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Design of a mastitis indicator sensor

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Abstract

Mastitis is one of the most common and costly diseases for dairy farms. The early detection of this disease can prevent many economic losses. Mastitis is associated with an increase in white blood cells in the milk. Cells behave as small capacitances till a specific frequency. At this frequency, the cells capacitances decrease. Measuring capacitances at specific frequencies enables the estimation of cell concentration in liquids. By using electrodes not connected to the liquid, capacitances of liquids at specific frequencies linked to the cell decay is measured. This method is able to detect differences in the capacitance of yeast concentrations. However, the milk measurements do not show the same response as the yeast concentration. Other substances in milk mask the decay in capacitance, making it unable to detect the cell concentration in milk.

Contents

List of Figures

List of Abbreviations

ADC	**A**nalog **D**igital **C**onverter
CDC	**C**apacitance **D**igital **C**onverter
DAC	**D**igital **A**nalog **C**onverter
FIR	**F**inite **I**nput **R**esponse
IC	**I**ntergrated **C**ircuit
MSA	**M**easurement **S**ystem **A**nalysis
SCC	**S**omatic **C**ell **C**ount
VISA	**V**irtual **I**nstrument **S**oftware **A**rchitecture

Chapter 1

Introduction

Nedap develops automation devices for the livestock industry for years. Their primary focus is on dairy farming and pig farming. Within the dairy farming, they sell sensors for individual dairy cow monitoring and management. This research is for the development of a new sensor in its current line-up of products.

In intensive livestock farming, the need to detect an animals' health without physical presence is becoming more relevant with the introduction of automated machines and increasing herd size. The question arises to design a sensor that measures specific values in the milk to give an indicator of the health of a cows' udder and gives warnings when suspicious mastitis situations arise.

This research focuses on the design and implementation of a sensor that can determine the Somatic Cell Count (SCC) using capacitance measurement.

1.1 Problem Description

Mastitis is one of the most common and costly diseases in dairy cows in developed countries. It is an inflammation of the mammary gland and udder tissue [1]. Affected cows create less revenue due to reduced milk production and require treatment, resulting in increased labor costs [51]. The infection activates the defense system of a cow, leading to an increased amount of white blood cells in the milk. Damage to the gland and tissue caused by the infection increases the concentration of sodium and chlorine ions [41]. Detection of mastitis at an early stage using somatic cell count as parameter, would decrease or even prevent antibiotic usage, decreasing losses.

By determining the probability of an infection per cow, the farmer can perform animal-specific actions on treating mastitis and preventing the spread of the infection. For example, if a cow is in the late stage of its lactation period, a cow can be selectively dried off to treat the infection preventing chronic mastitis. Also, if a sensor indicates an increased probability of mastitis, the milking setup can be flushed with water with a temperature higher than 80 °C before the next milking session to prevent the spread of the disease [55].

The somatic cell count is also used by the industry as an indicator on milk quality. Milk with a cell count below a certain value is ranked as higher quality and is of higher value. A sensor with the ability of measuring somatic cell count can therefore let the farmer focus on higher quality of milk increasing the price of milk [2, 52].

1.2 Project Goal

Since the farmer has many advantages of an sensor that can measure somatic cell count during milking, the project is about a conceptual method of measuring the somatic cell count that can eventually be used within current milking equipment. Using existing literature, a new method of determining the somatic cell count is developed and tested keeping mind of the limitations that are introduced by the usage within current milking setups.

Chapter 2

Background

This chapter contains background topics used throughout the paper. The first paragraph describes somatic cells, an important aspect throughout this whole research. The last two paragraphs describe dairy industry-related topics, namely the milking setup and some general notes about a cows' udder.

2.1 Somatic Cells

The formal definition of a somatic cell is the following; "any cell of a living organism other than the reproductive cells.". It is a broad definition that contains the majority of cells. E.g., organs, skin, and blood cells fall. Germ cells and stem cells do not fall into this definition [27].

Within this paper, somatic cell count (SCC) refers to the number of somatic cells in a fluid using ml^{-1} as units. SCC gives an indicator of the number of white blood cells per milliliter. 50% of the healthy cows have an SCC below 100.000 cells/ml, and 80% of the healthy cows have an SCC below 200.000 cells/ml. Infected cows have an SCC of over 300.000 cells/ml. These properties indicate that cows with an SCC below 200.000 cells/ml are likely not infected, and cows with an SCC above 300.000 cells/ml are likely to be infected. Furthermore, an SCC above 100.000 cells/ml is also associated with a loss in milk production [31]. SCC also functions as a general milk quality indicator. The European Union has set a limit of 400.000 cells/ml in the bulk milk, which has become the international standard [37, 47].

Other factors known to influence the SCC on cows is limited. Currently, only stress is known to influence SCC. Other important factors in a dairy cows' life are the length of lactation, age of a cow, and the milk yield. These factors do not affect the SCC [16].

2.2 Milking Setup

Deployment of the sensor is within existing milking machines. There are a large number of variables to be taken into account for the design of the actual sensor. While this research does not focus on the product design itself, the placement and properties of the milking process are being considered, in particular, uncontrolled variables which affect the measurement method and measurement results, as described in the following paragraph.

Placement of the sensor is of high importance to ensure stable readings and compatibility with modern milking machines. A milking session starts by placing the teat cups on the cows' udder and apply a vacuum to start the flow of milk and

keeping the teat cups to stay on the udder. This vacuum stays constant throughout the whole milking process. A second vacuum, applied to the side of the teat cups, controls the flow of milk. This second vacuum splits the milking process into two phases, a milking phase and a resting phase, known in the industry as pulsation. The resting phase is introduced to prevent injuries to the cows' udder. One cycle, containing a milking and resting phase, takes about one second. The time ratio between the two phases differs. However, 50/50, 60/40 and 70/30 are commonly used ratios. These ratios determine the division of time between the milking phase/resting phase in percentage.

The milk comes out of four udders, called quarters. An infection can exist in only one udder, which makes it preferable to predict the presence of mastitis per udder. Measuring per quarter also raises accuracy because the milk from the infected quarter does not get mixed with milk from healthy quarters, which would lead to a lower cell count and decreased ion concentration [41].

An abrupt reduction of vacuum can let air together with small droplets of milk to flow towards the udders. In the case of one or more mastitis infected udders, these droplets can infect other udders, spreading the disease. A completely cut off vacuum also causes the teat cups to fall of the udder. The sensor cannot disturb the vacuum applied to the udder due to these problems. During the milking phase, the milk does not fill the cross-section of a hose, leaving significant gaps of air in the tube [44]. Also, teat cups do not perfectly fit on the udders, causing smaller air bubbles to go into the milk. These properties make it difficult to get stable readings from the milk during the milking process.

The milking setup requires regular cleaning. Flushing the whole system with a disinfectant solution and water at around 85°C cleans the system [45]. While the sensor does not have to operate at these temperatures, it has to withstand the disinfectant and temperatures up to 85°C. The machine might be warmer than the average temperature after cleaning, increasing the milk's temperature. The increased temperature affects the measurement, in particular, the resistance measurement due to increased conductivity.

2.3 Udder Health

Ideally, the milk coming directly from a cows' udder is sterile [18], meaning free from bacteria or other living microorganisms. Any contamination in the form of cells or bacteria is being considered unwanted. A higher concentration of cells indicates a poor udder-health.

Contamination in the udder can occur in dirty circumstances, where bacteria can get in the inside of a teat. Once bacteria are inside an udder, they can proliferate. Milk is a nutrition-rich substance with a neutral pH and ideal temperature for bacteria to grow [42].

Conductivity only increases when the milk is in direct contact with blood. Contact between milk and blood forces ions to move from the blood to the milk, increasing ion concentration and therefore increasing conductivity. However, direct contact only occurs at damaged tissue within the udder itself. Only significant damaged udders are detected using conductivity as the measurement.

2.4 Conclusion

The previous sections describe the basic knowledge required for this paper and to understand some of the upcoming topics. The next chapter goes more in-depth about these topics and the problems to overcome for designing the sensor.

Chapter 3

Analysis

First, this section describes current sensors used by the livestock industry for mastitis indication. Later on, it introduces design challenges to overcome for designing the new sensor, together with the advantages of using a sensor compared to the current situation.

3.1 Current Measurement Methods

Measuring SCC is a labor-intensive process that requires samples taken from the milk. Sampling introduces errors due to variation in the SCC in the milk during the milking process. Measuring SCC per quarter requires a sample per quarter, requiring an analysis per quarter and increasing labor [40]. The SCC during lactation may change. Generally, the SCC is the highest at the beginning of the lactation, although this is not always the case [16].

Milk samples are analyzed using optical measurement systems. Techniques used are the Neubauer counting chamber principle [8], flow cytometry [19], or infrared techniques like Fourier transform infrared analysis [20]. These devices measure multiple samples quickly with a high resolution. However, due to the limitations incorporated with these techniques, smaller devices that enable measuring within current milking equipment are not yet developed.

A producer of milking setups, GEA, introduced a sensor which can estimate the SCC during milking for each quarter [21, 26]. This sensor is the first and only sensor that can measure SCC during milking. It received awards at trade shows [22, 33]. This development indicates the need for a sensor that can measure SCC during milking. Still, it is the only product that exists which can measure SCC during milking. The technique used appears to be similar to the four-probe technique described by [49].

Other mastitis sensors that sense during the milking process purely rely on conductivity. It can measure conductivity during the whole process, excluding the variation problem. However, it measures symptoms that are caused by damage done by the infection, making it only able to detect mastitis in a later stage. Furthermore, conductivity differs per cow per milking session, reducing accuracy [41].

3.2 Conductivity Sensors

The previous methods of online measuring rely on ionic conductivity measurements. Conductivity requires direct contact between the electrode and milk. While it is possible to have a sensor that is in direct contact with the milk, it is

considered as unpractical due to other reasons. The electrodes in contact need to be cleaned more thoroughly compared to the milking setup itself, requiring additional maintenance for the sensor.

A previous sensor designed in house at Nedap Livestock Management measured conductivity using two electrodes, which measures the current flowing through these electrodes via milk using a voltage source. Other methods rely on four electrodes. The two most outer electrodes are connected to a constant current source. The two other electrodes measure the voltage in the milk. Both methods are comparable to the two and four-terminal impedance measurement techniques.

3.3 Capacitance Sensors

Capacitance indicates the charge a material can store in relation to the electric potential over the material. It primarily depends on the geometry of the electrodes and the permittivity of the material between the electrodes. By applying a voltage to the electrodes, the material in between the electrodes starts to polarize. Due to this polarization effect, the electrodes can store a limited amount of electrons.

Capacitive sensors consist of two or more conductors which measure the capacitance of the dielectric material in the environment. These sensors have been used in the industry for quite some time now. Major differences in the design of the sensor rely on the return path of the currents applied to the sensor. In some applications, the environment is used as the return path, like a human touching a capacitive button. Still, the majority of applications use a regular wire as the return path [7].

The sensing principle of a capacitive sensor can be divided into two main methods. The first principle depends on the change in the environment utilizing the dielectric constant of the environment. Examples of this are the detection of metal near an electrode [28] or the presence of a human [7]. The other principle relies on the change of geometry of the electrodes. The electrodes physically move, which changes the capacitance. Examples are, for instance, pressure sensors or accelerometer. A pressure sensor has two electrodes, which can flex when the pressure outside the sensor changes, changing the distance [35]. The electrodes in the accelerometer shifts when it accelerates or decelerates. It also changes the distance or the overlapping area between the two electrodes [39].

3.3.1 Polarization Mechanisms

The property that creates capacitance in materials is called polarization. The polarization of materials can occur due to different mechanisms. These mechanisms determine the strength of the polarization, and the frequency range the polarization occurs. Also, each dielectric mechanism has a characteristic cutoff frequency. All materials have a unique magnitude and cutoff frequency per mechanism [11].

When the frequency is higher than the cutoff frequency of a specific polarization mechanism, it does not contribute to the total capacitance anymore, causing decay in capacitance. A uniform suspension has a clear cutoff frequency, causing a high rate of decline. A larger frequency span indicates less uniform suspensions.

A mechanism that differentiates the influence caused by the cells and the conducting liquid is required for the sensor. The following sections describe the four polarization mechanisms. Figure 3.1 shows the corresponding polarization to the frequency range. It also shows the expected permittivity ε' and conductivity ε'' of an unspecified medium.

