DESIGN AND DEVELOPMENT OF A NOVEL ELECTRONIC SENSOR FOR DETECTING MASTITIS BASED ON CONDUCTANCE/IMPEDANCE MEASUREMENTS

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DECLARATION

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Master of Science, is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: Valerie Fawcy ID No.: 51187191

Date: 24 June 2004

To my husband and son

&

" A Mes chers parents"

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LIST OF SYMBOLS AND UNITS

A	Area (m^2)
B	Susceptance (Ω)
C	Capacitance (F)
C_{dl}	Double layer capacitance (F)
E	Potential (V)
E_{app}	Applied potential (V)
F	Faraday constant
f	Frequency (Hz)
G	Conductance (Ω)
i	Current (A)
i_{dl}	Double layer capacitance current (A)
k	Initial specific conductivity (Sm^{-1})
R	Resistance (Ω)
T	Temperature (K)
Y	Admittance (S)
Z	Impedance vector (Ω)
Z_w	Warburg Impedance (Ω)
Z'	Real part of Impedance (Ω)
Z"	Imaginary part of Impedance (Ω)

LIST OF ABBREVIATIONS

AC	Alternating current
CV	Cyclic voltammetry
DC	Direct current
EC	Electroconductivity
EDL	Electrical double layer
EIS	Electrochemical Impedance Spectroscopy
BMSCC	Bulk tank somatic cell count
CMD	Clinical mastitis detection
ICSCC	Individual cow somatic cell count
IMI	Intramammary infection
ppm	Parts per million
SCC	Somatic Cell Count
SCMD	Subclinical mastitis detection

ABSTRACT

Mastitis, inflammation of the mammary glands of dairy cows, remains one of the costliest diseases in cattle and its early detection remains a major goal of the dairy industry. It has been established that mastitis causes changes in the conductivity of milk, by damaging the mammary membrane leading to alteration of the balance of sodium, potassium and chloride ions. Previous studies have demonstrated that these changes in milk conductivity can be used as a direct indicator of the presence and degree of mastitis infection but currently available commercial devices do not appear to be sensitive enough to detect mastitis accurately on-line.

The objective of this project is to design a novel electrochemical sensor to diagnose mastitis on-line in milking systems for cow herds. The study includes some preliminary experiments to assess the feasibility of developing such a sensor, the fabrication of the sensing system including the probe, the instrumentation and related software and the investigation of integrating the system into milking equipment.

A number of primary experiments were carried out in order to investigate what parameters can be used to develop the sensor based on conductivity or impedance measurement. Milk properties such as dielectric properties, conductivity and oxidation behaviour have been investigated using various electrochemical techniques. Cyclic voltammetry was used to characterise the milk initially, electrochemical impedance spectroscopy was carried out to establish the specific electrical properties of milk and basic conductivity measurements were done to calculate the overall conductivity of milk. These methods were cross-referenced against Somatic Cell Count (SCC) measurements which were determined using a bench top instrument in Teagasc National Centre Research laboratories.

Electrochemical impedance measurements showed that measurements should be taken above an operating threshold frequency to avoid polarisation effects and to improve the sensitivity of the conductivity sensor. Above this frequency, a good correlation between Somatic Cell Count (SCC) and impedance in milk has been found.

The first prototype portable instrument was designed and built in collaboration with a UK partner to make the impedance measurements following the specifications which were determined based on the preliminary studies. For the development of the sensing system, various electrochemical probes were developed after investigating the most suitable electrodes to be used in the system and taking into consideration technical and economical aspects such as cost and easy or low maintenance. The probe used comprises a two-electrode cell similar to those used in a normal conductivity sensor. The results from the instrument correlated very well with the benchtop instrument results. The sensitivity of the detection method is satisfactory even using absolute values of impedance. The sensitivity of the detection system is improved when ratioing of the highest quarter instead of the absolute values. This method allows better prediction of mastitis and lower false positive results.

For the integration of the system in the milking parlour, prototype claws with integrated electrodes have been developed and tested in field studies. Two designs of claws in which the measure of conductance takes place while the milk is flowing have been studied but they are not yet satisfactory. Following integration of the system on-line, early detection of mastitis in cows will be possible and this project is ongoing.

The first chapter is a discussion about the issue of detection of mastitis disease in dairy cattle. It comprises a short overview of the main characteristics of milk, a description of mastitis disease and its treatment in cattle and finally a discussion of the means of detecting mastitis both off-line and on-line.

1.1 Overview on cow's milk

1.1.1 Definition of milk

Milk is an extremely complex fluid synthesised by the mammary glands of female mammals to nourish their young [1]. Cow's milk, which is produced for human consumption, is known as a high nutrition value food. Similar to all animal products, milk requires rigorous quality control due to the fact that it may transfer diseases to humans [1,2].

Milk is defined by the United States Code of Federal Regulations (USCFR) as "*the lacteal secretion, practically free from colostrums, obtained by the complete milking of one or more healthy cows, which contains not less than 8.25% of milk solids-non-fat and not less than 3.25% of milk fat*" [3]. From this definition it is understood that some biochemical processes produce milk and a minimum amount of solids-non-fats and fats is required to get a milk of acceptable quality. The colostrum mentioned in the definition above corresponds to the first stream of milk, which transfers the immunity to the calf, and after a few days (that can vary from 3 to 5 days) the composition changes to give eventually the white liquid called milk. The details of the mechanisms of secretion are discussed in the next section.

1.1.2 Milk production

This section details the lactation mechanisms and describes the milking machine where on-line measurements for quality control are usually set up.
Some figures on dairy worldwide production are also given to demonstrate the importance of the dairy industry.

1.1.2.1 Secretion of milk

Milk is secreted after a sequence of complex chemical and physical reactions in the mammary gland. The udder positioned on the ventral surface of the animal consists of four mammary glands in which each gland has one teat to secrete the milk. The teat is constitute of fragile tissues and gets easily infected or damaged.

The milk-producing units are called alveoli (plural of alveolus) which are covered from inside by a layer of epithetial secreting cells [4]. The anatomy of the bovine mammary gland is illustrated in Figure 1.1. The central area of the alveolus serves as a storage area. The alveolus is connected by the lumen to the duct system by which the milk reaches the teat cistern and finally the teat. Blood capillaries and nerves surrounding the alveoli play an important role in the milking procedure.

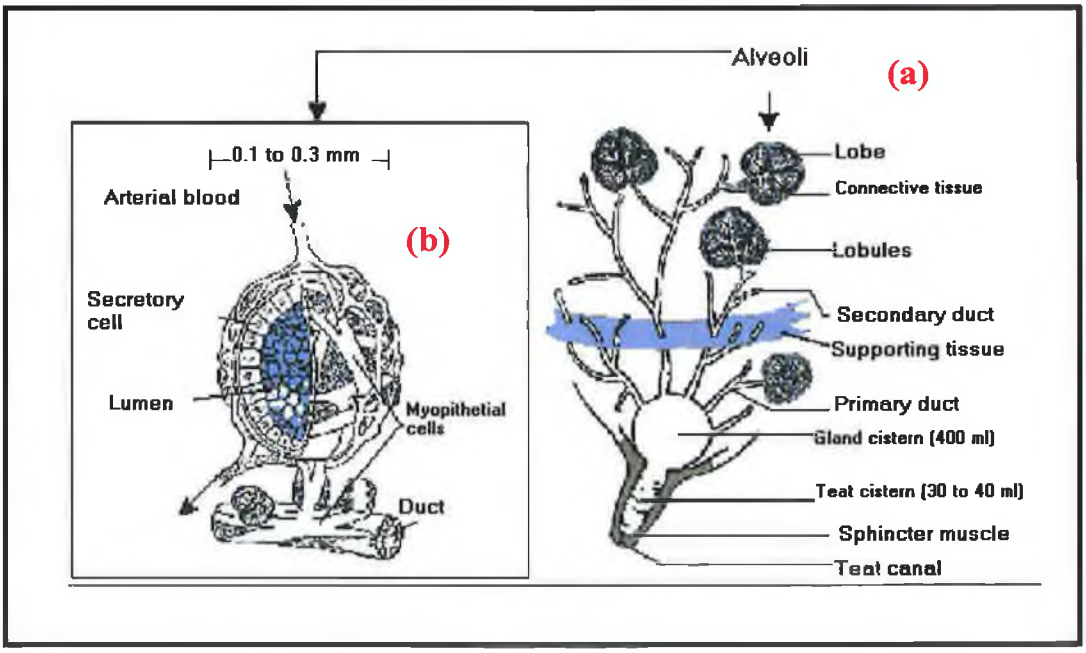

Figure 1.1: **(a)** Structure of the bovine mammary gland; teat, milk ducts.
(b) Magnification sketch of an alveolus showing secretory cells, capillaries blood and the storage area called lumen.

(Figure taken from Dairy Essentials n°20, Babcock Institute for international dairy research and development with the kind permission of the author Michel. A Wattiaux) .

1.1.2.2 Milking

Milking is simply the act of collecting milk after proper stimulation of a cow udder to release milk from the mammary. Upon triggering the milk ejection reflex, the milk is squeezed out of the alveoli by the muscular cells and flows into the duct system and the teat canal [1,4]. Cows are usually milked every 12 hours for about 5 minutes. Production yield is highly dependent on the milking process used.

It was observed that during the first six weeks of lactation, milk production increases continuously. After a peak is attained, milk yield gradually declines. The monitoring of milk production is usually followed by constructing the lactation curve that represents the dairy milk yield (kg) versus time of lactation.

1.1.2.3 Milk production

In the year 2000, the world production of milk was evaluated at 579.4 million tonnes. Dairy products such as cheese, yoghurt count for 68% [5]. Milk originating from cows constituted the highest percentage (84.5%), with buffalo milk in second place at 12%. On the world stage, the European union is the main milk producer with 25%, followed by the United States with 19% [5].

The statistics given by the Food and Agriculture Organisation (FAO) also indicate that Ireland is the world's largest consumer of liquid milk with 156.7 kg of liquid milk per capita per year [5]. In other words, each Irish individual consumes around 3 litres of milk a week!

1.1.2.4 Milking machines

A milking machine is defined as a complete installation for milking, usually comprising of a vacuum system, pulsation system, one or more clusters and other vacuum components. The vacuum produces the forces necessary to move air and milk through the system. Thus, it is the atmospheric pressure that makes air and intra-mammary milk pressure exert forces on milk in the system and the combination of these forces causes the flow.

Figure 1.2: Photographs showing **(a)** the milking unit (or cluster): the claw, the teatcup (steel), the milk tubes (black rubber) and the pulses tube (transparent plastic). **(b)** The milking unit in operation: the mammal is shown in the photograph. (Taken from *www.milkingmachines.co.uk*).

