modulus versus frequency for each considered sample (DI water, ethanol and isopropyl alcohol). The influence of each element of the equivalent circuit (C_{DL} , C_{SOL} , R_{SOL}) with respect to the frequency is shown [29]. (b) Theoretical impedance phase versus frequency for each considered samples. The model of the equivalent circuit associated with the curves is also provided.

Figure 4.19 provides a comparison between the evolution of the impedance theoretically estimated over the considered frequency range and the evolution of this impedance measured on unsolidified nitrocellulose. The theory and the measurements are far from a perfect fit. However, the general shape of the curves is similar and this similarity is sufficient to analyse the results of Chapter 5 and draw conclusions.

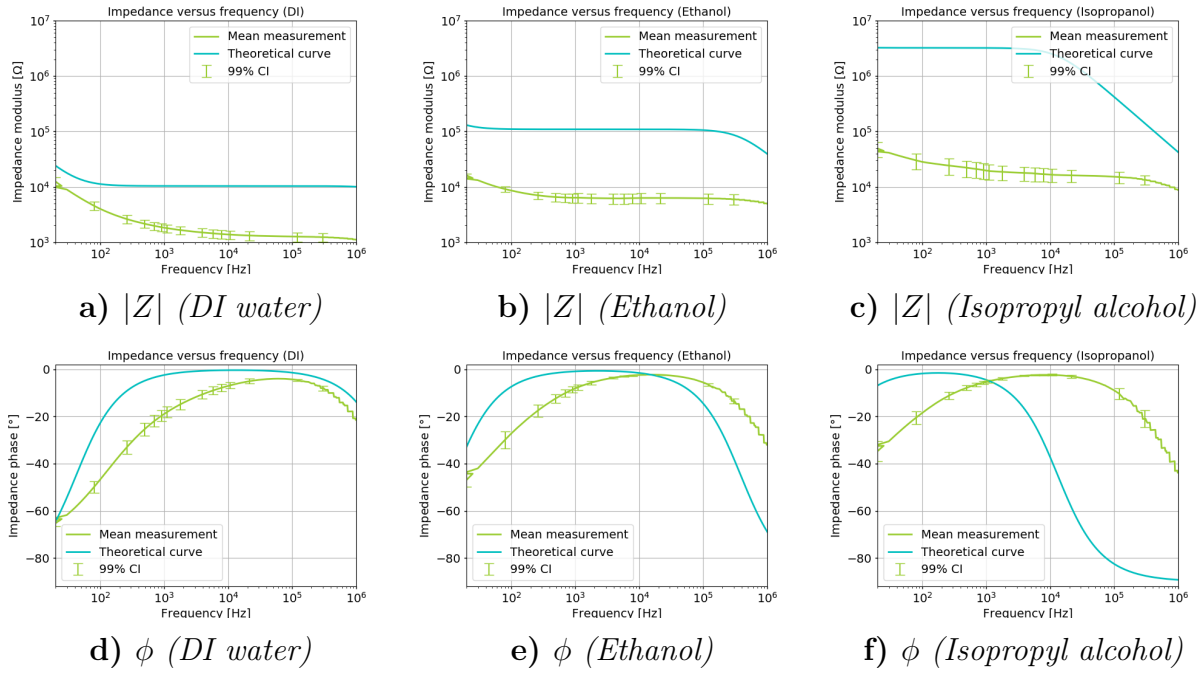


Figure 4.19: comparison between the evolution of the impedance theoretically estimated over the considered frequency range and the evolution of this impedance measured on unsolidified nitrocellulose.

4.4.3 Permeability variation

The lumped circuit model of the spiral planar PCB inductors is given in Figure 4.20. The model is a simplification of the more complex model presented in Figure 2.18(a) (see Section 2.4) [37]. The inductor system used here has neither a SiO_2 oxide layer nor a silicon substrate. The capacitances C_{ox} and C_{si} and the resistors R_{si} from more complex circuits are therefore not present.

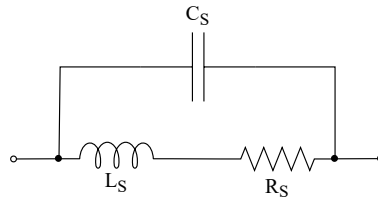


Figure 4.20: Simplified lumped circuit model of a spiral planar PCB inductor [37].

Series resistance R_S

R_S value depends on the skin and proximity effect. At low frequencies R_S is computed using Equation 4.18 [36, 38, 39, 40]. As frequency increases, the current within the inductor wires becomes non-uniform due to the formation of eddy current within it. This effect is called skin effect and is explained in more detail in Section 2.4 of the state of the art. Since measurements are performed at a frequency lower than $1GHz$ and because

each segment of the inductance is in the same plane, proximity effects can be neglected [40].

$$R_s = \frac{\rho l}{wt} \quad (4.18)$$

where

- ρ is the copper resistivity in $[\Omega.m]$,
- l is the overall length of the spiral inductor in $[m]$,
- w is the width of the copper conductor trace in $[m]$ and
- t is the thickness of the copper conductor trace in $[m]$.

R_s at high frequency is given in Equation 4.19.

$$R_s = \frac{\rho l}{wt_{eff}} \quad (4.19)$$

where t_{eff} is the effective thickness of the metal wires obtained from Equation 4.20[38, 39, 40]. The parameter t_{eff} allows the skin effect to be taken into account in the resistance computation.

$$t_{eff} = \delta(1 - e^{-\frac{t}{\delta}}) \quad (4.20)$$

where δ is the skin depth of the metal. Figure 4.21 illustrates the distribution of current flow in a cylindrical conductor, shown in cross section. The skin depth, δ , corresponds to the depth where the current density is just 1/e (about 37%) of the value at the surface [43].

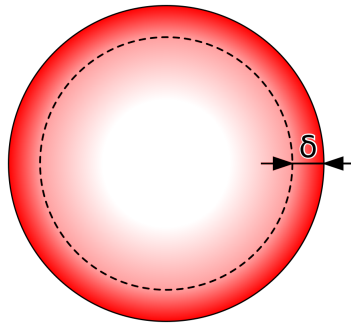


Figure 4.21: *Distribution of current flow in a cylindrical conductor, shown in cross section. The skin depth, δ , is defined as the depth where the current density is just about 37% of the value at the surface [43].*

Skin depth δ is given in Equation 4.21 [43].

$$\delta = \sqrt{\frac{2\rho_{cop}}{\omega\mu_{cop}}} = \sqrt{\frac{2\rho_{cop}}{2\pi f\mu_0\mu_{r,cop}}} \quad (4.21)$$

where

- ρ_{cop} is the copper resistivity in $[\Omega.m]$,
- ω is the angular frequency in $[rad.s^{-1}]$,
- μ_{cop} is the permeability of the conductor in $[H.m^{-1}]$ and equals $\mu_r.\mu_0$,
- $\mu_{r,cop}$ is the relative permeability of the conductor,
- μ_0 is the vacuum permeability in $[H.m^{-1}]$,

Equation 4.21 is valid as long as frequencies are lower than $1/\rho_{cop}\epsilon_{cop}$, where ϵ_{cop} is the copper absolute permittivity and ρ_{cop} the copper resistivity. Equation 4.21 is therefore valid up to a frequency of $27 THz$. Since the frequency range of the measurements reaches a maximum of $1 MHz$, Equation 4.21 can be used to determine the skin depth within the inductance conductor.

Table 4.11: *Copper wires parameters.*

Symbol	Description	Value	Unit	Ref
ρ_{cop}	Copper resistivity	1.67×10^{-8}	$\Omega.m$	[68]
$\epsilon_{r,cop}$	Relative permittivity	250×10^3	/	[69]
$\mu_{r,cop}$	Relative permeability	999×10^{-3}	/	[70]
t	Thickness of the copper conductor trace	17×10^{-6}	m	[37]

Parasitic capacitance C_s

The parasitic capacitance has two components. A component C_{tt} which characterises the crosstalk capacitance between adjacent turns and a component C_{su} which characterises the overlap capacitance between the spiral and the underpass. C_{tt} and C_{su} are illustrated in Figure 4.22.

However, since the adjacent turns are almost equipotential, the effect of crosstalk capacitance is assumed to be negligible. The impact of C_{su} on the other hand is much more significant given the large potential difference between the metal traces and the underpass. C_s can therefore be modelled as the sum of all overlap capacitances [36, 38, 40].

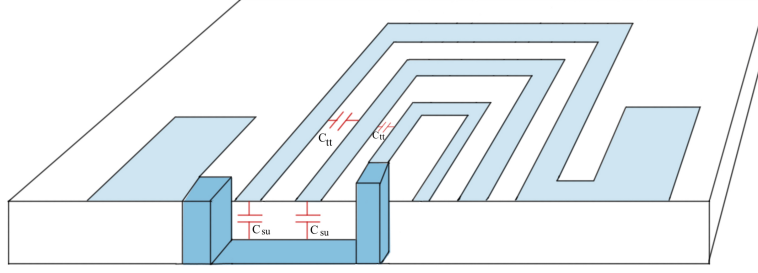


Figure 4.22: Cross section of a planar spiral inductor to illustrate the parasitic capacitances C_{tt} and C_{su} .

$$C_s = \epsilon_{FR4} \frac{mw^2}{t_{FR4}} \quad (4.22)$$

where

- m is the number of overlap,
- w is the metal traces width in $[m]$,
- ϵ_{FR4} is the permittivity of the FR4 layer in $[F.m^{-1}]$ and
- t_{FR4} is the FR4 thickness in $[m]$.

All planar inductors were patterned on an FR-4-type board. The thickness of the FR4 board t_{FR4} is approximately $1mm$, its relative permittivity ϵ_{FR4} can be approximated as 4 and the number of overlap m for each inductor is 6 [71].

Inductance L_s

Two approximation methods were chosen to compute L_s . These two methods are compared afterwards, in order to choose the best one.

1. Modified Wheeler Formula

The formula presented by Wheeler was suitable for discrete square spiral inductors. By modifying Wheeler's formula, it was possible to find a valid expression for square spiral integrated inductors [36, 44].

$$L_s = K_1 \mu_0 \frac{n^2 d_{avg}}{1 + K_2 a} \quad (4.23)$$

where

- a is the fill ratio. This is defined by the following formula:

$$a = \frac{D_{out} - d_{in}}{D_{out} + d_{in}} \quad (4.24)$$

- K_i are layout-dependent factors. For a plane and rectangular integrated inductance, these factors have the following values:

$$\begin{cases} K_1 = 2.34 \\ K_2 = 2.75 \end{cases}$$

- d_{avg} is the average diameter. It is defined by the following formula:

$$d_{avg} = \frac{1}{2} (D_{out} + d_{in}) \quad (4.25)$$

2. Bryan's Formula

Bryan's formula is an empirical equation and it is given in Equation 4.26.

$$L_s = 6.025 \times 10^{-7} (D_{out} + d_{in}) n^{\frac{5}{3}} \ln \left(4 \left(\frac{D_{out} + d_{in}}{D_{out} - d_{in}} \right) \right) \quad (4.26)$$

The difference between the inductance computed by Bryan and Wheeler is maximum of 7%. Figure 4.23 shows the resulting impedance of the equivalent circuit C(RL) comparing the case where L_s is approximated by Bryan or Wheeler. The results are similar despite the 7% difference. Further developments are done by approximating L_s with Wheeler's formula.

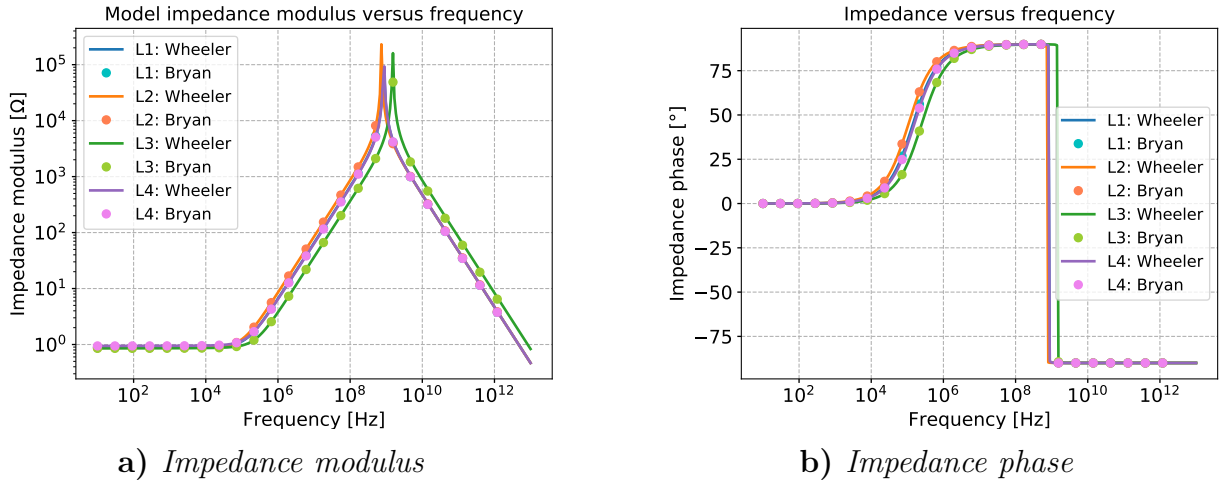


Figure 4.23: *Equivalent circuit model impedance of a planar spiral inductance as a function of frequency. Comparison of the equivalent circuit inductance approximation by Bryan's formula and Wheeler's formula, for each inductance.*

At approximately 10^9 Hz, the phenomenon observed is called the Self-Resonant Frequency (SRF) and it is expressed in Equation 4.27. Before SRF, the inductance increases more rapidly before dropping sharply to negative. After SRF, the inductance resonates with its parasitic turn-to-turn capacitance and the inductance becomes equivalent to pure capacitance [37, 39]. Above SRF, the inductance is not supposed to be used [37, 38, 41].

$$SRF = \frac{1}{2\pi\sqrt{L_s C_s}} \quad (4.27)$$

In order to verify the accuracy of the developed equivalent circuit model, Figure 4.24 is provided. The theoretical curve of the impedance computed with the model is compared to the impedance of the planar spiral inductance measured with the LCR meter. These measurements are made on the four inductors used in Chapter 5. An open and short calibration was performed. The open calibration was performed by locking up the BNC connectors connected to the LCR meter away from each other by a distance equal to that between the two BNC connectors soldered to the inductors. For the short calibration, the two female BNC connectors connected to the LCR meter were joined by a male BNC connector. Measurements were made with an applied voltage of $20mV$ and a frequency range of $20Hz$ to $1MHz$.

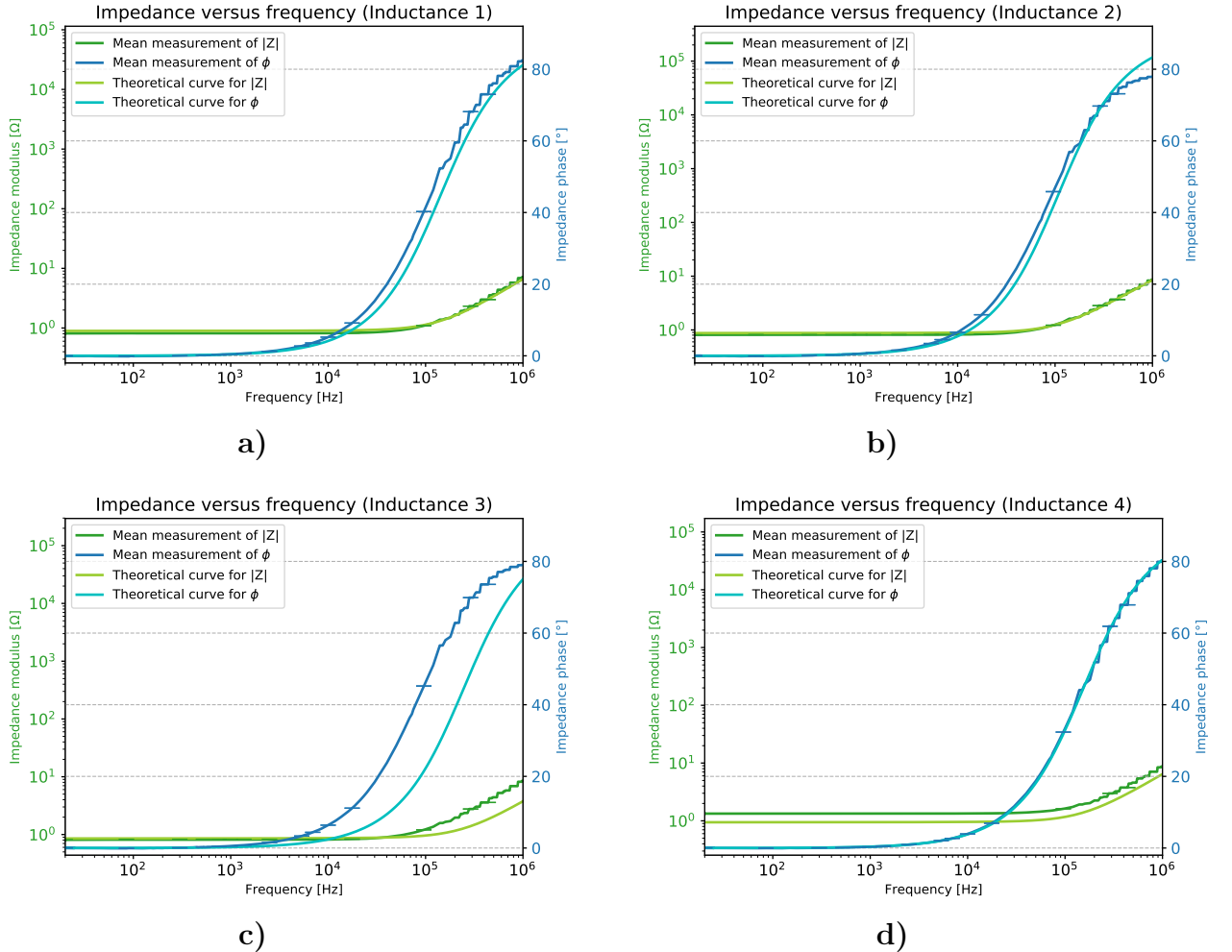


Figure 4.24: Comparison between impedance measurements and theoretical impedance curve calculated using the developed equivalent circuit. Measurements on LCR meter with an applied voltage of 20 mV over a frequency range of 20 Hz to 1 MHz . (a) Impedance of inductor 1. (b) Impedance of inductor 2. (c) Impedance of inductor 3. (d) Impedance of inductor 4.

From the graphs in Figure 4.24, it is assumed that the developed equivalent model is sufficiently close to the measurements.

In order to vary the impedance of the inductor two Fe-based alloy foils are deposited on the inductor. The coil of the inductor and the Fe-based alloy foils are separated by a thin layer of plastic film. This tuning method can be related to the Metal Shielding Tuned (MST) inductor method where a removable metal plate is brought close to the inductor in order to vary its magnetic flux. The variable inductance consists therefore in the planar spiral inductor and the metal foil as illustrated in Figure 4.25. The magnetic field induced in the planar spiral inductor by the variation of the potential has its field lines changed when the metal foil is placed on the inductor. Furthermore, these field lines penetrate the Fe-based alloy foil. Lenz's law predicts that a counteractive magnetic field is induced and that this reduces the magnetic flux penetrating the inductor, thus causing the inductance to change [37, 72].

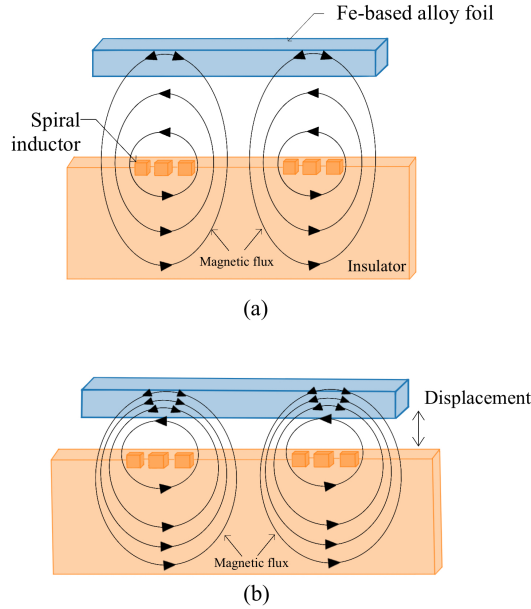


Figure 4.25: *Principle of Metal Shielding Tuned (MST) [37, 72].*

The equivalent circuit of the variable inductance is shown in Figure 4.26 and is a two-port π equivalent circuit model a bit different from the traditional two-port π equivalent circuit model developed hereabove.

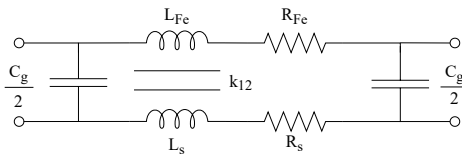


Figure 4.26: *Equivalent circuit model of the planar spiral inductor combined with a Fe-based alloy foil.*

L_s is the series inductance of the spiral inductor and R_s is the series resistance. But to this is added L_{Fe} the equivalent inductance and R_{Fe} the equivalent resistance of the metal foil. k_{12} is the equivalent coupling coefficient between spiral inductor and metal foil and is expressed in Equation 4.28. C_g is the parasitic capacitance between spiral inductor and metal foil. The existence of C_g is due to the fact that the electric field generated by the spiral inductor also penetrates the metal foil, in addition to the magnetic field lines [37, 72].

$$k_{12} = \frac{M_{12}}{\sqrt{L_1 \cdot L_2}}, \quad (4.28)$$

where M_{12} is the mutual inductance between the spiral planar inductance and the metal foil.

Chapter 5

Results

Throughout this chapter, **impedimetric** and **potentiometric** detection methods have been studied. They were analysed in the case of a major physical constraint: the **solidification** or not of a nitrocellulose (NC) membrane. Indeed, in the design of the Lateral Flow Assay (LFA) integrated in the biosensor system, the nitrocellulose membrane used can be combined with a polyester layer. The polyester layer solidifies the membrane in the sense that it is easier to handle but it doesn't allow conduction paths.

The behaviour of impedimetry and potentiometry to three types of incoming signal variation was studied: a variation in **conductivity**, **permittivity** and **permeability**. The capabilities of the two detection methods are analysed according to three merit figures: **sensitivity**, **consumption** and **repeatability**.