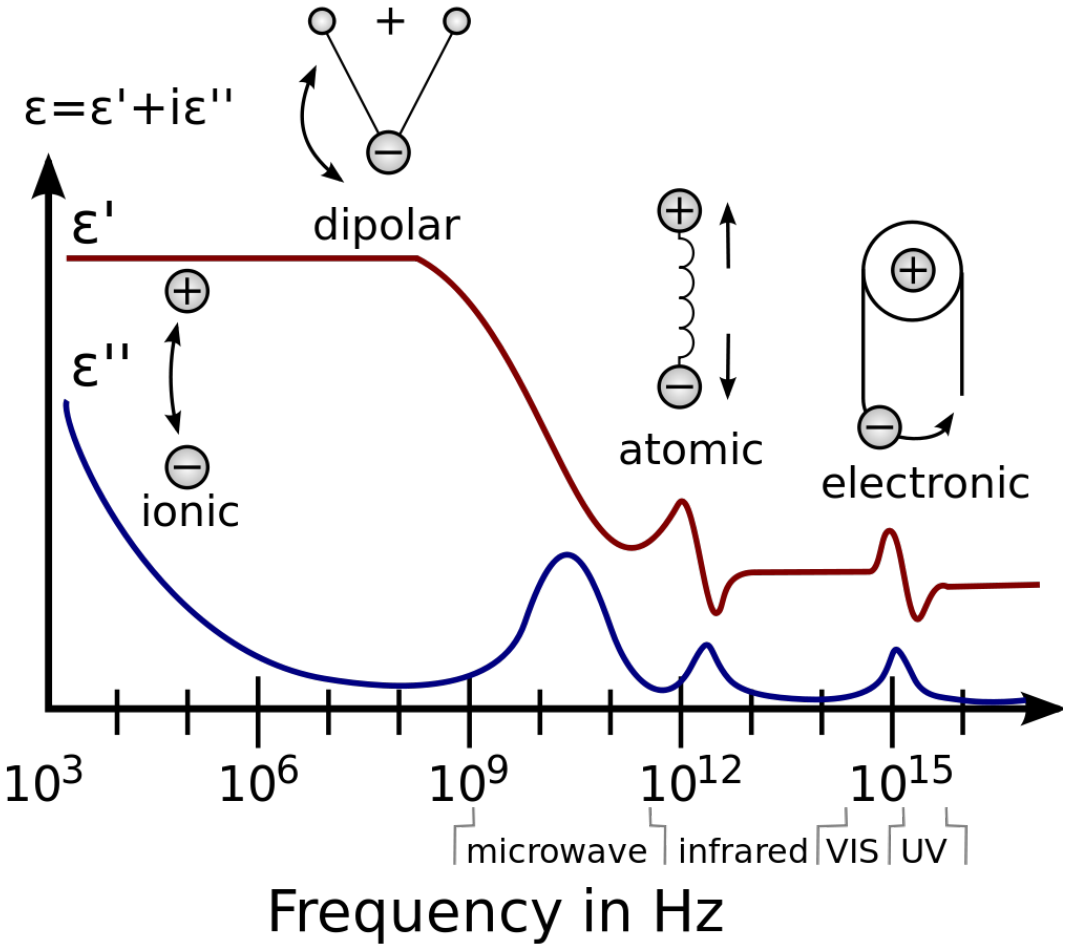


Figure 3.1: Overview of polarization mechanisms at specific frequencies [11]

Ionic Polarization This type of polarization is due to the displacement of ions in materials, for instance, in crystal elements or cells. The materials contain solved ionic elements like NaCl. In the absence of an electric field, the location of these ions causes for zero charge in the materials. However, these ions move under the influence of an electric field, causing a potential difference within the material itself.

Dipolar Orientation Polarization Materials can have a permanent dipole due to its molecular structure. These molecules' shapes are in such a way that a single molecule has a permanent dipole moment. For example, the water molecule H_2O shown in Figure 3.2 shows an angle between the two OH bond dipole moments, a measure of polarity in chemical bonds, resulting in a permanent dipole moment. These materials are also known as natural dipoles.

When no electric field is applied, and there exist no thermal differences in the bulk material, the molecules are orientated randomly causing zero dipole moment per molecule. However, when applying an electric field, the molecules orientate in such a way that the dipole moments add up to each other, causing a total dipole moment [43].

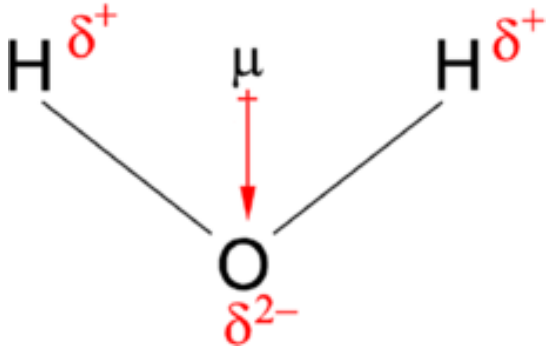


Figure 3.2: H_2O Molecule [36]

Atomic Polarization Atomic polarization comes from the change in the mean position of atoms in the molecule, which increases the bond dipole moment and the total dipole moment. Due to the dependence on the bond dipole moment, it only has an influence on molecules with a permanent dipole. This phenomenon only occurs at radio frequencies [10].

Electronic Polarization Electronic polarization displaces the electron relative to the atom nucleus. It stretches the atom. This electronic polarization only occurs at really high frequencies, around 10^{15}Hz. While the atom size fluctuates for many frequencies, it only provides a noticeable difference at the resonance frequency. Above the resonance frequency, this mechanism does not contribute to the permittivity [11].

The polarization method best used for determining the SCC is the ionic polarization. The ionic polarization focuses on larger and slower items, which can differentiate cells and water. The other polarization mechanisms occur at smaller items, like water molecules, which exist in both cells and the surrounding liquid.

3.3.2 Cell Capacitance

Milk is a conducting liquid containing immersed biological cells. Biological cells ionically polarize and have in contrast to ionic solutions, a frequency-dependent permittivity, and conductivity in the range of DC to several GHz. This frequency-dependent permittivity is also referred as the α, β and γ-dispersion, shown in figure 3.3. Each dispersion happens in a different frequency range and has different causes due to the structure of cells [13, 15]. A graphical explanation of the polarization based on the cells is shown in Figure 3.4. α-dispersion happens in the frequency range from DC to 0.1MHz, which occurs due to tissue interfaces, such as membranes. β-dispersion happens in the range of 0.1MHz to 100MHz. Mammalian cells generally have the β-dispersion at 1MHz [24]. This phenomenon is due to the structure of a cell, namely the cell walls causing a

higher permittivity at this frequency [13]. Both α-dispersion and β-dispersion fits in the ionic polarization description, but at different levels. α-dispersion happens due to the movement of whole cells, while β-dispersion occurs due to the submerged ions in the cells. γ-dispersion is caused by the polarization of water molecules and happens at a GHz frequencies [15]. γ-dispersion is a dipole orientation polarization and is neglected due to the high portion of water in milk and, therefore, unable to differentiate cells from milk.

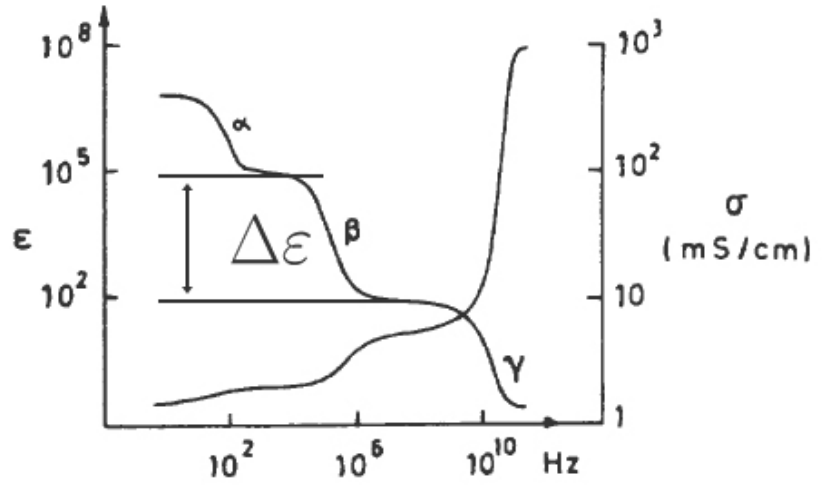


Figure 3.3: Dielectric constant ε (decreasing) and conductivity σ (increasing) as function of frequency [48]

The change in capacitance before and after the β-dispersion is proportional to the cell concentration. The change in permittivity for spherical cells in the β-dispersion is given in equation 3.1, for which P is cell volume fraction, r cell radius and C_m represent membrane capacitance [4].

$$\Delta\varepsilon = \frac{9PrC_m}{4} \tag{3.1}$$

The influence of these phenomenons is so significant that the influence of other particles, like oil-droplets or dissolved gasses, does not affect the capacitance. This property increases the practical usage of this method due to the varying composition of milk. The magnitude of the dispersion is directly proportional to the concentration of cells in the volume [13].

Figure 3.5 shows the expected shape of the capacitance measurement of water and two concentrations of cells. Note also that the values are in the graph are estimated. At higher frequencies, the measurements of the cell concentrations should be equal as the water capacitance. Lower frequencies give higher capacitance values compared to the water capacitance. Therefore, measuring at two frequencies, one lower frequency, and one higher frequency than the dispersion frequency gives an estimation about the change in permittivity, giving an assumption on the cell concentration.

An estimation of the concentration of cells within liquids has been researched. [48] researched the capacitance and conductivity of cells at specific frequencies. This research opened the way for determining cell concentration, also known as biomass, within liquids. It resulted in research about the ability of an electrical

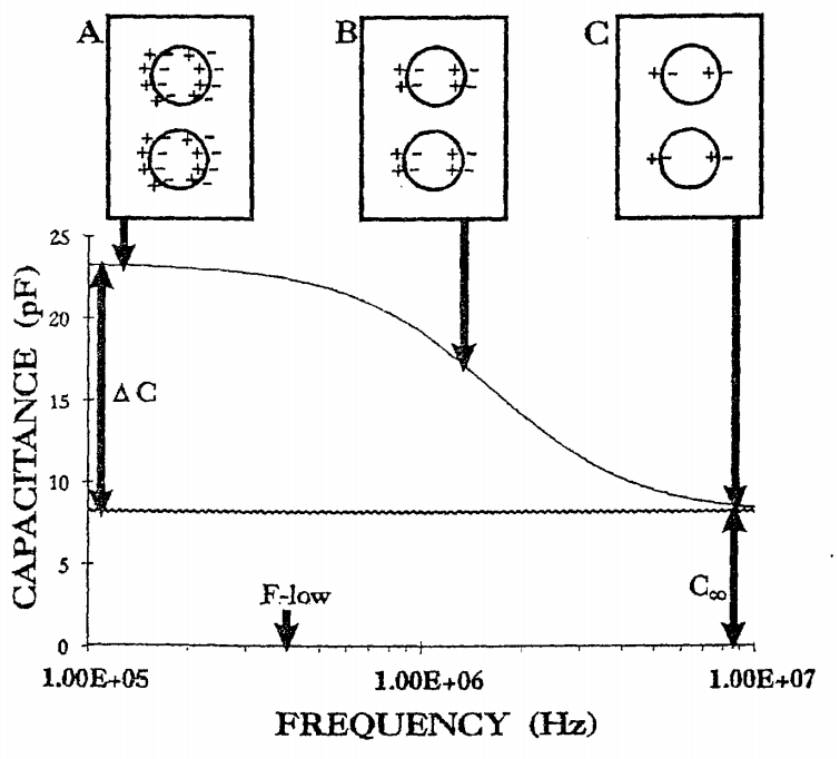


Figure 3.4: Varying capacitance of cell suspensions depending on the frequency [14]

circuit, which functions as a real-time sensor for determining the cell concentration. [13] indicates that there is a linear relationship between the concentration of yeast cells and capacitance, as shown in Figure 3.6. Also, other methods of determining cell concentration are compared to the capacitance method. The paper concludes that the measurement of RF permittivity can measure the concentration of cells in real-time. [4] used a commercially available machine to research the ability to measure cell concentration in high conducting liquids.

All these methods do rely on direct contact with the liquid. For this sensor, it is preferable to contact-less electrodes. Since capacitance usually requires an insulator between the electrodes a dielectric, the sensor can be isolated from the liquid, so that the whole liquids' capacitance is measured.

3.3.3 Cell Size

As described in the previous section, the change in permittivity depends on cell sizes. Table 3.1 shows the composition of cells found in the milk. It indicates that the majority of the cells are from the category of white blood cells. These fall in the category of the largest category of cells, the eukaryotic cells, as shown in Figure 3.7. Bacteria, which causes the infection, are of simpler construction and size. They fall into the category of prokaryotic cells. The cells of interest within the SCC are bigger, and therefore easier to detect due to the larger capacitance influence.

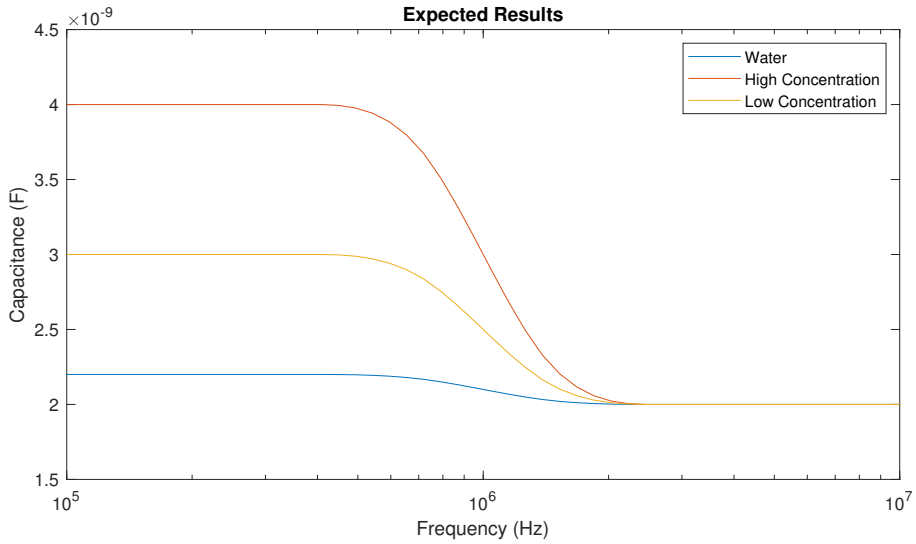


Figure 3.5: Expected results of different concentrations with an assumed β-dispersion at 1MHz.