The improvement of the milking machine is a constant preoccupation for farm managers. Recently, a new generation of automatic milking systems has appeared. Many research projects are now aimed at increasing the milking efficiency and preventing herd disease. For instance, a new design of flow simulator developed at Teagasc Research Centre (Moorepark, Ireland) allows the recording of vacuum in the claw at the end of an artificial teat in the pulsation chamber and in the milk pipeline [6]. This simulator is then very useful in the practical understanding of vacuum loss processes and mechanisms.

1.1.3 Chemical and Physical properties of milk

This section describes the specific structure of milk and also some of its properties. Milk is a nutritious product that contains more than 100 substances that are either in solution, suspension or emulsion in water. It is important to understand the structure of the milk before analysing it.

1.1.3.1 Structure of milk

Milk is a complex fluid containing components with different physical states of dispersion [1,2,7,8]. Milk is described as:

- A colloidal suspension. This is due to casein, the major protein of milk, which is dispersed as tiny solid particles that are continuously in suspension. These particles are called micelles, and the dispersion of the micelles in the milk is referred to as a colloidal suspension.

- An emulsion. This is due to the presence of fat and fat-soluble vitamins in the milk that form a suspension of small liquid globules that do not mix with the water in milk.
- A solution. This solution is mainly composed of lactose (milk sugar), some soluble proteins which are called whey proteins, mineral salts and other substances.

1.1.3.2 Composition and principal properties of milk

An approximate composition of milk with its principal constituents expressed in percentage terms is given in Figure 1.3.

The principal component of milk is water [1]. This important aqueous phase constitutes the continuous phase and is useful to allow the milk to flow in the teat canal. Indeed, without water, milk would only be a viscous secretion composed of fats and proteins. The milk solids contain lactose, fats and proteins. Lactose is unique to milk and it plays a major role in milk synthesis.

The 0.65% of minerals mentioned is mainly salts of phosphates, citrates, chlorides, sulphates, carbonates and bicarbonates of sodium, potassium, calcium and magnesium. Other elements are found in milk in trace amounts, including copper, iron, silicon, zinc and iodine.

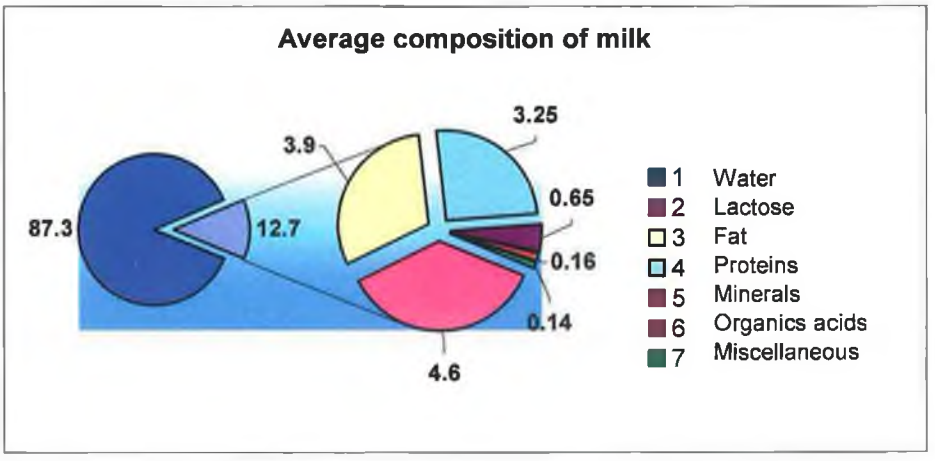

Figure 1.3: Approximate composition of milk in percent *(Plotted from data taken from a table in ref 1).*

The 3.9% fat component of milk is composed of a complex mixture of lipids. Triglycerides are the major type of lipid in milk fat. Triglycerides are composed of three fatty acids covalently bound to a glycerol molecule by ester bonds. Milk fat is essential for the neonate that is able to synthesise milk fat as an energy source only a

few days after birth. Others components of milk are susceptible to changes in concentration during different stages of lactation. Some of these components include: Oligosaccharides, urea, ammonia, oxygen, cells (epithelial cells, or bacteria present when the cow suffers from udder infection) and viruses.

The huge percentage of water in milk explains why milk has mainly aqueous properties and hence it can be analysed using analytical techniques suitable for aqueous samples. Milk solids give milk its principal properties. These are summarised in Table 1.1.

Properties	Values	Conditions
Freezing point	-0.518 to -0.534 °C	Lower than that of pure water (0°C) due to dissolved substances in milk.
Boiling point	100.17°C	Greater than that of pure water (100°C).
Electrical conductivity	45-55*10^4 mho	In milk, fat and colloidally dispersed substances decrease conductivity.
pH (at 25°C) (with pHmeter)	~6.6	Fresh milk is slightly acid. Generally the pH is lower (pH 6.0) in colostrums and higher (up to 7.5) during mastitis.
Oxidation-reduction potential (25°C, pH 6.6, in equilibrium with air)	+0.25 to +0.35V	
Surface tension	50-52 dynes at 20°C	Cow's milk surface tension is about 70% of that of water. Factors involved in absorption and formation and stability of emulsions.
Specific gravity	~1.0321 at 20°C	Ratio of the density of the product and the density of water at the same temperature.
Viscosity	2.0-2.1 cp at 20°C	Refers to resistance to flow measured in centipoises. Used for design dairy equipment.
Ionic strength	0.08 Molar	
Refractive index, n_D^{20}	1.3440-1.3485	

Table1.1: General physical properties of milk

(Adapted from The Doyle Pharmaceutical Company (Minneapolis, USA) from ref 1 and ref 8).

Any addition of water to milk can be easily identified because solids concentrations in milk will no longer be in the normal ranges. The casein micelles and the fat globules give milk most of its physical characteristics, and give taste and flavour to dairy products such as butter, cheese and yoghurt.

1.1.3.3 Other remarks

Due to its physical state, milk is very susceptible to adulteration and deterioration. Consequently, milk is quickly cooled down to 4°C after collection for conservation until processing such as sterilisation or ultra heat treatment are carried out. Indeed, at cold temperatures the micro-bacteria have lower growth rates and this reduces contamination of the bulk milk [1].

Many factors influence cow's milk composition such as the breed type, diet, season, herd-to-herd variation, health condition (oestrus, mastitis) and of course milking procedure [9]. Humans can interfere with and control some of these factors in order to produce milk of desired quality such as: health condition and milking procedure.

Factors that influence cow health are illustrated in Figure 1.4. It is clear that careful monitoring of cow's udder health can improve milk quality.

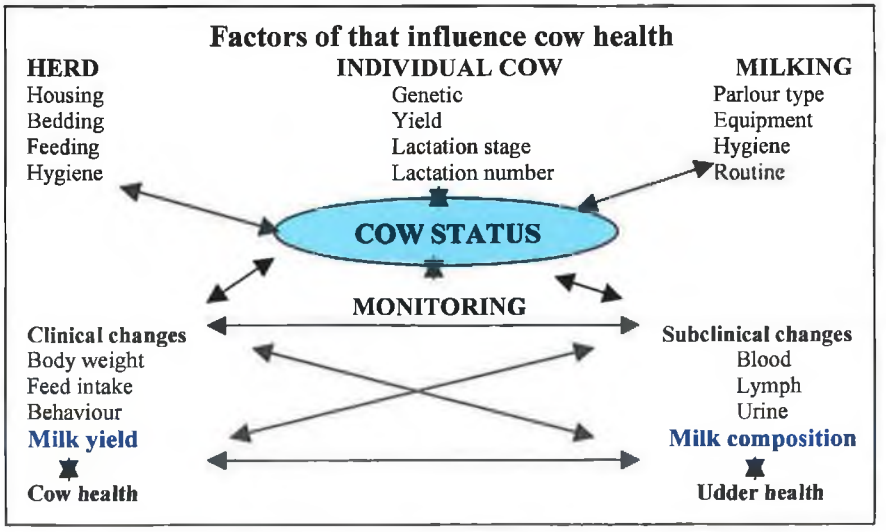

Figure 1.4: Possible factors for evaluation of cow health *(Adapted from ref 9)*.

1.2 Mastitis and its treatment in cattle

1.2.1 Mastitis definition

1.2.1.1 Definition of mastitis

Etymology: Greek: *Mastos* = breast and *itis* = inflammation.
In the Encyclopaedia Britannica, mastitis is described an inflammation of the mammary gland (the breast or udder) occurring in dairy cattle such as cows, goats, and ewes.
Medically, mastitis is defined as an inflammatory reaction of the parenchyma of the mammary gland due to bacterial, chemical, thermal or mechanical injuries [10].

1.2.1.2 Development of the disease

Mastitis infection may occur when bacteria or irritant microorganisms penetrate into the mammal of the animal. A healthy teat is tightly closed during the no-milking periods such that bacteria present in the environment cannot enter it. However, while the cow is being milked the teat canal remains dilated for a short period of time afterwards (one to two hours) which can allow the microbes to infiltrate the teat canal and multiply inside the organ. The same effect can be also observed after the udder tissue has been damaged, as the sphincter muscle becomes unable to close the canal fully anymore.

The immunological response to fight against the infection is marked by a massive production of leukocytes (or white cells) in milk. The purpose of these leucocytes is to destroy the irritant microorganisms or the bacteria using a phagocytose mechanism, and then to repair the damaged tissue and return to normal function. Nevertheless, when a leukocyte engulfs bacteria, some toxins that facilitate the transfer of other white cells from the blood to the milk are released leading to a high permeability of blood vessels. Eventually, leucocytes move from the blood to the milk easily. Hence, the change of the amount of leucocytes in the milk enables researchers to evaluate the degree of disease spreading by counting the number of somatic cells in milk. Moreover, the blood passing into the milk may form some clots that may finally block the teat canal or duct systems.

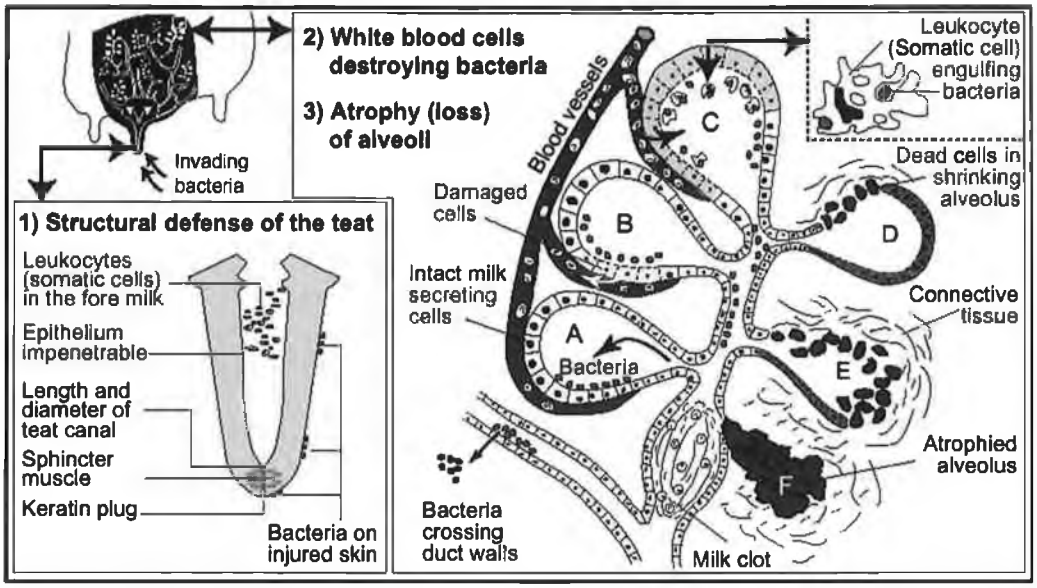

Figure 1.5: Development of mastitis and the cow's defence against the infection.