The impedimetric measurements were performed using an Agilent LCR meter. Potentiometric measurements were carried out using a Methrom Autolab potentiostat. The LCR meter and Autolab potentiostat behaviour to a change in the signal input was investigated in four situations, as illustrated in Figure 5.1:

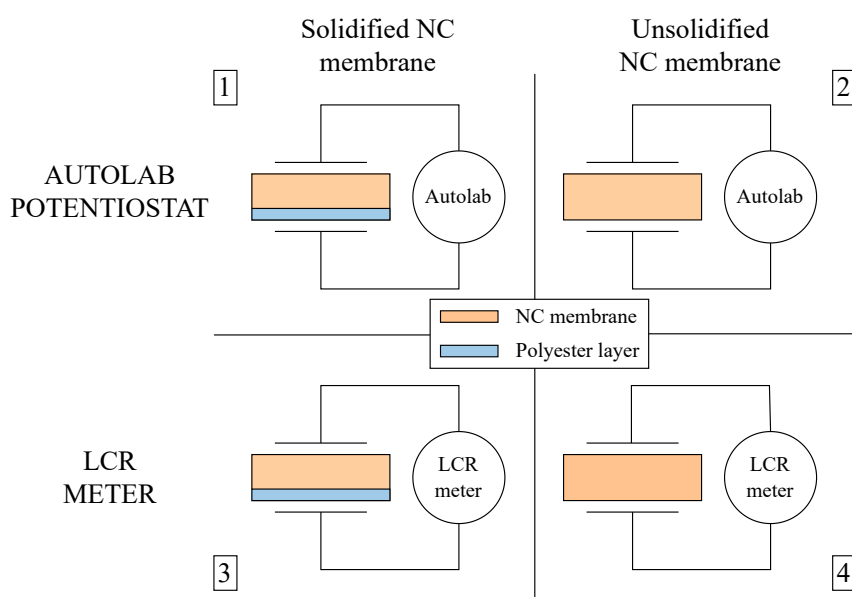


Figure 5.1: *The four situations studied in the result chapter.*

1. A first set of measurements is performed with the **Autolab potentiostat** on a **solidified** NC membrane.
2. A second set of measurements is performed with the **Autolab potentiostat** on an **unsolidified** NC membrane.
3. A third set of measurements is performed with the **LCR meter** on a NC membrane **solidified** with polyester.
4. A fourth set of measurements is performed with the **LCR meter** on an **unsolidified** NC membrane.

All the measurements presented below are performed over a frequency range of 20Hz to 1MHz , in order to study the behaviour of the system over the maximum available frequency range.

5.1 Autolab potentiostat measurements on solidified NC membrane

Figure 5.2 shows an **Autolab potentiostat** impedance measurement of a **solidified** NC membrane.

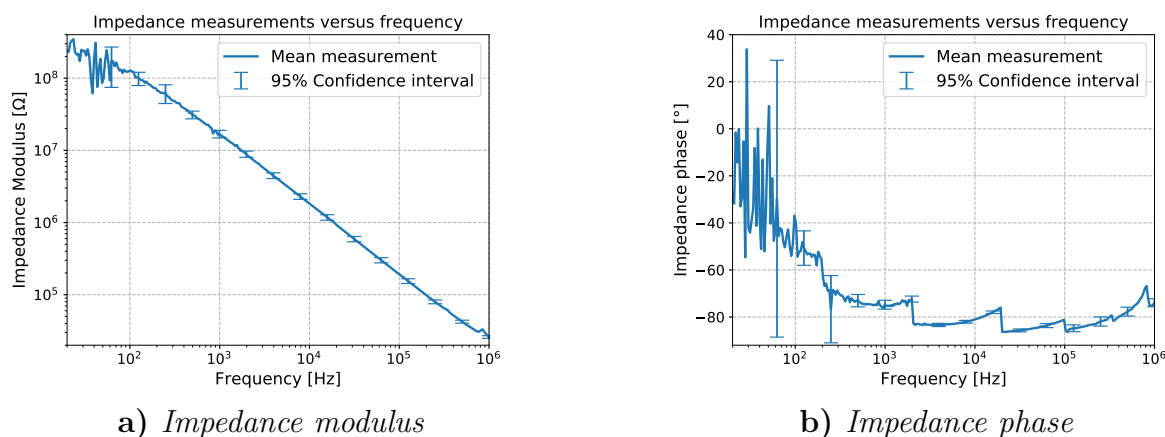


Figure 5.2: *Autolab potentiostat measurements on a solidified nitrocellulose (NC) membrane with an applied potential of 20mV and a frequency range of 20Hz to 1MHz . Twelve tests were performed to derive the modulus and phase impedance mean. The results are not usable because the irregularities in the measurements are too considerable.*

Impedance phase measurements show "breaks" at regular intervals. These breaks occur because the measured impedance between the counter electrode and the reference electrode is greater than $35\text{k}\Omega$, as explained in more detail in Section 4.1.2 of *Materials and Methods* Chapter. The measurements are therefore unusable.

Measurements on the **Autolab potentiostat** with **solidified NC** membrane are therefore **not carried out** any further.

5.2 Autolab potentiostat measurements on planar spiral inductor

The potentiometric detection method is not tested for permeability variation because the Autolab potentiostat is not capable of making conclusive measurements on spiral planar inductors, as shown in Figure 5.3. Indeed, measurement "jumps" are observable.

Measurements on the **Autolab potentiostat** with **permeability variation** of the sample measured are therefore **not carried out** any further.

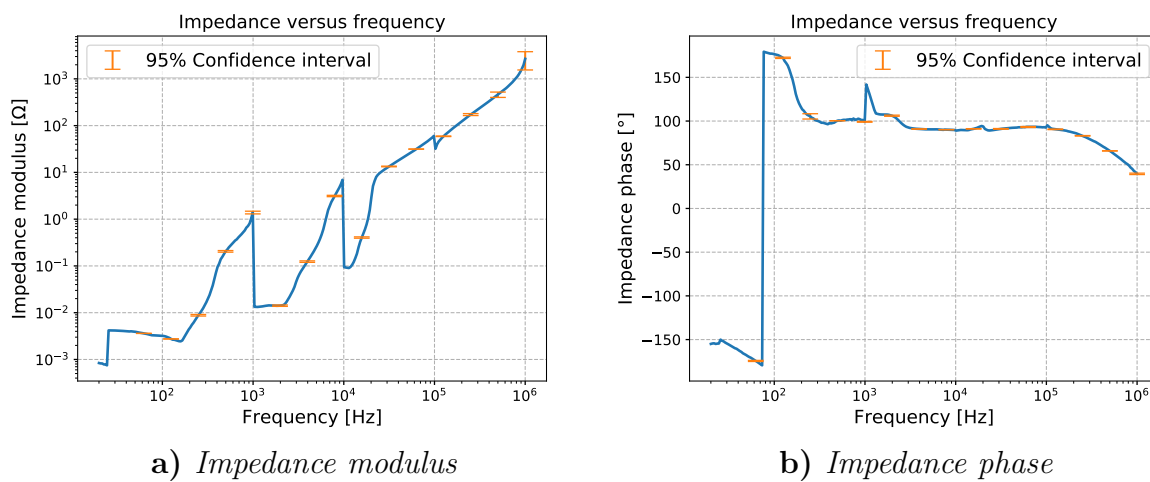


Figure 5.3: *Autolab potentiostat measurements on a spiral planar inductor without any tuning. Applied potential of 20mV and a frequency range of 20Hz to 1MHz. Six tests were performed to derive the modulus and phase impedance mean. The results are not usable because the irregularities in the measurements are too considerable.*

One explanation for these jumps could be related to one of the limitations of the Autolab potentiostat, the limited measurement resolution, listed in Section 4.1.2 of Chapter *Materials and methods*.

Indeed, as the Autolab measurement recording is digital, the resolution is limited. This has negative impacts when measurements coincide with low currents that vary over a wide range. In this situation, if we assume that the current is inversely proportional to the impedance, a large value of impedance induces a small value of current and since the impedance varies by a factor of $10^6\Omega$, from 20Hz to 1MHz, it is assumed that the current also varies greatly. Therefore, it is assumed that the situation affected by the resolution limit is encountered. The breaks would therefore be caused by the latency (0.5ms to 2ms) between the change of current range set-up covered by the machine.

5.3 Sensitivity for conductivity variation of the sample

The variation in conductivity within the measured sample was carried out using Phosphate-Buffered Saline (PBS) diluted with DI water five times to obtain five samples of different conductivity. This liquid was then deposited on a NC membrane. Saturation of the membrane with the liquid was checked visually, ensuring that no film of liquid remained on the surface of the membrane.

Sensitivity of the LCR meter and Autolab to impedance variation is computed using Equation 4.3 developed in Section 4.3. Sensitivity is evaluated for an increase in sample conductivity relative to a reference conductivity. A sample of Deionized (DI) water is chosen as conductivity reference because its permittivity is close to the samples studied and its conductivity is lower than those of the samples. Similar permittivity ensures that it is the variability of the conductivity that is being studied and not the variability of the permittivity. In addition, the variation in conductivity is maximised, giving the devices a better chance of perceiving the variations in conductivity and making it easier to compare their respective behaviour in response to these variations.

5.3.1 Gradual disappearance of minimum sensitivity levels with samples of increasing conductivity

Figure 5.4 shows the evolution of sensitivity as a function of frequency for the three situations studied. Figure 5.4(a) shows the smallest increase in conductivity (by a factor of 28 ($= \sigma_{PBS}/\sigma_{DI}$)), while Figure 5.4(b) shows the largest increase in conductivity (by a factor of 319×10^3).

The steep minima observed in 5.4(a) for the three curves more or less coincide with the first cut-off frequency of the measured system equivalent circuit. Derivation of cut-off frequencies and their dependency on the equivalent circuit elements is explained in Section 4.4 of *Materials and Methods* Chapter.

The relationship between abrupt sensitivity minimum and cut-off frequency is consistent with the fact that at the first cut-off frequency, the modulus impedance reaches a plateau. Therefore $Z_{PBS,meas}$ stops decreasing and $|Z_{DI,meas} - Z_{PBS,meas}|$, which is proportional to sensitivity (see Equation 4.3), stops increasing. Thus, S_i , the sensitivity, decreases.

The notable difference is the gradual disappearance of the abrupt minimum as the conductivity of the sample increases. Figure 5.4(b) illustrates that for a conductivity variation around 10^4 times higher than in Figure 5.4(a), sensitivity curves for LCR meter measurements on solidified NC membrane and Autolab measurements on unsolidified NC membrane (blue and green) no longer show steep minima. As the conductivity increases, the first cut-off frequency f_1 rises. Indeed, f_1 is inversely proportional to the resistance solution R_{SOL} , and R_{SOL} decreases as conductivity increases. The arrival of the modulus impedance

plateau therefore comes later and later. The steep minima disappears when the plateau reaches the megahertz. LCR meter measurements with unsolidified NC membrane reacts in the opposite way with a steep minimum that moves to the left as the conductivity increases.

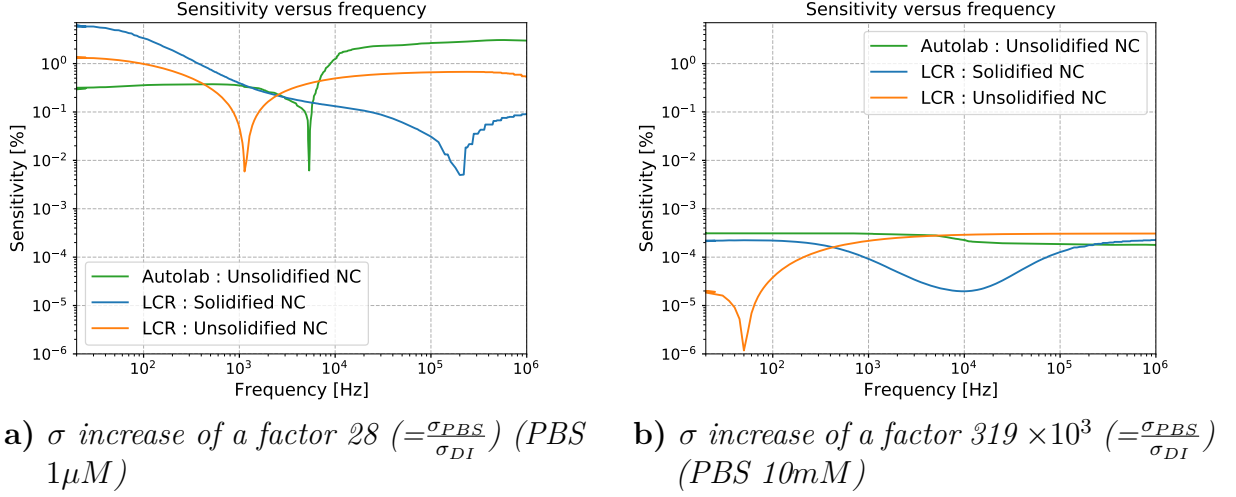


Figure 5.4: Comparison between LCR meter and Autolab sensitivity when NC membrane is either solidified or not solidified. Data to obtain the sensitivity as a function of frequency was gathered by averaging 12 impedance modulus measurements. Each measurement was performed with the same calibration, an applied potential of 20mV and over a frequency range of 20Hz to 1MHz. The repeatability of these measurements is discussed in Section 5.6.

A first general trend can be highlighted. The **overall sensitivity** of the three cases considered **decreases progressively** as the difference between the reference conductivity and the conductivity of the PBS sample considered increases.

Decrease of sensitivity induced by increase in conductivity can be explained by the term $|\sigma_{DI} - \sigma_{PBS}|$ in sensitivity Equation 4.3, which is inversely proportional to the sensitivity. Indeed, as σ_{PBS} increases, $|\sigma_{DI} - \sigma_{PBS}|$ increases and the sensitivity decreases. The increase in $|Z_{DI,meas} - Z_{PBS,meas}|$ is not sufficient to compensate the increase in $|\sigma_{DI} - \sigma_{PBS}|$.

5.3.2 Highest sensitivity results from unsolidified NC membrane

In order to provide an overview of the evolution of sensitivity over the frequency range and to classify the three studied cases according to their detection performance in response to a variation in conductivity, Figures 5.5 is provided. These Tables are based on the sensitivity versus frequency Figures. They are all available in Appendix D.1 (the lowest and highest conductivity cases are given in Figure 5.4). Each line corresponds to a certain factor of increase in conductivity (28, 301, ...) with respect to the reference conductivity of DI water sample. Depending on the conductivity variation and the position on the frequency range, the colours in Figure 5.5(a) indicate which case has the highest sensitivity.

The colours in Figure 5.5(b) show which case has the lowest sensitivity.

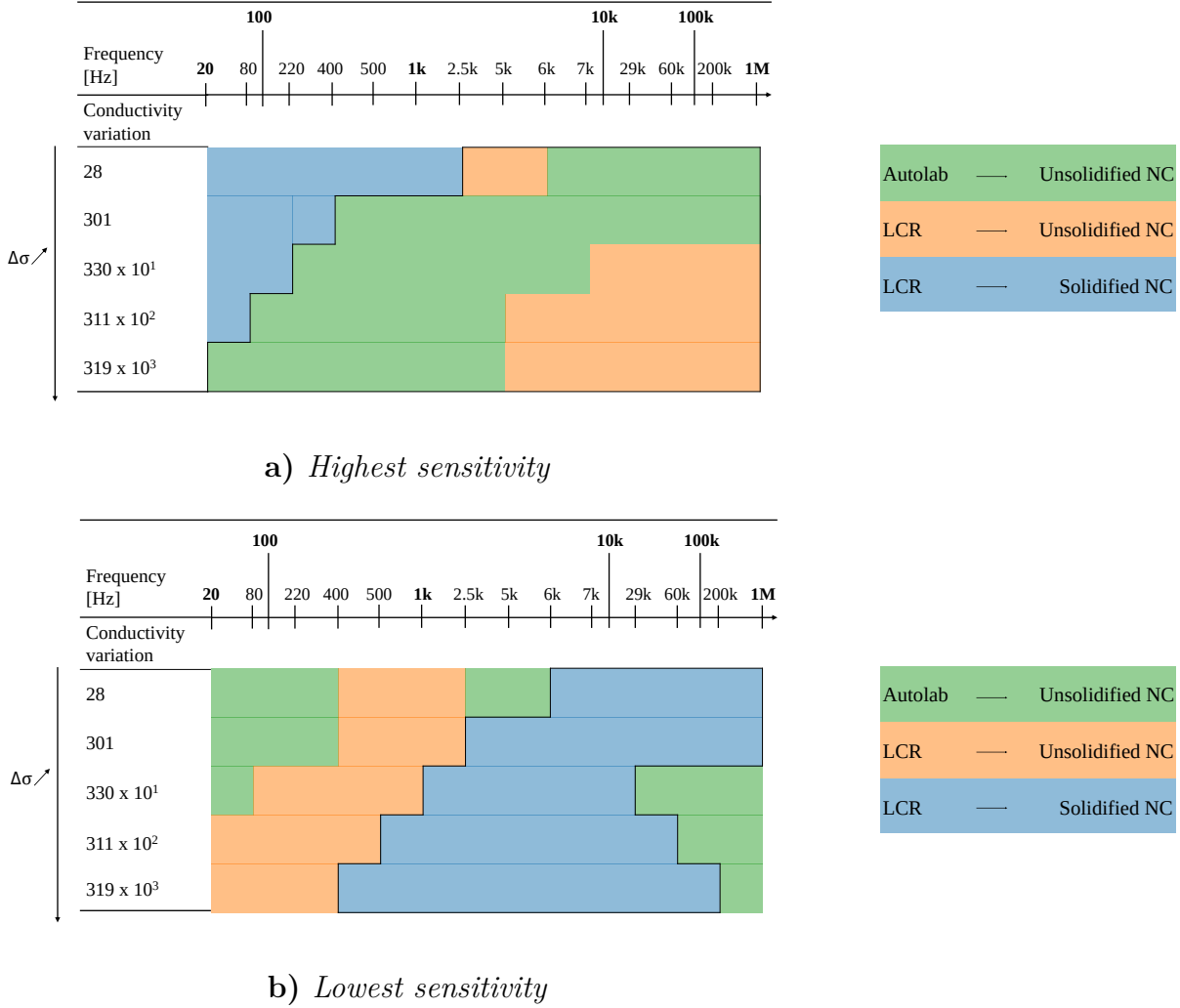


Figure 5.5: Evolution as a function of frequency and conductivity variation of the sensitivity. Tables based on the sensitivity versus frequency Figures. They are all available in Appendix D.1. From top to bottom, the lines correspond to the progressive increase in the conductivity difference between the DI water reference and the PBS sample under consideration. From the left to the right, the increase in frequency can be observed as illustrated by the arrow. (a) Shows which of the three cases (see legend on the right) has the highest sensitivity throughout the frequency range. (b) Shows which of the three cases (see legend on the right) has the lowest sensitivity throughout the frequency range.

The main conclusions that can be drawn from the observation of Figure 5.5(a) are:

- Measurements on **unsolidified NC membranes** generally show the **highest sensitivity** over the frequency range studied. As the conductivity difference increases, Autolab measurements have better sensitivity at low frequencies (**green**). LCR meter measurements on unsolidified NC membranes reach the top of the ranking, at high frequencies, for an increase factor of 330×10^1 (**orange**).

- Detection via the **LCR meter** on a **solidified NC membrane** is recommended at **low frequencies** (20 to 500Hz) when the conductivity variation is increased by a factor of 28 to 301 (**blue**).

The main conclusions that can be drawn from the observation of Figure 5.5(b) are:

- **LCR meter** measurements on **solidified NC membrane** (**blue**) have the **lowest sensitivity** in general.
- **LCR meter** measurements on **unsolidified NC membrane** (**orange**) show the **lowest sensitivity** at **low frequencies**, up to about 2.5kHz. Autolab measurements on unsolidified NC membrane (**green**) exhibit the lowest sensitivity least often.

The advantage of unsolidified setups over solidified setups when the variation in conductivity is significant can be explained by several factors.

- Predominance of unsolidified measurements in Figure 5.5(a) as the conductivity increases is explained by the influence of the double layer capacitance on the total impedance.

First of all it should be remembered that C_{DL} variations depend on the ions in the solution because permittivity depends on the number of ions. It may be concluded that the higher the conductivity, the more ions are available to create conduction paths, the more C_{DL} is likely to vary. Since the NC membrane is unsolidified, the double layer capacitance C_{DL} is no longer shielded by the capacitance created by the polyester layer and C_{DL} variations are therefore perceived more clearly by the measuring device .

This is demonstrated by the following mathematical proof. Development of the impedance expressions is available in Appendix F.

$$S_{i,without\ backing} > S_{i,with\ backing}$$

$$|Z_{DI,meas} - Z_{without\ backing}| > |Z_{DI,meas} - Z_{with\ backing}|$$

$$Z_{without\ backing} < Z_{with\ backing}$$

$$[...]$$

$$1 + j\omega R_{sol}C_{DL} + j\omega R_{sol}C_{sol} < \frac{C_{backing} + C_{DL}}{C_{backing}} + j\omega R_{sol}C_{DL} + j\omega R_{sol}C_{sol} \frac{C_{backing} + C_{DL}}{C_{backing}}$$

$$\text{QED since } \frac{C_{backing} + C_{DL}}{C_{backing}} > 1.$$

- Sensitivity predominance of Autolab measurements on unsolidified NC membranes compared to LCR measurements on unsolidified NC membranes is probably also explained by the influence of the double layer capacitance. Focusing on Figure 5.5(a), it can be seen that the Autolab measurements lose sensitivity around $5kHz$. This frequency is close to the first cut-off frequency of the system's equivalent circuit. Frequencies lower than the cut-off frequency define the area where C_{DL} has the greatest influence on the total impedance of the system. So C_{DL} variations are better perceived by the Autolab than by the LCR meter. This can be explained by the fact that potentiometric measuring devices such as the Autolab are usually equipped with Ion-Selective Electrodes (ISE), the principle of which is explained in Section 2.2.1 of the state-of-the-art. It can be assumed that with these electrodes, the variability at the ion level is more perceptible than with a set-up of parallel plates.

The **conclusion** to be drawn from this Section is that measurements on **unsolidified NC membranes** generally show **better sensitivity** than measurements with solidified NC membranes.

5.4 Sensitivity for permittivity variation of the sample

Potentiometry and impedimetry are now studied, through LCR meter and Autolab potentiostat measurements, in the case of a significant variation in permittivity of the measured sample. This change in measured sample permittivity is achieved by the following three liquid models: deionized (DI) water, ethanol and isopropyl alcohol. DI water has the highest permittivity and isopropyl alcohol the lowest. Each measurement is repeated 12 times with the same measurement conditions except for the nitrocellulose membrane and the liquid drop which are changed for each test.