Table 3.1 also shows the percentage of living cells in the milk, called the viability. The dead cells are unable to form a potential difference over their membranes, making them immeasurable using this method. Although the percentage of living cells drops from 92 % to 71 % when a cow suffers from mastitis, the total number of measurable cells, the living cell count, still increases.

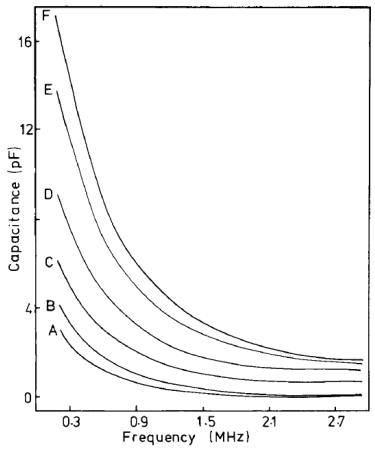
Parameter	Healthy	Subclinical mastitis	Clinical mastitis
SCC (10^5 cells/ml)	1.60 ± 0.38	4.60 ± 0.39	7.50 ± 0.54
milk neutrophils (%)	19.27 ± 0.24	43.12 ± 0.37	75.83 ± 0.40
segmented neutrophils (%)	98.00 ± 0.19	96.00 ± 0.18	93.00 ± 0.24
band neutrophils (%)	2.00 ± 0.18	4.00 ± 0.19	7.00 ± 0.24
milk lymphocytes (%)	14.88 ± 0.24	11.43 ± 0.24	7.80 ± 0.39
milk macrophages (%)	65.53 ± 0.48	45.45 ± 0.47	16.95 ± 0.36
viability of neutrophils (%)	92.53 ± 0.31	80.40 ± 0.36	71.47 ± 0.63

Table 3.1: Milk cell composition [3]

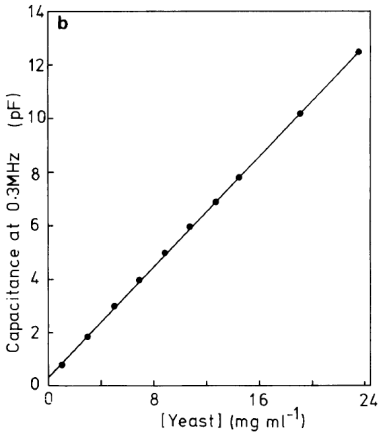
3.4 Temperature Dependant Permittivity

The milking process is a process that incorporates many temperature fluctuations, as described in Section 2.2. The properties of milk and cells under different temperatures is essential to take into account for proper readings. While conductivity increases when the temperature increases for a solution due to increased mobility of ions [6], there does not exist a simple rule for the behavior of materials relating to their permittivity value. The permittivity of a material depends on three factors [25];

- A decreased concentration of polarizable particles, due to an increased volume caused by increased temperature.
- An increase in polarizability of particles due to an increase in the volume.



(a) Capacitances of different cell suspensions at different frequencies [13]. Concentrations used are A, 0; B, 1.7; C, 4.4; D, 7.1 ; E, 13.6; F, 18.9 (mg/ml)



(b) Capacitance of yeast cell suspensions at 300kHz [13]

Figure 3.6: Influence in capacitance of cell suspensions at radio frequencies

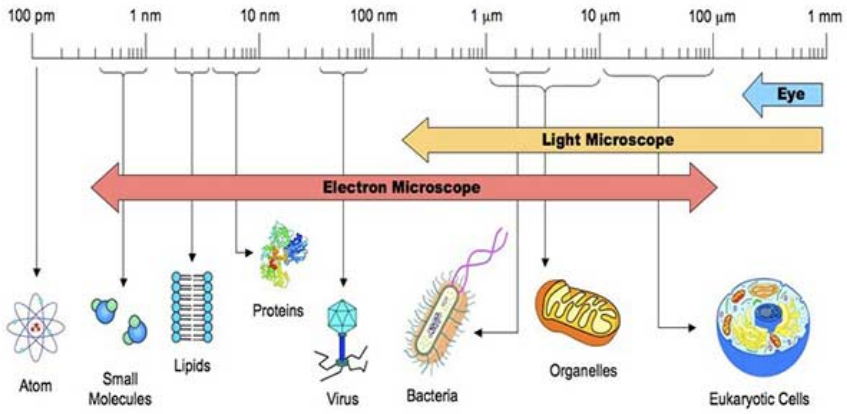


Figure 3.7: Size comparison [5]

- The temperature dependability of polarizability.

The expected temperature T of the milk is between 20°C and 40°C. The volumetric expansion for a ΔT of 20 is about 0.428% with thermal expansion of water at $0.000214C^{-1}$ [53]. In this case, volumetric expansion is, therefore, considered as negligible.

The research about cell capacitance does not mention any form of temperature dependence on the cell dispersion and, therefore, assumed that there is no difference in cell capacitance depending on the temperature.

3.5 Electrode Polarization

Conductive liquids contain dissolved free ions. These ions move under the influence of an electric field. The charged particles move towards the electrode of

opposite charge, forming an ionic double layer around the electrode. The ionic double layer, as the name suggests, consists of two layers. The first layer, also called the inner Helmholtz plane, consists of a layer of ions of opposite charge relating to the attached electrode. The second layer, the outer Helmholtz plane, consists of ions of similarly charged particles compared to the electrode, which sticks to the first layer. These charged particles provide for a negative potential at the electrodes, which decreases the total potential in the liquid. The thickness, called the Debye length, determines the potential decrease in the liquid. In some cases, this polarization prevents the formation of an electric field within the liquid completely. Electrode polarization is particularly of high importance in highly ionic liquids due to the ease of the double layer buildup. A polarized electrode causes for higher measured capacitance compared to real capacitance [12, 30].

The properties of this double layer depend highly on the properties of the ion dissolved in the liquid. Material properties that determine the strength of the double layer are the valence and diffusion constant of the ion, concentration of ions in the liquid, and temperature. The frequency also affects the double layer. The ionic double layer breaks down at frequencies higher than 1MHz. However, this is also the region in which the β-dispersion takes place. The polarization can, therefore, obscure the region of interest for measuring SCC [9, 12].

3.5.1 Polarization Correction Methods

Multiple methods have been introduced to overcome the problem of electrode polarization. These methods are divided into two categories; prevention and correction.

Prevention Polarization takes place at electrodes, which has a potential difference compared to the liquid. The ions move towards the electrode. However, if the electrode has the same potential as the liquid itself, the ions do not bond to the electrode. So if the sensing electrodes have the same potential as the liquid, it does not polarize. This technique exploited by the three [23, 54] and four [4, 49] probes method, for which one or two electrodes ensures a potential within the liquid and the other electrodes measures the voltage. From this method, voltage drop and phase difference are used to determine the resistive and capacitive component of the liquid.

Correction In some cases, when the potential drop is not significant, the polarized electrodes are still able to measure some potential within the liquid. Methods have been determined to correct the measurements to remove the polarization effect. One method used assumes a constant polarization effect, modeled as a resistor and capacitor in series. This method always gives a constant phase difference at a specific frequency [12, 30]. However, the composition and temperature of the milk are never the same. We cannot assume a constant polarization layer at the electrode every time. This correction method is, therefore, unsuitable for this application.

Another method is to use electrodes at different distances, for which both distances are larger than the Debye length to ensure that the electrodes are not within the polarization layer itself. The two electrodes give different capacitance measurements, from which the real capacitance can be extracted based on the

distance difference. The change in capacitance is only due to the difference in distance between the electrodes [30].

3.6 Conclusion

Since SCC is an important measure of quality within the dairy industry, both for the cows' health and milk quality, as written in Section 2, a sensor that can estimate the SCC in real-time would be useful in the industry.

The sensor to be designed focuses on the measurement of SCC in terms of cell concentration. Research in this area is conducted and concluded that capacitance is a powerful method of determining cell concentration, as described in Section 3.3.2.

The previous sections describe the design challenges to overcome for the new sensor. It sets the frequency range in which the sensor operates based on the polarization methods cell capacitance, as described in Section 3.3.1 and 3.3.2. The method to overcome the electrode polarization, as described in Section 3.5 determines the sensor and hardware design. These properties form the basis for the design of the hardware in the next paragraph.

Chapter 4

Design

As described in the previous paragraph, the sensor measures the capacitance of milk at specific frequencies. The following paragraphs describe the design of the actual sensor and acquisition hardware.

4.1 Requirement Analysis

Since the primary goal of the sensor is to determine when suspicious situations arise relating to milk capacitance. The range of the sensor, in terms of SCC, is in between a capacitance relating to an SCC of 100.000 cells/mL and 300.000 cells/mL. The range of the liquids' capacitance is yet to be determined together with the required resolution.

Testing of the acquisition hardware is based on the following criteria; accuracy, precision, and stability of the whole measurement system, which refers to the performance of the system. Good performing systems have excellent linearity and stability, a small bias, and high repeatability. Below stands the exact definition of these criteria.

- High repeatability ensures for the same measured value under different environmental circumstances. A high repeatability system performs well on a Round-robin test, a test with multiple independently performed measurements.
- A stable system produces a signal with little fluctuations. A stable system has a low variance.
- A linear system has a linear relation to the returning signal and the measured value. It also indicates whether the bias is constant over the whole measurement region.
- A small bias indicates a small difference between the measured value and the actual real value.

Accuracy of the data acquisition hardware is determined in the ability of accurate measuring known capacitances. The main focus shall be on stability and repeatability. Those properties ensure predictable behavior of the hardware. If the hardware performs as predicted, the other aspects, namely bias and linearity, can be corrected by calibration.

Determining the accuracy of the sensor is more difficult compared to the hardware. While there are reference samples available for calibrating existing SCC measurement devices, these samples only ensure the total cell count, including dead cells. Also, living cells tent to die quickly while out of the udder.

The exact relation between the SCC and capacitance can be determined, but it requires manual analysis of the measured milk using other methods, for instance, manual counting using a Neubauer chamber and a microscope, as described in Section 3.1.

Company Requirements Nedap has given some requirements for the actual sensor. While this research does not design the actual product itself, the method chosen has to meet these requirements to be suitable to be incorporated into a product. The main goal for the prototype is to conduct a proof of concept of measuring SCC using capacitance.

The sensor needs to be able to measure per quarter, measuring per teat of the udder. It, therefore, has to fit in the section of the milking machine, which does not hold the mixed-milk. Ideally would be a sensor that fits in the milk-claw, the region where the four suction-cups come together and mix the milk from the four teats.

The sensor to withstand the regular temperature shifts and cleaning-solvents used when cleaning the setup. Favored is a method of measuring without direct contact due to the additional design requirements when the sensor is in direct contact with the milk.

Furthermore, some requirements are expressly set up for only the sensor or acquisition hardware.

4.2 Sensor Design

A capacitance sensor measures the capacitance between two conductors and a dielectric material. The sensing principle utilizes the change in the dielectric property of the milk, which results in a different capacitance.

The following requirements are set up for the design of the sensor.

1. Capacitance range

   Capacitance in series causes for the lowest capacitance to be measurable. The liquid must have a much lower capacitance compared to the isolation layer between the electrodes and liquid. The measurable theoretical capacitance is limited to the capacitance of the sensor itself.

2. Size

   Capacitance is closely related to the thickness of the cross-section of the material under test. The dimensions of the two electrodes determine the area which forms an electrical potential to the dielectric material. While a larger sensor area might be preferable due to the higher capacitive value, smaller sensors are easier to place within the preferred location of the sensor. The sensor also needs to be able to deal with the electrode polarization problem. The ideal size is the minimum size required while still able to provide for an accurate measurement. The exact size is yet to be determined by the tests.

3. Prototype Manufacturability

The sensor operates submerged in a liquid. The prototype needs to be resistant to water and other liquids. It is preferable to use basic materials to iterate with designs quickly.

4.2.1 Probe Design Space Exploration

There are different techniques for measuring dielectric of materials. Figure 4.1 shows an overview of these techniques compared to the operating frequency range. It indicates two possible methods for determining the materials' capacitance. Applicable methods indicated in this graph are the Capacitor and Open Coax Probe method. The main difference between these methods is the placements of the electrodes, as the next paragraphs explain.

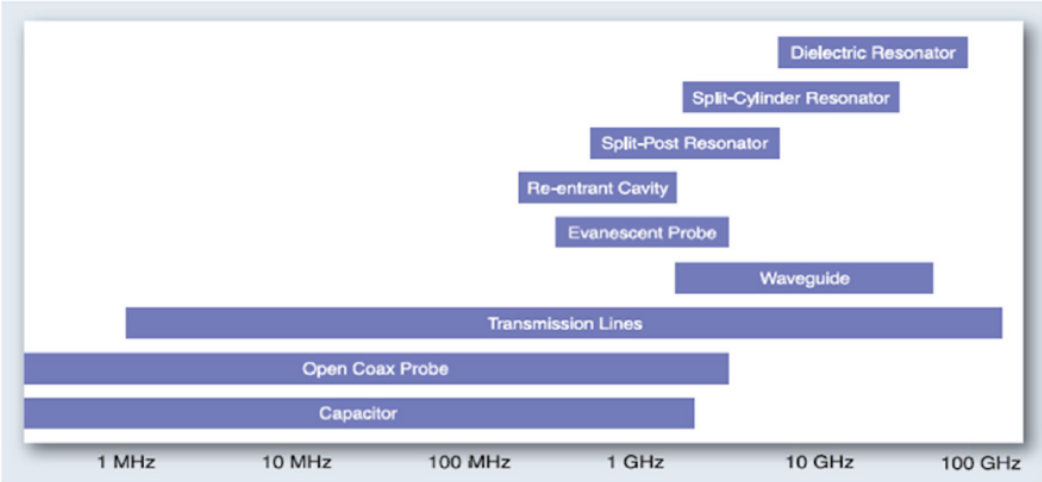


Figure 4.1: Different types of sensor compared to the operating frequency [11]

Capacitor The capacitor principle creates a parallel plate capacitor with the material under test as its' dielectric. The electric field applied is transferred directly to the opposed electrode via the material under test. The capacitance depends on the area of the electrodes and the dielectric. If the distance between the electrodes varies, the reading varies as well, decreasing the repeatability.