(Figure taken from Dairy Essentials n°23, Babcock Institute for international dairy research and development with the kind permission of the author Michel. A Wattiaux).

To summarise, once bacteria enter the teat canal, two phenomena might take place. In the first instance, the bacteria are killed by the cow's defence system (leucocytes); the infection is cleared so the secretion of the milk goes back to normal. In the second instance, the infection is persistent and the bacteria invade the tissue of the teat canal before entering into the mammary gland and multiply. The system defence is ordered to kill all bacteria by destroying the infected organ and replacing the tissues by some non-secreting or connective tissues. Production of milk is stopped and the alveoli start to shrink, therefore more and more blood is observed in the composition of the milk, the milk becomes unfit for human consumption and the disease status is advanced.

1.2.1.3 Causes and types of mastitis

The causes of mastitis are disputed. One theory places the blame on certain predisposing causes (injury to the udder, milking practice, etc.) that reduce the animal's resistance and permits organisms to produce the disease. In this case, the best control for mastitis is the elimination of predisposing causes. Other theories believe that micro pathogens transmitted from cow to cow cause mastitis. Thus, mastitis is generally classified into two groups: "contagious" and "environmental".

A variety of pathogens can induce mastitis infections. A total of 137 microbial species have been referenced in the bovine mammary gland [11]. The mode of entering has been already described in the previous section 1.2.1. Pathogens known to cause mastitis

are of different kinds: bacteria, coliforms, yeast (present in milk after an overuse of antibiotics), and viruses. The most common bacteria stake are "*Streptococcus agalactiae*" and "*Staphylococcus aureus*".

Streptococcus agalactiae cannot live for long outside the organism and thus it is generally easy to eradicate their spread. It spreads during milking via the milking machine and herd person's hands; hence a good control of cleanliness should be enough to avoid this type of contamination.

Staphylococcus. Aureus is known to be the major bacteria responsible for bovine mastitis, especially subclinical and chronic mastitis. Milk production losses are reported to be 45% per quarter and 15% per infected cow. It is recognised to influence greatly the somatic cell counts (SCC) fluctuations [12]. These sorts of organisms can live inside and outside the udder. It is observed that for such bacteria the infection induces scarring and it is difficult to treat without heavy antibiotics.

The coliform bacteria "*Escherichia Coli*" are commonly involved in environmental mastitis infection; they are present in environments where cleanliness is not respected. The coliforms are normal inhabitants of soil and the intestines of cows, and their action is different than for contagious bacteria, as they mix with milk and multiply secreting some toxins that transfer into the blood stream. The consequence is a severe form of mastitis. The infection is general and the animal is usually in extreme danger.

1.2.1.4 Degrees of mastitis

The degree of the infection is defined according to the symptoms of the animal. There exist three degrees of infection: clinical, subclinical and chronic.

In the clinical form of mastitis, some gross abnormalities in the udder are noticed. The infected mammal usually presents swelling, heat, redness, pain and disturbed function [10,11]. In addition, some flakes or clots are observed in the milk.

For severe cases (acute mastitis), the animal may suffer from different but related symptoms, for instance: fever, rapid pulse, loss of appetite and importantly a decrease in milk production. Furthermore, it has been noticed that for one clinical case diagnosed there are 20 to 40 times as many subclinical case [10].

Subclinical mastitis is a real challenge to detect for dairy farmers; it is a major issue because of the possibility of contaminating the rest of the herd. Indeed, the diagnosis is not straightforward as no visible changes occur in the appearance of the milk or the udder.

A subclinical milk sample is changed in composition but no gross abnormalities are observed. The only changes that may be noticed are the detection of pathogenic agent during analysis, an increase somatic cell count or a decreasing yield in milk from the cow.

Finally, chronic mastitis is diagnosed when the inflammatory process persists for months at a time.

1.2.1.5 Cost of mastitis in a dairy herd

Mastitis is among the most important [13] and costly disease found in herd cattle. Worldwide, annual losses due to mastitis have been estimated to be approximately 35 billion US dollars [14]. In the US, the annual costs of mastitis have been estimated to be 1.5–2.0 billion US dollars. The economical impact is huge and is due to different consequences related to the disease such as production losses when the yield decreases, non-deliverable milk due to antibiotics that may remain in the milk and reduced milk quality with a resultant decrease in price. Sometimes there can be a udder damage is irreversible and severe, treatment and labour costs can be high, problems with subsequent cheese processing and manufacturing and of course, there is always the risk of early death of the infected cow [15,16].

1.2.2 Effect of mastitis on milk

1.2.2.1 Somatic cells and their relation to mastitis

Etymology: *Somatic*: Derived from the body

Milk contains white blood cells or leukocytes that make up the majority of somatic cells. As stated in the description of the disease development, these cells move into the udder during inflammation. The measurement of the number of somatic cells in milk is defined as somatic cell count (SCC) [17]. SCC has firmly established itself as a good indicator to detect and determine the degree of mastitis infection [11,15,17,18], and also to predict monetary losses due to intramammary infection [19].

The bulk tank somatic cell count is usually performed to evaluate the milk contamination in the tank. Although it is not really accurate, it is generally accepted that for healthy milk bulk tank SCC should be less than 200,000 cells /ml in the European union. In the case of mastitis infection, this cell count increases and the milk is considered to be suspect or abnormal. Indeed, dairy cows have evolved a complex internal body system to combat infection. When mastitis occurs, the amount of

leukocytes (or fighting cells) increase in the infected area to combat bacteria by phagocytose [10,11].

A change in SCC does not always indicate an infection; other factors have been found to affect the somatic cell amount, for instance season, age, stage of lactation, stress, and technical aspects. However when SCC reaches more than 200,000 cells/ml, it could be a sign of primary infection. SCC can be counted in laboratories with direct analysis methods (See section 1.2.3). Under EU standards 92/46/EEC directives [20], milk intended for human consumption, for drinking or for food processing, must have a total bacteria count of not more than 100,000 per ml and a somatic cell count of not more than 400,000 per ml. Hillerton suggests that milk quality is acceptable if < 10% of the cows have SCC between 200,000 and 400,000 cells/ml and none above this level [21]. To encourage farmers to respect these quotas some bonus payment schemes based on low somatic cell count and on measurable microbiology results have been set up [15,20].

1.2.2.2 Changes in milk composition

Milk composition is reported to change with the onset of mastitis. These changes results from, first, the reduction in synthesis activity for the main components of milk (fat, lactose and casein) and second, the increase of presence of blood elements due to the intramammary infection (proteins, chloride and sodium)[22].

The types of proteins change dramatically. Casein, the major milk protein declines, and this milk of lower quality which in turn impacts the quality of the dairy products such as cheese affecting its yield, flavour and quality. The disruption of casein synthesis contributes also to lowered calcium in milk. Also, levels of glucose in milk are found to be decreased when cell counts rise above 500, 000 cells/mL.

Table 1.2 reports the effect on the milk composition caused by mastitis.

Constituents	*Normal Milk (%)*	*High SCC Milk (%)*	*Percent of Normal*
Milk non fats solids	8.9	8.8	99
Fat	3.5	3.2	91
Lactose	4.9	4.4	90
Total protein	3.61	3.56	99
Total casein	2.8	2.3	82
Whey protein	0.8	1.3	162
Sodium	0.057	0.105	**184**
Chloride	0.091	0.147	**161**
Potassium	0.173	0.157	91
Calcium	0.12	0.04	**33**

Table 1.2: Change in milk constituents associated with elevated somatic cell counts. *(Adapted from Harmon [21]).*

The concentration of minerals is found to change with mastitis infection because of increased blood capillary permeability, the destruction of tight junctions, and the destruction of ion-pumping systems [23]. The concentrations of sodium and chloride increase. On the contrary, the concentrations of lactose, calcium and potassium ions are significantly reduced [21]. Also, concentrations of fat, solids non-fat, lactose, and casein are lowered and concentration of blood serum albumin increased.

The decrease in lactose concentration leads to a decrease in milk yield, as lactose is a major osmotic regulator of milk volume. The sodium and chloride ions come from the blood to compensate osmotically for the depressed lactose synthesis or vice versa. They are related by the Koestler number [8]:

$$\text{Koestler number} = \frac{100 \times \%Cl}{\%\ \text{Lactose}} \qquad \textbf{(Eq 1.1)}$$

This number is normally 1.5-3.0 but increases on mastitis infection and has been used as an index for mastitis.

Also, the activity of hydrolytic enzymes in milk is higher which leads to the breakdown of blood constituents into milk such as sodium and chloride and milk constituents into blood which explains the decrease in potassium levels.

The pH is also seen to change showing as well a conductivity change. The pH of normal milk is about 6.6-6.7 but during mastitis infection the pH increases to as high as 7.2. Indeed, milk pH approaches the pH of blood 7.4 due to degeneration of the mammary cell membrane and the influx of constituents from blood [8,11].

The changes in composition are difficult to understand as various salts in milk are interrelated and these relationships are also affected by pH.

1.2.2.3 Other alterations /clinical manifestations

In many cases a reduced milk yield can be observed. The appearance of clinical milk may change to a yellowish colour and have a watery "look" instead of a white-to-white yellow appearance for healthy milk. Moreover, some flakes or clots of blood can be found. The flakes are usually congealed leucocytes, secretory cells and protein. Such gross alterations appear as a result of bacterial infection of the mammary gland.

The variation of proteins, quality and ionic concentrations has critical effects on the subsequent cheese or dairy products processing. For example, sodium chloride concentration is important for the development of proper flavour, texture and quality of cheese. Hence, processing time is extended and shelf life is reduced. Other characteristics that may change are the organoleptic characteristics and due to a higher concentration of sodium and chloride the milk tends to have a salty taste.

1.2.3 Treatment of mastitis in cattle

There are two types of proper treatment procedures (recommended by the National Mastitis Council) for mastitis: lactation or dry cow therapy. The appropriate choice of therapy is important to achieve the best cure rate.