Sensitivity of the LCR meter and Autolab is computed using Equation 4.3 developed in Section 4.3. Sensitivity is evaluated for an increase in sample permittivity relative to a reference permittivity. The sample of Deionized (DI) water is arbitrarily chosen as permittivity reference.

5.4.1 Steep sensitivity minimum for Autolab measurements

Figure 5.6 shows the evolution of sensitivity as a function of frequency for the three situations studied. Figure 5.6(a) shows the smallest decrease in permittivity (by a factor of 3 ($= \epsilon_{DI}/\epsilon_{ET}^1$)), while Figure 5.6(b) shows the largest decrease in permittivity (by a

¹ET = ethanol

factor of 4 ($= \epsilon_{DI}/\epsilon_{IS}^2$)).

Figure 5.6 shows a steep minimum of sensitivity for Autolab measurements (**green curves**). This steep minimum shifts to the right as the permittivity decreases (see Figure 5.6(b)). It coincides more or less with the third cut-off frequency of the measured system equivalent circuit. Derivation of cut-off frequencies and their dependency on the equivalent circuit elements is explained in Section 4.4 of *Materials and Methods* Chapter.

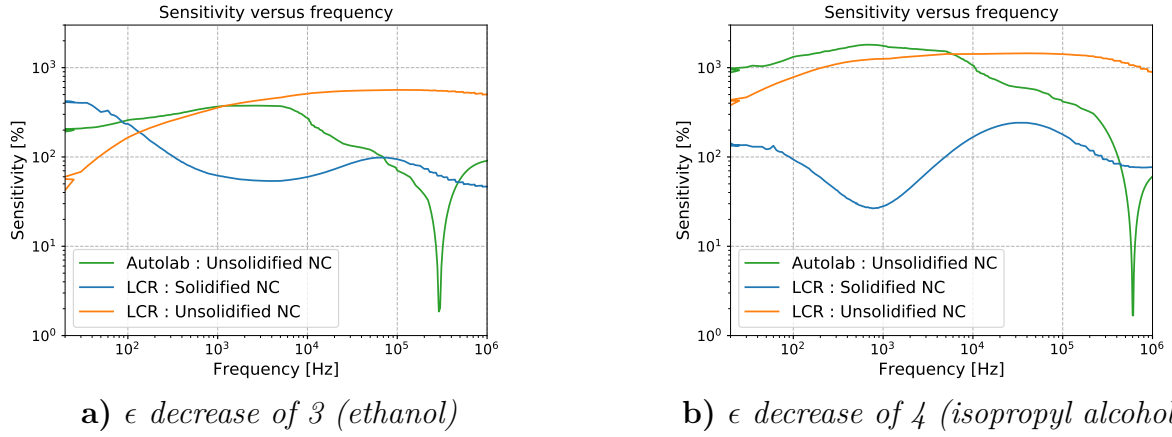


Figure 5.6: Comparison between LCR meter and Autolab sensitivity when the nitrocellulose membrane is either solidified by a polyester layer or not solidified. Data used to obtain the sensitivity as a function of frequency was gathered by averaging 12 impedance modulus measurements. Each measurement was performed with the same calibration, the same applied potential (20mV) and over the same frequency range (20Hz to 1MHz). The repeatability of these measurements is discussed in more detail in Section 5.6.

Figure 5.7 helps to explain this steep minimum. It shows the variation of the impedance modulus with respect to frequency during an Autolab measurement of an unsolidified NC membrane saturated with isopropyl alcohol.

For frequencies smaller than the third cut-off frequency ($\approx 400kHz$), the impedance modulus tends to become independent from frequency variations. Once past this third cut-off frequency, the frequency dependence of the impedance modulus reappears, causing it to decrease. Around the third cut-off frequency, the impedance modulus increases from its constant position before dropping. It is this increase that causes a decrease in the term $|Z_{DI,meas} - Z_{IS,meas}|$. Since it is proportional to sensitivity (see Equation 4.3), sensitivity decreases shortly and suddenly.

The increase in impedance modulus can perhaps be explained by measurement errors in the Autolab. Indeed, the impedance phase measurements show breaks around the third cut-off frequency ($\approx 400kHz$) (see Figure 5.7).

²IS = Isopropyl alcohol

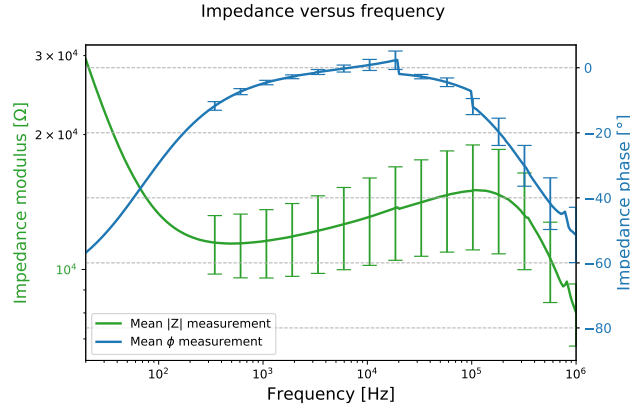


Figure 5.7: *Evolution of impedance as a function of frequency. Measurements carried out using the Autolab potentiostat on an unsolidified NC membrane saturated with an isopropyl alcohol solution. Means were obtained on the basis of 12 tests performed at a potential of 20mV.*

5.4.2 Increase in sensitivity from decrease in permittivity

Sensitivity of measurements with isopropyl alcohol (see Figure 5.6(b)) is greater than the sensitivity of measurements with ethanol (see Figure 5.6(a)), except for measurements with solidified NC membrane. Sensitivity formula (see Equation 4.3) illustrates that a decrease in permittivity ϵ_x leads to an increase in $|\epsilon_{DI,meas} - \epsilon_x|$. This should cause the sensitivity to decrease, but it actually increases. This means that $|Z_{DI,meas} - Z_{x,meas}|$ increases more than $|\epsilon_{DI,meas} - \epsilon_x|$ increases. This can be explained by a lower $|Z_{x,meas}|$ term for measurements with isopropyl alcohol than with ethanol.

This phenomenon is not observed for unsolidified membrane measurements. The main difference between these two cases is the presence of a capacitance induced by the polyester layer, called $C_{backing}$. Without the polyester layer, there is no backing capacitance and therefore the double layer capacitance, in series with $C_{backing}$, is no longer shielded by $C_{backing}$. Ethanol has a higher conductivity than isopropyl alcohol. Ethanol therefore has more ions. Its double layer capacitance is therefore greater. A large double layer capacitance is thus detrimental to the sensitivity.

The general **conclusion** to be drawn from these observations is that measurements on **unsolidified NC membranes** offer **better sensitivity** as the **permittivity variation** becomes larger.

5.4.3 Highest sensitivity attributed to LCR meter with unsolidified NC membrane

In order to provide an overview of the evolution of sensitivity over the frequency range and to classify the three studied cases according to their detection performance in response to a variation in permittivity, Figure 5.8 is provided. These Tables are based on Figure 5.6. Each line corresponds to a certain factor of decrease in permittivity (3, 4) with respect to

the reference permittivity of DI water sample. Depending on the permittivity variation and the position on the frequency range, the colours in Figure 5.8(a) indicate which case has the highest sensitivity. The colours in Figure 5.8(b) show which case has the lowest sensitivity.

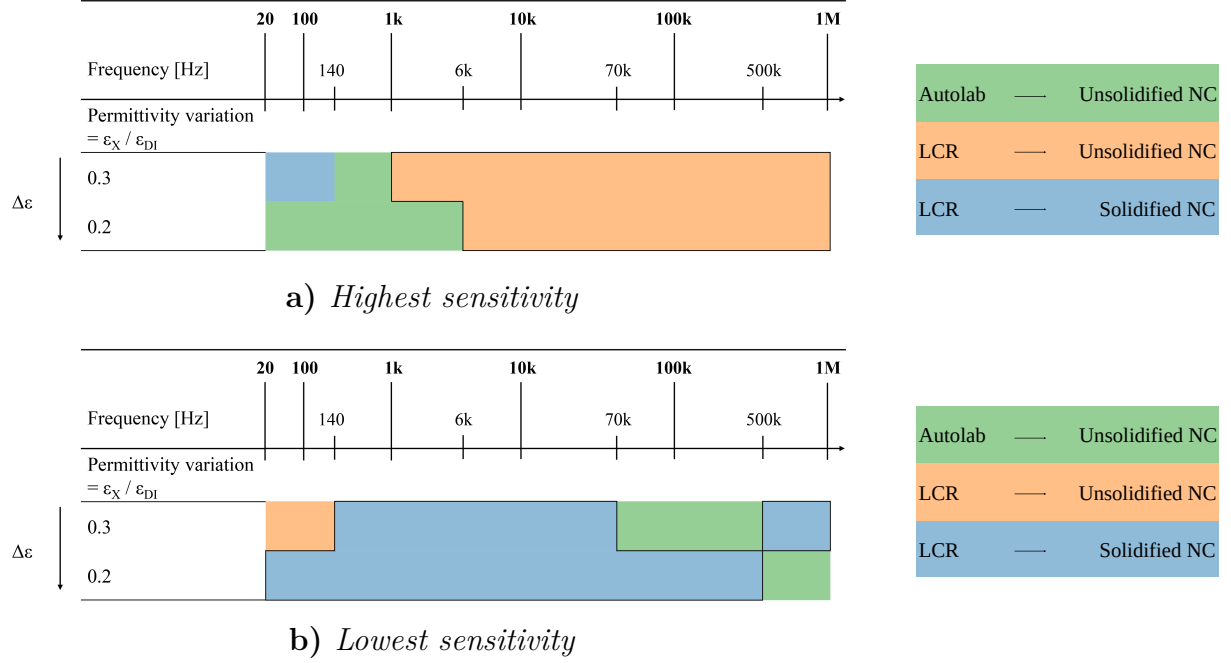


Figure 5.8: Evolution as a function of frequency and permittivity variation of the sensitivity. These Tables are based on the sensitivity versus frequency Figures 5.6. From top to bottom, the lines correspond to the progressive increase in the permittivity difference between the DI water reference and the ethanol and isopropanol samples. From the left to the right, the increase in frequency can be observed as illustrated by the arrow. (a) Shows which of the three cases (see legend on the right) has the highest sensitivity throughout the frequency range. (b) Shows which of the three cases (see legend on the right) has the lowest sensitivity throughout the frequency range.

The main conclusions that can be drawn from the observation of Figure 5.8 are:

- **LCR meter** measurements on **unsolidified NC membranes** generally show the *highest sensitivity* over the frequency range studied (**orange**).
- **LCR meter** measurements on **solidified NC membrane** have the lowest sensitivity in general (**blue**).

The advantages of LCR meter measurements on unsolidified NC membrane when the variation in permittivity is significant can be explained by several factors.

- Predominance of measurements on unsolidified membranes compared to measurements on solidified membranes is explained by the influence of the capacitance of the

equivalent circuit associated with the electrode-membrane-electrode system. Indeed, the capacitance is proportional to the permittivity of the measured sample. A change in permittivity is more noticeable in the solution C_{sol} and electrical double layer capacitance C_{DL} . Since there is no polyester layer, there is no backing capacitance involved and the variations in C_{DL} and C_{sol} are no longer shielded. They are therefore much more perceptible to measuring devices.

- In the case where the sample varies in conductivity, the unsolidified Autolab measurements predominate over the unsolidified LCR measurements. In the case where the permittivity varies, the opposite is observed. Indeed, the unsolidified LCR measurements predominate. It had been assumed that the advantage of Autolab over LCR in the case of conductivity variation came from its Ion-Selective Electrodes (ISE). Here, the conductivity of the samples is very low. The amount of ions present is therefore assumed to be very low and the Ion-Selective Electrodes no longer allow the Autolab to provide the best sensitivity over the widest frequency range.

5.5 Power consumption of an electrochemical system

Theoretically, since the systems all satisfy Ohm's law, the impedance measured should remain the same regardless of the applied potential. However, situations have been encountered where this law is no longer respected. An experiment conducted in the 1930s observed an increase in the resistance measured by a Wheastone bridge when the direct potential was increased [73]. An explanation for this phenomenon is discussed in *Discussion Chapter*. The Point-of-Care device studied would be more interesting if it provided the same quality of information regardless of the voltage applied. Indeed, the consumption of the device could be reduced. To study the behaviour of the detection methods under the effect of the variation of the applied potential, measurements are carried out, in the case of solidified or unsolidified NC membrane, with an applied voltage of $20mV$ and $200mV$. All other measurement conditions are kept the same, such as calibration, frequency range and saturation of the NC membrane.

Impact of voltage variation on LCR meter measurements is investigated in this Section.

5.5.1 Difficulty of comparison due to sample evaporation

Measurements carried out to compare the two detection methods during a variation in permittivity, suffer from the evaporation of the isopropyl alcohol over time. Indeed, in order to compare the measured impedance as a function of voltage, a NC membrane saturated with isopropyl alcohol is placed between the two electrodes. A first measurement is taken at $20mV$. Then, on the same paper, a second measurement is made at $200mV$. By not changing the membrane between two measurements, the aim is to eliminate any variation except that of the voltage. However, over the time of the two measurements, the isopropyl evaporates, biasing the results. Figure 5.9(a) shows a LCR meter test on an NC membrane saturated with isopropyl alcohol, with 267 measurement points. Figure 5.9(b) shows the

same measurement but with six times fewer measurement points. The measurement time is therefore greatly reduced. The shift to the left of the 200mV measurements compared to the 20mV measurements between 10^3 Hz and 10^6 Hz is significantly reduced.

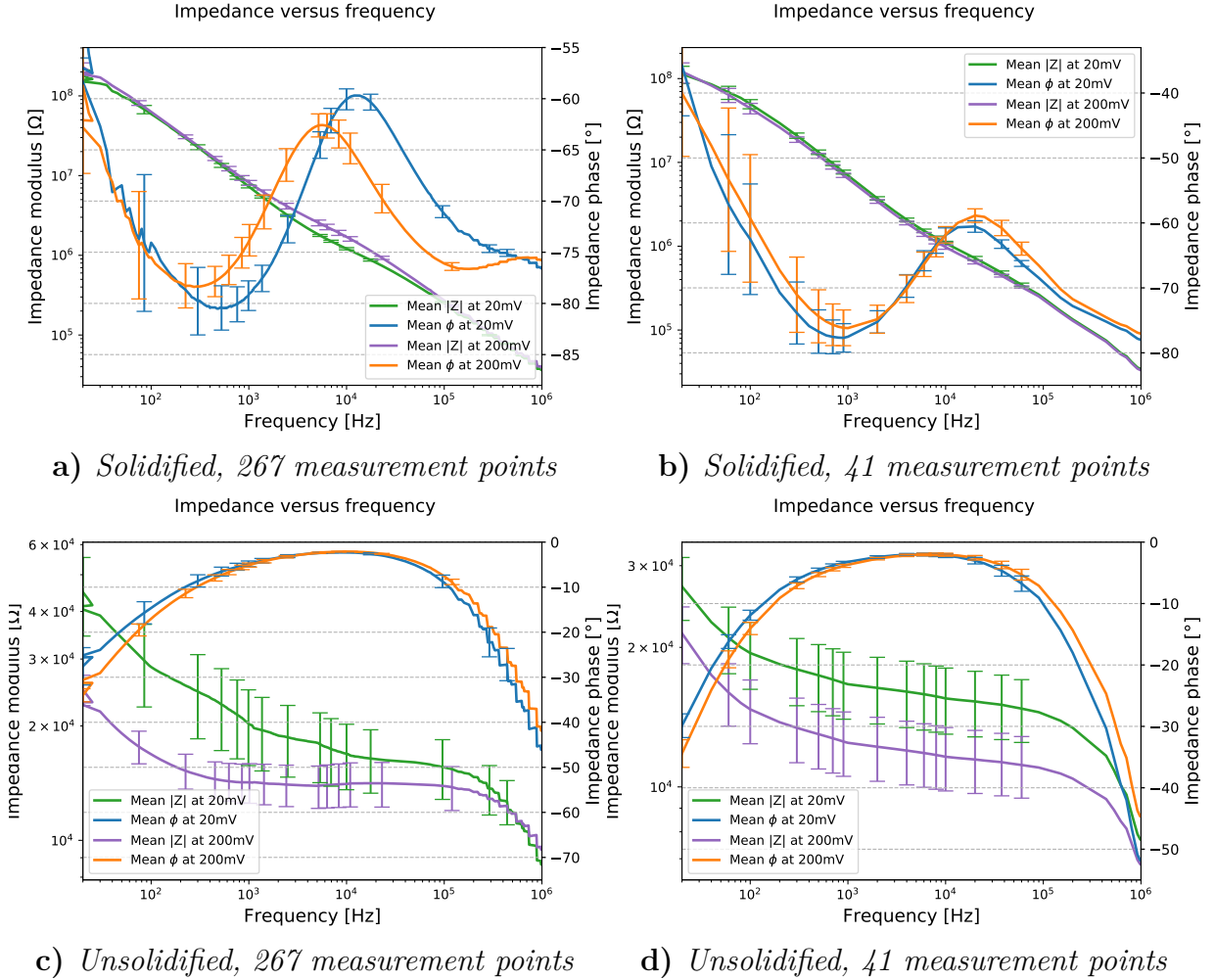


Figure 5.9: Measurements carried out on the LCR meter with a NC membrane saturated with **isopropyl alcohol**. Comparison between measurements carried out with an applied potential of 20mV and of 200mV. The two figures illustrate the same experiment, but one of the measurements is made on 267 measurement points and the other on 41. The aim is to vary the measurement time.

It is assumed that these shifts are caused by the evaporation of isopropyl alcohol because measurements with deionized DI water do not undergo this same shift (see Figure 5.10(f)) and isopropyl alcohol evaporates much faster than DI water.

In the case where the nitrocellulose membrane is unsolidified, reducing the number of measurement points to reduce the measurement time does not counteract the evaporation of isopropyl alcohol, as shown in Figure 5.9(c) and 5.9(d). Figure 5.9(c) shows LCR meter measurements with 267 measurement points on unsolidified membrane saturated with isopropyl alcohol. Figure 5.9(d) shows the same experiment but with only 41 measurement points. The reason may be that as the membrane is not solidified, it is less likely to retain

liquid within its pores when electrode pressure is applied. Evaporation occurs even more quickly.

Comparison between measurements at $20mV$ and $200mV$ is not performed for the case of unsolidified membrane saturated with isopropyl alcohol since the measurements are biased by evaporation. The measurements on solidified membrane saturated with isopropyl alcohol will be analysed in the case where the number of measurement points is 41.

5.5.2 Impact of voltage on a solidified membrane measurement

Figure 5.10 illustrates the evolution of measured impedance as a function of frequency. Figures 5.10(a), 5.10(b) and 5.10(c) show measurements made on unsolidified NC membrane, saturated with $1\mu M$ PBS, $10mM$ and DI water, respectively. Figures 5.10(d), 5.10(e) and 5.10(f) show measurements made on solidified NC membrane, saturated with $1\mu M$ PBS, $10mM$ and DI water, respectively.

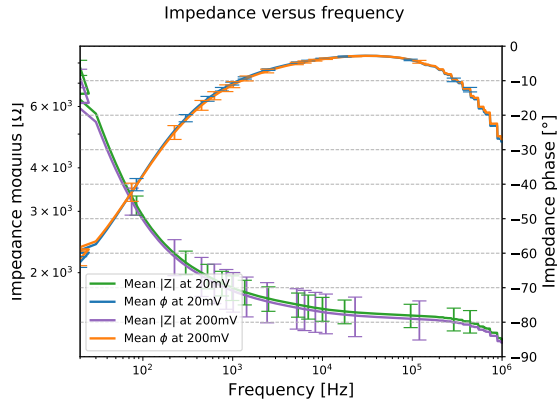
Measurements performed at $20mV$ are compared to those performed at $200mV$.

The main observations of Figure 5.10 are:

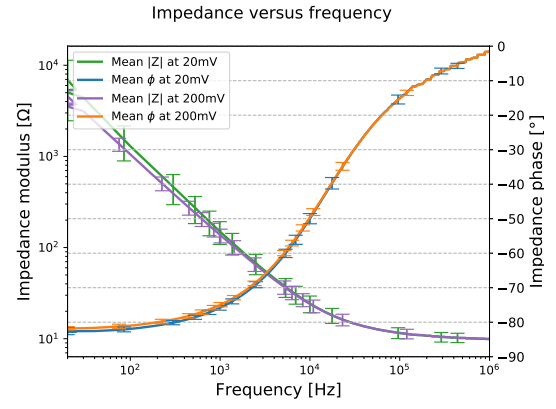
- When using an **unsolidified NC membrane**, LCR meter measurements are the same whether the applied potential is $20mV$ (blue and green) or $200mV$ (orange and purple). However, Figure 5.10(b) and 5.10(c) show a slight difference between impedance modulus measurements at $20mV$ and $200mV$. For PBS $10mM$, the difference arises at low frequencies.
- When measurements are made on a **solidified NC membrane**, Figure 5.10(d), 5.10(e) and 5.10(f) reveal a difference in impedance phase measured when applied potential varies between $20mV$ and $200mV$. This difference is observed at low frequencies.
- When **NC membrane is solidified**, the 95% confidence interval of impedance phase measurements is wider at low frequencies compared to measurements on unsolidified membrane. This is illustrated in Figures 5.10(e) and 5.10(f).

One reason for the lower repeatability of measurements at low frequencies in the case where the NC membrane is solidified may be that the measurement limits of the LCR meter are reached. Indeed, the measurable impedance range of the LCR meter starts at $0.01 m\Omega$ and ends at $99.99 M\Omega$. Its frequency range is from $20 Hz$ to $1 MHz$.