Open Coax Probe The Open Coax Probe relies on the fringing effect of an electric field. This effect bends the electric field at the edges of an electrode. This field can be picked up by another electrode. An example of this effect is shown in Figure 4.2, which shows a cross-section of a circular probe. This example places the electrodes in the same plane. The center electrode is the electric field applying electrode, while the outer electrodes, B and B', are the sensing electrodes. The sensing electrode can be in the same plane as the electric field applying electrode, which drastically decreases the complexity of the prototype. The distance between the two electrodes is fixed, which decreases variations in different readings, increasing the repeatability. For example, Figure 4.3 shows a sensor that can measure the height of ice. This sensor uses the interdigitated comb electrodes geometry [57].

This principle is referred to as the Fringing Effect principle because the prototype is not based on the original coax probe, but merely a sensor which utilizes the fringing effect.

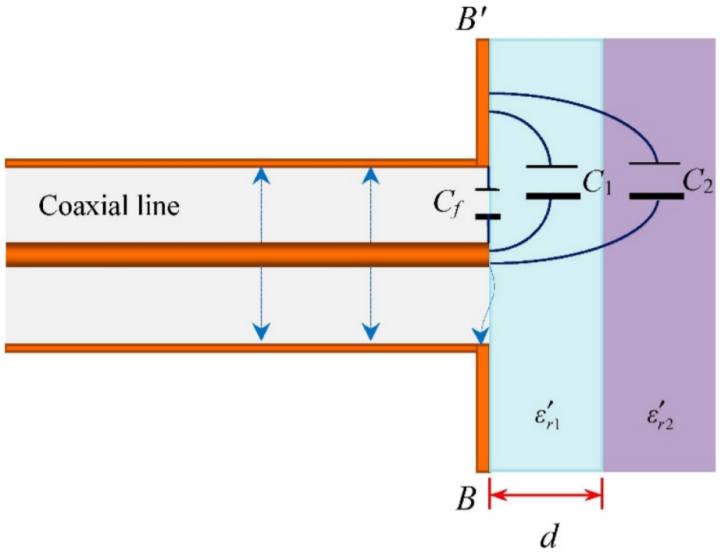


Figure 4.2: Fringing effect coax probe sensor [56]

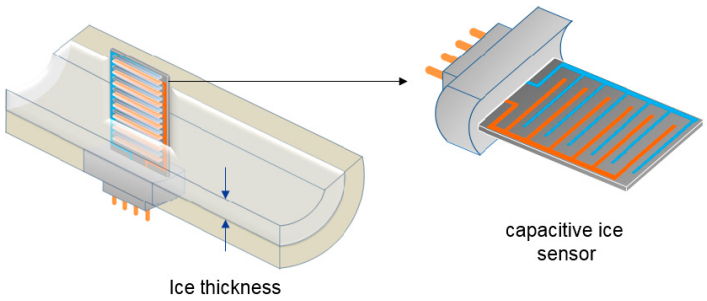


Figure 4.3: Ice forming sensor [57]

Trade-off analysis Both methods have their drawbacks. It is yet unclear which drawback will have the most significant influence on the measurement. Due to the simplicity and similar hardware, both methods are going to be tested to determine the preferred method.

4.3 Acquisition Hardware

The acquisition hardware transforms the electrical signals from the sensor to a value that is interpretable to a capacitance value. It also provides some signal conditioning to produce a clean output signal.

The following requirements are set up for the design of the acquisition Hardware.

1. Frequency range

   The hardware needs to perform in the frequency range where the α and β cell-dispersion takes place. It also needs to measure the capacitance at one given frequency to determine the change in capacitance over multiple frequencies. If the hardware is not able to measure in the range of the cell-dispersion, it is not able to detect the SCC. Assumed is that the desired frequency range is in the range of 10kHz and 100MHz due to the α and β dispersion.

   If a specific method is unable to meet this criterion, it is unsuitable for this application.

2. Measurement range

   The range of capacitance is yet unknown. However, the expected range will be in the picofarad range [14]. Due to the fixed frequency range and capacitance depending on the sensor size and milk value, the hardware chosen needs to be flexible enough to produce a valid output.

3. Data acquisition

   The ideal sensor is able to measure the capacitance of the sensor continuously. This creates a time continues measurement of capacitance over time, in which the valuable data is extracted digitally.

   The hardware also needs to process the samples quicker than the sampling period. This ensures no delays in the data processing comparing to the data acquisition. However, this property can be optimized when the exact frequencies are known by acquiring just a sufficient amount of samples at a specific sampling frequency.

4. Signal conditioning

   Signals from the sensor need to be conditioned to remove noise, which can disturb the measurement. It also isolates the sensor from the signal acquisition hardware.

4.3.1 Hardware Design Space Exploration

All of the criteria above is used to determine the preferred method for data acquisition. Each of the following paragraphs describes a method which might be suitable for this application.

Voltage divider The simplest method of determining the sensors' capacitance value is to place it in series with a known value. The voltage in the middle indicates the relation between the known value and sensor value.

Another option that uses the same principle is the De-Sauty bridge. It uses two voltage dividers consisting of known resistors and one known capacitor placed in parallel, as shown in Figure 4.4. It is an improvement over the Wheatstone bridge to ensure capacitive measurements [34, 38]. The unknown capacitor is determined using the potential over D. Another method is to use variable resistors and capacitors to set the potential over D to zero. However, this requires fine-tuning of the variable components to get an accurate reading. Another

downside of these bridges is the inability to correctly estimate a lossy capacitance due to the large number of system parameter that needs to be tweaked [32].

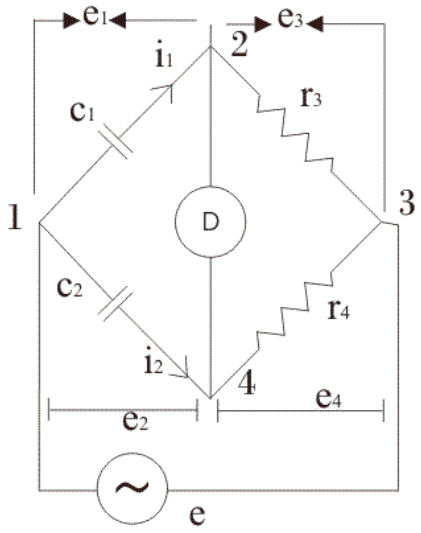


Figure 4.4: De-Sauty bridge [17]

Amplification Amplification relies on the same principle as the voltage divider. However, amplification is an active method of determining the relation between the two capacitances. Acquisition hardware based on an inverting OpAmp circuit utilizes this principle. The OpAmps' output indicates the ratio of the two resistances, which forms a linear relationship between the known resistance and sensor value. This method has two advantages over the voltage divider principle. The main advantage of the amplification principle is the increased potential difference over the sensor. The input is directly connected to the incoming signal and the other to virtual ground. Therefore the potential difference is maximized, which is a useful property when the sensor is polarized, as described in Section 3.5. The voltages at the electrodes make shielding easy. As one electrode is connected to virtual ground, a grounded shield is sufficient. The shield close to the electrode close to the signal generator can include capacitances. However, the potential of this electrode is known, so the parasitic capacitance added by this shield can be corrected for. Also, the shield can be connected directly to the same signal to create an active shield. This removes the parasitic capacitance due to the lack of a potential difference. In the case of the single voltage divider, at least one shield should have the same voltage as the voltage between the two reactances. Also, the phaseshift enables calculating the capacitive and resistive parts of the sensor. [32] adopts the same principle. The researches perform lots of analog modifications to the signal to produce an in-phase and quadrature component of the signal to determine the resistive and capacitive component. However, this can also be calculated digitally, significantly reducing the systems' complexity. [7] indicates that for the detection of material properties, this method of data acquisition is preferable over the other options.

Resonance frequency A coil and capacitor placed in series results in an oscillating circuit. This circuit has a specific frequency in which the circuit starts to

resonate. At this frequency, the coil and capacitor reactance is the same. By determining the frequency at which the resonating is happening, the capacitive value can be determined together with the known coil value. Unfortunately, for this purpose, this method is not usable due to the dependence on the frequency. This method cannot determine the capacitance at any given frequency.

CDC Companies have made IC's, which can read a capacitance sensor and produces a digital output signal. These IC's are typically used in combination with off-the-shelf sensors. CDC's uses an excitation signal to temporary charge the capacitor and measure the voltage on the capacitor. From the stored charged together with the voltage on the capacitor, the capacitance can be determined.

Unfortunately, these chips perform at a specific predefined frequency. It lacks the flexibility needed to be able to detect the correct cell dispersions and is, therefore, unsuitable for this project.

Another disadvantage of this method is that capacitive loss results in reduced accuracy. Some energy is not stored in the capacitor, resulting in a lower voltage on the capacitor, and therefore decreases the measured capacitance. Highly conducting milk results in less accurate measurements due to its increased conductivity.

Trade-off analysis Based on the frequency requirement, only two methods are suitable for determining the capacitance, namely the voltage divider and amplification. The advantages of the known potentials at both electrodes of the amplification makes this a preferable method over the voltage divider method.

4.4 Conclusion

The first prototype relies on the amplification principle. It also provides flexibility to modify the measurable range of the sensor by replacing the feedback resistance and capacitance. It provides enough flexibility to test both the Fringing Effect principle and the Capacitor principle. The next chapter describes the implementation of these principles and the corresponding results.

Chapter 5

Methodology

The main focus is to determine the probability that an udder is infected based on the capacitance of milk. The methodology is shown in Figure 5.1. The milk flows over the sensor from which enables measuring of the total capacitance. Utilizing a signal containing two frequencies, the change in capacitance can be determined, which links to the SCC.

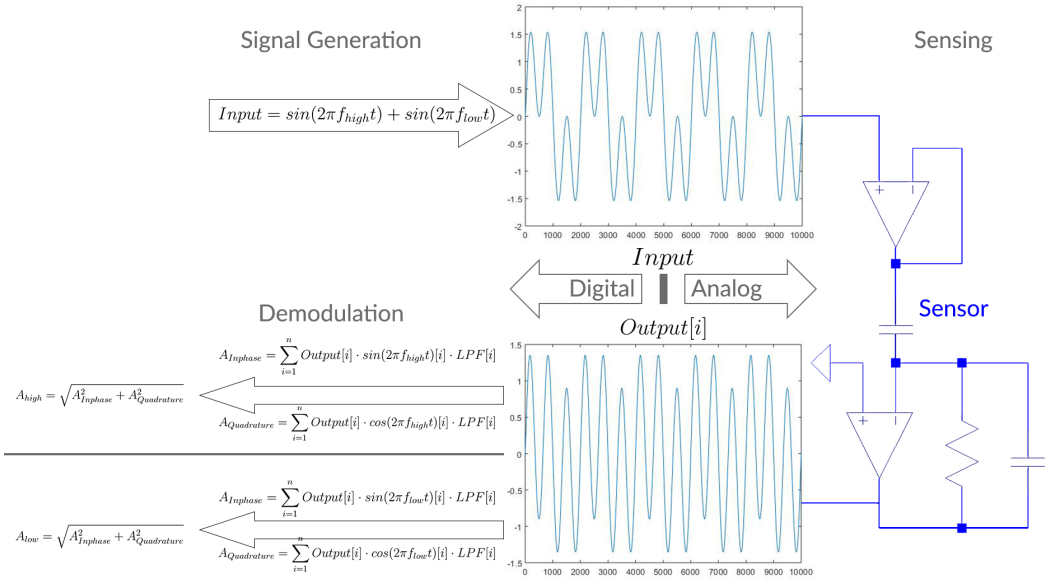


Figure 5.1: Measurement Method

5.1 Signal Generation

This step provides the signal which enables the estimation of the SCC using a single measurement. One frequency below the β-dispersion frequency and one frequency higher enables the measurement of the change of capacitance caused by the cell dispersion.

After the sensor, the amplitude of both frequencies is determined, leading to two different gains indicating two different capacitances.

5.2 Sensing

Sensing happens in the analog domain. The signal generator functions as the DAC, converting the digital signal to an analog signal. The analog signal is then set into a buffer for impedance matching. The signal passing the sensor is passed through an inverting OpAmp, providing for the amplification. The output of the amplifier is connected to an oscilloscope, which functions as the ADC within the circuit. Further processing rest on digital signal processing in the form of demodulation as filtering.

Only analog filtering in the circuit is the low pass filter at the inverting OpAmp to increase the stability of the circuit. The cut-off frequency of this filter is significantly higher than the region of interest, so it does not affect the measurement.

5.3 Demodulation

Demodulating returns the amplitude of a signal at a specific frequency. First, the signal is multiplied by both an in-phase and quadrature signal at the same frequency as the input signal to create two different signals. These two signals represent the in-phase amplitude and quadrature amplitude. Multiplications create a signal containing two frequencies, in this case, DC and two times the carrier frequency. A low pass filter removes the higher frequency component and returns a DC signal, which represents $\frac{1}{2}$ amplitude of the in-phase or quadrature signal. Taking the square root of the squared sum of the amplitude returns the original phase-insensitive amplitude.