The first method involves the administration of an intramammary antibiotic during lactation. Several factors are important in the selection of antibiotic treatments. For instance, the type of pathogens involved is crucial. Indeed, antibiotics for use in lactating cows are ineffective against coliforms bacteria though antibiotic uses against environmental pathogens (see section 1.2.1.3) allow curing about 60% of the cases. Depending of the degree of infections, the cure rate during lactation can be very low.

The second technique is dry cow therapy. Dry cow therapy is the use of intramammary antibiotic therapy after the last milking of lactation. It is nowadays consider as the best option for mastitis treatment. It eliminates the majority of infections present at drying off and reduce rate of new infections during the first week of the dry period. Dry cow therapy has many advantages over the lactation therapy indeed a higher cure rate is noticed as a higher dose of antibiotics can be used safely, the retention time in the teat is longer, the tissue damages can be sometimes regenerated and the risk of contaminating milk is reduced saving thus the possibly discard milk when using lactation therapy. This technique is particularly attractive as the cost to the producer should be minimal [10].

The Food and Drug Administration (FDA) must approve the antibiotics used for either of the treatment procedure. The products used should be formulated specifically for lactation or dry cow therapy. In case of other off-label antibiotics use (e.g. home remedies), a careful veterinary control should be assured and the milk has to be discarded during seven days.

For both of these treatments, the procedure should be specific and very precise for a herd person, as careless treatment procedure would result in infection resistant to treatment.
For extreme cases of high SCC in a herd, the method of culling is necessary and the selection of the animal is usually done on the basis of the age. But this extreme solution is very costly for the farmer.

The best solution to prevent clinical mastitis is to follow rigorously proper milking procedure such as post-milking teat disinfections with a germicidal dip and respect of a clean environment and bedding (no stagnant water, etc...). Vaccine prevention uses reduce the severe cases causes by coliforms but is still not effective against *Staph. Aureus* and *Strep. agalactatie*. The detection by cow-side testing (see section 1.3.1.2) at subclinical stage also decreases the occurrence of clinical mastitis in herds, monitoring individual cow SCC and bacteriological culture allow to detect some subclinical affected animal however it is important to notice that some herds are affected even a low SCC results. Electrical detection based on the ionic concentration variation is suggested to check-cross the SCC diagnosis.

1.3 Detection of mastitis in cattle

Mastitis is a severe disease for the herd and therefore it is imperative to diagnose the breast infection at a subclinical stage. This section will discuss the problem of detecting mastitis at early stage and also will describe briefly the means both off-line and on-line of detection currently used in milking parlours to diagnose the infection.

1.3.1 Detection methods in general

1.3.1.1 Difficulties with diagnosis of mastitis

In the past, the herd person usually noticed the appearance of the fist clinical symptoms of mastitis on the animal.
Nowadays, the detection of abnormal milk is required before milking according to the EU directive: "*Before milking of the individual cow, the milker must inspect the appearance of the milk. If any physical abnormality is detected, milk from the cow must be withheld from delivery to the dairy*". Therefore, foremilk of each animal is now visually examined directly in the parlour using a strip cup or a plate. However, as no symptoms are really obvious, farmers or herdspersons rarely identify the primary signs during a routine inspection or during milking in individual cows [15]. Hence, the

biochemical properties of milk turn out to be very useful in order to evaluate the cow's metabolic status, in particular, the udder condition [24]. In the last few decades, a variety of cow side tests and laboratory tests have been developed for detecting mastitis such as the strip cup test, the California Mastitis Test, etc. Thanks to these methods of detection, foremilk control is usually followed by owners of small herds, but it takes a lot of time and money for large herds.

Currently available in-line mastitis detectors are fitted to the long milk tube. They have a wire mesh filter through which milk passes. Mastitis clots clog the filter and clot-free milk is able to pass through the filter.

Figure 1.6: Photograph of a mastitis detector on-line. (*Taken with permission from www.ambic.co.uk*).

Some assume that all cases of mastitis can be detected using this method; in fact those in-line filters can pick up 'clotty' forms of mastitis but will miss 'watery' cases and certainly subclinical degree of the infection. Therefore, the development of a reliable and automatically monitored on-line detection system has attracted the attention of a wide variety of researchers in fields like dairy production, microbiology and sensor research.

1.3.1.2 Cow-side testing

A variety of diagnostic tests for mastitis are available and are used as indicators of udder inflammation. They cannot, however, assess the degree of infection accurately. As the name indicates they are performed directly on the animal. These experiments are useful to indicate suspect samples but they cannot be accepted as definitive procedures as many false positives cases remain.

The **California Mastitis Test** (CMT) is an on-farm subjective screening test based on scoring the degree of gel formation of the milk and reagent mixture. The reagent consists of a detergent and an acid-base indicator. The detergent ruptures the somatic cells, which releases their DNA. Afterwards, the DNA mixes with the detergent and forms a precipitate or gel. The gel consistency is directly related to the amount of leucocytes present in the milk sample.

Figure 1.7 shows the special CMT paddle and a bottle of reagent. The test is simple and quite cheap. In order to perform it, 2 mL of milk are placed in each cup in the paddle and the same quantity of reagent is added. The paddle is then gently rotated in circular pattern for 10 s. The gel formation is recorded right after the test because the gel changes consistency with time [25].

Figure 1.7: The equipment required to perform a CMT test (bottle of reagent and paddle).

The **Wisconsin Mastitis Test** (WMT) is based on the same principle as the CMT but is for laboratory use only. The results are more quantitative than those of CMT [25].

Hand held meters based on conductivity measurements are also a cow-side test. This test is easy and rapid to perform. Depending on the commercial meters used, a few drops of cow's foremilk are collected in a cup into which some electrodes are built in. The results are displayed digitally. If few samples from the same cow exhibit high conductivity, the milk could be abnormal. The results may indicate a suspect cow but they need to be repeated and confirmed using further investigations using laboratory techniques.

1.3.1.3 Laboratory testing

When an animal is suspected of having mastitis symptoms or gives a positive result with a cow-side test, its milk is analysed using lab-based test methods to measure the degree of infection by counting the somatic cells or detecting the presence of bacteria. There are a number of tests based on bench measurements such as Direct Microscopic Somatic Cell count, Electronic Somatic Cell, Counting Methods or other microbiological methods [3].

One such system for measuring somatic cell count is the **Fluoro-opto-electronic method** and two machines that employ this method are Fossomatic and Bentley machines [26]. These instruments use a laser based flow cytometry system. The milk is mixed with some ethidium bromide, a toxic fluorescent dye solution used to disperse fat globules and stain DNA in the somatic cells. The somatic cells are separated by the stream of a carrier fluid and exposed to a laser beam. As the stained cells pass through the excitation source they fluoresce. The energy emitted by each nucleus is measured as an electronic pulse that is then converted to a count corresponding to the number of somatic cells in the sample analysed. Dead or partly degenerated bacteria absorb dye but produce signals of such low intensity as to be included in the background of the apparatus.

1.3.2 Electrochemical detection methods for mastitis

This section presents a short review of the main detection systems for mastitis using electrochemical means. It focuses on methods based on conductivity, conductance or impedance measurement for the development of hand held meters or on-line systems in milking machines. It does not, however, present the theory of conductivity that will be discussed in chapter 2. Also, it introduces the issue of threshold determination for "dumping" mastitic samples.

1.3.2.1 Electroconductivity sensor

An electrochemical sensor is essentially an electrochemical cell consisting of two or more electrodes in contact with a solid or a liquid electrolyte [27].

Electrochemical sensors are classified according to their mode of measurement: potentiometric (voltage), amperometric (current), and conductimetric (measurement of conductivity) sensors.

An electroconductivity sensor measures the change in conductivity of an electrolyte in an electroanalytical cell. Conductibility or Electrical Conductivity (EC) (symbol σ, units **S** (Siemens) or **Mho**) is defined as the ability a solution has to tranmit an electric current. The conductivity of a solution depends on the ionic concentration, type of ions, temperature and the geometry of the area in which current is carried.
Measurements of conductivity are usually made with glass cells in which the solution is contained or dipped between platinum electrodes. The resistance between the electrodes is measured (See section 2.3).

1.3.2.2 Electroconductivity of milk

Typical electroconductivity (EC) of normal milk appears to be between 4.0 and 5.5 mS/cm at 25°C [28]. The EC of milk has also been expressed as a concentration of NaCl with the same conductivity as the examined milk which reflects the total ionic concentration of the milk [29].
The electrical conductivity of milk is determined by:

- Concentration of charge carriers
- Interactive influence of ions
- Type of electrolytes

Dissociated inorganic salts are the main contributors to conductivity, with some 60% of the total conductivity being due to sodium chloride and potassium chloride [30].
Puri and Parkash [31] determined a empirical linear correlation between chloride content and conductivity:

$$\text{E.C of milk (at } 25^{\circ}\text{C)} = 0.685 + 0.1089\ [\text{Cl-}] \qquad \textbf{(Eq 1.2)}$$

The most accurate published measurements of the conductivity of milk as a function of fat and solids-non-fat are those of Prentice [32], who found that fat-free milk behaved electrically as a homogenous electrolyte. The fat globules of milk reduce the conductivity by occupying volume and by impeding the mobility of ions. Thus the conductivity of whole milk is less than that of skim milk by about 10%. Prentice [32] as well noticed that homogenisation of milk does not measurably influence conductivity of milk. EC changes with concentration or dilution of milk but the relationship is not simple because of the effects of concentration on the distribution of minerals between colloidal and dialysable phases.

Temperature control is important in conductivity measurements since the conductivity of milk increases about 0.113mS per degree Celsius rise in temperature [33,34]. Prentice [35] confirmed the high dependence of electroconductivity on temperature throughout the range from 15 to 40°C. He determined empirically that conductivity of milk increases with temperature according to a polynomial expression of quadratic form (within the limits of experimental error):

$$\kappa=\kappa_o (1+a\theta+b\theta^2) \qquad \textbf{(Eq 1.3)}$$

Where κ_o is the conductivity at 0°C

θ is temperature in °C

a,b are arbitrary constant.

Prentice noticed also that the values for the temperature coefficient (κ) are not constant and decrease with increasing temperature. Table 1.3 shows some values for the temperature coefficient at various temperatures.

Temp (in °C)	Normal milk	Mastitic milk	0.1M KCl
15	0.0241	0.0238	0.0224
20	0.0223	0.0220	0.0208
25	0.0208	0.0205	0.0195
30	0.0195	0.0192	0.0183
35	0.0183	0.0180	0.0173
40	0.0173	0.0171	0.0164

Table 1.3: Temperature coefficient ($^oC^{-1}$) of electrolytic conductivity at various temperatures. (Average of 6 samples). Adapted from [35].

The incidence of severe mastitis in a cow did not bring the temperature coefficient of milk outside the range of variation encountered in normal milk.