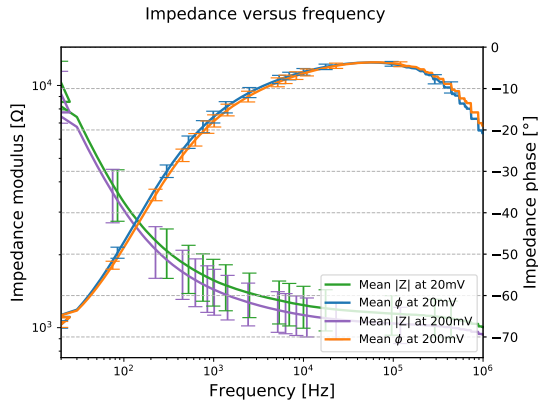
To understand the LCR meter's behaviour when it reaches its limits, a measurement of seven polyester layers, as used to solidify nitrocellulose membranes, is shown in Figure 5.12. It is performed 12 times with an applied potential of $20mV$. As illustrated by the theoretical curve, the measurement should be purely capacitive and from $10kHz$ the



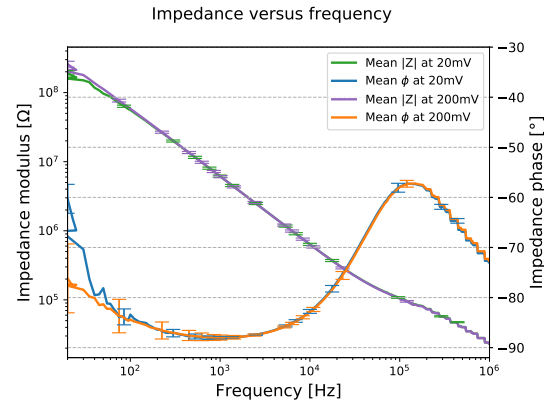
a) *Unsolidified NC membrane saturated with PBS 1 μ M*



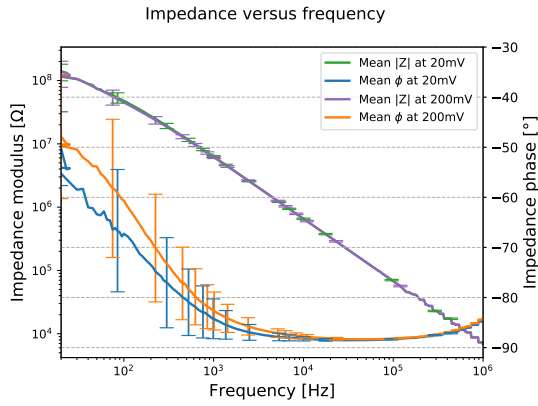
b) *Unsolidified NC membrane saturated with PBS 10mM*



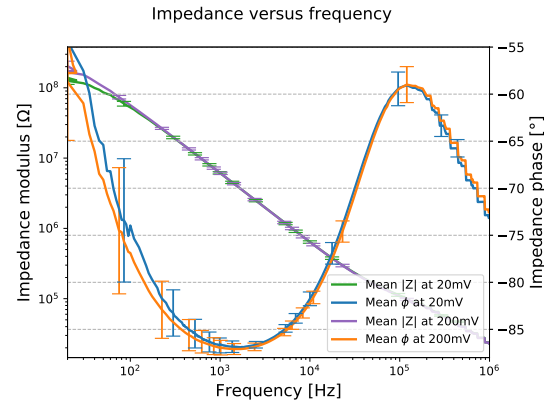
c) *Unsolidified NC membrane saturated with DI water.*



d) *Solidified NC membrane saturated with PBS 1 μ M*



e) *Solidified NC membrane saturated with PBS 10mM*



f) *Solidified NC membrane saturated with DI water.*

Figure 5.10: Comparison between LCR meter measurements carried out with an applied potential of 20mV (green) and of 200mV (blue). Each curve is obtained by averaging 12 measurements of the system impedance. The repeatability of the measurements is illustrated by the 95% confidence interval and can be assumed to be sufficient to judge the measurements obtained as relevant.

impedance modulus value should exceed 99.99M Ω .

The theoretical curve is obtained by assuming the electrode-polyester layer-electrode system to be similar to a parallel-plate capacitance whose dielectrics are the polyester layers.

The equivalent circuit and the corresponding impedance expression are given in Figure 5.11.

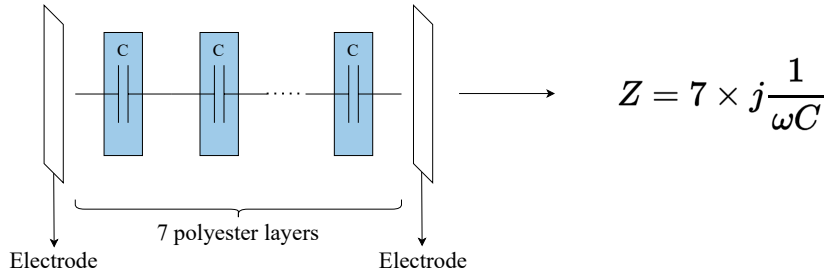


Figure 5.11: *Equivalent circuit and the corresponding impedance expression of the electrode-polyester layer-electrode system measured to study the LCR meter measurements limits.*

Figure 5.12(a) illustrates that once the impedance value has exceeded the limits of the LCR meter, at 10kHz , the curve tends to plateau and deviates significantly from the theoretical curve.

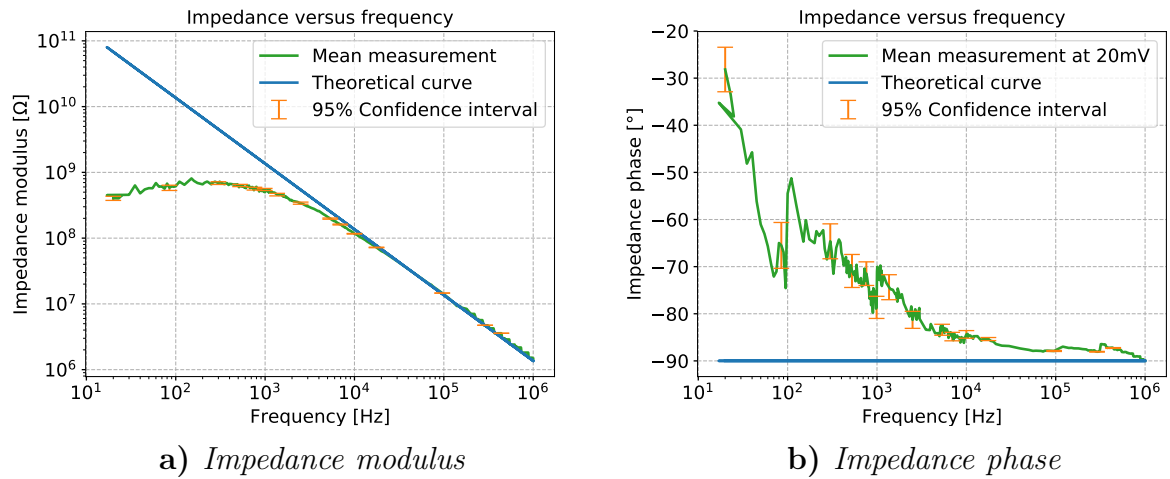


Figure 5.12: *Measurements carried out on the LCR meter with seven layers of polyester stacked to form a $700\text{ }\mu\text{m}$ thick layer. Each curve is obtained by averaging 12 measurements of the system impedance. The repeatability of the measurements is illustrated by the 95% confidence interval and can be assumed to be sufficient to judge the measurements obtained as relevant.*

With regard to power consumption, the conclusion is that for measurements on an **unsolidified NC membrane**, the applied voltage does not matter. The **power consumption** of the biosensor **can therefore be limited** by performing the measurements with a low voltage.

When applied voltage varies and measurements are performed on a **solidified NC membrane**, a **difference between measurements at 20mV and 200mV** is observed in the impedance phase. It is observed that the measured impedance depends on the

frequency but also on the conductivity. Indeed, Figures 5.10(d) and 5.10(f) illustrate that at low frequencies, the phase of the measured impedance decreases as the voltage increases. Figure 5.10(e), on the other hand, shows the opposite. As the voltage increases, the phase of the measured impedance increases, also at low frequency. The difference is in the conductivity of the measured sample. A more detailed analysis is carried out in the next Section.

5.5.3 Link between conductivity and voltage variation at low frequencies

In order to study the relationship between conductivity, applied voltage and frequency, Figure 5.13 is provided. It depicts the evolution of impedance phase as a function of frequency and compares measurements made at 20mV and 200mV. Each graph represents a measurement made on a sample of different conductivity. The conductivity is increased by a factor of 100 between each graph.

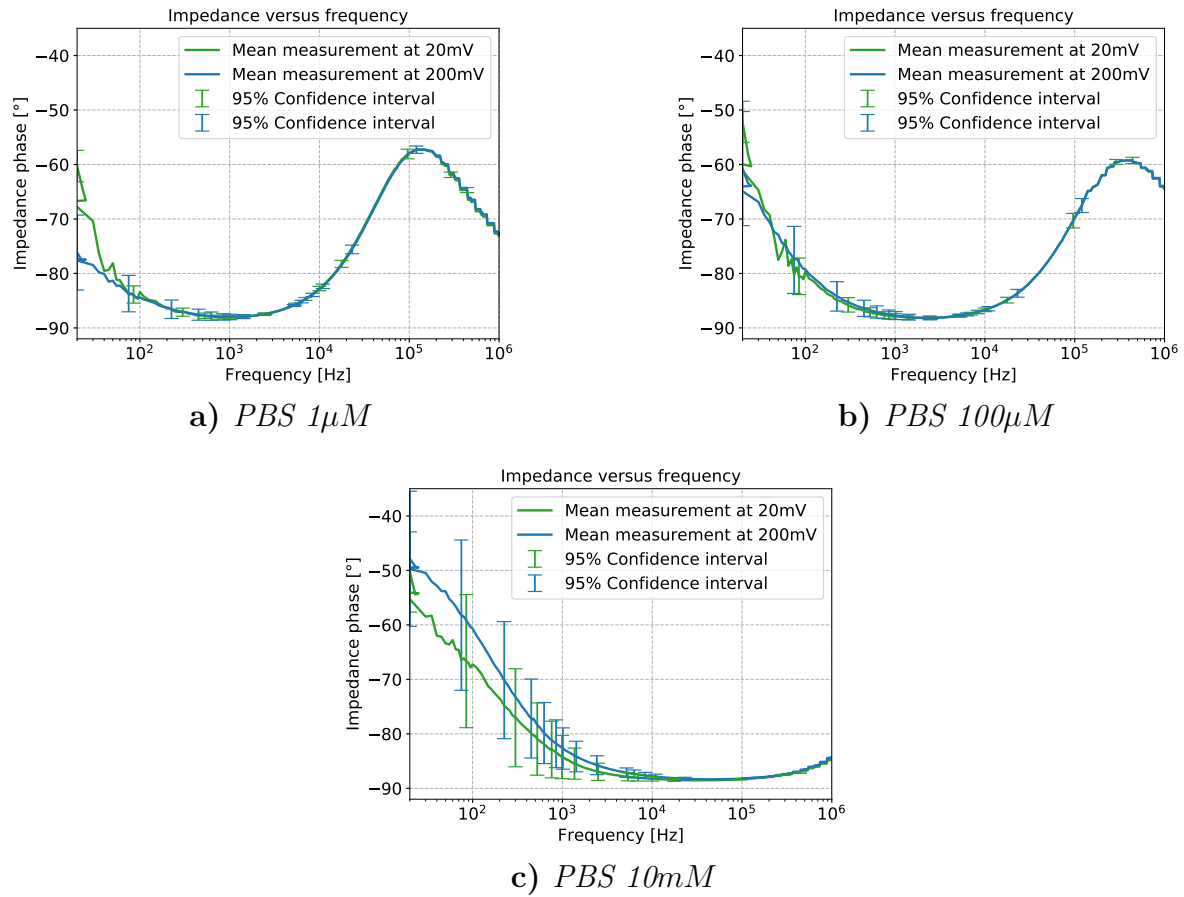


Figure 5.13: Evolution of the impedance phase as a function of frequency. Comparison between measurements made at 20mV and 200mV. The means were obtained from 12 tests. The repeatability of the measurements is illustrated by the 95% confidence interval. The repeatability of the measurements decreases as the conductivity increases.

The observations are gathered hereafter.

- The 95% confidence interval expands as the sample conductivity increases but only at low frequencies. Reliability of measurements therefore decreases as the conductivity increases. The extent of the 95% confidence interval appears to be less for measurements at $20mV$ than at $200mV$.
- For the lower conductivity case (see Figure 5.13(a)), an increase in voltage results in a decrease in the impedance phase measured at low frequency. The system then tends towards a more capacitive behaviour. When the conductivity increases by a factor of 100 (see Figure 5.13(b)), the voltage increase has very little impact on the measurements. Indeed, the measurements at $20mV$ and $200mV$ are similar. When the conductivity increases by a further factor of 100 (see Figure 5.13(c)), an increase in voltage from $20mV$ to $200mV$ results in an increase in the measured phase impedance. The system then tends towards a more resistive behaviour.

The reasons for variations in repeatability of measurements are as follows:

- The difference between the 95% confidence interval for measurements at $20mV$ and $200mV$ can be explained by a measurement error. Indeed, the measurements are taken 12 times. Each test involves a nitrocellulose membrane saturated with the liquid sample. One test consists of four measurements. Two measurements at $20mV$ and then two measurements at $200mV$. The measurements at $200mV$ are therefore more likely to be negatively affected by the drying of the paper.
- The explanation for the increase in the 95% confidence interval as the conductivity increases, for the $20mV$ and $200mV$ measurements, may lie in the electrical double layer, as this occurs at low frequency. An increase in conductivity leads to an increase in the value of double layer capacitance C_{DL} . A hypothesis may be that this increase in C_{DL} leads to an increase in the measured impedance modulus which then pushes the measurements into the limits of the LCR, resulting in a decrease in the repeatability of measurements.

The link between conductivity and applied voltage will be further explored in *Discussion* Chapter.

5.6 Repeatability performance for an electrochemical system

Repeatability of measurements is crucial to judge of their reliability. The three considered cases of detection methods (LCR meter with solidified and unsolidified NC membrane and Autolab potentiostat measurements on unsolidified NC membrane) are compared

here with respect to their repeatability factor R , defined in Equation 4.4 (see Section 4.3.2).

In the case of the electrochemical system (variation of conductivity and permittivity), measurements are repeated 12 times for each case (LCR with solidified NC, LCR with unsolidified NC, Autolab with unsolidified NC) and by liquid samples (five PBS samples for conductivity variation and three samples for permittivity variation (DI water, ethanol and isopropyl alcohol)).

Repeatability accounts for the statistical dispersion of measurements. Factor R oscillates between 0 and 1. It is equal to 1 when there is no dispersion with respect to the calculated mean. This means that the measurements are perfectly repeatable.

The measured value used to judge the repeatability of the measurements is the impedance modulus. Impedance modulus measurements provide graphs that most closely match the equivalent circuit developed in Materials and Methods. Impedance phase measurements deviate more from the theory. Studying repeatability via the impedance modulus allows one to rely more securely on the equivalent circuit to justify this or that behaviour.

The study of repeatability in electrochemical systems is divided into two frequency areas, referred to in this explanation as low frequency LF and high frequency HF. The boundary of the two zones is approximately at the first cut-off frequency of the systems studied. The graphs in Figure 5.14 show the evolution of the repeatability of measurements as a function of frequency. Each graph compares the three cases studied and the first cut-off frequencies associated with each measurement case are shown as dotted vertical lines.

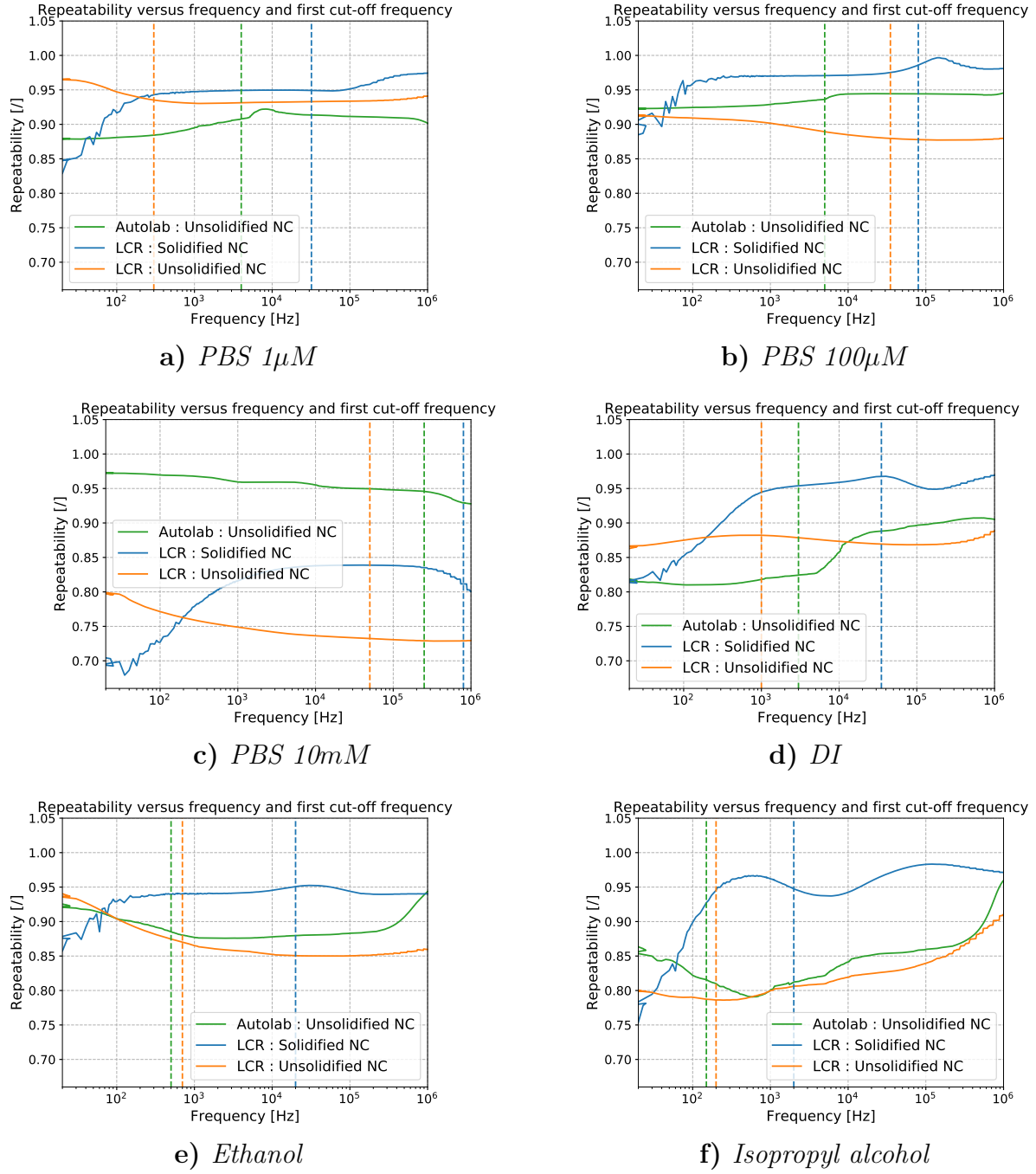


Figure 5.14: Evolution of the repeatability factor R as a function of frequency for the measurements of the impedance modulus. Comparison between the three detection cases studied: LCR meter with solidified NC membrane (*blue*), LCR meter with unsolidified NC membrane (*orange*) and Autolab with unsolidified NC membrane (*green*). The first cut-off frequencies associated with each case for the six liquid sample types considered are represented by vertical dotted lines. On the left of the first cut-off frequency is the area referred to here as low frequency and on the right the high frequency area. The method to obtain the cut-off frequencies is provided in Section 4.4. The exact values of these cut-off frequencies are given in Appendix F

5.6.1 Low frequency repeatability in electrochemical systems

The performance of the three situations in the so-called low frequency zone is analysed hereafter.

Influence of the LCR meter measurement limits

It is observed that in the six cases of liquids considered, repeatability of the **LCR meter** measurements on **solidified NC membrane (blue)** drops in the low frequency range. There are two possible explanations for this decrease in repeatability. One of them is related to the LCR meter measurement limits. Indeed, the impedance modulus measured in the case where the nitrocellulose is solidified is higher than the $10^8 Hz$ impedance limit of the LCR meter in the low frequency zone. Therefore measurement's reliability decreases and so does repeatability.

Influence of the double layer capacitance C_{DL}

The drop in repeatability of **LCR meter** measurements on **solidified NC membrane** may also have a second cause. This cause is the influence of the double layer capacitance C_{DL} at low frequency.

To understand this, it is important to remember that the capacitance characterising the polyester layer $C_{backing}$ is in series with the double layer capacitance within the NC membrane C_{DL} , as recalled in Figure 5.15 developed in Section 4.4.1 of Materials and methods Chapter.

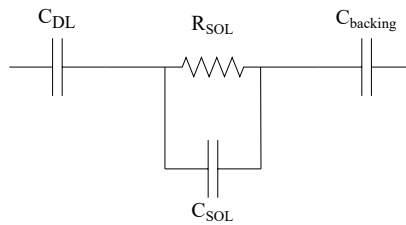


Figure 5.15: *Equivalent circuit of the electrochemical system when the nitrocellulose membrane is solidified by a polyester layer. This polyester layer induces a capacitance called $C_{backing}$.*

It is in the low frequencies that C_{DL} has the greatest influence. Since C_{DL} varies more between each measurement than $C_{backing}$, it introduces more irregularity in the measurements. These irregularities decrease with increasing frequency, as the influence of C_{DL} decreases. The first cut-off frequencies of LCR measurements with solidified NC correspond to the beginning of the zone where C_{DL} has lost its influence. From the graphs in Figure 5.14 it can be seen that these frequencies also correspond more or less to the time when repeatability reaches a maximum.

C_{DL} also influences **Autolab** measurements on **unsolidified NC membrane**. The predominant influence of C_{DL} at low frequencies tends to decrease the repeatability in this area. However, this is not the case for Figures 5.14(e) and 5.14(f). C_{DL} is proportional to the permittivity of the sample. This permittivity is low for ethanol and isopropyl alcohol. C_{DL} is therefore very low and this leads to better repeatability. Indeed, the variations in C_{DL} are less important compared to the "constant" values of R_{SOL} and C_{SOL} between each measurement. Permittivity of DI water, on the other hand, is higher and therefore C_{DL} is greater, so these irregularities negatively affect repeatability (see Figure 5.14(d)).

Influence of the sample's low conductivity

Figures 5.14(d) and 5.14(f) illustrate measurements with very low conductivity samples compared to the others. This low conductivity coincides with a relatively constant low frequency repeatability factor K for the **LCR meter** measurements on **unsolidified NC membrane** (**orange curves**).

This influence of the conductivity is reflected from an equivalent circuit point of view in the influence of R_{SOL} (see Figure 5.15). Indeed, resistance of the solutions, being inversely proportional to the conductivity, is high when conductivity is low. C_{SOL} has very little influence in the low frequencies compared to R_{SOL} and C_{DL} . It is therefore assumed that the system can be modelled as an RC circuit with C_{DL} and R_{SOL} in parallel. The simplified impedance expression is obtained by:

$$Z = j\omega C_{DL} + \frac{1}{R_{SOL}} \quad (5.1)$$

A large value of R_{SOL} increases the influence of C_{DL} on the total impedance. The repeatability at low frequencies is therefore mainly influenced by the variability of C_{DL} before increasing when C_{DL} gives way to C_{SOL} at high frequencies.