The filter used is an FIR window filter generated by Matlabs' build-in filter design tool. An FIR filter uses a fixed amount of samples to get a valid result. An filter of order $n-1$ requires n samples to generate one valid value. A DC signal requires just one filtered sample for determining the complete signal. During measurement, only n amount of samples are taken.

The filter method is derived from a regular convolution. However, it is simplified only to require one step, skipping the whole shifting aspect of a convolution. It exists of element-wise multiplication of all the samples with the reference signal and FIR filter. Finally, summing all the samples to produce the one sample required.

Equation 5.1 shows the demodulation process to calculate the amplitude of an single frequency, modelled as a time-discreet sine wave with frequency f, reference frequency f_{ref}, n samples and a low pass filter LPF of order $n-1$. The same algorithm can be used to determine the amplitude of other frequencies, as shown in figure 5.1.

$$\begin{aligned} f &= f_{ref} \\ Input &= sin(2\pi ft) \\ A_{Inphase} &= \sum_{i=1}^{n} Output[i] \cdot sin(2\pi f_{ref} t)[i] \cdot LPF[i] \\ A_{Quadrature} &= \sum_{i=1}^{n} Output[i] \cdot cos(2\pi f_{ref} t)[i] \cdot LPF[i] \\ A &= 2\sqrt{A_{Inphase}^2 + A_{Quadrature}^2} \end{aligned} \tag{5.1}$$

The total noise power N of a signal depends on the noise density N_0 and the bandwidth B, as shown in equation 5.2. Since the bandwidth decreases drastically because of the low pass filter, the total noise power decreases as well. Therefore this demodulation has excellent resistance against noise.

$$N = BN_0 \tag{5.2}$$

5.4 Capacitance Calculating

Calculating capacitance is based on the amplification rate of the amplifier. The difference in input and output amplitude, the gain is calculated. The components in the feedback loop of the OpAmp are exactly known. Therefore a simple calculation is necessary to estimate the reactants of the sensor at that specific frequency. The calculation is shown in Equation 5.3.

$$\begin{aligned} Gain &= \frac{A_{Out}}{A_{In}} \\ X_f &= \frac{1}{\frac{1}{R_f^2} + (C_f 2\pi f)^2} \\ X_{sensor} &= \frac{Gain}{2\pi f X_f} \end{aligned} \tag{5.3}$$

5.5 Dispersion Influence

The change in capacitance is linear dependent on cell concentration. Due to the significant size difference of white blood cells compared to other cells, as described in Section 3.3.3, this dispersion will be caused mainly by the concentration of white blood cells, i.e., SCC.

5.6 Conclusion

The methodology provides a general overview of the whole sensor system. The next chapter explains the implementation of the sections described in this chapter and the resulting test results.

Chapter 6

Implementation and Testing

Now that the most favorable method is determined, it needs to be constructed and tested in real-world environments. This chapter first describes the implementation of the prototype, followed by the measurement method. The last section describes the results of the prototypes.

6.1 Implementation

This paragraph describes the realization of the prototypes, development of the script, and signal processing.

6.1.1 Sensor Realisation

The realization of the sensor appeared to be a challenge. Multiple methods are tested to design a sensor that can work within a liquid. The main reason for this difficulty is the waterproofness of the sensor, together with the thin walls required to maximize the theoretical measured capacitance, as written in Section 4.2. For instance, 3D printing resulted in thick walls, which were unable to detect any differences in capacitance, whether the container was empty or full. Using small watertight bags as containers on which electrodes placed on the side, did result in better measurements but appeared to be unstable due to environmental influences. The capacitance measurement of the sensor continuously increased over time. The best results came from a simple office-grade lamination machine. Copper tape is cut into the desired shape of the sensor and glued to the plastic used for lamination. Wires are soldered to the copper before lamination for the contact points on the sensor itself.

6.1.2 Hardware Realisation

The main challenge within the hardware realization is to get a linear frequency response at very low reactances. Creating hardware which minimizes parasitic capacitances and parasitic inductances are essential to increase linearity. As the results later indicate, the measured value has a significant impact on the frequency response of the whole hardware.

The OpAmp used is a Texas Instruments OPA659 that can handle high-frequency signals up to 650MHz signals and is unity-gain stable [29]. The amplification is set very low at around -6dB to improve linearity and stability at higher frequencies due to the gain-bandwidth product of the setup.

6.1.3 Signal Generation

Determining the ideal frequency in which the dispersion takes place requires a signal generator that can produce multiple frequencies.

A Rohde & Schwartz SMC100A signal generator is used to generate the input signal. It can generate a signal with a broad range of frequencies, from 9 kHz up to 3.2 GHz [50], which is sufficient for the desired range introduced in Section 3.3.2. VISA commands enable communication between the signal generator and Matlab. It enables Matlab to control the output of the signal generator, like output frequency. The script sends for each measurement a different frequency, creating a script that performs a frequency sweep test.

6.1.4 Data Sampling

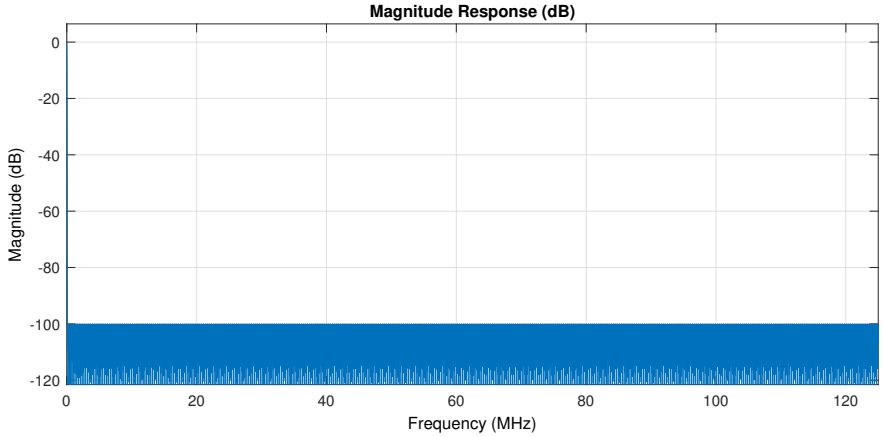
The signal requires conversion from an analog signal to digital values for further processing of the data. A digital oscilloscope connected to the PC enables the data stream from the prototype to Matlab. The digital oscilloscope, a PicoScope 5204, have drivers to connect and control the oscilloscope via Matlab. It enables control of the measurement range, sample frequency, amount of samples, and triggering of the oscilloscope. It provides enough flexibility and accuracy in measuring over a wide range of frequencies up to 250MHz, and measurement ranges from $100mV$ to $5V$, with an accuracy of 8 bit [46].

The oscilloscope is set at a sampling frequency of 250MHz. This sampling frequency ensures that high-frequency signals, up to 125MHz, are correctly sampled. In total, 10000 samples are taken for one measurement. The total measurement takes 40μs, enough to fit one period of a 25kHz signal. The measurement has a bandwidth suitable to measure signals within the whole β-dispersion region.

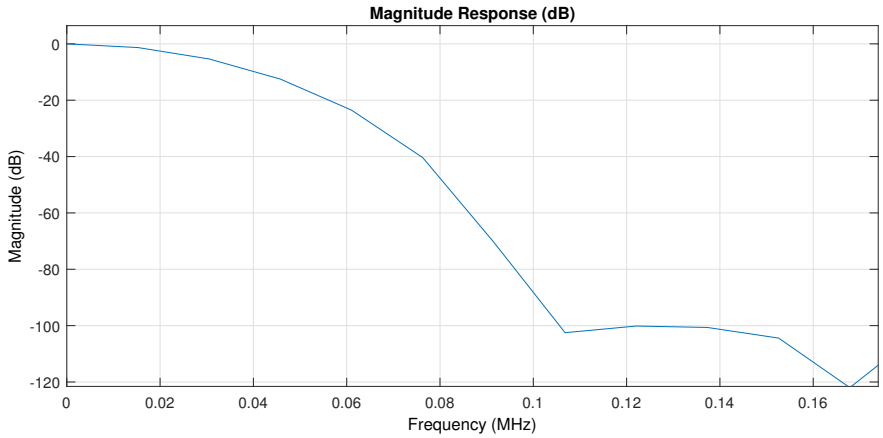
6.1.5 Filtering

The demodulation shifts the carrier frequency to DC. An ideal filter filters all frequencies except DC, minimizing the bandwidth of the filtered signal.

The designed filter uses a Chebyshev windows FIR filter of order 9999. A Chebyshev filter has a high roll-off compared to other filters, filtering the majority of the high-frequency components. The used filter has a -3dB point at 21.6kHz. The magnitude response of all the frequencies is shown in Figure 6.1. The maximum decay of magnitude of 100dB is at a frequency of 100kHz. The input signal used can contain multiple frequencies, each at least 100kHz apart from each other to ensure no interference of input signals. This range is sufficient for the actual sensor; however, for the frequency sweep, these frequencies are closer apart. Therefore only signals containing one carrier frequency are used per measurement.



(a) Chebyshev windows FIR filter



(b) Chebyshev windows FIR filter at cutoff frequency

Figure 6.1: Generated Matlab Filter magnitude response

6.2 Measurement System Analysis

Validation of the prototype is only as proper as the measurement technique. This section describes methods to ensure valid readings throughout all tests.

First of all, the environment variables need to be as constant throughout measurements of the same samples. It includes temperature and uniform distribution of cells in the liquid. Samples are kept at room temperature to ensure a nearly constant temperature. Milk samples are first kept outside the refrigerator for a while to warm up. Samples which has water as the basis comes from a large bottle of water at room temperature. Stirring the liquid before measuring also ensures an evenly distributed concentration.

Another important aspect is to validate whether the gotten results do represent the real value. It appeared to be a though as the measured properties, namely living cells, tend to die quickly. Real white blood cells from cows die in a matter of hours, for which the decay in a few hours is significant, which would result in different measurements. The commercially available reference samples gotten from Qlip B.V. do not contain living cells and are therefore unsuitable. The best alternative is yeast. Living yeast cells have a membrane to form a potential. Yeast is also readily available, cheap, and easy to test whether the cells are still alive. Living yeast cells, together with sugar, form CO_2 bubbles in the

liquid. Yeast gives the freedom to create a large volume of reference samples so that the electric field does not exceed outside the liquid. There are multiple mixes created to detect differences in measured capacitance. Many similar techniques to determine the biomass of a liquid, i.e., cell content, can determine yeast using capacitance measurements [4, 9].

6.3 Hardware Tests

The performance of the hardware and script is determined using known capacitances. Three known capacitances are used to determine the accuracy, stability, and linearity. Figure 6.2 shows the average capacitance measurements at the frequency region of interest. Figure 6.3 shows an accuracy plot of these measurements at the expected β-dispersion of the SCC.

First, notice the excellent stability of the frequency response till 20MHz. At higher frequencies, the measurements are not accurate enough. This peak is due to the decreased reactance of the capacitor at higher frequencies.

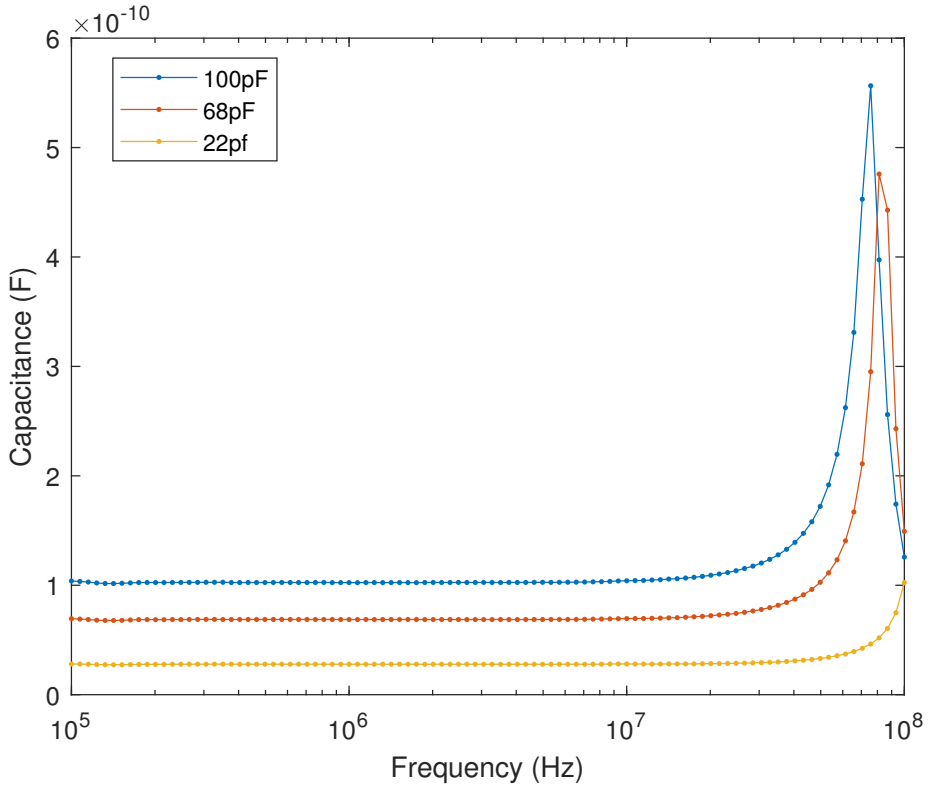
Figure 6.2b shows the measurements against the real values. It shows that the error does not increase linear compared to the real value. Considering the stability and repeatability shown in Figure 6.3, it can be corrected when using more reference values to calibrate the system response.