1.3.2.3 Electroconductivity of mastitis milk

In dairy cattle, EC increases following mastitis infection [36]. This increasing is due to changes of concentration of Na^+, Cl^- concentrations in the cow's milk [16].

Although somatic cell counts do correlate with specific conductivity, the cell counts appear to be more sensitive for mastitis detection [37,38] it has also been found that the ratio of specific conductivities of fore- and post milk was an effective index of mastitis due to the sharp rise in conductivity of the postmilk from infected quarters.

In addition to mastitis, other factors have been reported to have an influence on EC of milk such as oestrus, genetic group [39], milk fever [40] and intramammary antibiotic treatment of uninfected quarters [41]. Hormones like oxytocin appeared to change milk composition in a way similar to mastitis [42].

An increased milking interval can also be associated with a rise in EC of milk [43]. This could be explained by the increase in Na^+ and Cl^- and the decrease of K^+ found after incomplete milking and after an extended milking interval.

1.3.3 Electrical Devices for mastitis detection

The use of EC forms the basis of detection of abnormal milk in many automated milking systems and a few hand-held meters marketed internationally. Early studies have reported that there was a high correlation between conductivity and mastitis and this encouraged the use of milk conductivity variation as an accurate and a rapid method for detecting intramammary infections [44,45,46]. Researchers have stated in the literature that conductivity varies directly with changes in SCC. These results were and still are widely discussed as other studies could not confirm them [47,48].

These different conclusions are due mainly to errors in measurement, as the milk conductivity data collection requires some important considerations in carrying out the measurement. For example, true milk conductivity is measured when a contact between the milk and electrode is complete [49] and the correct temperature compensation is maintained.

Moreover, commercially available products have presented a sensitivity that is not high enough to be powerful sufficiently to detect the early stages of mastitis. Finally, the most difficult problem for routine application is the defining of a limit or threshold for diagnosis of abnormal milk samples.

1.3.3.1 Electrical Devices for mastitis detection

In the seventies, Linzell and Peaker [41] decided to compare electrical conductivity of first streams of milk from each quarter. To identify infected quarters the conductivity should exceed 16% of the lowest value observed.

This study was followed by many projects aimed at designing devices to monitor on-line udder health using this same principle [50,51,52,53,54]. Most studies concluded that the use of conductivity for detecting subclinical mastitis is effective but that the sensitivity based on this single detection factor is quite low. Onyango et al. stated that

the relationship between SCC and EC is not direct [55]. Other researchers proposed a multifactorial approach instead of one single parameter. Nielen et al. provided an extensive review of this aspect [15,16] and stated that there are a number of other parameters related to mastitis that can be automatically detected during milking such as milk production, cow temperature, cow activity, etc [16].

These deviations can be recorded and analysed by different algorithmetric filters to detect mastitis [56]. De Mol and al. uses one of these possible algorithmetric filters in a few recent studies [56,57]. The detection model uses conductivity, milk yield and temperature measurements to generate mastitis alerts. A time-series analysis combined with a mathematic filter is used to calculate expected values for yield, EC and temperature. An alert is given when a combination of deviations fall outside the confidence intervals. The multivariate approach and algorithmetric-based predictions have proven to be useful for mastitis detection, but the results also showed that the number of false positive alerts may still be too high for practical applications. Maatje at al. found that nearly 100% of the cases of clinical mastitis and over 50% of the cases of Subclinical mastitis could be detected by this method [58]. De Mol suggests that further research into sensor improvements may be worthwhile [24].

1.3.3.2 Hand–held meters

Only few hand held meters are currently available on the market. These devices measure the conductivity of milk samples and are used on quarter milk samples.

The device **MAS-D-TEC®** (Wescor, Logan Utah, USA) is claimed to be the world's largest selling electronic mastitis detector. It assesses the level of sodium and chloride in the milk. The analysis is fast as within two seconds the conductivity is analysed and displayed on a graphic scale. The maker suggests that absolute EC scores of ≥ 5 is a sign of the presence of subclinical mastitis. It is claimed that the apparatus does not need any washing between quarters and between cows. There is no temperature sensor as it is assumed that the milk going out from the animal has the same temperature as its body. A few studies evaluated the use of this instrument on farms to detect subclinical mastitis and the results showed that the device did not achieve sufficient accuracy to be used for screening [59].

Other studies deal with hand-held meters [60,61,62]. Milner et al [61] observed (in U.K conditions) that EC increased in cows subclinically infected with *S.Aureus* pathogens but did not show major variation in cows infected with *S.uberis* [60,61]. The results suggested that clinical mastitis caused by both pathogens could be detected earlier by conductivity methods than by waiting for a herdsperson to detect visible changes in the milk or cow. Moreover, the hand-held meter (Milk Checker, Eisai, Tokyo, Japan) used in his study (see Figure 1.8) was revealed to be impractical for commercial applications since it needed careful cleaning between each measurement, and it was concluded that reliable automated sensors and decision-making algorithms would be required.

Seguya and Mansell evaluated a hand-held resistance meter and found out that the detection was generally poor [62]. With current technology in hand-held meters, it appears that cow-side tests (see section 1.3.1.2) are more useful than the use of such electronic devices.

Figure 1.8: Photograph of a hand held Milk Checker Eisai, Tokyo, Japan. *(Taken from www.sentinelproducts.com)*

Recently, Ferrero et al. presented a new design of low cost mastitis detector to be used as a hand-held meter using AC conductivity measurements. The sensor is described as a two-electrode cell made of stainless steel, and it is equipped with an internal memory where the results are stored. Three detection levels have been determined: healthy cows, infected cows and mastitis cows [63]. This new design would be capable of detecting subclinical mastitis but at this stage only a small prototype is available and further tests on farm are necessary to make conclusions in regard to the efficiency of the device.

1.3.3.3 On-line systems

Several studies have evaluated milk conductivity for detecting subclinical mastitis, many with the view of incorporating such a system into the automated milking machine [49,64]. The following features characterise on-line conductivity measurement: repeated and reliable measurements of each quarter during milking; measurement errors such as low or high values; external influences on parameter values; non-disease fluctuations of parameters [56]. Many herds now rely on in-line mastitis detectors. Automated systems collect almost continuous electrical conductivity data but unfortunately in parlour systems, measurements are usually of whole udder milk and thus the detection of any events at the individual quarter is immediately reduced [65].

Rossing and Maatje [50], Maatje et al. [51] first proposed a milking claw where the milk flowed over sensor cells. Figure 1.9 represents the claw equipped with electrodes made of polished stainless steel. EC was continuously monitored by measuring the loss of voltage every 8 s. Reliability and repeatability of the results were found to depend on uniform sensor cells with clean electrodes. The correlation of the diagnosis with laboratory equipment was found to be from 83 to 89%. Highest conductivity was found at the start and at the end of milking. Using this same device, about 75% of infected quarters were identified in an experimental farm [66]. For information, an acceptable system would detected above 85% of the infected cases.

In a more recent study [64], the same group of researchers investigated the efficacy of daily QMC (quarter milk conductivity) measurements combined with daily milk yield and milk temperature. The instrumentation consists of a sensor built into the claw; the electrodes were located in four small tubular cells each containing a pair of stainless steel electrodes. In this system, the milk from each quarter flows through the claw. EC is measured by applying a constant 50-kilohertz AC signal (0.8 volts peak to peak) between two electrodes. EC is taken 7 times per second; the values are averaged and stored by a processor. The number and status of lactation caused variation of comparable levels. They detected 100% of 25 clinical cases when EC levels increased. Also, some differences have been found between farms where come from milk samples [64].

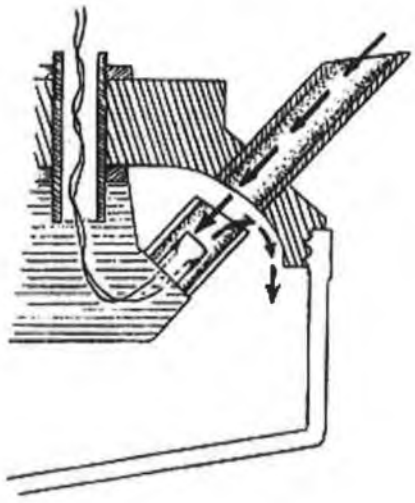

Figure 1.9: Drawing of the claw equipped with electrodes for measuring electroconductivity of milk [50].

Puckett et al. also reported on experiments in the milking parlour [52]. Milking claws were equipped with sensor electrodes for quarter. To minimise the influence of milk level in the sensor cell, which was proportional to milk flow, various designs of electrodes were investigated. Best results were obtained with a flat sensor surface at the end of the electrode. Integrating data from up to three milking procedures avoided false positive and false negative results. Using the quarter with the lowest conductivity was found to be the best reference.

Lake designed a sensor without electrodes, measuring electrical conductivity in each quarter by induction [53,67]. Milk flow was split into two channels with an annular configuration. This design was used to avoid the fouling problem of electrodes exposed to the milk and in order to get reliable and rapid measurements. Two coils and the annular flow of milk formed an electrical transformer, transmitting a signal with a frequency of approximately 50kHz. The voltage in the secondary circuit was proportional to the electrical conductivity of milk. By avoiding phase shifts from primary to secondary circuits; the influence of temperature on signals could be avoided. The device only worked correctly if milk was flowing without interruption.

Jones developed another system [49] adapted from a previous study from Datta et al where the conductivity cell consisted of a well, where milk accumulated, and a central stainless steel electrode [68]. The stainless conductivity probe was insulated so that only the tip of the probe was uninsulated. A resistor was built in series with the electrode to form a voltage divider. For determination of milk conductivity, an AC

signal of 2V peak to peak and 100kHz was applied across the circuit, and the voltage at the electrode was measured. The peak voltage at the electrode in each quarter was accumulated for 50μs (five cycles of the input signal). The data acquisition system collected milk conductivity at 6-s intervals to decrease the variability of the results. As milk flow increased, apparent conductivity also increased but was quite erratic. These results were explained by fluctuations that can occur in the contact region between milk and the electrodes; indeed, turbulence in milk or insufficient volume to cover the electrode may also cause fluctuations.

Afimilk system ®, developed by Afikim, Israel, is a computerised milking system for monitoring and breeding management [54,69]. Besides monitoring parameters such as milk yield or cow activity it can measure milk conductivity. The measuring device includes a unit for measuring the EC of milk from the individual cow on each of a number of successive days, a memory for storing the measured values, a computer and a comparator for determining an average value of stored data and for comparing these averages with the respective values to determine deviations. The EC is measured +- 1% using a low frequency AC current between stainless steel electrodes located in the milk meter. An infected cow is detected if there is a sharp rise in conductivity compare to the value for healthy milk. In the US patent, it is stated that a deviation of approximately 15% of the daily conductivity constitutes a first indication of the onset of mastitis [54]. Furthermore, it has been noticed that the main obstacle of reliable measurements is the presence of air bubbles and foam. Under Israeli conditions, the technique showed that the mean electrical resistance was lower in infected cows and particularly in cows infected by *St. Aureus.* [69]. The system still experienced some problems in detecting mastitis but combining EC with other parameters such as a cow's activity may help in detecting mastitis in an automated milking system.