5.6.2 High frequency repeatability in electrochemical systems

The performance of the three situations in the so-called high frequency zone is analysed hereafter.

Influence of the capacitance induced by the polyester layer, $C_{backing}$

LCR meter measurements on **solidified NC membrane** (**blue**) have the highest repeatability factor in the high frequency area, as illustrated in Figure 5.14, except for measurements with the most conductive sample, PBS 10mM (see Figure 5.14(b)). This can be explained by the fact that the capacitance induced by the solidifying polyester layer dominates the expression of the impedance, since its value is much larger than the other impedance components. And since it dominates and its value is approximately invariant

between each measurement, repeatability is favoured.

Influence of R_{sol} and C_{sol} over C_{DL}

Figure 5.14 illustrates that repeatability of **Autolab** measurements on **unsolidified NC membrane (green)** is increased in high frequency area, except for the most conductive sample (see Figure 5.14(b)), whose repeatability decreases as the frequency increases. The high frequency area, delimited by the first cut-off frequency, coincides with the region where the resistance and capacitance of the solution, R_{SOL} and C_{SOL} , respectively, dominate the impedance expression compared to C_{DL} . An assumption to be made is that a C_{DL} measurement is less repeatable than an R_{SOL} or C_{SOL} measurement. The detection process should therefore focus on R_{SOL} or C_{SOL} detection to draw conclusions about the sample if it wishes to maximise repeatability.

Influence of the frequency independence of R_{sol}

Figure 5.14 illustrates that repeatability of **Autolab** measurements on **unsolidified NC membrane (green)** reaches a plateau in high frequency area, except for the most conductive sample (see Figure 5.14(b)). Each plateau occurs after the first cut-off frequency, except for the least conductive case, the isopropyl alcohol measurement (see Figure 5.14(f)), where the plateau occurs at around 10^4 Hz, while the cut-off frequency is around 200 Hz.

This quasi-stagnation of repeatability is due to the resistance of the solution R_{SOL} . Indeed, R_{SOL} dominates the expression of the impedance starting from the first cut-off frequency and it does not vary with frequency, which is why repeatability no longer varies either.

5.6.3 Conclusion on repeatability in electrochemical systems

A general ranking of the repeatability of impedance modulus measurements is as follows:

- **LCR meter** measurements with a **solidified NC membrane (blue curve)** generally show the best repeatability over the entire frequency spectrum studied.
- In second place are the measurements on an **Autolab potentiostat** with an **unsolidified NC membrane (green curve)**. This is especially the case for measurements with conductivity variation. Indeed, for the system that studies **permittivity variation**, measurements on **unsolidified membranes** give more or less similar repeatability factors with **both the LCR meter and the Autolab potentiostat**.
- Measurements with the **LCR meter** on an **unsolidified NC membrane (orange curve)** generally show the lowest repeatability when the system studies conductivity variation.

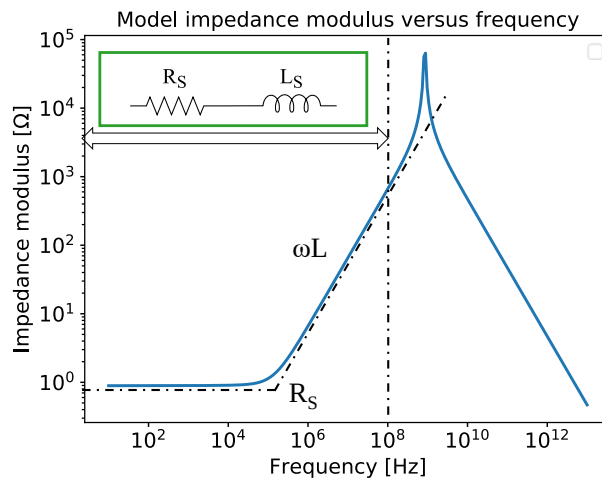
It should be noted, however, that the lowest repeatability factor R achieved is 0.68. This minimum is reached for the highest conductivity case (see Figure 5.14(b)). The maximum deviation of a measurement from the calculated mean is therefore never more than $0.47 (= (1 - R)/R)$ times this mean value. But this is an extreme case at very low frequency. On average, the repeatability factor does not fall below 0.8. The deviation of a measurement from the average over all measurements is therefore never more than $1/4 (= (1 - R)/R)$ times the computed mean. This means that there is no need to favour one detection method over another as all give very good repeatability factors.

5.7 Performance of impedimetric detection under permeability variation

Within the biosensor field, it is common to study the change in conductivity or permittivity of a sample to deduce what is or is not contained in it. But it is also possible to carry out detections using variations in permeability of a sample.

The impedimetric detection method is tested using four planar spiral inductors. Two of these inductors are on the same support and have a copper core in the centre of their spiral. The permeability variation is performed by depositing Fe-based alloy foil on the spiral inductors. The foils are either made of nanocrystalline or amorphous soft ferromagnetic Fe-based ribbons. Each measurement is repeated six times with the same measurement conditions. Nanocrystalline ribbon has the highest permeability.

To understand the observations that are made in the following subsections, the equivalent circuit developed in Chapter *Materials and Methods* is recalled, as well as the expression of the associated impedance and the model impedance graph expected from this equivalent circuit (see Figure 5.16 and Equation 5.2).



$$Z = R + j\omega L \quad (5.2)$$

Figure 5.16: *Equivalent circuit of a planar spiral inductance when stray capacitances are not taken into account and its graphical representation [24].*

From 20Hz to around 100kHz , it is the resistance of the inductor R_S that has the most influence on the total impedance of the system.

5.7.1 Sensitivity to permeability variation

Sensitivity of the LCR meter to a change in inductance permeability is studied by means of the sensitivity Equation 4.3 developed in *Materials and methods* Chapter.

Figure 5.17 illustrates the evolution of sensitivity of the LCR meter, with respect to a change in permeability, along the frequency range studied (20Hz to 1MHz). Figure 5.17(a) shows the case where permeability is changed by an amorphous Fe-based ribbon while Figure 5.17(b) shows the case with a nanocrystalline Fe-based ribbon.

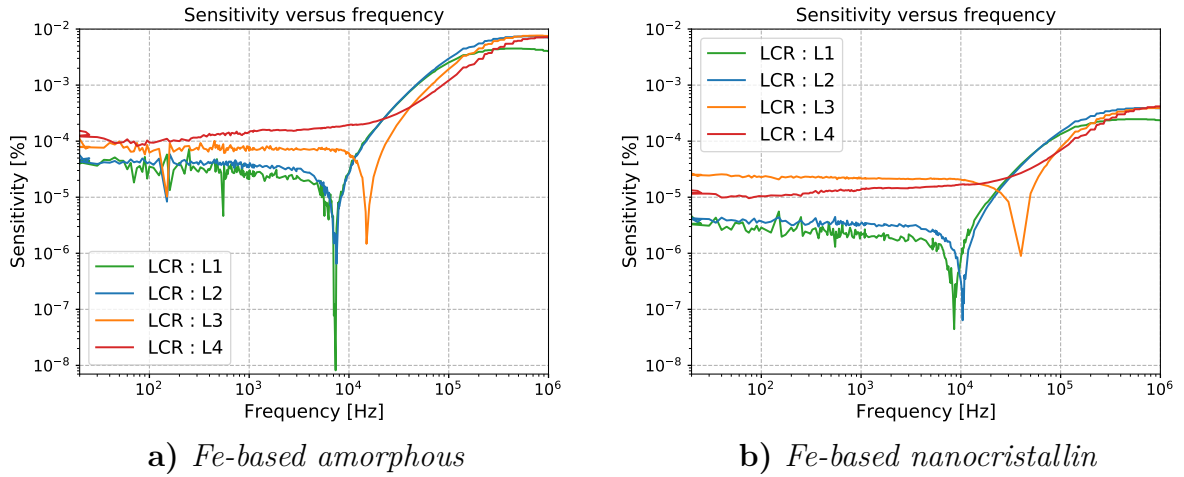


Figure 5.17: Comparison between the sensitivity LCR meter measurements of four different spiral planar inductors. The permeability of the flat inductor varies when an iron alloy foil is added to the inductor spirals. The iron is either amorphous (left figure) or nanocrystalline (right figure). It is the sensitivity of the LCR meter to this permeability variation that is studied. The sensitivity is calculated using Equation 4.3.

The main trend in these graphs is the low sensitivity value over the entire frequency range studied at the LCR meter.

This is consistent since the change in permeability translates into a change in inductance and the impact of the inductance on the total impedance is low at frequencies less than 10^5 Hz . Figure 5.17 confirms that as we approach 10^5 Hz , and therefore the area where the inductance has more influence on the impedance expression, the sensitivity increases.

It is assumed that a higher frequency measurement logically promises greater sensitivity since the inductance has a larger influence on the total impedance expression at high frequencies. To confirm the hypothesis a measurement of the inductances is carried out using a Vector Network Analyzer (VNA). These measurements were carried out by a

member of the research group. Figure 5.18 compares LCR meter and VNA sensitivity. Figure 5.18(a) is the LCR meter sensitivity over the frequency range 20Hz to 1MHz . Figure 5.18(b) is the VNA sensitivity over the frequency range 100kHz to 100MHz .

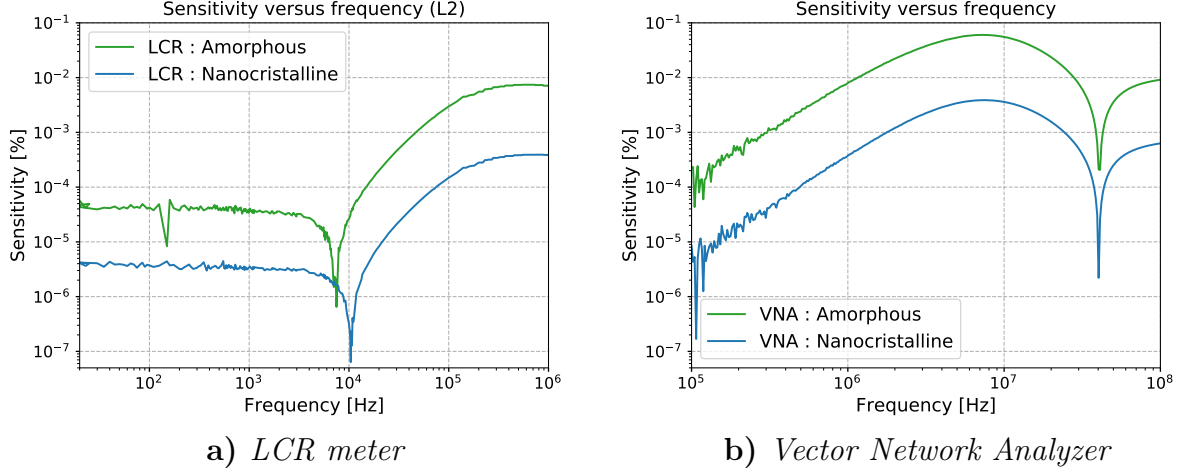


Figure 5.18: *LCR meter sensitivity to permeability change (a) compared to Vector Network Analyzer (VNA) sensitivity to permeability change. The devices do not work at the same frequency range. LCR meter frequency range starts from 20Hz to 1MHz . Usable VNA frequency range starts from 100kHz to 100MHz . LCR meter tests were repeated six times for each case (amorphous and nanocrystalline). VNA tests were repeated only once.*

It can be seen that the sensitivity of the VNA measurements is greater than that of the LCR meter measurements. Maximum sensitivity of the VNA is reached when the frequencies approach the Self-Resonant Frequency (SRF) of the inductor, defined by Equation 5.3 [37, 39].

$$SRF = \frac{1}{2\pi\sqrt{L_S C_S}} \quad (5.3)$$

5.7.2 Repeatability performance to permeability variation

LCR meter measurements on the four inductances considered are compared here with respect to their repeatability factor R (see Equation 4.4 in Section 4.3.2). Measurements are repeated 6 times for each case (Inductance without tuning, inductance tuned by a Fe-based amorphous ribbon and by a Fe-based nanocrystalline ribbon).

Repeatability of measured impedance modulus is studied. Figure 5.19 shows the evolution of the repeatability factor R as a function of frequency for the four inductances. Each sub-figure presents the comparison between the three permeability variation cases studied. A general observation is the very high repeatability of all inductance measurements in all situations. Indeed, factor K varies between 0.988 and 1.

To explain the reason for this high sensitivity, three statements must be put forward:

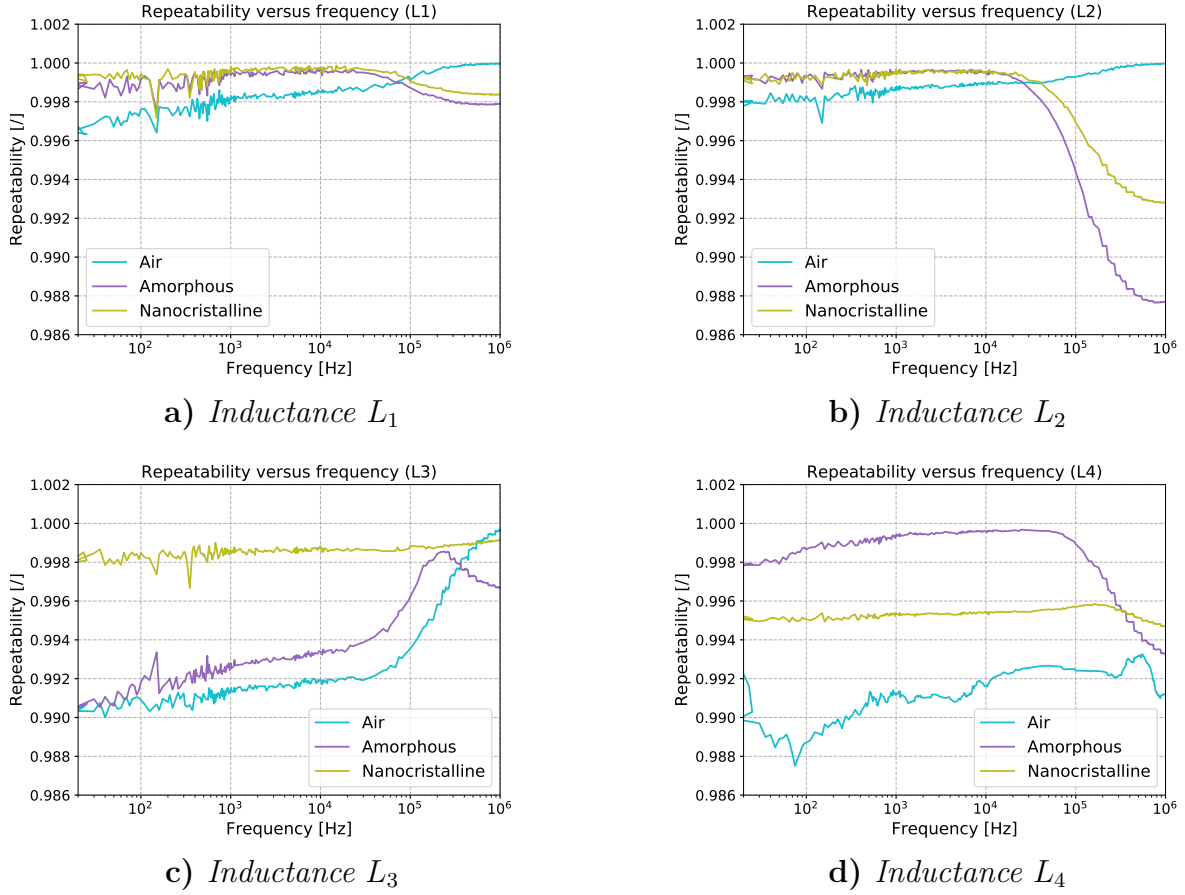


Figure 5.19: Repeatability factor R versus of frequency for the four inductances under study. Repeatability for each inductance is evaluated according to the three cases of permeability variations: inductance is not tuned (blue), inductance is tuned by an Fe-based amorphous ribbon (purple) and inductance is tuned by an Fe-based nanocrystalline ribbon (yellow). Means for the three cases were obtained on the basis of tests repeated six times.

- Measurements are performed at low frequencies and as shown in Figure 5.16, the influence of the inductor resistance predominates over the total impedance.
- At low frequencies, the influence of the skin effect on the current density within the conducting wires of the inductor is small.
- The value of the resistance depends on the skin effect.

Thus the resistance of the spiral planar inductance is almost constant at low frequencies since skin effect has no major effect on current density. And since resistance is constant and predominates over the expression of the impedance, the impedance value measured in each test is almost constant. And this is responsible for the very high repeatability.

One conclusion on the repeatability of the system is that it is much higher than the repeatabilities of the electrochemical systems studied in Section 5.6. An impedimetric measurement aiming to maximise its repeatability should turn to the detection of permeability variation.

Chapter 6

Discussion

This chapter provides a summary of the main findings from *Results* Chapter used to answer the research question. A critique of the strengths and limitations of the research is then developed. The Chapter concludes with an overview of the prospects for future work.

6.1 Conclusion on biosensor design

Given the results obtained and the constraints encountered in the comparison of the two detection methods, the research question must be divided into four parts to address it. As a reminder, the research question can be summarised as: « *Which **detection method** should be preferred, between **potentiometry** and **non-faraidic impedimetry**, in the design of a paper-based biosensor, depending on the **sample variation** that is to be analysed and the type of **paper** used in the Lateral Flow Assay **detection pad**?* ». The four parts are therefore:

1. For a **detection pad** consisting exclusively of a **nitrocellulose membrane unso-lidified** by a polyester layer, what **detection methods** should be favoured, at high and low frequencies, when the **conductivity** or **permittivity** of the sample being measured changes?
2. And if the **nitrocellulose membrane** is **solidified**, what about it?
3. Potentiometry has not been tested in the case of **permeability variation**, does this mean that **impedimetry** is **the right choice** for the biosensor design?
4. The power consumption of potentiometry has not been tested. The voltage range of the LCR meter covers a range of $5mV$ to $2V$ [45]. The voltage range of the Autolab on the other hand ranges from $0.2mV$ to $0.35V$ [74]. Working at high voltages is therefore much less suitable for the Autolab than for the LCR meter. The only question that arises from the analysis of the figure of merit that is the power consumption is: which **type of membrane** to favour (solidified or non-solidified) to **minimise the power consumption** of the biosensor?

6.1.1 What detection method with unsolidified nitrocellulose membrane and conductivity or permittivity variation?

Table 6.1 answers the first question.

Firstly, in the case of a variation in sample conductivity, low frequency detection is best done with a potentiometric method. In this way, the sensitivity and repeatability of the measurements is maximised. At high frequencies, the choice is less straightforward as maximum sensitivity is obtained via non-faraidic impedimetry while maximum repeatability is obtained via potentiometry. However, in both cases, the repeatability is very high, so a compromise is made: it is better to favour impedimetric detection.

Then, for permittivity variation of the sample, the case of low and high frequencies requires a compromise. As with conductivity variation, repeatability is always very important, regardless of the method, the choice of method will therefore be the one that maximises sensitivity. This is potentiometry for low frequency detection and impedimetry for high frequency detection.

Table 6.1: Summary of the best performance in sensitivity and repeatability of impedimetric and potentiometric detection in the case of a variation in conductivity or permittivity of the sample. The performance in relation to the type of variation is classified into two situations: low and high frequency. Maximum and minimum sensitivity and repeatability are also provided. If the choice of detection method is obvious because it maximises repeatability and sensitivity, the "TOTAL" line is completed with the name of the chosen method. If it is not straightforward because a trade-off between repeatability and sensitivity must be made, the "TOTAL" line includes "/".

		Conductivity variation $\Delta\sigma$ [0.0016 ; 17.49] $mS.cm^{-1}$		Permittivity variation $\Delta\epsilon_r$ [17.9 ; 80]	
		LF [20 ; 5k] Hz	HF [5k ; 1M] Hz	LF [20 ; 5k] Hz	HF [5k ; 1M] Hz
Sensitivity	Method	Potentiometry	Impedimetry	Potentiometry	Impedimetry
	Maximum [%]	0.3	0.6	1846	1447
Repeatability	Minimum [%]	3×10^{-4}	3×10^{-4}	210	900
Repeatability	Method	Potentiometry	Potentiometry	Impedimetry	Potentiometry
	Maximum [%]	98	95	94	96
TOTAL	Minimum [%]	88	88	78	82
TOTAL		Potentiometry	/	/	/

6.1.2 What about detection with solidified nitrocellulose membrane and conductivity or permittivity variation?

The use of a nitrocellulose membrane bonded to a polyester layer in the LFA detection pad of the biosensor makes it impossible to use potentiometric detection.

However, the opinion is more nuanced with regard to impedimetric detection. Indeed, the use of solidified nitrocellulose membrane offers the highest sensitivity when the sample varies little in conductivity (conductivity increase from 30 to 300) and at low frequency (from $20Hz$ to about $500Hz$).

Above $500Hz$, the use of a solidified membrane is not recommended.

As far as detection of permittivity variations is concerned, the choice of a solidified membrane is not recommended in all situations because sensitivity is always the lowest.

The solidification of the nitrocellulose membrane brings a major advantage: the repeatability of measurements. It is always better when the nitrocellulose membrane is accompanied by its polyester layer.

6.1.3 Is impedimetric detection the right choice for permeability variation?

The answer is yes, but only if the impedimetric detection is based on the Vector Network method and not on the auto-balancing bridge method. Indeed, an LCR meter such as the one used in *Result* Chapter operates over a maximum frequency of $20Hz$ to $1MHz$. A Vector Network Analyzer operates over a much wider frequency range ($100kHz$ to $100MHz$). A measurement performed up to $100MHz$ already provides better sensitivity levels.

6.1.4 How to minimise biosensor power consumption by adjusting the nitrocellulose membrane of the sensor pad?

One way to minimise the power consumption of the biosensor is to ensure that it can operate at a minimum voltage. The choice of solidifying the nitrocellulose membrane with a polyester layer influences this choice. To minimise the voltage to be applied, it is advisable to choose an unsolidified nitrocellulose membrane. A voltage of $20mV$ then provides the same results as detection with an applied potential of $200mV$.