Increasing reactance can improve the frequency response of the hardware. If this appears to be the case, smaller sensors decrease the capacitance, which increases the reactance. Expected is that the SCC gives the β-dispersion at 1 MHz. So the frequency response appears not to be a problem for these measurements. However, if the β-dispersion region is at higher frequencies, smaller sensors can be used instead to move the peak towards higher frequencies.

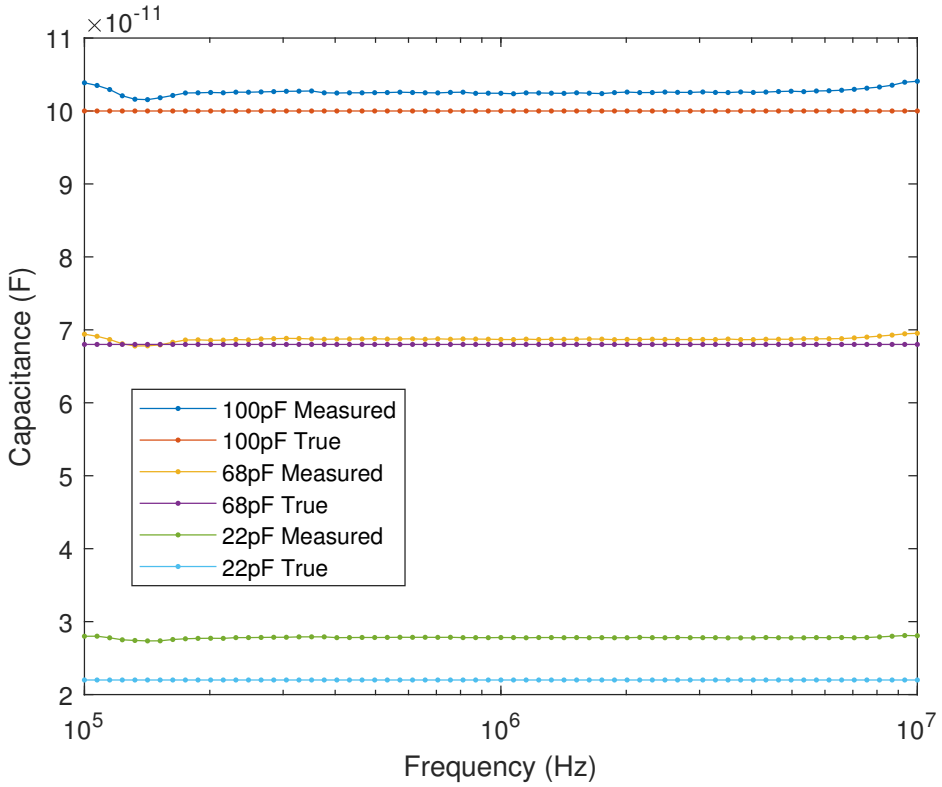
Overall, the performance of the prototype is sufficient to determine the expected dispersion, as shown in Figure 3.5. When the exact frequency of the dispersion is known, the hardware can be designed around this frequency so that the performance is increased compared to this general approach.

6.4 Conclusion

The prototype designed can estimate the capacitance. While accuracy is not yet ideal, it does enable it to measure within the expected range of change in capacitance necessary to determine the cell dispersion. The next chapter will discuss the test results obtained using reference liquids and the relation of the substances together with the measured capacitances.

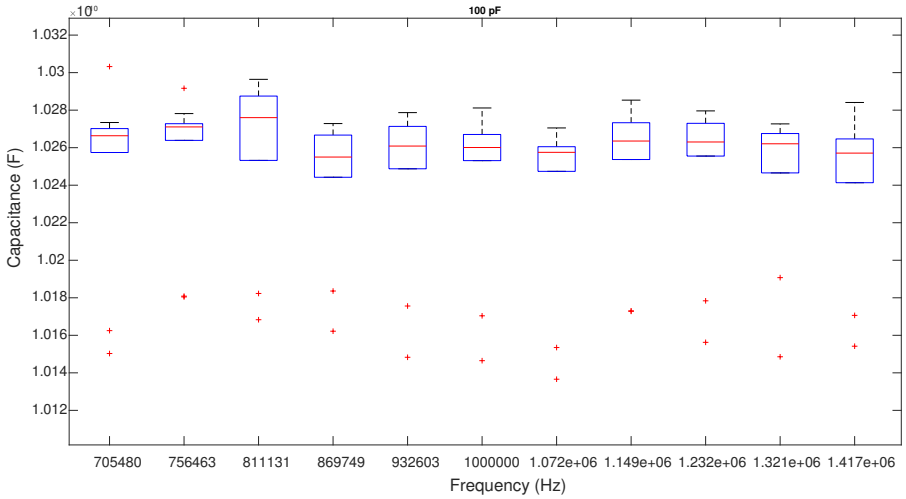


(a) f = 100kHz to 100MHz

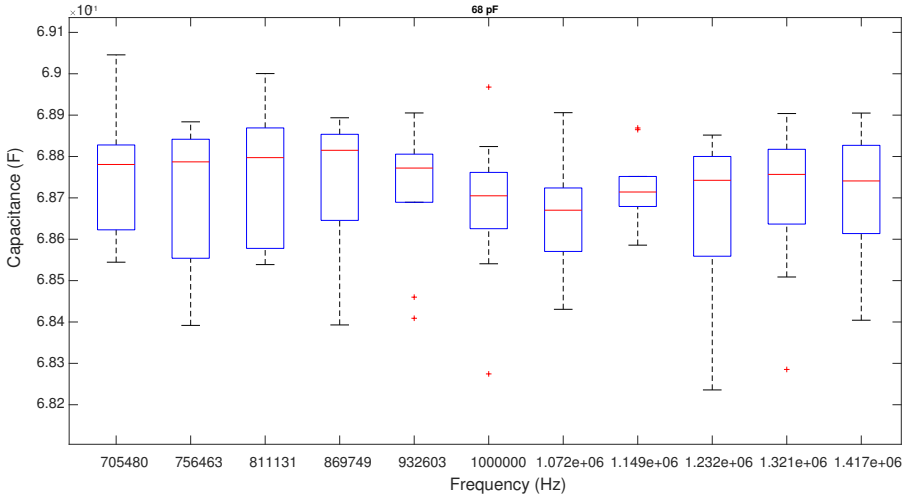


(b) f = 100kHz to 10MHz including plotted real values

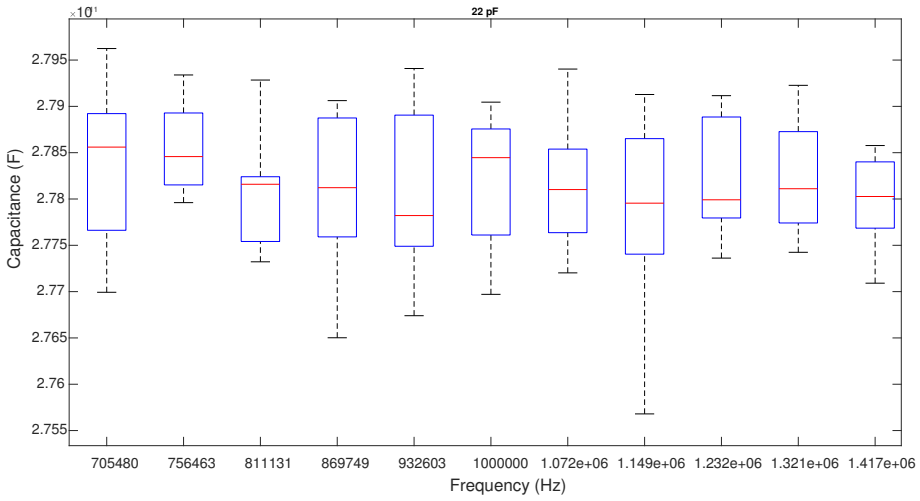
Figure 6.2: Measurements of known capacitors



(a) 100pF Boxplot



(b) 68pF Boxplot



(c) 22pF Boxplot

Figure 6.3: Accuracy of reference capacitors. Boxplots determined using 10 measurements at each frequency.

Chapter 7

Results

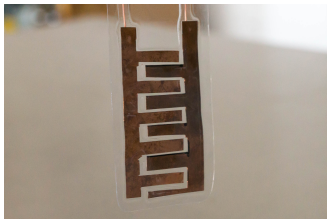
The previous chapter tested the functionality of the hardware using real capacitances. This chapter describes the experiments and results to test the influence of cells on the total liquids' capacitance.

7.1 Experimental Setup

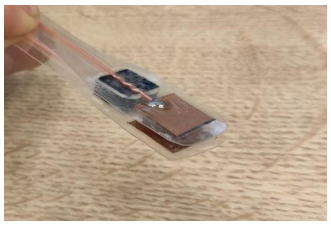
This section described the general idea of the tested methods and the corresponding results. It uses a simulated testing environment that has similar properties as the real world. These tests used yeast as the reference liquid. Since previous research indicate the success of determining the concentration of yeast in liquids, it is a suitable reference liquid to test the method itself is also successful in getting the same results as the literature indicates.

7.1.1 Two Electrode Principle

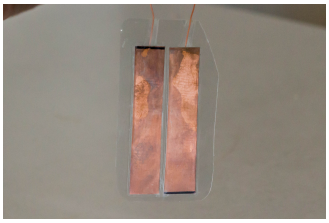
The first method used is based on the most straightforward capacitance test. It uses two electrodes, which determines the capacitance of the medium. The two sensor principles, as described in 4.2, are used. Figure 7.1 shows a few sensors used for these experiments. Figure 7.2 shows the inverting amplifier schematic used, as described in Section 4.3.



(a) Fringing Effect Principle, Prototype "Kam"



(b) Capacitor Principle, Prototype "Par"



(c) Fringing Effect Principle, Prototype "Long"

Figure 7.1: Two Electrodes Principle

Container Capacitor Principle

This principle is designed to maximize the sensor area relative to the liquid used. Figure 7.3 shows the two components of this setup. It consists of two separate electrodes. The inner electrode is connected to the signal generator and the outer shell to the virtual ground on the OpAmp.

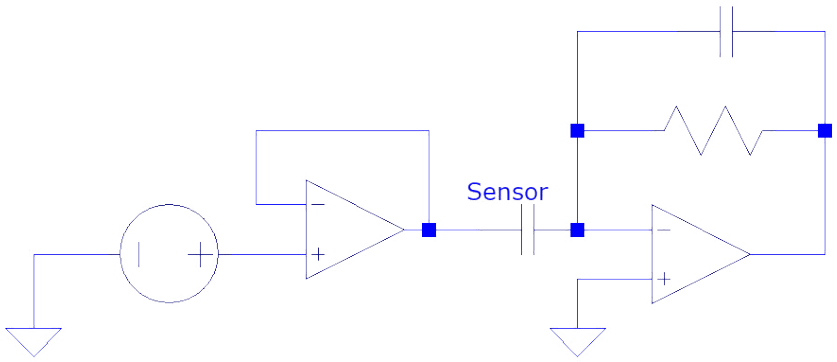
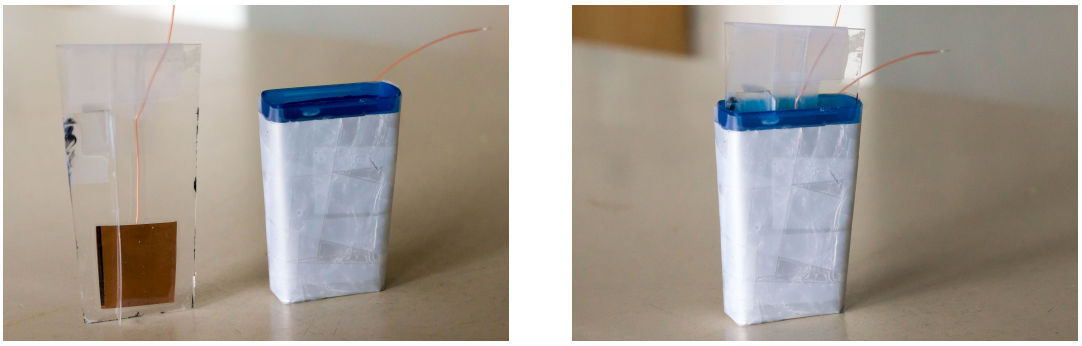


Figure 7.2: Acquisition Hardware



(a) Sensor and Container (b) Assembly

Figure 7.3: Capacitance Principle as a container, Prototype ”Bak”

Results

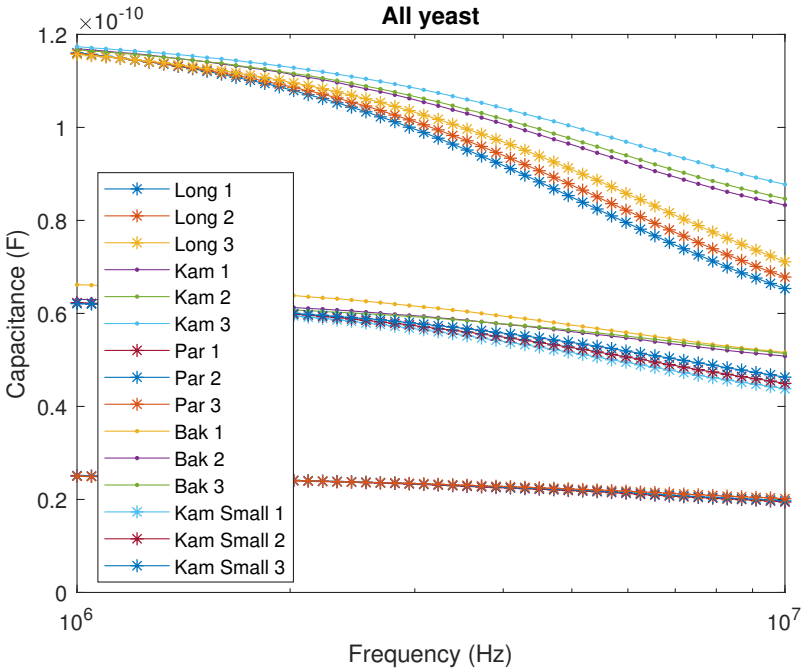
The first few prototypes generated promising results. There is a slight difference in capacitance at β-dispersion frequencies. It also correctly indicates the yeast concentration, yeast 3 being the highest, and yeast 1 being the lowest. Surprisingly, the capacitor principle gave much lower results compared to the fringing effect principle. While the area of the electrode is indeed much smaller compared to the fringing effect, assumed was that the change in position compromised for this.