1.3.4 Conclusion to electroconductivity-based mastitis sensor

The conductivity of milk has been researched extensively as a means of detecting mastitis in dairy cows and commercial systems are available as hand-held meters and on-line systems. However, conductivity of milk is not affected in the same way by all mastitis-causing pathogens and some infections may be defeated by the immune response of the cow before veterinary intervention is required. Milner et al (1996) have shown experimentally that subclinical infections from certain primary pathogens were

detected but other types were not [61]. But the method is nevertheless promising and the negative cases remain few. Automated detection is therefore suitable for cows milked in an automatic milking system [57].

1.3.4.1 Definitions

Sensitivity: Probability that an EC positive sample is a true indicator of disease.
Specificity: Probability that an EC negative sample is a true negative result
Positive predictive value (PPV): Proportion of true positives amongst the apparent positives.
Negative predictive value (NPV): Proportion or true negatives amongst the apparent negatives.

		Sample tested		
		Positive	**Negative**	*Total*
E.C class	**Positive**	a (true positive)	b (false positive)	a+b
	Negative	c (false negative)	d (true negative)	c+d
	Total	**a+c**	**b+d**	**n**

Sensitivity	**a / (a+c)**
Specificity	**d / (b+d)**
Positive predictive value	**a / (a+b)**
Negative predictive value	**d / (c+d)**
Prevalence	**(a+c) / (a+b+c+d)**

Table 1.4: Probability of the sample. Adapted from [70].

1.3.4.2 Setting thresholds/limits

The threshold values for eliminating mastitic samples are chosen in such a way that the numbers of false positive and false negative detections be minimised at an optimal ratio. The effectiveness of the system is influenced by the choice of threshold or limit. Increasing the threshold value introduces the probability of missing cases suspected of mastitis but it also decreases the number of false positives.

To distinguish between normal and mastitic milk using EC, several methods of calculation have been investigated. The first one is known as "**absolute threshold**". Such a threshold is used for a quarter or animal that has mastitis when EC exceeds a certain value. Several researchers have reported a higher mean EC in diseased quarters or abnormal milk than in normal quarters or normal milk [70]. This threshold is useful for detecting high milk EC samples like clinical mastitic milk but is unlikely to be efficient. The second threshold is known as "**differential conductivity**" threshold or "**Inter Ratio Quarter**" (IRQ) [71]. It is defined as the ratio between the quarter with the lowest conductivity and the other quarters of the same cow [41,43,72]. According to Linzell, a quarter with EC≥16% above the lowest quarter has mastitis [41]. The principle behind these terms is that sources of variation in EC other than mastitis would be the same for all quarters, so a comparison of EC values between quarters should reduce extrinsic variation. One assumption was that a cow would almost never have mastitis in all four quarters at the same time. For on-line detection, IRQ was proposed, based on means within milking or running averages over milking [66]. Practically, The use of differential EC has been shown to improve both sensitivity and specificity of EC. Nielen et al. observed that the sensitivity increased from 57% to 68% and specificity increased from 91% to 96% when differential values were used rather than an absolute threshold [16]. An other definition of IRQ states that the parameter should first be normalised by logarithmic transformation and then corrected for the mean, thus creating a normally distributed parameter with mean zero [71].

However, a combination of absolute and differential methods has been used in many studies and it seemed that it is the most effective method to detect abnormal milk [41,43]. This method can avoid error from intrinsic factors such as the breed of the cow or the time of lactation. Indeed, Fernando et al. observed that in uninfected cows, conductivity of strippings was higher than foremilk conductivity in those quarters infected [43]. Using logistic regression, the combination of both the absolute value and "Differential EC" without using threshold, seemed to provide the best model fit [73].

Hamman and Zeconni performed an evaluation of EC as mastitis detection method (using absolute thresholds) from a selection of published papers [70]. They found that EC is not a good screening test for either clinical or subclinical mastitis. The statistics reveal that when EC indicates that 100 cows are infected clinically only 58 would truly have clinical mastitis and 15-30% of animals diagnosed as mastitis free would actually be infected.

1.3.4.3 Possibilities for improvement

With the current information it is not possible to predict whether it will be possible in future to overcome the current difficulties encountered in the diagnosis of mastitis. Some researchers consider that the errors of detection are due to the low sensitivity of the measuring devices and systems. For instance, ideas such as developing further sensors [70], avoiding the fouling effect of the milk proteins by regular cleaning or using new materials for the electrodes such as graphite, using highly sophisticated computerised cell programmes to evaluate conductivity data are believed to be able to improve the results. Other people think that using better arithmetic model would help in the analysis of the results [57]. But mathematical methods and sensor technologies are not easy and economical to integrate into an on-line system As described earlier, milking systems have encountered difficulties with fouling of the electrodes by fat and protein, and regular cleaning necessary to maintain accuracy throughout milking. This could not be used in an automated system and use of a non-contact sensor or sensor operating where no polarisation effect occurs turns out to be the preferred solution. Alternating current impedance techniques appear to be a new promising method for analysing and probing certain medium or electrolyte such as milk [74,75,76,77]. In the last decade, many researchers investigated and used this technique to develop new bio- or chemical sensors such as urea sensor [78] or for controlling the amount of added water in milk [74,75]. The question is: "Could it be used for detecting mastitis on-line? ", this is the question this project tries to answer.

1.4 References

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Chapter II

Principles of experimental techniques

The purpose of this chapter is to describe the analytical methods that are mentioned in the experimental chapters. This section mainly deals with the principles and definitions electrochemical methods.

2.1 Electrochemical methods in general

This section is focused on the phenomena occurring on the surface of an electrode when it is in contact with an electrolyte. On order to avoid spurious results when using electrochemical methods such as electrical impedance spectroscopy or conductivity measurements, it is important to understand these processes. The following sections will focus on two processes that occur on the electrode surface - faradaic processes and polarisation effect. An understanding of polarisation effects enables a decision to be made regarding the lowest frequency that can be used to measure the bulk conductivity of electrolyte solutions.

2.1.1 Processes occurring on electrode surface

2.1.1.1 Faradaic processes

Faradaic or charge transfer processes occur at the electrode's surface. They involve the transfer of electrons that allows oxidation to take place at the anode and reduction to take place at the cathode. Since Faraday's law governs these reactions, they are called Faradaic processes. Under some conditions a given electrode-solution interface will show a range of potentials where no charge transfer reactions occur because such reactions are thermodynamically or kinetically unfavorable. Processes such as absorption and desorption where the structure of the electrode solution interface may vary with changing potential or solution composition are called *non-faradaic processes*. Conductimetric measurements are based upon non-faradaic process. However, when non-faradaic currents are imposed on the cell, it involves the formation of *the double layer* adjacent to each of the electrodes.

2.1.1.2 Electrical double layer and Polarisation effect

Methods such as conductimetric measurements do not involve faradaic currents (no oxidation or reaction to take place onto the electrode surface). When no faradaic processes occur, it involves the formation of an **electrical double layer** at the electrode solution interface and its surrounding electrolyte. This double layer is formed by the

solution immediately adjacent to the electrodes when it acquires an opposing charge. The separation is very small about the order of few angstroms.

It consists of two parts shown in Figure 2.1:

(1) A compact inner layer, in which the potential decreases linearly with distance from the electrode surface. This layer d_0d_1 consists of a layer of water molecules called the hydratation sheath and predominantly ions with an opposite charge to that of the electrode surface.

(2) A more diffuse layer d_1d_2 of solvated ions, in which the potential decreases exponentially [1].

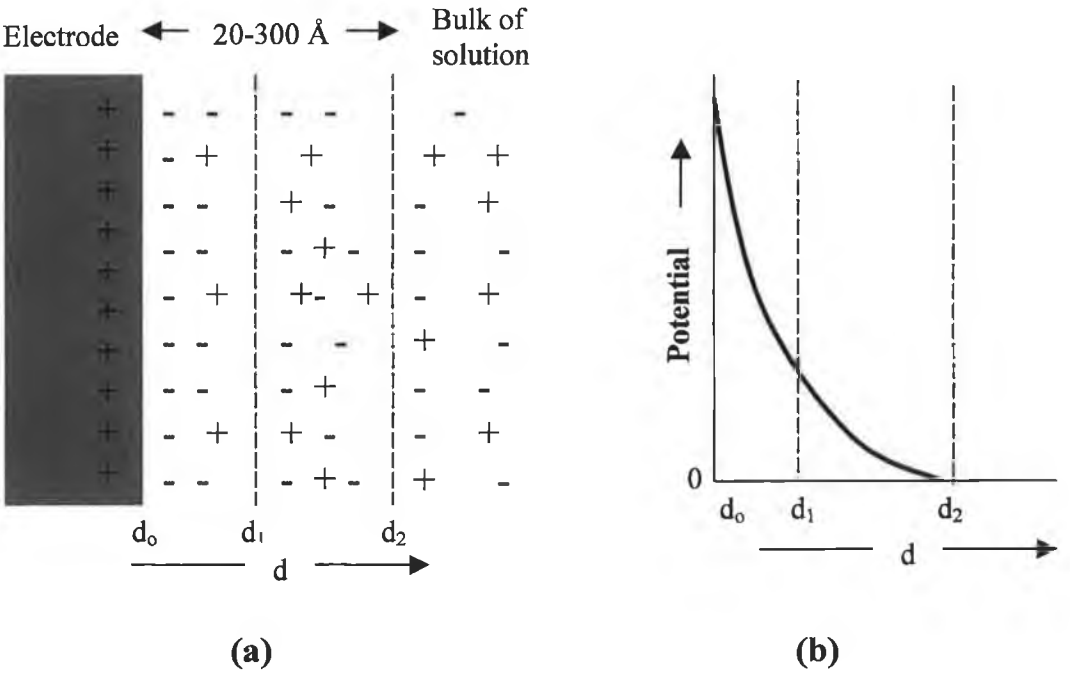

Figure 2.1: Electrical Double Layer formed at the electrode surface as a result of an applied electric potential [1].

In the 1850s, Helmholtz first attempted to explain the capacitive behaviour of this interface [2]. He viewed the electrode solution interface as a parallel-plate capacitor. The double layer capacitance is a result of a charge separation in the metal solution interface. The value of the double layer capacitance depends on electrode potential, temperature, solution composition and electrode material. The capacitance current increases with frequency and with the electrode size. It can reach from few hundreds to few thousands microfarads per cm^2.

The expression of the double layer capacitance is expressed as follows:

$$C_{dl} = \frac{\varepsilon_o \, \varepsilon_r \, A}{\lambda} \qquad \textbf{(Eq 2.1)}$$

Where ε_o is the permitivity of free space ε_o=8.854 pF/m

ε_r is the relative permitivity

A is the macroscopic area of the electrode

λ is the thickness of the electrode double layer.