That a measurement at $200mV$ on a solidified nitrocellulose membrane gives better results than a measurement at $20mV$ has not been discussed in the *Result* Section. The difference between $20mV$ and $200mV$ measurements on solidified nitrocellulose membrane is discussed in Subsection 6.2.3 of this Discussion. The question is whether a measurement at $200mV$ gives better results and if so, how are they better?

6.2 Strengths and limitations of the study

This Section presents the strengths and limitations of the research conducted in this master thesis. Firstly, the impact on measurements of exceeding the LCR meter limits is discussed. Then the equivalent circuit fitting will be briefly criticized. The low frequency influence of a non-zero electric present in the absence of an AC signal is discussed in the case of measurements with solidified NC membrane. Finally, the set-up of the Autolab measurements is looked at more closely to highlight the parasitic elements encountered.

6.2.1 Influence of exceeding the limits of the LCR meter at low frequency on high frequency measurements

LCR meter measurements on nitrocellulose membrane combined with a polyester layer exceed the impedance measurement capability of the instrument at low frequencies. In order to ensure that the measurements obtained at higher frequencies were usable and not affected by early measurement errors, a measurement over a smaller frequency range (1kHz to 100kHz) is performed. Figure 6.1 illustrates a comparison of LCR meter measurements carried out from 20Hz to 1MHz and from 1kHz to 100kHz . Measurements are made on a nitrocellulose membrane saturated with $1\mu\text{M}$ PBS with an applied voltage of 20mV .

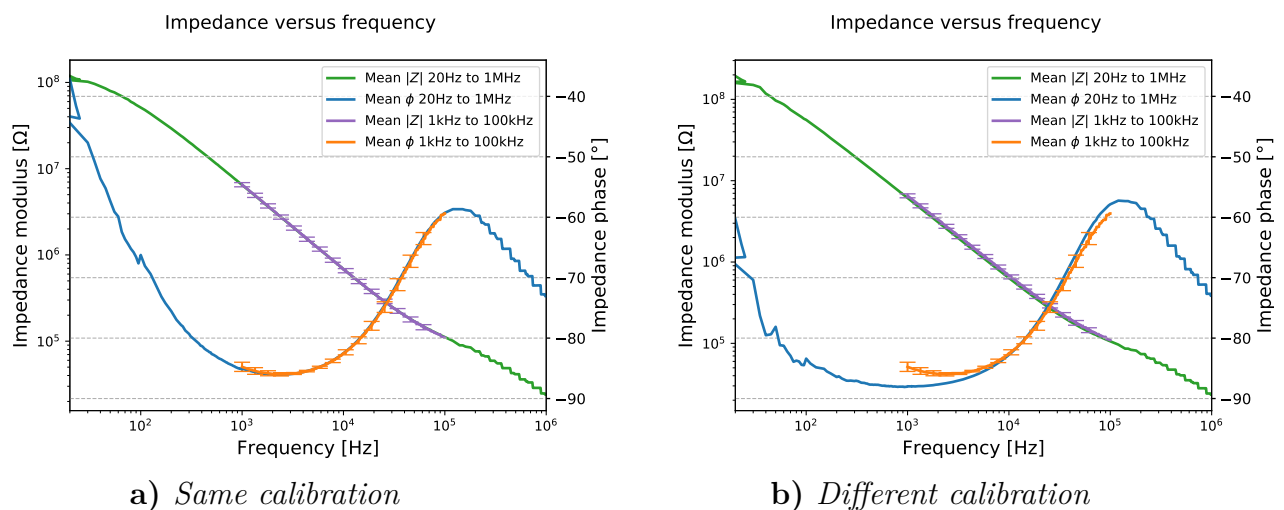


Figure 6.1: *Impedance versus frequency for LCR meter measurements. Comparison of measurements from 20Hz to 1MHz and measurements from 1kHz to 100kHz , in order to find out whether measurement errors occurring at low frequencies influence the result of high frequency measurements. Measurements are made on a nitrocellulose membrane saturated with $1\mu\text{M}$ PBS with an applied voltage of 20mV . Tests can be performed with the same calibration or not.*

Figure 6.1(a) shows this measurement when the calibration of the instrument is identical for all tests. The good news is that the LCR meter is not affected by measurement errors occurring upstream.

Figure 6.1(b) shows the same situation except that the calibration of the instrument between the measurements in the 20Hz to 1MHz frequency range and the 1kHz to 100kHz range is not the same. A difference between the two experiments exists. This highlights the importance of calibration when measuring with the LCR meter. As all the experiments conducted during this year were spread over a long period of time and the calibration of the instrument was reset at each start-up, comparisons of the Chapter result may be slightly biased by varying calibrations.

6.2.2 Fitting of the analytical model

In order to understand the physical behaviour behind the measurements, an equivalent circuit of the different systems studied was developed. In the case of the equivalent circuit for the solidified nitrocellulose membrane measurements, major deviations were observed between the fitting and the measurements, as illustrated in Figure 6.2.

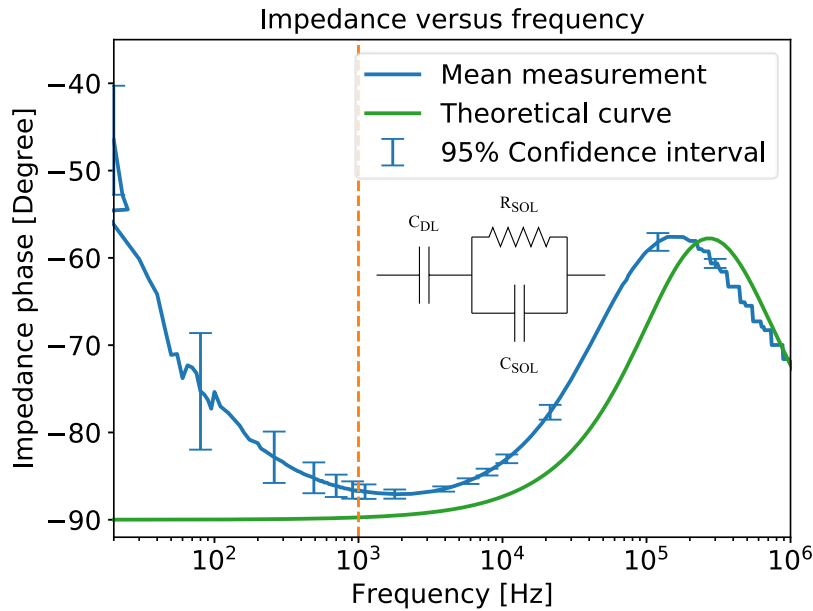


Figure 6.2: Major deviation between fitting and low frequency measurements ($< 10^3$ Hz). Measurements were made with a LCR meter on a solidified nitrocellulose membrane saturated with $10\mu\text{M}$ PBS. Measurements were repeated 12 times before taking the average.

Two measurements fittings, from 20Hz to 10^3 Hz and 10^3 Hz to 10^6 Hz, are carried out thanks to the software « Zview for Windows ». This tool is used to analyse EIS data. It takes as input a .txt files which provides the real and imaginary parts of the measured impedance, at each frequency considered. A form of equivalent circuit can be chosen and Zview compute accordingly the best values that will fit the measured impedance [75]. The fittings are shown in Figure 6.3. It can be concluded that the developed C(RC) equivalent circuit matches the measurements, but that the values of this circuit vary with frequency. The values for both circuits are provided in Table 6.2.

Table 6.2: Comparison of the equivalent circuit values of Zview fitting as a function of the frequency range covered by the fitting (20 to 10^3 Hz or 10^3 to 10^6 Hz).

Frequency [Hz]	C_{DL} [pF]	C_{SOL} [pF]	R_{SOL} [Ω]
20 - 10^3	86.2	35.6	1.01×10^8
10^3 - 10^6	23.6	8.46	5.61×10^4

For measurements on PBS-saturated nitrocellulose membrane, three main simplifying assumptions were made:

- The relative permittivity of NC membrane saturated with PBS is constant over the frequency range and equals 80.
- The conductivity of NC membrane saturated with PBS is constant over the frequency range and has been computed thanks to a conductivity probe.

The permittivity of PBS was approximately equal to the permittivity of the deionized water and the deionized water has a permittivity that decreases as the frequency increases. This explains why the double layer capacitance C_{DL} and solution capacitance C_{SOL} decreases with increasing frequency (see Table 6.2). The fitting developed in *Materials and methods* Chapter fits better with measurements from 10^3 Hz to 1 MHz than with measurements from 20 Hz to 10^3 Hz (see Figure 6.2). This makes sense since the constant permittivity approximation is very close to reality in the upper frequency range.

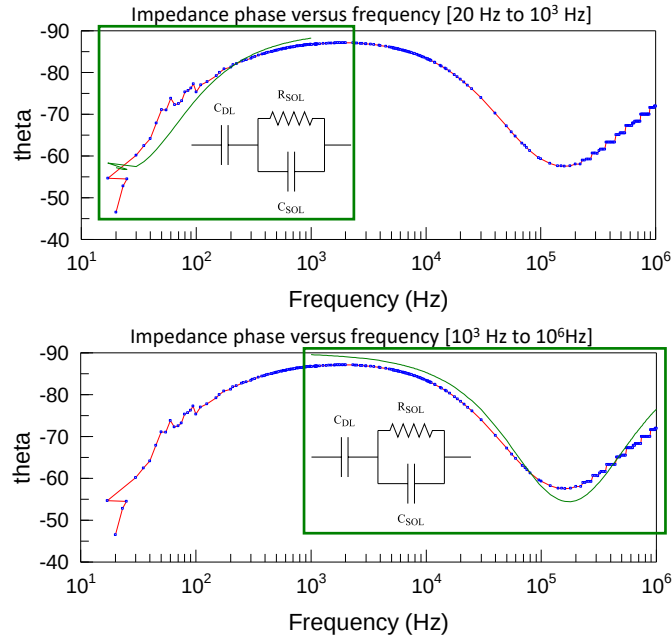


Figure 6.3: Zview fitting of measurements on solidified nitrocellulose membrane saturated with $10\mu\text{M}$ PBS. The associated fitting circuit is also supplied. The fitting is performed from 20 Hz to 10^3 Hz (upper graph) and from 10^3 Hz to 10^6 Hz (lower graph).

The conductivity of the samples was also assumed to be constant, but a search of the scientific literature shows that the conductivity of PBS increases with frequency [54]. The

solution resistance R_{SOL} is inversely proportional to the conductivity, which explains why it decreases with increasing frequency (see Table 6.2).

6.2.3 Link between conductivity, applied potential and frequency

When measurements were performed at $200mV$, a relationship between sample conductivity, applied voltage and phase impedance was observed at low frequencies. Indeed, for a voltage of $200mV$ and a sample of low conductivity, the phase impedance measurement was lower than the values obtained at $20mV$. For a 100 times more conductive sample, the phase impedance measured at $200mV$ and at $20mV$ was more or less similar. For a sample that was still 100 times more conductive, the phase impedance at $200mV$ was then greater than that at $20mV$.

A possible explanation has been found in the scientific literature. G. Barbero, L. Alexe-Ionescu and I. Lelidis (2005) state that at low frequencies, the electrolytic cell acts as a linear system as long as the amplitude of the applied potential remains below the thermal voltage ($U_t = k_B T / q$), where $k_B T$ is the thermal energy and q the modulus of the electrical charge of the ions [76]. C.Chuang and M.Shaikh add that the amplitude of the AC voltage must be less than about $50mV$ to obtain a linear current response. If this condition is repeated, the electrical impedance defined by the ratio of voltage to current is independent of the applied voltage [16].

When the applied voltage is higher than $50mV$, the influence of a non-zero electric field present in the absence of an AC signal becomes relevant in the characterisation of the electrochemical system [77].

Barbero et al. (2018) explain that this non-zero electric field can be due to polarisation caused by electrodes with different absorption properties depending on whether the ions are positive or negative. But it can also be caused by a DC bias [77]

The presence of bias is responsible for an increase in the resistance plateau of the electrochemical cell. This can be explained by the decrease of the bulk concentration of ions, due to the presence of the bias, responsible for an increase of the surface density of ions [77].

For my master thesis, I will tend to advise working at a voltage of $20mV$ instead of $200mV$ to keep the system linear and therefore as simple as possible to study.

6.2.4 Parasitic elements in autolab set-up

The measurement set-up for the Autolab potentiostat introduces parasitic elements that are not taken into account in the research. These parasitic elements are illustrated in Figure 6.4 and explained hereafter.

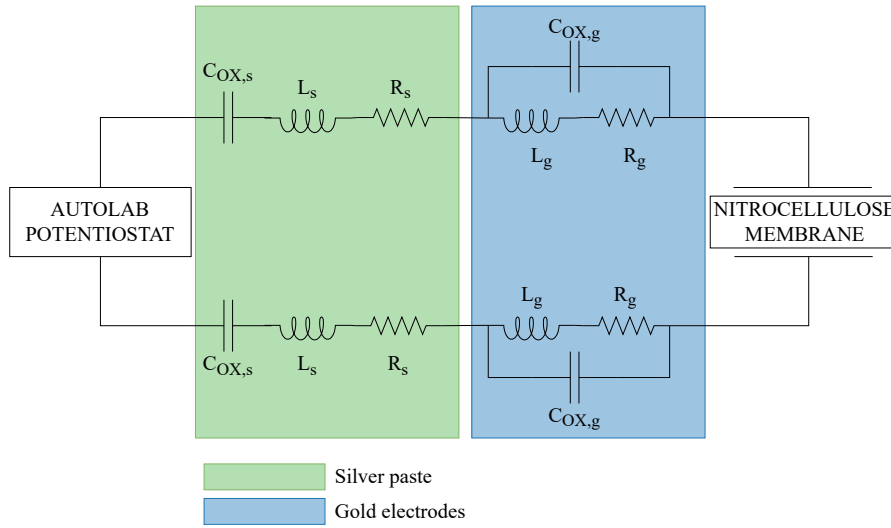


Figure 6.4: *Equivalent circuit of the autolab measurement set-up including the parasitic elements introduced by the gold electrodes and the silver paste between the autolab electrodes where the automatic calibration plan of the instrument stops and the nitrocellulose membrane.*

Oxidation layer

An oxide layer on the surface of a metal can form when it is in contact with the ambient air or with an aqueous electrolyte. The use of silver in electrical contacts is common because silver has a high resistance to oxidation [78].

Gold is regularly used in the field of electrochemistry because it is assumed that it does not exhibit an active redox species. It only exhibits a non-faraidic or double layer behaviour over a potential range from 0V to 1.3V. However, it is possible that oxyspecies are formed in the double layer region. This is due to the fact that all metal surfaces have defects that offer the possibility for electrocatalytic reactions to occur [79]. Gold oxide even seems to have a two-layer structure [80].

Equivalent inductance

The equivalent inductance induced by the silver paste and the gold electrode can be approximated by the formula for the inductance of a straight wire. This is given in Equation 6.1 [81]. This formula is valid for a low frequency range, where the skin effect still has little impact [82].

$$L = \frac{\mu_0}{2\pi} [l.\log(2l) - l.\log R - l + R] \quad (6.1)$$

where

- μ_0 is the permeability of free space in $[H.m^{-1}]$,
- l is the length of the wire and
- R is the radius of the wire.

Equivalent resistance

The equivalent resistance may be approximated by Equation 6.2 or 6.3, when frequencies are low or high, respectively. These Equation were detailed in Chapter 2 and 4.

$$R_s = \frac{\rho l}{wt} \quad (6.2)$$

$$R_s = \frac{\rho l}{w\delta(1 - e^{-\frac{t}{\delta}})} \quad (6.3)$$

The capacitance representing the oxidation layer at the silver paste is in series because the current must pass through this layer before reaching the silver paste. The capacitance associated with the oxidation layer of the gold electrode is in parallel since it has the same voltage across it as the gold electrode.

These differences between the Autolab and LCR meter setup lead to a slightly biased comparison of the measurements. It was nevertheless interesting to study the Autolab set-up because it corresponds more closely to a real implementation of a biosensor equipped with an LFA than the parallel-plates used with the LCR meter.

6.3 Towards miniaturisation

The biosensor developed in the thesis to which this master thesis is attached aims to be a Point-of-Care biosensor. The scientific and technological obstacles to be overcome in order to develop a biosensor that is operational in the field lie in particular in the miniaturisation of the transducers and transmitters [83]. Consequently, a miniaturisation of the detection methods studied in this master thesis is necessary. This section briefly discusses some possibilities for future miniaturisation.

Low-cost and miniaturized potentiostat

In the scientific literature there are still few low-cost miniaturized potentiostats capable of being integrated into Point-of-Care (PoC) devices. Therefore, Pruna et al. set out to develop one [84]. In summary, the potentiostat can be divided into three parts: the power supply, the digital stage and the analogue stage. The power supply consists of four low-power integrated chips. The digital stage contains two microcontrollers that generate the waveform, ensure the acquisition of the responses, optimal post-processing of the signal *in situ* and communication with an external device such as a computer. The analogue stage includes operational amplifiers and feedback loops. It is also equipped with eight working gold microelectrodes and integrated reference and counter electrodes. This potentiostat has clear advantages in the development of PoC respecting the ASSURED criteria. Indeed, it has dimensions adapted to a field use ($12 \times 7 \times 2 \text{ cm}^3$), weighs only 70g and its manufacturing cost is close to 300USD. In terms of performance, it operates at frequencies below 10Hz .

Smartphone-controlled electrochemical impedance spectroscopy

Zhang et al. developed a smartphone-controlled biosensor using Electrochemical Impedance Spectroscopy (EIS) [85]. The biosensor is fast, sensitive, selective and portable. It is equipped with a miniaturised biosensor, a hand-held EIS detector and a smartphone. The hand-held EIS detector converts biomolecular interactions into electrochemical variations. This detector then transmits the impedance signals to the smartphone via bluetooth. The purpose of the smartphone is to control the electrochemical measurement process. Then it analyses the impedance signals. It is equipped with an Android application providing a user-friendly interface between the users and the smartphone-controlled system.

The main advantage of this biosensor is its connection to smartphones. Indeed, in 2021, 6.648 billion people owned a smartphone, which is about 83.37% of the population [86]. This simplifies access to the biosensor for everyone. Moreover, the connection of a biosensor to a smartphone allows the biosensor to access the advantageous features of the smartphone such as its touch screen or its powerful data storage [85].

From a result point of view, the impedances are recorded on 25 different frequency points evenly distributed on a logarithmic axis from $10Hz$ to $10kHz$. The magnitude of stimuli signals was set at $200mV$ [85]. This second biosensor corresponds more closely than the potentiostat of Pruna et al. to the specifications studied in this master thesis.

Increasing the sensitivity of a biosensor with an AC/DC bridge

Table 6.1 shows that the sensitivity of the potentiostat and LCR meter to a change in conductivity is very low. An example of an AC/DC bridge structure, developed by Neshani et al. (2020), can enhance the sensitivity of sensors that produce small fractional changes (1%) at the electrode-solution interface in capacitance for fast, real-time sensing applications [19].

In summary, the capacitive differential bridge structure consists of two functionalized electrodes (one working and one counter), an AC and DC excitation and a balancing capability. An electrochemical capacitance electrode requires a DC bias in addition to the AC excitation to operate optimally [19].

One of the strategies developed by Neshani et al. to adapt the detection to a PoC device is the use of readout amplifiers commercially available, fully differential operational amplifiers which are therefore low cost and provide low complexity [19].

The sensor developed by Neshani et al. is a non-faradaic one. The advantage of non-faradaic or capacitive biosensors is that they are simple as they do not include a redox probe and are therefore particularly suitable for PoC applications. A particular property of this type of sensor is that they have the ability to make measurements at a single frequency. It is therefore easier to implement them than a potentiostat or a faradaic sensor [19]. However, since any element is detected via this method without any specificity, the biofunctionalisation of the membrane is particularly important to ensure that it is the

target that is detected. In addition, the use of negative controls in parallel is essential [16].

Chapter 7

Responsible approach: Eco-design

The emergence of PoC biosensors and the spread of their use around the world is increasing the impact they can have on the environment. Figure 7.1 presents the research questions for a multidimensional analysis of the design of a PoC water quality sensor that meets the ASSURED (Affordable, Sensitive, Specific, User friendly, Rapid and robust, Equipment free, Deliverable to end users) criteria [6, 49].

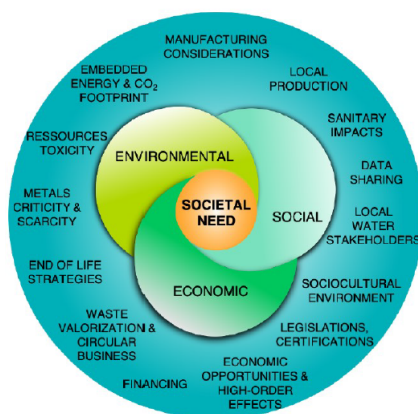


Figure 7.1: *Emergence of research questions for the multidimensional analysis of affordable water quality sensors [49].*

This Chapter deals with environmental issues and their close link with economic issues. Recycling, which was embryonic in the 1970', has since spread throughout Europe and is an integral part of the economy [87]. The search for profitability is therefore a key factor.

7.1 Eco-design definition

Eco-design is one of the seven pillars of the circular economy defined by Ademe, the French agency for ecological transition. Eco-design is itself part of circular design, which takes into account not only the environmental impact of a product but also its social and economic impact [88, 89].

Eco-design aims to design products that respect the environment throughout their life cycle. It aims to integrate the notion of the environment from the product design

stage. Indeed, this method is based on the assumption that, as the manufacturing stages of a product progress, the possibilities of reducing environmental impacts decrease due to the reduction of available technical choices. It is claimed that about 80% of a product's impacts are determined at the design stage [88]. Eco-design is therefore a preventive method, which operates upstream of product manufacture [89].

This method includes a Life Cycle Assessment (LCA) of the product, but this is only one of the six stages of eco-design, as illustrated in Figure 7.2 [88, 90].