Multiple electrodes are used to compare the generated capacitance. Figure 7.4a shows the measurements of the different probes and 7.4b the normalized values. The ideal electrode maximizes the differences between the different yeast mixtures.

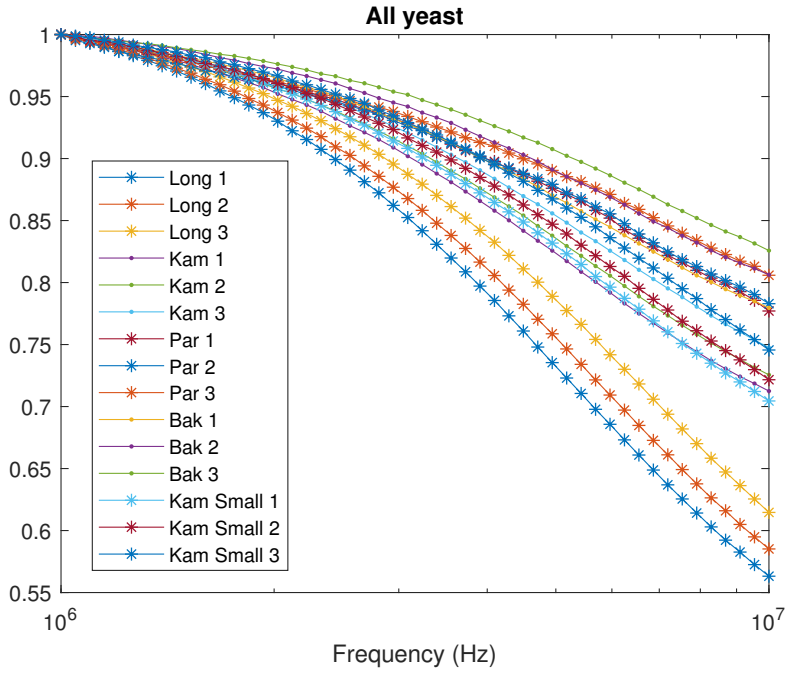
Prototype ”Long” gave the largest measurable change in capacitance. What did surprise me is the fact that the ”Kam” and ”Long” prototype gave the same capacitance measurements at lower frequencies, but the decay in the ”Long” prototype is larger compared to ”Kam”. This change in decay might be due to the longer length in which the electrodes are close, having a larger inter-electrode capacitance compared to the ”Long” prototype. The liquid has, therefore, less influence on the total capacitance measurement.

The Container principle did not produce the expected results. The sensor area is larger compared to all the other electrodes. Unfortunately, the sensor

also measures the permittivity of the relatively thick plastic wall of the blue container, which decreases the permittivity drastically.



(a) Probe comparison real values



(b) Probe comparison normalized values

Figure 7.4: Measurements using different geometries and yeast mixtures, 3 indicating a high concentration. 1 indicating the lowest concentration

7.1.2 Stability

The repeatability of the sensor is determined by taking multiple measurements over time without interfering or modifying the sample. A new prototype is built to minimize environmental factors which influence the measurements.

The test-setup has the following parameters optimized;

- Interference

  Because of the broad frequency range increases the influence of the environment. Shielding ensures isolation from the environment and minimizes the influence of interference.

- Sensor Placement

  The sensor is glued in place to prevent changes in the prototype, which could interfere with the measurement itself. Using a sufficient amount of liquid ensures that the sensing region is saturated.

- Cell Sediment

  Cells are not solved in the liquid but submerged. The higher density of cells compared to water causes the cells to sink to the bottom. This sediment will form a layer of higher concentration of cells on top of the sensor. The sensor is placed on the bottom to measure the influence of sediment.

- Parasitic capacitance

  The main focus is on the capacitance of the liquid. Ensuring that the measurement represents the liquids' capacitances means that environmental capacitance is set to a minimum. The container, therefore, requires a low capacitance. Tempex is an excellent material to hold the sensor and liquid because it has a very low capacitance value due to the large portion of air within Tempex. Thick walls also decrease capacitance introduces by the shield due to increased distance from the sensor.

- Temperature

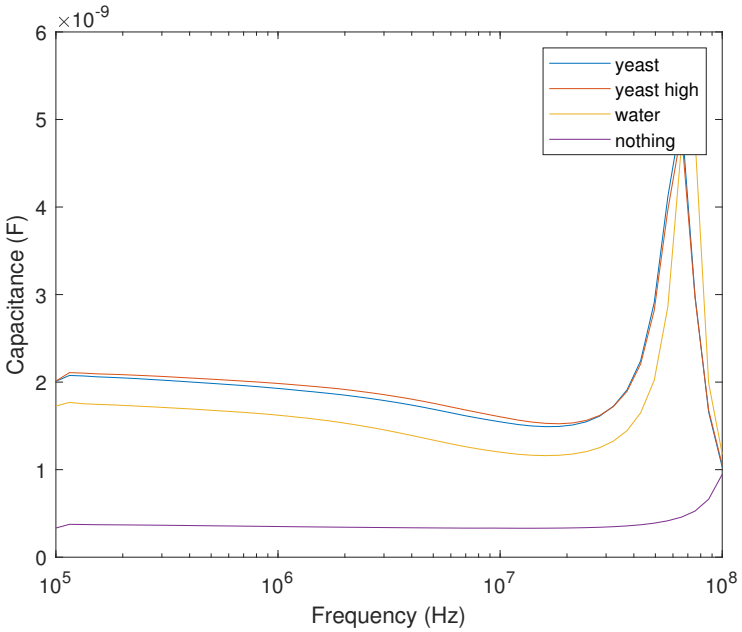
  Even though the exact influence of the temperature is unknown and likely does not influence the measurement, it is chosen to set stable. The liquids used are kept at room temperature to minimize temperature shifts. Only minor temperature shifts can occur due to temperature shifts in the environment. Within the measurement time, it is unlikely to cause significant shifts in temperature, also due to the temp-ex walls, which also function as temperature isolation.

Taking 100 frequency sweeps over the whole frequency range determines the stability of the system. One complete measurement takes roughly 15 minutes to complete.

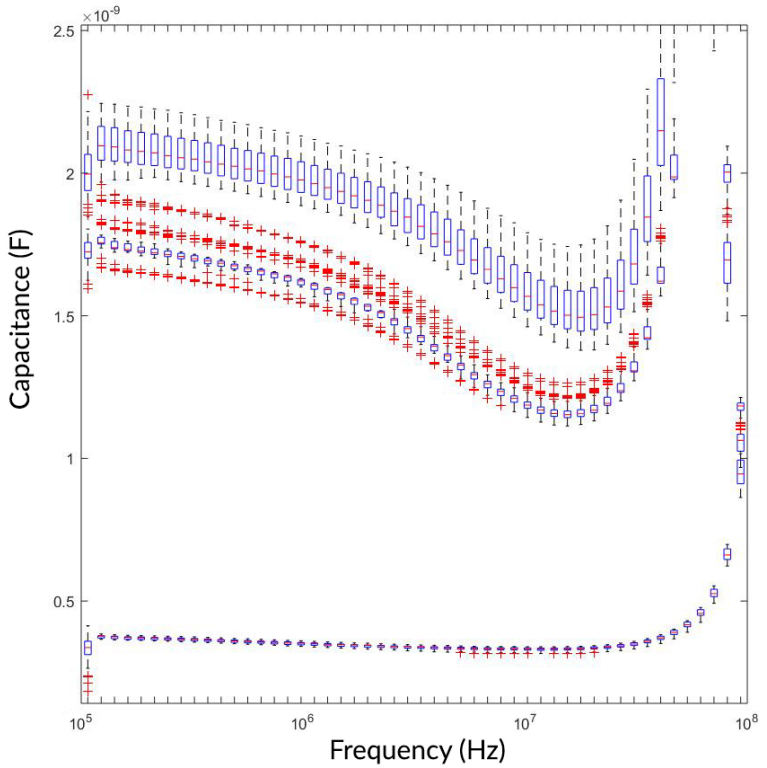
Four different contents are measured; air, water, small yeast concentration and, large yeast concentration. The average measurements are as expected, with the high concentration having the largest value, followed by the small concentration, water, and at last, air. Figure 7.5a shows the measurements of the four concentrations.

Unfortunately, as the box plots indicate, the measurements of yeast do fluctuate more compared to the air and water measurements. Figure 7.5b shows the

box plots of the high yeast concentration (highest value), water, and air (Lowest value). As the graphs indicate, the yeast concentration is less stable compared to the other measurements.



(a) Average values of all the mixtures



(b) Box plots of High yeast concentration, water, air (from high capacitance value to low capacitance value)

Figure 7.5: Different liquids measurements

7.1.3 Electrode Polarization

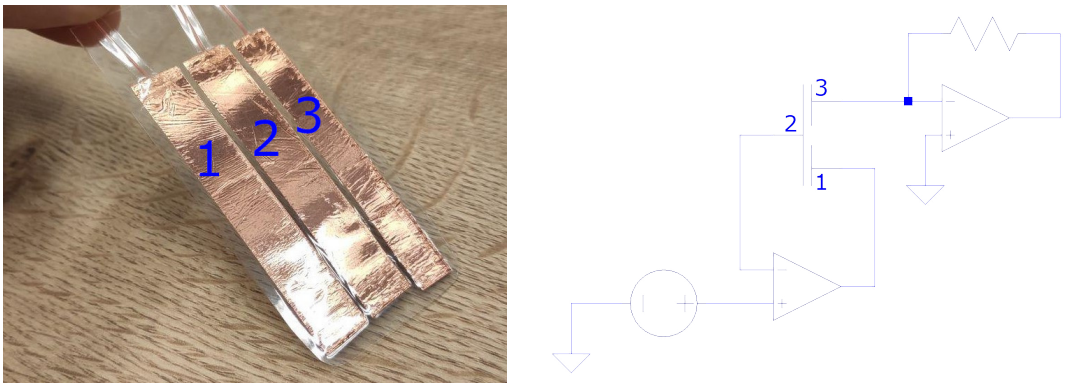
Preventing polarization requires smart use of the electrodes. These methods use three electrodes. It uses the theory described in Section 3.5 for the prevention of the polarization.

Prevention Principle

In the prevention principle, the measurements electrodes are not polarized, taking out the polarization effect. Figure 7.6a shows the electrode used and Figure 7.6b shows the corresponding schematic.

This approach is to minimize the electrode polarization at two electrodes, the reference electrode and the sensing electrode, referred to as electrode 2 and 3 in Figure 7.6a.

The potential field is applied by electrode 1, which is the polarized electrode. If the amplification is strong enough, electrode 1 will cause a potential in the liquid to form, in which the voltage at electrode 2 is similar to the voltage at the input of the OpAmp. The capacitance is between electrodes 2 and 3, which are non-polarized, using the voltage at the input as the reference voltage.



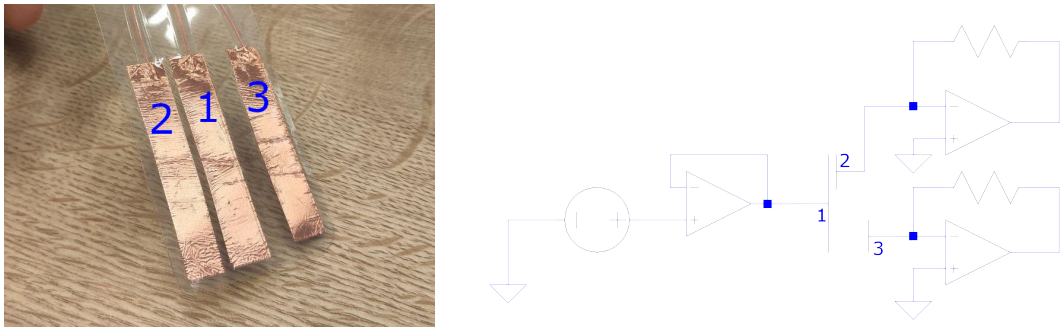
(a) Polarization Prevention (b) Polarization Prevention Schematic

Figure 7.6: Polarization Prevention Realization

Unfortunately, having the sensor also in the feedback loop makes the setup susceptible to noise. The output of the first OpAmp was not able to produce a clear signal. It cannot be ensured that there is a clear signal at the reference electrode. This setup is considered unstable and, therefore, not able to produce valid results.