The term "polarisation" comes from the analytical discipline called "polarography", in which the electrodes must be polarisable. There are a few types of polarisation that are interesting to focus on in this study: interfacial and orientational polarisation. The interfacial polarisation effect, referred as **electrode polarisation**, occurs when there is inhibition of the passage of potential determining ions through a phase boundary i.e. the electrolyte double layer. Polarisation is due to a modification of the molecular structure of the sample under the effect of the applied electric field. With direct current, formation of the double layer involves the passage of a momentary current which then drops to zero (that is, the electrode becomes polarised) unless some faradaic processes occur. With AC current, however, the reversal of the charge occurs at each cycle as negative and then positive ions are attracted to the electrode surface.

The phenomenon of electrode polarisation has been conventionally described and analysed in terms of equivalents circuits modelling.

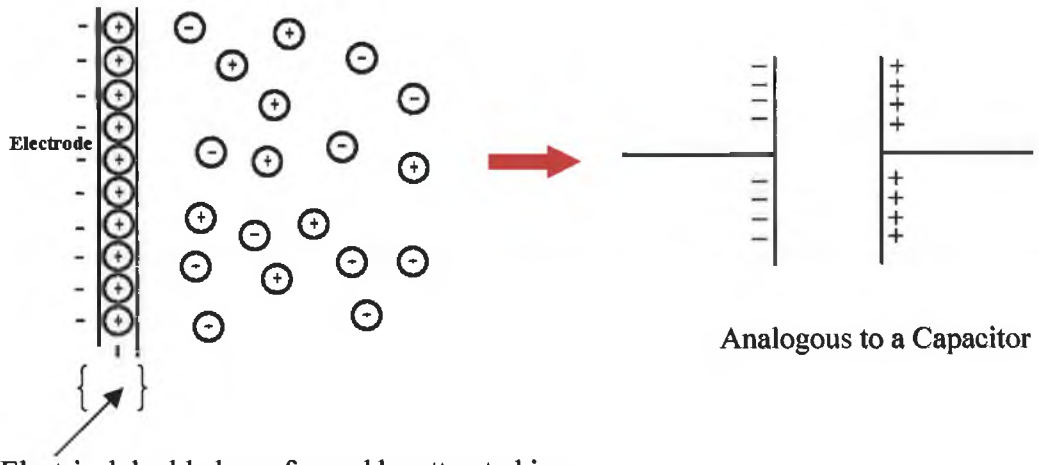

Figure 2.2: Polarisation effect occurring at the electrode surface leading to an inhibition of the passage of the potential

As it is shown in Figure 2.2, the double layer acts as a capacitor which is frequency dependent, in many cases an equivalent circuit of Resistor-Capacitor (RC) in series is used to model the electrochemical system at the electrode surface. This frequency dependence occurs because of reduction of the polarisation effect as the frequency is increased. Also, it is observed that increasing the effective surface area helps to reduce electrode polarisation.

The electrode polarisation effect can under certain circumstances be greatly increased by the presence of foreign substances on the cathode surface. These may consist of electrolyte anions and cations, oxides or hydroxides, or other organic or inorganic components of the electrolyte. They are adsorbed at the electrolyte surface and when the electrolyte is completely covered by foreign substance, it is passivated. Polarisation increases with increasing applied current, but decreases with increasing temperature and increasing agitation of the bath.

At very high frequencies, an important part of the current is carried by a second mechanism resulting from electrical **orientational polarisation** of the dielectric electrolyte. The conduction of an AC current through an electrolyte involves ionic motion as well as induced and orientation polarisation of the molecules in the bulk solution. Localised charge distributions occur in the material and the positive charges are oriented towards the anode and the negative charges to the cathode as shown in Figure 2.3. This phenomenon of orientated molecules leads to an increase in the stored charge.

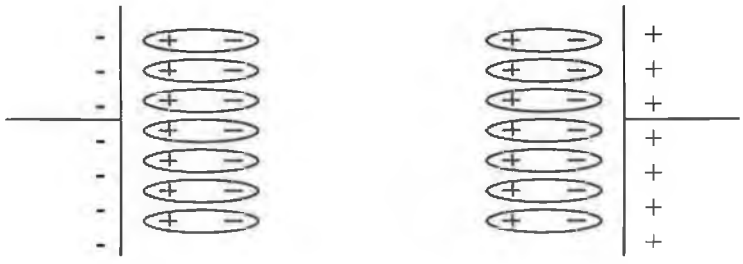

Figure 2.3: Localised charged distributions in the material as orientational polarisation occur at high frequencies.

This type of polarisation usually takes place over the frequency range from 100 kHz to 10 MHz for macromolecules such as proteins in solution or higher (up to 20 GHz) for small molecules such as amino-acids and water. [1,3].

2.1.1.3 Fringe field effect

When a potential is applied, the electric field between the electrodes is not homogeneous over the cross section of the cell. As illustrated in Figure 2.4, the electric field lines do not end at the edge of the electrode. This phenomenon is called the fringe field effect.

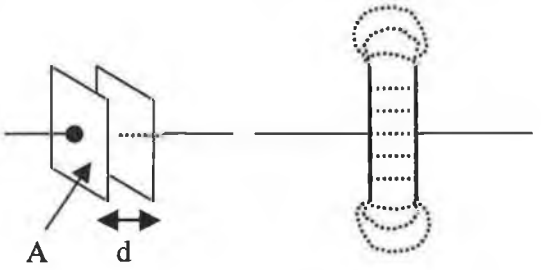

Figure 2.4: Schematic representing the fringe field effect, the electric field is not homogenous over the cross section of the cell [4].

In the case of conductimetric methods, the cell constant cannot be calculated from the geometric dimensions but must be found with calibrations. (See Section 2.3)

2.1.2 Useful definitions

Resistance: is a measure of the extent to which a substance opposes movement of electrons among its atoms. The more easily the atoms give up/accept electrons, the lower the resistance (R). It is defined in certain conditions by Ohm's Law named after a German physicist who stated that the current (I) through a conductor is directly proportional to the potential difference (E) between its ends if the temperature and other physical conditions are constant.

$$R = \frac{E}{I} \qquad \textbf{(Eq 2.1a)}$$

It is expressed in positive real number ohms. R is manifested with AC current or DC current.

Conductance: denoted *G* is an expression of the ease with which electric current flows through a substance. It is the inverse of resistance (G = I/E) and it is measured in Siemens (S). Therefore, when the applied voltage is a direct-current (DC) (current held constant), the current is directly proportional to the conductance.

Conductivity: Conductivity, Conductibility or Electrical Conductivity (EC) (symbol σ, expressed in Siemens per meter) is related to conductance, but involves a specific geometrical arrangement of the substance. It therefore also involves space parameters *A* (electrode area of contact with the substance) and *d* (distance the current passes through the substance i.e. electrode separation). See section 2.3.1.2 for more details.

Impedance: Impedance is an expression of the opposition that an electronic current circuit or system offers to alternating and/or direct electric current. Impedance was first proposed by Warburg [5]. He stated that when two metal electrodes are placed in a conductive medium, the system behaves as a resistor and capacitor either in series or in parallel. The symbol of the impedance is Z, and it is a vector quantity consisting of two independent phenomena: Resistance and Reactance.

For a system in series combination, the resulting impedance is the following:

$$Z = \sqrt{R^2 + \frac{1}{2\pi f C^2}} \qquad \textbf{(Eq 2.2)}$$

Where R is the resistance of the system, f the applied frequency and C the capacitance of the system.

Reactance: denoted χ, is an expression of the extent to which an electronic component, circuit, or system stores and releases energy as the current and voltage fluctuate with each AC cycle. Reactance is expressed as an imaginary number in ohms. It is manifested with AC but not with DC supplies.When AC passes through a component that contains reactance, energy might be stored and released on the form of a magnetic field, in which case the reactance is inductive noted ($j\chi_L$); or energy might be stored and released in the form of an electric field, in which case the reactance is capacitive noted ($j\chi_C$). Reactance is conventionally multiplied by the positive square root of –1, which is the unit imaginary number called j operator, to express the impedance as a complex number of the form: $\mathbf{R + j\,\chi_C}$ or $\mathbf{R + j\,\chi_L}$

Susceptance: denoted B is an expression of the ease with which alternating current (AC) passes through a capacitance or an inductance. It is defined as the reciprocal of the reactance of a circuit and thus the imaginary part (Im) of its admittance. It is measured in Siemens. $B = \mathrm{Im}(\frac{1}{Z})$ **(Eq 2.3)**

Admittance: Reciprocal of impedance and it is considered as the AC equivalent of the conductance, measured in Siemens.

Rms amplitude: The RMS voltage amplitude (V_{RMS}) is the DC voltage which will deliver the same average power as the AC signal.

$$V_{Rms} = \frac{V_P}{\sqrt{2}} \qquad \textbf{(Eq 2.4)}$$

V_P is the peak voltage of the signal.

2.2 Electrochemical Impedance Spectroscopy

The electrochemical impedance spectroscopy (EIS) is a technique used to evaluate the electrical behaviour of electrodes and electrolyte materials. The use of Impedance Spectroscopy has been reviewed by MacDonald [6,7]. But it was Sluyters who introduced the use of the complex plane plot for the analysis of electrochemical reactions in a long series of papers beginning in 1960 [8,9].

The impedance of an electrochemical cell is determined by the impedances of the various cell "components"; for example, the double layer capacitance of the interfacial region at the electrode surface, the charge transfer reaction, and the mass transport. Hence, measurement of the cell impedance can be used to determine the impedance of a given component and its contribution to the electrochemical reaction.

2.2.1 Theory

In a controlled-potential EIS experiment, the system is held at equilibrium at a fixed DC potential and a small amplitude (5-10 mV) AC waveform is superimposed on the DC potential (Figure 2.5). The small amplitude ensures that there is a linear relationship between the current and the potential, and that the response is steady state; that is, it does not change with time and many measurements can be averaged.

The response of the system to this perturbation from equilibrium is measured in terms of impedance Z of the system. The frequency of the AC waveform is varied, and hence the impedance of the system is obtained as a function of frequency. The response can be analysed as a sum of sinusoidal functions (a Fourier series).

The procedure is performed over a range of frequencies and can be quite slow as measurements have to be made one frequency at a time. Impedance experiments are usually conducted in the range of 10^{-3}-10^{5} Hz (to stay in linearity range); therefore it is possible to distinguish between processes having different relaxation times. Care must be taken when evaluating data acquired at extreme frequencies, as measurements at very low frequencies take a long time during which the interface may change rendering the result meaningless, while those at the high frequency end are frequently unreliable due to stray capacitances and inductances.