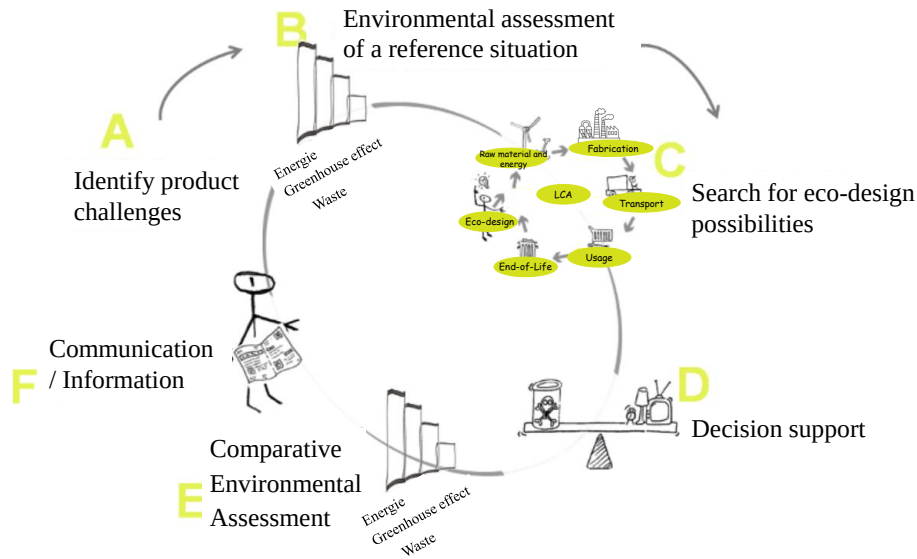


Figure 7.2: *The six cyclical stages of product eco-design [90].*

The six eco-design steps illustrated in Figure 7.2 are explained hereafter [90].

1. A - Identify product challenges

Identification of the challenges related to the product in order to size the eco-design project best suited to the context.

2. B - Environmental assessment of a reference situation

This stage occurs after the product challenges have been put into context. It therefore includes a first draft of the design which is taken as a design reference for the rest of the eco-design.

3. C - Search for eco-design possibilities

Eco-design ideas are searched and analyzed.

4. D - Decision support

The eco-design options found in the previous stage are placed in parallel with each other in order to help in the decision-making process.

5. E - Comparative environmental assessment

This third part aims to compare the chosen reference situation with the supposedly

improved design. An important element in this step is to ensure that the environmental impacts of the new design have not just been displaced. For example, if whether a reduction in manufacturing impact would have resulted in new environmental impacts at the recycling stage [89].

6. F - Communication/Information

This stage concerns the marketing of the product. The objective is to promote the environmental approach behind the manufacture of the product.

7.2 Objectives of responsible design assessment

The eco-design of the envisaged biosensor requires an analysis of the three parts of the biosensor, namely the Lateral Flow Assay (LFA) and its container, the electrical reading circuit responsible for the electrochemical detection and the plastic cassette containing the whole, as illustrated in Figure 7.3.

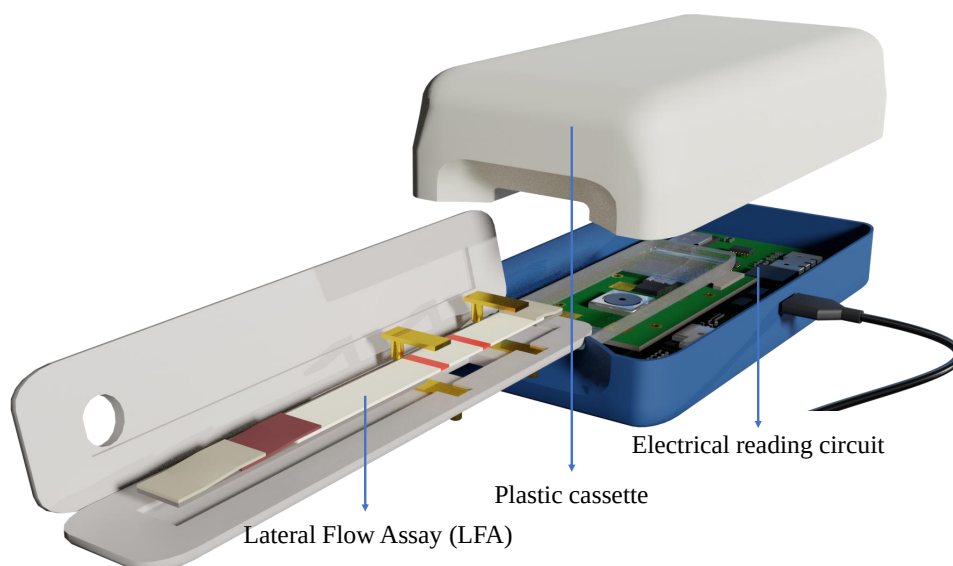


Figure 7.3: *Prototype including the three parts of the biosensor, i.e. the Lateral Flow Assay (LFA) in its container, the integrated circuit responsible for the electrochemical detection and the plastic case containing the whole (illustration by Nicolas Moreau)*

This chapter will only take a first look at eco-design by analysing, as a priority, the design of the plastic cassette. It is oriented around the three points of eco-design highlighted above. Firstly, eco-design possibilities are studied. Then, once all the design choices have been made, the prototype is compared with the reference prototype using RecyClass. RecyClass is an online tool developed by Valipac, at the initiative of the Plastics Recyclers Europe association. This online tool allows the evaluation of the recyclability of industrial packaging. The recyclability study of the prototypes via Recyclclass is summarised in the comparison section.

Valipac was established in 1997 on the initiative of the Belgian business world as a collective response to the Extended Producer Responsibility (EPR) programme for industrial packaging [91]. EPR is a policy approach in which producers are assigned a responsibility, financial and/or physical, for the treatment or disposal of post-consumer products. The aim is to provide an incentive to prevent waste at source and to encourage the design of environmentally friendly products [92].

7.3 Search for eco-design possibilities

The eco-design envisaged is a biosensor composed of three parts, each of which is perfectly independent of the others. The plastic housing is therefore designed as a unit, without taking into account the LFA or the integrated circuit.

An important step in the choice of housing design is the choice of plastic used. There are seven main families of plastic [93, 94]:

- **Polyethylene Terephthalate (PET)** common in the manufacture of food packaging.
- **High-Density Polyethylene (HDPE)** common in household product bottles.
- **Polyvinyl Chloride (PVC)** used in cheese and meat packaging.
- **Low-Density Polyethylene (LDPE)** show same properties as HDPE but more commonly used in plastic bags and packaging.
- **Polypropylene (PP)** common for medical packaging.
- **Polystyrene (PS)** used for food packaging, furniture, etc.
- **Polycarbonate (PC), epoxy resins, Teflon (PTFE), melamine or the rubber family** (latex, neoprene foam).

PP is selected because it is water-repellent, thus providing good protection from surroundings for the LFA and the integrated circuit. In addition, it is a common plastic used in medical packaging [95]. Moreover PP has the advantage of being light, resistant to chemicals, electrically insulating, cheap. The main drawback of PP is its sensitivity to UV light which can damage the mechanical properties of the plastic when used externally, which will be the case for the biosensor [93, 96]. Therefore, UV protection should be implemented.

A second design choice is the manufacturing method of the plastic cassette. In order to limit waste, an additive manufacturing technique is envisaged. It is also called 3D printing. Polypropylene has the disadvantage of being difficult to print because it requires constant temperature control. Nevertheless, it is commonly used in 3D printing [96].

The next design steps are inspired by the different issues addressed in the RecyClass analysis of the case. Each choice will be made with the aim of maximising the recyclability

of it.

In a plastic recycling stream, waste will be sorted according to its main polymer, its shape (rigid or flexible) and its colouring. A plastic is considered coloured when a mixture is used to colour the main polymer resin. Even a white colouration is considered a colouration [97].

Recycling efficiency is closely related to the main polymer of the packaging. The casing is therefore designed to be 100% PP and transparent. Indeed, the box must be transparent in order to be effectively identifiable by optical sorting devices. The most common optical method in Europe is Near-Infrared (NIR) Spectroscopy. Optical identification is faulty and costly when the surface of the plastic is coloured by undetectable carbon black, due to light absorption. Moreover the housing has only right angles, since a rounded design makes recycling more complex. The box is rigid but not extremely so as not to make the compaction difficult [97].

Other secondary choices that maximise the recyclability of the box are [97]:

- No labels glued to the case to ensure optical identification.
- No sleeves because complex box geometries make it harder to recycle.
- No multi-layer structures because different polymers welded together is to avoid.
- No metallisation (e.g. adding a layer of aluminium to the inside of the case) since it will leave the plastic recycle stream and join the aluminium recycle stream.
- No mixing of rigid and flexible plastics.
- No locking system. The two parts of the case are designed so that they clip together.

Moreover, the consumer should be able to separate the case from the LFA and the integrated circuit itself with a simple downward whip of the open case to place the housing in a plastic recycling line afterwards. The plastic case, the LFA container and the integrated circuit are therefore not welded together [97].

Particular attention must be paid to isolating the case from the nanoparticles contained in the LFA. Indeed, a contamination of the plastic would lead to a contamination of the recycling chain and thus of the recycled plastics [97].

PP is not chosen to be biodegradable or oxo-degradable in this case as it becomes incompatible with existing plastic recycling chains. Indeed, the polymers making up biodegradable plastics cannot be mixed with standard polymers as they have a negative impact on the properties of the recycled plastic [97].

7.4 Comparative environmental assessment

The reference design is a 100% PP box, bicolour and the integrated circuit is not removed until it enters the recycling chain. The optimized design is also a 100% PP box but the integrated circuit is removable. The criteria that play a role in the recyclability score of the design and reference boxes are shown in Table 7.1. The final score of the box is also shown. The definitions of the scores are [97]:

- **CLASS A** - «The packaging does not pose any recyclability issues and the recycled plastics can potentially feed a closed-loop scheme to be used in the same quality application ».
- **CLASS D** - «The packaging has significant design issues that highly affect its recyclability or imply large material losses. In both cases the recycled plastic can only be fed into low-value applications ».

Table 7.1: *Comparison between the criteria that differ between the reference and the optimised case (without the integrated circuit) in order to obtain their recyclability score. These scores were obtained by following the RecyClass methodology for both cases (see [97]).*

Reference container	Designed container
Bicolour (dark colours)	Transparent
Rounded geometry	Simple geometry (right angle)
Rigid	Low stiffness
Net weight > net weight PP	Net weight < net weight PP
	

It can be seen that even minor changes can lead to big changes in the recyclability of the product.

7.5 Conclusion

This analysis is only a preliminary to the eco-design of the biosensor studied. The challenge ahead is to design the integrated circuit in a circular dynamic.

We are aware that there is a de facto gap between conceiving, or just stating, that a material is recyclable and the fact that it is actually taken care of after use. Indeed,

although the designated box is maximally recyclable, it still needs to be recycled. The following map (see Figure 7.4) shows the different European countries where a box like the one designed could be recycled.

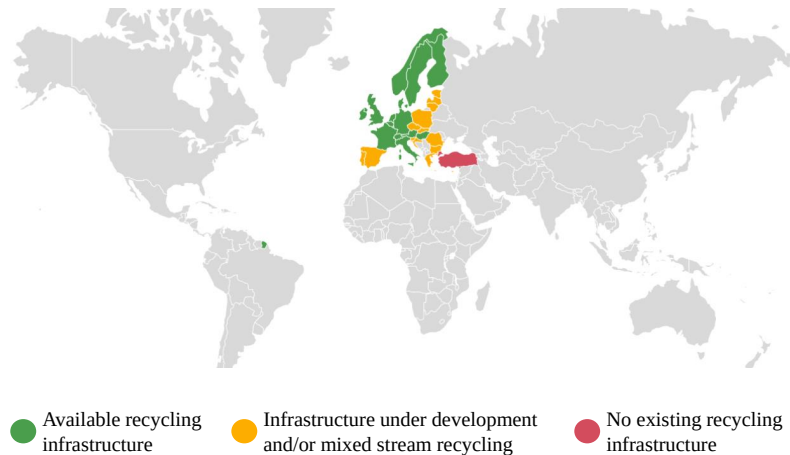


Figure 7.4: *Recycling possibilities of the designed casing depending on the European country where the biosensor is used [97].*

The economic argument takes precedence: the simple fact of collecting, storing and handling the waste incurs a basic cost. A logistical study of this part of recycling is necessary. Recycling yards, bonding, relocation of processing (with the risk of losing control of the process)...

The multiplicity of components further increases the risk of additional costs. Components that are easy to disassemble are a guarantee of both acceptable cost and quality of the finished recycled product.

The best waste is that which is not produced. This is a very current leitmotiv. And, in view of the few decades of experience worldwide, we are all aware that the only effective recycling must be EASY and CHEAP.

And because recycling can be expensive and random, it has become obvious that we need to move from "design" to "eco-design" of products, integrating ecology at the very beginning of the design process.

Chapter 8

Conclusion

Water quality assessment is a priority to ensure optimal life conditions for all human beings. In order to support the right to water as defined by the UN, water quality monitoring needs to move beyond the domain of laboratory to field measurements carried out by anyone [98].

In order to go one step further in this objective, a Point-of-Care biosensor project is currently developed by Jean-Pierre Raskin's research team at the Ecole Polytechnique de Louvain. My master thesis is integrated in this development. More specifically, it consists in a comparison between the performances of potentiometry and non-faradaic impedimetry detection. It was conducted on a simplified system composed of nitrocellulose membrane similar to the detection pad of a classical Lateral Flow Assay, which is used in the developed biosensor. The comparison is done using three different types of sample variation. These are variation in conductivity, permittivity and permeability. In order to simplify the approach, permeability variation was not derived from a nitrocellulose membrane soaked with a liquid sample, as it is the case for conductivity and permittivity, but by a variable spiral planar inductance. The nitrocellulose membrane was either solidified with a polyester layer on one side or not solidified. This choice will be relevant to the LFA design of the biosensor. It is therefore necessary to understand the impact it has on potentiometric and non-faradaic impedimetric detection.

The results of the measurements are analysed using a simplified equivalent circuit developed for each investigated system.

A first result allows the following conclusion: if the nitrocellulose membrane is unsolidified and the sample variation is conductive or permissive, at low frequencies, it is advisable to perform the detection with a potentiostat type device. On the contrary, non-faradaic impedimetry is recommended at high frequencies. These settings are used to maximize the sensitivity of the measurements. This maximization is done at a slight expense of the repeatability factor.

It was shown that the use of a nitrocellulose NC membrane combined with a polyester layer makes measurements with potentiostat impossible. Consequently, if a solidified NC membrane is adopted for the LFA design in the future, only non-faradaic impedimetric

detection is available.

Regarding the detection of permeability variation, it is revealed that the potentiostat is not suitable for this type of detection either. Concerning non-faradaic impedimetry, measurement sensitivity was particularly low. It is suggested that a device such as the Vector Network Analyzer (VNA) could be used to study permeability variations. Indeed, permeability variations are more significant at very high frequencies and VNA rises much higher in frequency than the LCR meter.

A first step in minimising the power consumption of the biosensor has been stated. Indeed, the use of a unsolidified nitrocellulose membrane offers similar measurements performance whether the voltage applied is $20mV$ or ten times higher.

An introduction to eco-design is also presented in this work. It is applied to the simplified case of a plastic cassette that can contain the future LFA and electrical reading circuit. The RecyClass tool is used in the evaluation of the design choices for the plastic cassette. It was found that the container should be as simple as possible to promote recycling. The biosensor should be composed of three distinct parts (LFA, electrical reading circuit and container). This should allow a separation of the three parts before recycling and thus improve the recyclability of the biosensor and its impact on the environment.

In conclusion, the results obtained in this master thesis aim to provide some guidelines for the future design of PoC biosensors equipped with an LFA. The future prospects concern the miniaturisation of the detection systems. Future work will also be supported by the scientific literature that provides some leads for the development of PoC devices based on potentiometry and impedimetry. Three technologies are suggested as possible candidates in this master thesis. In particular, one of them proposes the implementation of an AC/DC bridge that would increase the sensitivity of the biosensor. This could be implemented in the case of impedimetric and potentiometric detection when a change in conductivity occurs, which currently has a very low sensitivity. In addition, future research may aim to combine biosensors with the use of smartphones. Indeed, smartphones are widely used around the world (around 85% of the population own one). The use of biosensors could be encouraged as at least 4/5 of the population would have access to the tool responsible for post-processing. Through dedicated application, the interaction would be simplified between the biosensor and the user.

Appendices

Appendix A

Paper-based biosensors

Table A.1 provides the detection methods compatible with three different paper-based biosensors: Dipstick assays, Lateral Flow Assay (LFA) and μ PADs.

Table A.1: *Classification of various methods used with the different types of paper-based analytical devices [9, 11, 12, 99, 100, 101].*

Dipstick assays	Micropaper-based analytical devices	Lateral flow assays
Colorimetric	Colorimetric	Colorimetric
	Fluorescent	Fluorescent
	Electrochemical	Electrochemical
	Electrochemiluminescent	Chemiluminescent
	Surface-enhanced raman spectroscopy (SERS)	Surface-enhanced raman spectroscopy (SERS)
	Photoelectrochemical	
	Chemiluminescent	

Table A.2 shows the advantages and disadvantages of each of the three paper-based analytical devices.

Table A.2: *Advantages and disadvantages of all three paper-based analytical devices [9, 11, 12, 99, 100, 101].*

Device	Advantages	Disadvantages
Dipstick assays	Rapid detection (min) Simple design Ease of manufacturing and usage Lightweight Low-cost Equipment-free	Optical detection only Only qualitative analysis No multistep detection procedures No sophisticated assays
μ PADs	Simple fabrication Equipment-free Quick analysis (5-10min) Small sample volume (5-10 μ L) Real-time analysis Quantitative detection	Difficult mass production No flow control Limited precision Possibility of complex and multiple steps Long optimization time Require trained personnel
LFA	User-friendly Quick analysis (5-15min) Low-cost Multiplexed detection No sample pre-treatment required/ No washing steps necessary Equipment-free Good stability Long lifetime Possible quantification	Limited sensitivity Semi-quantitative detection Long fabrication Large sample volume (~ 100 μ L) Detection time influenced by viscosity of sample (Sample must be in liquid state)

Appendix B

Critical values of t for two-tailed tests

Table B.1: *Critical values of t for two-tailed tests [102].*

Critical values of t for two-tailed tests								
Significance level (α)								
Degrees of freedom (df)	.2	.15	.1	.05	.025	.01	.005	.001
1	3.078	4.165	6.314	12.706	25.452	63.657	127.321	636.619
2	1.886	2.282	2.920	4.303	6.205	9.925	14.089	31.599
3	1.638	1.924	2.353	3.182	4.177	5.841	7.453	12.924
4	1.533	1.778	2.132	2.776	3.495	4.604	5.598	8.610
5	1.476	1.699	2.015	2.571	3.163	4.032	4.773	6.869
6	1.440	1.650	1.943	2.447	2.969	3.707	4.317	5.959
7	1.415	1.617	1.895	2.365	2.841	3.499	4.029	5.408
8	1.397	1.592	1.860	2.306	2.752	3.355	3.833	5.041
9	1.383	1.574	1.833	2.262	2.685	3.250	3.690	4.781
10	1.372	1.559	1.812	2.228	2.634	3.169	3.581	4.587
11	1.363	1.548	1.796	2.201	2.593	3.106	3.497	4.437
12	1.356	1.538	1.782	2.179	2.560	3.055	3.428	4.318
13	1.350	1.530	1.771	2.160	2.533	3.012	3.372	4.221
14	1.345	1.523	1.761	2.145	2.510	2.977	3.326	4.140
15	1.341	1.517	1.753	2.131	2.490	2.947	3.286	4.073
16	1.337	1.512	1.746	2.120	2.473	2.921	3.252	4.015
17	1.333	1.508	1.740	2.110	2.458	2.898	3.222	3.965
18	1.330	1.504	1.734	2.101	2.445	2.878	3.197	3.922
19	1.328	1.500	1.729	2.093	2.433	2.861	3.174	3.883
20	1.325	1.497	1.725	2.086	2.423	2.845	3.153	3.850
21	1.323	1.494	1.721	2.080	2.414	2.831	3.135	3.819
22	1.321	1.492	1.717	2.074	2.405	2.819	3.119	3.792
23	1.319	1.489	1.714	2.069	2.398	2.807	3.104	3.768
24	1.318	1.487	1.711	2.064	2.391	2.797	3.091	3.745
25	1.316	1.485	1.708	2.060	2.385	2.787	3.078	3.725
26	1.315	1.483	1.706	2.056	2.379	2.779	3.067	3.707
27	1.314	1.482	1.703	2.052	2.373	2.771	3.057	3.690
28	1.313	1.480	1.701	2.048	2.368	2.763	3.047	3.674
29	1.311	1.479	1.699	2.045	2.364	2.756	3.038	3.659
30	1.310	1.477	1.697	2.042	2.360	2.750	3.030	3.646
40	1.303	1.468	1.684	2.021	2.329	2.704	2.971	3.551
50	1.299	1.462	1.676	2.009	2.311	2.678	2.937	3.496
60	1.296	1.458	1.671	2.000	2.299	2.660	2.915	3.460
70	1.294	1.456	1.667	1.994	2.291	2.648	2.899	3.435
80	1.292	1.453	1.664	1.990	2.284	2.639	2.887	3.416
100	1.290	1.451	1.660	1.984	2.276	2.626	2.871	3.390
1000	1.282	1.441	1.646	1.962	2.245	2.581	2.813	3.300
Infinite	1.282	1.440	1.645	1.960	2.241	2.576	2.807	3.291

Appendix C

Graphs of equivalent circuit impedance versus measured impedance

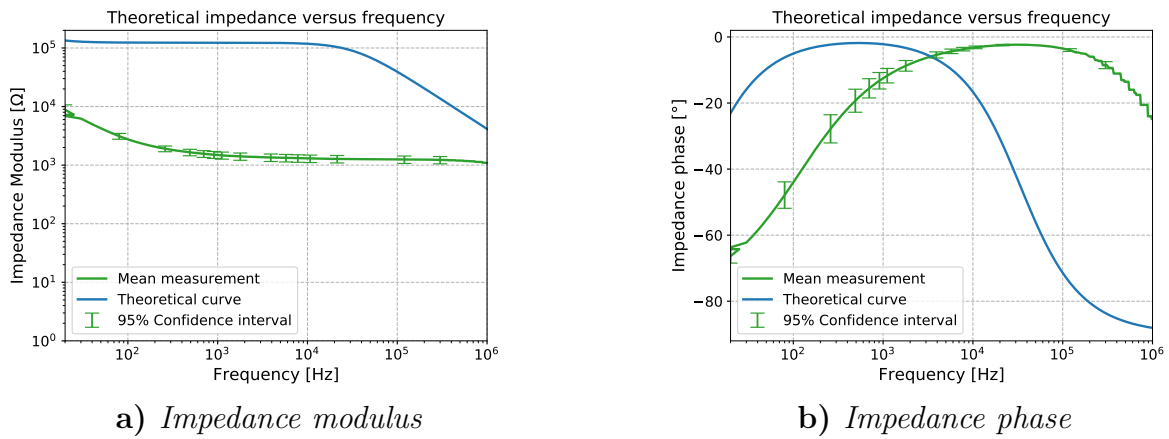
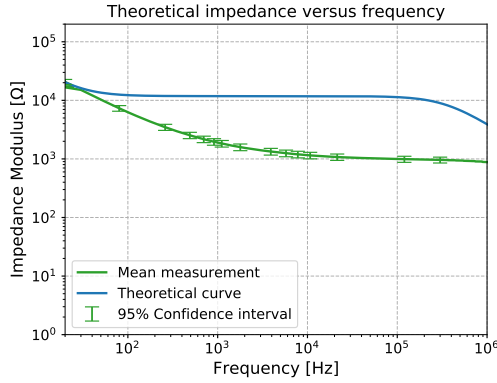
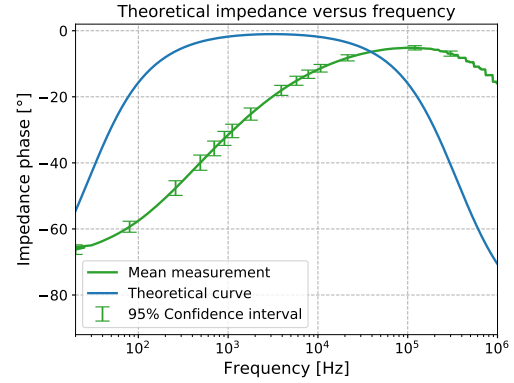


Figure C.1: Comparison between the equivalent circuit impedance and the impedance measurements of the electrochemical system consisting of a nitrocellulose membrane saturated with Phosphate-Buffered Saline $1\mu M$, both as a function of frequency. The LCR meter measurements were repeated 12 times before averaging the impedance.