Correction Principle

The correction principle uses the fact that polarization is constant so that it can be corrected when using two measurements. It cancels out the influence of the polarization by using electrodes at different distances. It results in two different capacitances, for which the change in capacitances relates to the change in distance. Figure 7.7a shows the electrode used and Figure 7.7b shows the corresponding schematic. The middle electrode, electrode 1 in the schematic, functions as the potential field applying electrode while the other two electrodes, electrodes 2 and 3, functions as the sensing electrodes.



(a) Polarization Correction (b) Polarization Correction Schematic

Figure 7.7: Polarization Correction Realization

Assumed is that both capacitances decrease when the frequency increases due to the β-dispersion and breakdown of the polarization. However, the difference in capacitance between the two different electrode pairs should change depending on the frequency and cell concentration. However, the measurements, shown in Figure 7.8, show no significant differences between the cell concentrations and water measurements.

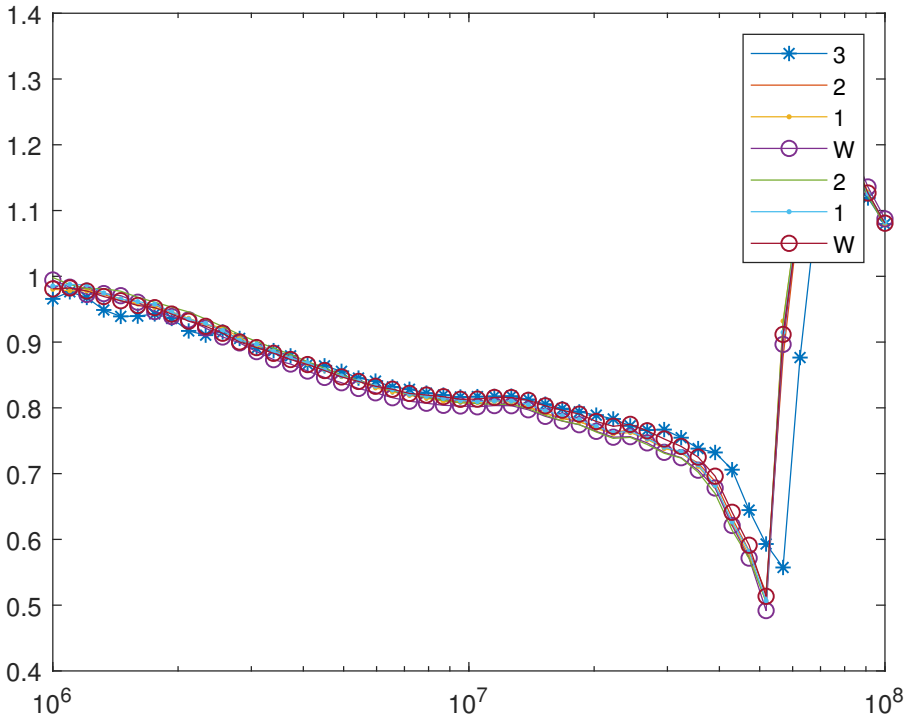


Figure 7.8: Differences between two electrodes readouts

7.2 Milk Tests

In this section, the results from the tests are validated using real milk just gotten from a cow. Since the desired cells to be measured form milk is significantly

larger compared to yeast cells, there might be a slight difference in the results.

However, if these results from real milk are similar to the results in the previous paragraph, it can be concluded that the other test setups used in the previous paragraph do represent the actual response of the system when used with milk.

7.2.1 Milk Results

Thanks to the colleges at Nedap, milk references are easily obtained. Hand milking a cow obtained the samples used in this test. The exact SCC of these samples is unknown. However, from the measurements, it should be able to measure differences between cows and the reference samples. The samples are referring to the cows' number. Some measurements are taken multiple times, as indicated by the single-digit after the cows' number.

Notice in these measurements that the capacitance of milk does not decay at the higher frequencies, while all of the other substances did decay. Pay additional attention in measurements "cow174 23 and "cow174 13". These are measurements of a milk and water mixture, for which "cow174 23" consist of $\frac{2}{3}$ of milk and "cow174 13" consist of $\frac{1}{3}$ of milk. The water component in the milk makes the capacitance decrease at higher frequencies. Interesting is that milk consists of 87% out of water [42]. There must exist some part in the milk, which increases the permittivity of milk.

The time from the farm to the office was still a few hours. Possibly enough to kill the majority of cells. Another test needs to be conducted to ensure a measurable amount of living cells.

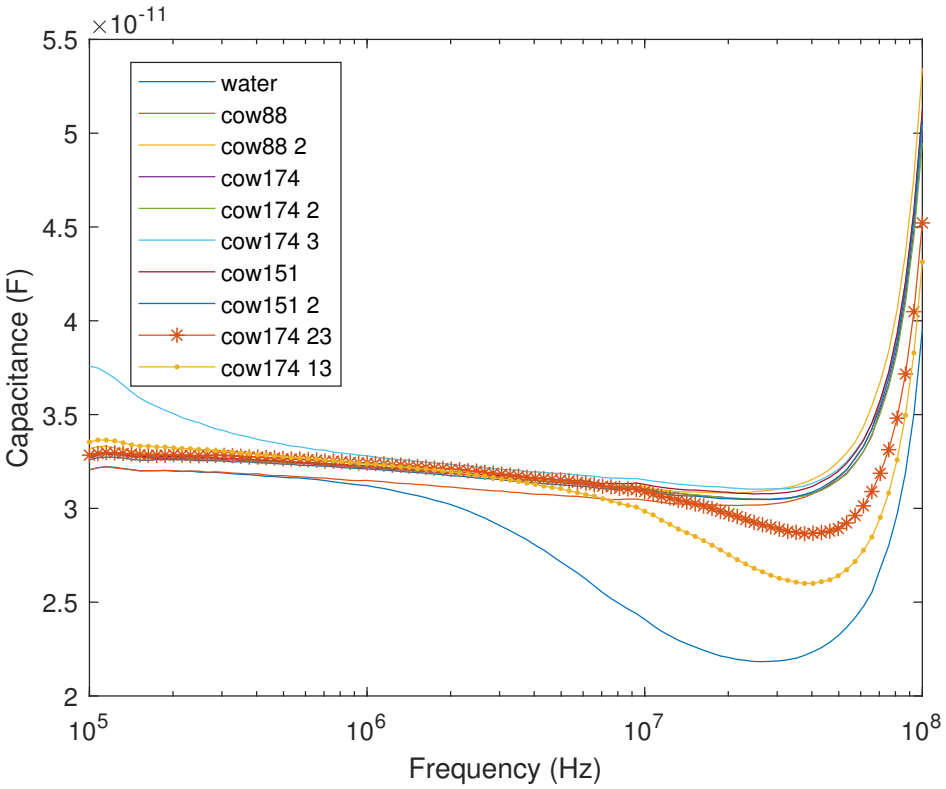
7.2.2 Milk Stability Results

The milk supplying farms are close to the office, from which the milk can be taken from the farms to the office without a significant cell-death. It took 15 minutes to get the milk from the farm to the measurement setup at the office. Milk samples used in Table 3.1 are measured within two hours after milking, Indicating that there is still a sufficient amount of time to perform transportation and measuring the samples. It implies that milk from the bulk-tank is not suitable for this application. The used setup is the same as in Section 7.1.2.

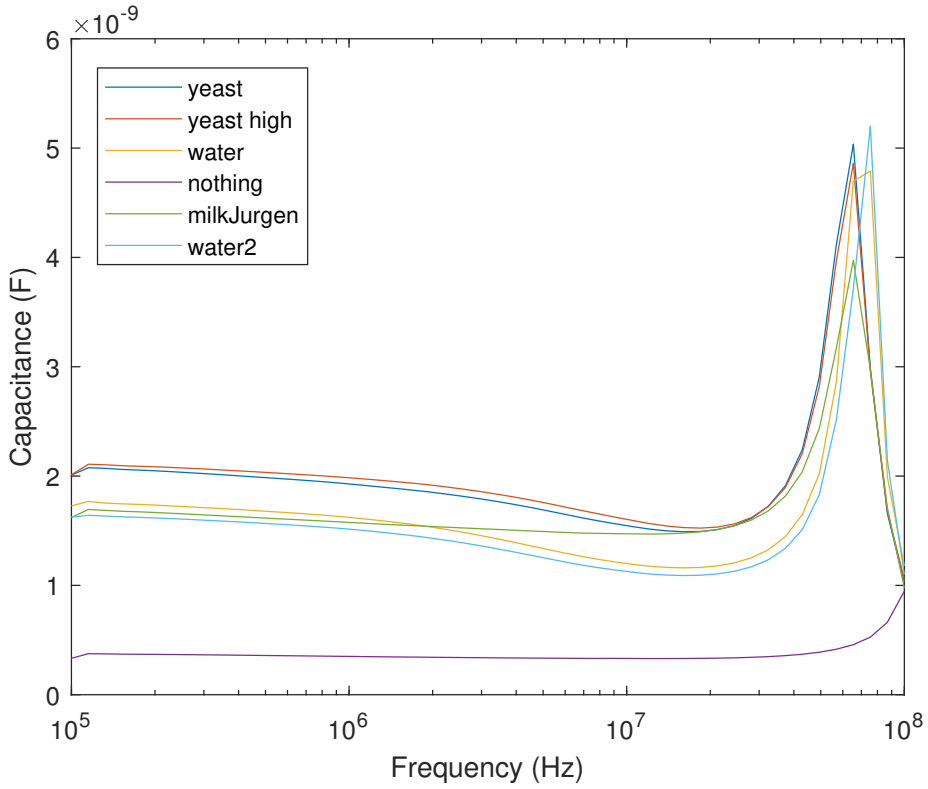
The results using the fresh milk correspond to the results described in the previous section. The results indicates that cell death, and cells in general, did not have any influence on the measurements, but some other aspect in milk keeps the measurements constant thought the whole frequency range.

7.3 Conclusion

While the experimental setup indicated some differences in measurements based on the concentration of cells, this appeared not to be the case using milk. The milk measurements of capacitances are stable throughout the whole region. These results do not correspond to the expected results. The next chapter will discuss the results together with the theory.



(a) Capacitance of small milk samples from different cows.



(b) Measurements including fresh milk.

Figure 7.9: Milk measurements

Chapter 8

Discussion

Considering the research focusses on the design of a sensor that can detect early symptoms of mastitis, some interesting results came to light. This chapter discusses the findings of this research in comparison with literature.

8.1 Cell Dispersion

Assumed was that the different cell dispersions are measurable when isolated electrodes are used. Unfortunately, this appears not to be the case. The total capacitance of the liquid is being measured, for which the cells do not contribute to the measurements.

The fact that the whole liquids' capacitance is high becomes especially noticeable when measuring real milk. The milk itself behaves differently than the test-mixtures used throughout this research. While research on cell capacitance is done for determining the cell concentration in controlled environments, the influences on other substances are yet unknown.

8.2 Current Sensors

The main difference between the approach in this research and the currently available sensors in this domain is the dielectric insulator. Sensors available all use electrodes that are in direct contact with the liquid itself and applies a potential over the liquid. It causes that the only insulator involved are from cells within the liquid. This also explains why these researches never discussed other materials that influence the capacitance. If the material is not insulated, it will react as a resistor instead of a dielectric.

8.3 Prototype Performance

All the circuits tested have similar problems at the higher frequencies. When the frequency increases, the reactance by the capacitances becomes very small, which makes the circuit susceptible to noise and resonance frequencies. Figure 7.5a indicates that this behavior is linked to the capacitance of the sensor. It shows that this peak is shifted towards higher frequencies when the measured capacitance is lower. Removing this behavior is quite hard. Parasitic components are removed to a certain level. Other methods to shift this pole more towards higher frequencies is to use a smaller sensor. This decreases the measurable capacitance. It does also decreases the effect, which makes it harder to

detect the dispersions. The performance of the prototype did not perform optimally over the whole range, although it functions sufficiently to detect whether this method was able to determine whether the method can determine the SCC.

8.4 Electrode Insulator

In Section 3 are some products and methods described that are able to measure the concentration of cells using capacitance as the parameter. The main difference is the placing of the electrodes within the liquid. All these products have the electrode in direct contact with the liquid under test, providing for a current trough the liquid itself. The main difference is the insulator — the cell-wall functions as the insulator instead of the plastic when current applied on the liquid. The lack of an insulator prevents other materials from polarizing due to the lack of an insulator. The capacitance of the cells is not significant enough to assume the concentration.

One major challenge to overcome using this method is the high conductivity of milk. Research has been done on measuring the biomass of cells in highly conductive medium [4]. They used a commercially available system made by Forgale, nowadays owned by the Hamilton company [24].

Chapter 9

Conclusion

As indicated in the introduction, this project is about the development of a sensor that can correctly estimate the SCC using capacitance as a parameter during milking. Unfortunately, the proposed method is unable to identify the influence of cells on the capacitance measurements. The capacitance of the cells did not have a significant influence on the total capacitance, making it unable to detect using all the liquid as the dielectric. Other aspects of milk have a significant influence on the total capacitance, masking the dispersion effect.

However, as the previous paragraphs indicate, there is still a method that uses the same principle, namely the β-dispersion to determine the concentration of cells in liquid. It primarily uses the same signal processing as the capacitance measurement. By implementing the optimizations described in the previous paragraphs, it can be optimized to be implemented on embedded devices.

9.1 Future Work

Research on estimating the concentration of cells in liquid-based on capacitance is proved to be successful. As written in Section 3.3.2, the relation between cell concentration and capacitance is linear. Instead of using insulated electrodes but conducting electrodes and applying a current in the liquid might result in measurements that can determine the total capacitance caused by the cells. This method could be a follow-up study for another student.

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