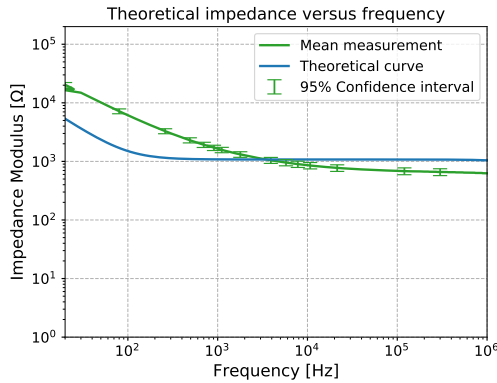


a) Impedance modulus

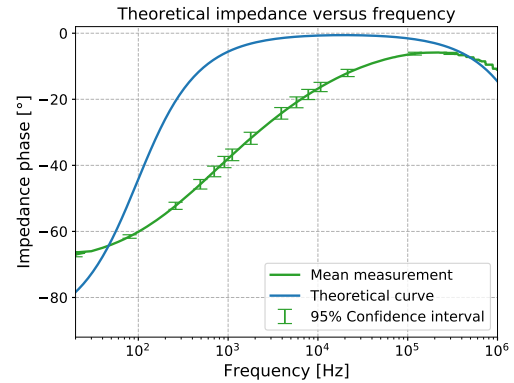


b) Impedance phase

Figure C.2: Comparison between the equivalent circuit impedance and the impedance measurements of the electrochemical system consisting of a nitrocellulose membrane saturated with Phosphate-Buffered Saline 10 μ M, both as a function of frequency. The LCR meter measurements were repeated 12 times before averaging the impedance.

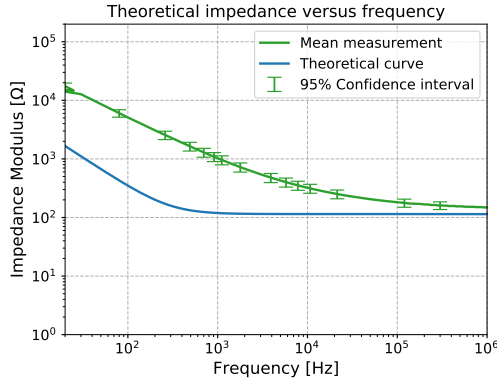


a) Impedance modulus

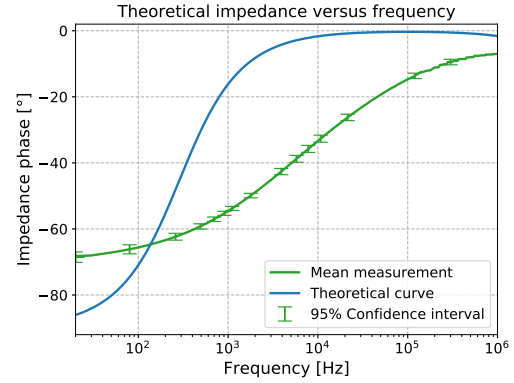


b) Impedance phase

Figure C.3: Comparison between the equivalent circuit impedance and the impedance measurements of the electrochemical system consisting of a nitrocellulose membrane saturated with Phosphate-Buffered Saline 100 μ M, both as a function of frequency. The LCR meter measurements were repeated 12 times before averaging the impedance.

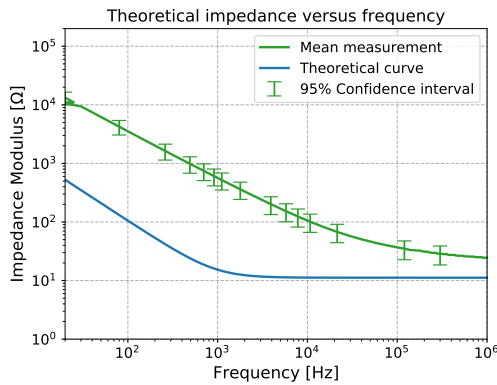


a) Impedance modulus

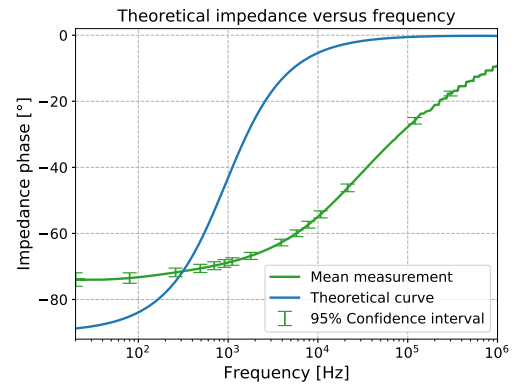


b) Impedance phase

Figure C.4: Comparison between the equivalent circuit impedance and the impedance measurements of the electrochemical system consisting of a nitrocellulose membrane saturated with Phosphate-Buffered Saline 1mM, both as a function of frequency. The LCR meter measurements were repeated 12 times before averaging the impedance.



a) Impedance modulus



b) Impedance phase

Figure C.5: Comparison between the equivalent circuit impedance and the impedance measurements of the electrochemical system consisting of a nitrocellulose membrane saturated with Phosphate-Buffered Saline 10mM, both as a function of frequency. The LCR meter measurements were repeated 12 times before averaging the impedance.

Appendix D

LCR meter sensitivity versus Autolab sensitivity

D.1 Conductivity variation of the sample

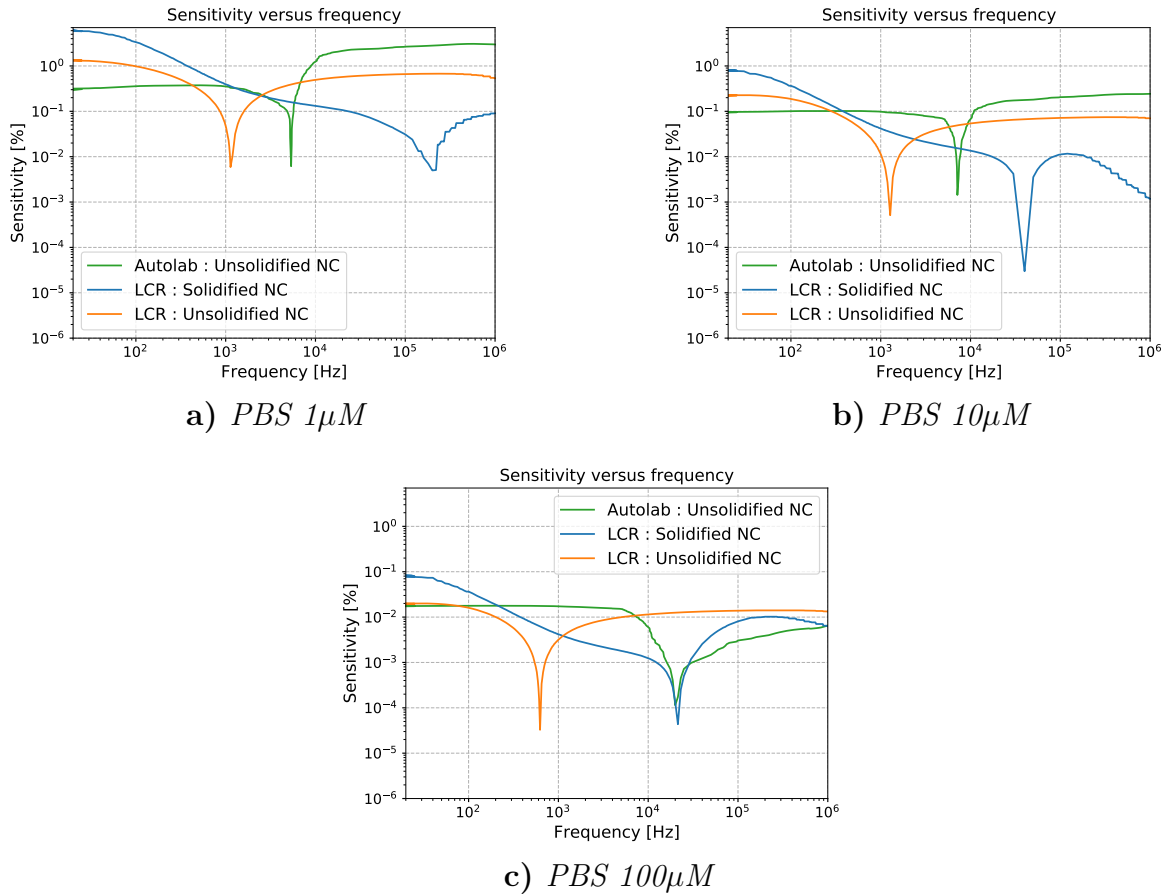
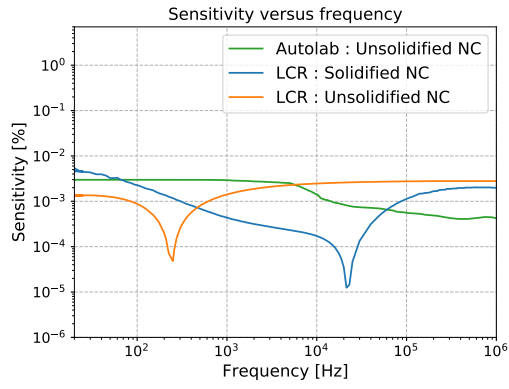
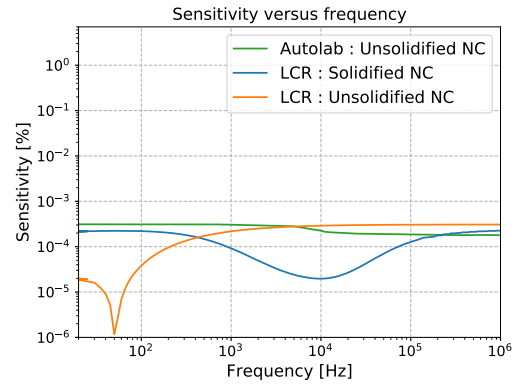


Figure D.1: Comparison between LCR meter and Autolab sensitivity when NC membrane is solidified or not solidified. Mean impedance used to compute S_i is obtained from 12 tests. Each measurement was performed with the same calibration, at 20mV and over a frequency range of 20Hz to 1MHz. Repetability factor is found in Section 5.6.



a) *PBS 1mM*



b) *PBS 10mM*

Figure D.2: Comparison between LCR meter and Autolab sensitivity when NC membrane is solidified or not solidified. Mean impedance used to compute S_i is obtained from 12 tests. Each measurement was performed with the same calibration, at 20mV and over a frequency range of 20Hz to 1MHz. Repetability factor is found in Section 5.6.

Appendix E

LCR meter repetability versus Autolab repetability

E.1 Conductivity variation of the sample

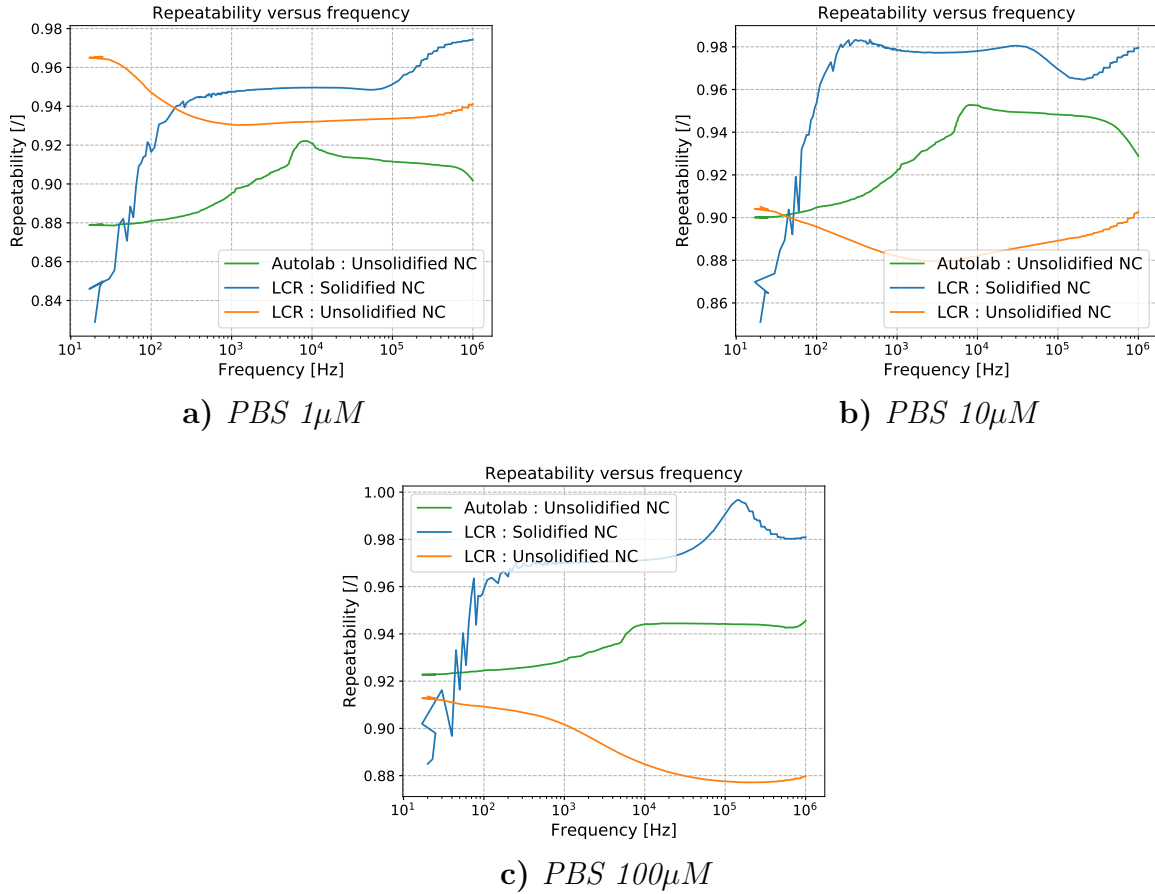
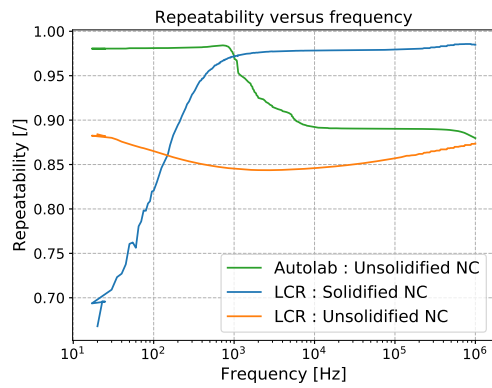
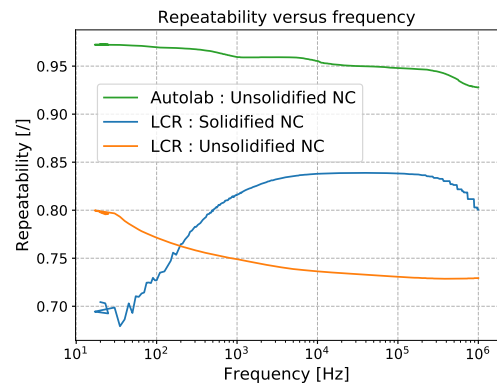


Figure E.1: Evolution of the repeatability factor R as a function of frequency for the measurements of the impedance modulus of the five PBS samples. Comparison between the three detection cases studied: LCR meter with solidified NC membrane (blue), LCR meter with unsolidified NC membrane (orange) and Autolab with unsolidified NC membrane (green).



a) PBS 1mM



b) PBS 10mM

Figure E.2: Evolution of the repeatability factor R as a function of frequency for the measurements of the impedance modulus of the five PBS samples. Comparison between the three detection cases studied: LCR meter with solidified NC membrane (blue), LCR meter with unsolidified NC membrane (orange) and Autolab with unsolidified NC membrane (green).

Appendix F

Impedance expression

Expressions of the impedances associated with the equivalent circuit are given, as well as the expression of the cut-off frequencies in each situation. Table F.1 shows all the cut-off frequency values for each case.

Measurements with unsolidified nitrocellulose membrane

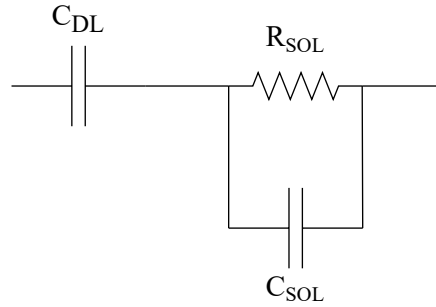


Figure F.1: Representation of the simplified Randles equivalent circuit model. It is used when measurements are carried out with unsolidified NC membrane.

$$Z = \frac{1}{j\omega C_{DL}} + \left(j\omega C_{SOL} + \frac{1}{R_{SOL}} \right)^{-1} \quad (F.1)$$

$$\Leftrightarrow Z = \frac{1 + j\omega R_{SOL} (C_{DL} + C_{SOL})}{j\omega C_{DL} (1 + j\omega C_{SOL} R_{SOL})} \quad (F.2)$$

$$\omega_1 = \frac{1}{R_{SOL}(C_{DL} + C_{SOL})} \quad ; \quad \omega_2 = \frac{1}{C_{DL}} \quad ; \quad \omega_3 = \frac{1}{C_{SOL}R_{SOL}} \quad (F.3)$$

Measurements with solidified nitrocellulose membrane

$$Z = \frac{1}{j\omega C_{DL}} + \left(j\omega C_{SOL} + \frac{1}{R_{SOL}} \right)^{-1} + \frac{1}{j\omega C_{backing}} \quad (F.4)$$

$$\Leftrightarrow Z = \frac{1 + j\omega R_{SOL} \frac{C_{backing}C_{SOL} + C_{SOL}C_{DL} + C_{backing}C_{DL}}{C_{backing} + C_{DL}}}{j\omega \frac{C_{DL}C_{backing}}{C_{backing} + C_{DL}} (1 + j\omega C_{SOL}R_{SOL})} \quad (F.5)$$

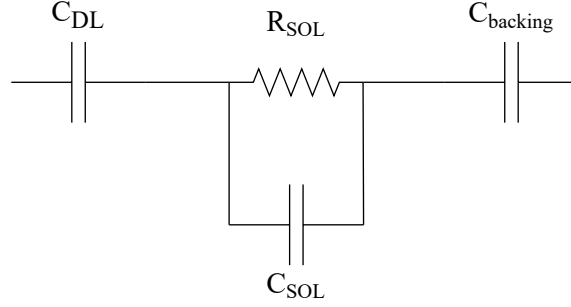


Figure F.2: Representation of the simplified Randles equivalent circuit model with an additionnal backing capacitance $C_{backing}$. It is used when measurements are carried out with solidified NC membrane.

$$\omega_1 = \frac{C_{backing} + C_{DL}}{R_{SOL}(C_{backing}C_{SOL} + C_{SOL}C_{DL} + C_{backing}C_{DL})} \quad (F.6)$$

$$\omega_2 = \frac{C_{backing} + C_{DL}}{C_{DL}C_{backing}} \quad (F.7)$$

$$\omega_3 = \frac{1}{C_{SOL}R_{SOL}} \quad (F.8)$$

Measurements with spiral planar inductance

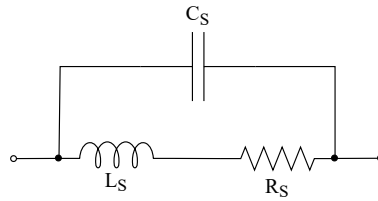


Figure F.3: Simplified lumped circuit model of a spiral planar PCB inductor [37].

$$Z = \left(j\omega C_s + \frac{1}{R_s + j\omega L_s} \right)^{-1} \quad (F.9)$$

$$\Leftrightarrow Z = \frac{j\omega L_s + R_s}{1 + j\omega C_s(j\omega L_s + R_s)} \quad (F.10)$$

Table F.1: *The cut-off frequencies of the different nitrocellulose membrane systems studied knowing that $\omega = 2\pi f$.*

		Solidified NC membrane			Unsolidified NC membrane		
		f1 [MHz]	f2 [GHz]	f3 [MHz]	f1 [Hz]	f2 [kHz]	f3 [MHz]
σ variation	PBS 1 μ M	0.0149	1.59	0.0537	8.49	1047.4	0.0336
	PBS 10 μ M	0.156	1.59	0.561	28.06	331.7	0.351
	PBS 100 μ M	1.71	1.59	6.14	97.20	104.7	3.842
	PBS 1mM	16.07	1.59	57.89	289.42	33.12	36.18
	PBS 10mM	164.5	1.59	592.5	936.66	10.47	370.26
		f1 [kHz]	f2 [GHz]	f3 [kHz]	f1 [Hz]	f2 [kHz]	f3 [kHz]
ϵ variation	DI water	0.516	1.59	1.86	0.038	136.437	1.162
	Ethanol	12.9	1.59	112.4	0.298	52.047	70.265
	Isopropyl alcohol	0.451	1.59	5.19	0.064	329.250	3.243

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