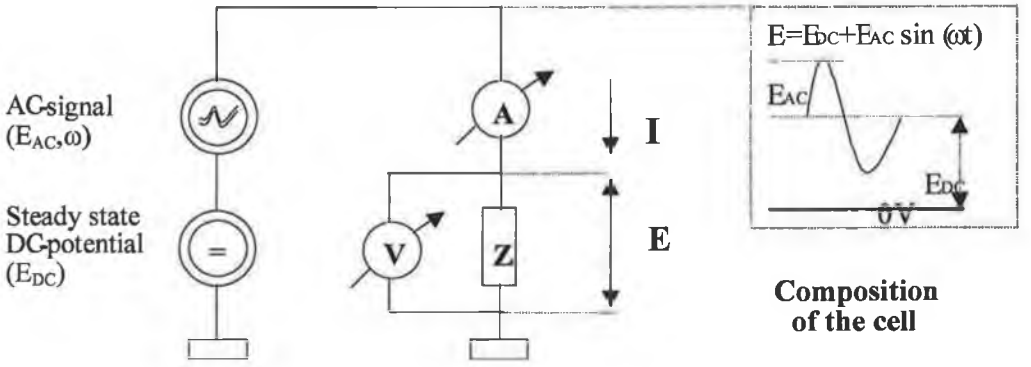

Figure 2.5: Principle of AC-Impedance Measurement.

If the AC potential is E= ΔE sin (ωt), then the AC current through a resistor of impedance R is given by:

$$I = \frac{E}{R} = \frac{\Delta E \sin(\omega t)}{R} \qquad \textbf{(Eq 2.5)}$$

Where ω is the angular frequency in rad.s^{-1} (=2π*f* where *f* is the frequency in Hz)

ΔE is the amplitude of the sine wave.

Trough a capacitor C the charge is given by:

$$q = C\,E \qquad \textbf{(Eq 2.6)}$$

On differentiating to get the current I:

$$I = C\frac{dE}{dt} = C\left(\Delta E\,\omega\cos(\omega t)\right)$$

i.e. :
$$I = C\Delta E\omega\sin(\omega t + \frac{\pi}{2}) = \frac{\Delta E}{\chi_C}\sin(\omega t + \frac{\pi}{2}) \qquad \textbf{(Eq 2.7)}$$

The term *Xc* (=1/ωC) is called the capacitive reactance and represents the impedance of a capacitor.

The above equations show that there is a phase difference between the applied AC potential and the AC current response for any circuit containing a capacitor. Therefore, the impedance can be represented using a vector diagram displaying the in-phase (Z') and out-of –phase (Z'') impedances, the total impedance and the phase angle. Impedances are often represented using a complex notation, Z' and Z'' being the real and imaginary axes, respectively.

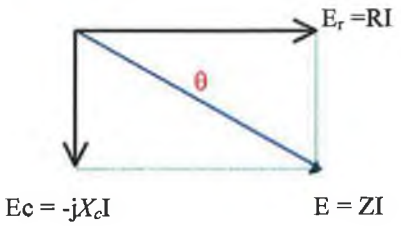

Figure 2.6: Phasor diagram for a series resistor and capacitor an AC current where j is the unit vector along the imaginary axis and $j^2=(-1)$.

The total voltage E across both resistor and capacitor is expressed by the vector sum of the two potentials:

$$E = E_r + E_c = IR + (-j\chi_c I) = I(R - j\chi_c) = IZ \qquad \textbf{(Eq 2.8)}$$

The total impedance (Z) is also expressed as:

$$Z = Z' + jZ'' \qquad \textbf{(Eq 2.9)}$$

It is characterised by a magnitude and phase angle, and is most represented as a function of frequency:

$$Z(\omega) = |Z|\, e^{j\theta} \qquad \textbf{(Eq 2.10)}$$

Where $|Z|$ is the magnitude, and θ is the phase angle.

There are also related to the real and imaginary components Re (Z) and Im (Z) by:

$$Z(\omega) = \sqrt{(\mathrm{Re}\, Z(\omega))^2 + (\mathrm{Im}\, Z(\omega))^2} \qquad \textbf{(Eq 2.11)}$$

For a capacitor, the impedance is purely imaginary. However, in a resistor, the impedance is real.

The data from an impedance experiment can be represented in a number of ways; the two most commonly used being the Nyquist and Bode plots. The Nyquist plots (or Argand diagrams) display the imaginary impedance vs. real impedance (Z'' vs. Z') at each frequency (the positive y axis generally correspond to -Z''). The Bode plots displays both the logarithm of the total impedance and the phase angle as a function of the logarithm of the frequency. Although the Nyquist plots are more commonly used for historical reasons, the data in a Nyquist plot is often poorly resolved (particularly at high frequency), and the explicit frequency dependence is not displayed. In contrast, the Bode plot directly displays the frequency dependence; in addition, the data is well resolved at all frequencies, since logarithm frequency scale is used.

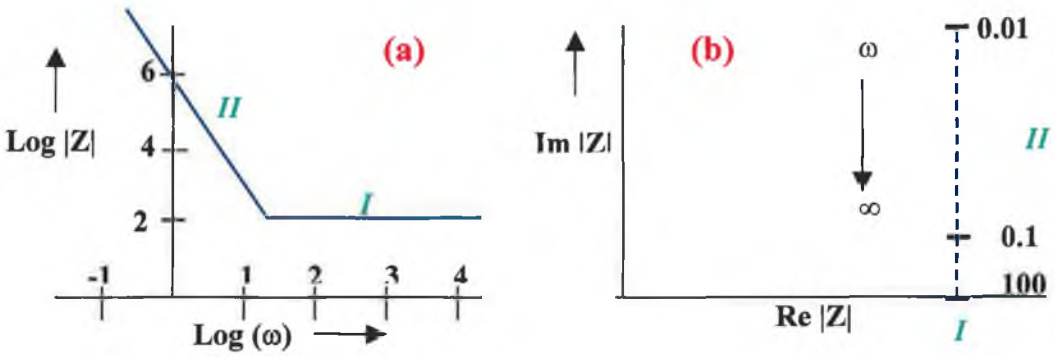

Figure 2.7: Typical representation of (a) Bode plots and (b) Nyquist (Argand) diagrams for a series RC circuit R=100 Ω and C=1 μF. *(I)* represents the Frequency independent zone and *(II)* represents the frequency dependent zone.

On the left hand side (Figure 2.7), the Bode plot is a log –log display of Impedance (log IZI) versus frequency (log (w)). In this example, the impedance decreases up to about 10 Hz, and is constant above this (frequency independent resistance) at around 100 W. Representing this as a Nyquist (Argand) diagram gives the plot on the right hand side which plots the frequency dependent (Imaginary component (Im IZI) against the real component (Re IZI). This is a line at 100 Hz, with the point of intersection on the Re axis representing the constant frequency independent component in (a); and the frequency dependent portions of (a) defining the line above this point in (b).

Cahan advises one to plot the data using a Bode plot which presents clearer results [10]. The relative contributions of the various components typically varies with frequency, for example, electron transfer kinetics may become dominant at high frequency, whereas diffusion may become dominant at lower frequencies. Measuring over a wide frequency range allows processes with different time scales to be detected within the same experiment.

2.2.2 The Principle of Equivalent Circuits

One common method to get quantative data from an EIS experiment is based on the principle of equivalent circuits; that is, the various cell components can be modelled using electronic and mathematical components (e.g. a resistor for electron transfer kinetics and solution resistance, and a capacitor for the interfacial (double layer) capacitance), and a circuit can be built that gives the same amplitude and phase angle as the real cell does under a given excitation. [11,12]. When using this approach, it is

important that each equivalent circuit element corresponds to a component of the electrochemical cell; otherwise the equivalent circuit has no meaning.
Essentially, the only way to generate an equivalent circuit appropriate for a given electrochemical system is to use simulation software. Once an appropriate equivalent circuit has been generated, the next step is to correlate the various electronic components of this circuit with the physical properties of the electrochemical system.

A common way to model an electrochemical cell is to use an equivalent circuit called the Randles equivalent circuit shown in Figure 2.8. In the absence of an electroactive species the double layer capacitance is nearly a pure capacitance; therefore it is represented simply by the element C_{dl}. When a diffusing electroactive species is present, the interfacial region is modelled as the series combination of R_{CT}, the resistance due to the heterogeneous electron transfer (the smaller the rate of electron transfer the larger R_{CT}) and Z_W, the Warburg impedance, (a frequency dependent reactance due to the mass transfer between the bulk solution and the electrode surface) in parallel with C_{dl}. All these combinations are combined in series with $R_{SO,}$ the solution resistance.

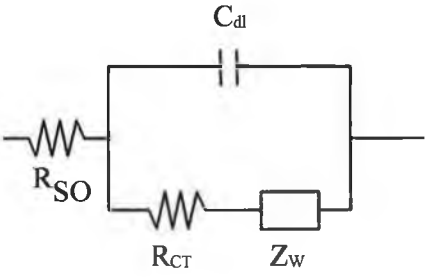

Figure 2.8: Randles Equivalent Circuit [4, 11,12]

The **charge transfer resistance R_{CT}** is derived from the small over potential limit of the Butler Volmer equation:

$$I = I_o \exp\left(\frac{-nFA}{RT}\right)\eta \qquad \textbf{(Eq 2.12)}$$

Hence

$$R_{ct} = \frac{RT}{I_o nF A} \qquad \textbf{(Eq 2.13)}$$

It should be noticed that this linear relationship only applies at low overpotentials between 5 and 10 mV. Hence, the AC amplitude used in EIS experiment is limited to 5 to 10 mV.

The mass-transport in the case of a semi-infinite diffusion can be modelled using the **Warburg impedance Zw**, which is given by the following equation:

$$Zw = \frac{W}{(j\omega)^{1/2}} \qquad \textbf{(Eq 2.14)}$$

Where W is a complex expression derived from Fick's diffusion law.

This element has a constant phase angle (the impedance versus log frequency is a straight line with a slope of -0.5) and gives a straight line in the Nyquist plot at an angle of 45°.

The expressions for Z' and Z'' for Randles circuit are complicated and it is often studied for two limiting cases:

(1): $\omega \rightarrow \infty$

In the high frequency limit, $R_{CT} >> Z_W$ and the control is purely kinetic. The expression of Z' and Z'' for an RC parallel circuit become:

$$Z' = R_{SO} + \frac{R_{CT}}{1 + \omega^2 C_d{}^2 R^2_{CT}} \quad \text{and} \quad Z'' = -\frac{\omega C_d R^2_{CT}}{1 + \omega^2 C_d{}^2 R^2_{CT}}$$

Combining and simplifying we obtain:

$$\left(Z' + R_{SO} - \frac{R_{CT}}{2}\right)^2 + (Z'')^2 = \left(\frac{R_{CT}}{2}\right)^2 \qquad \textbf{(Eq 2.15)}$$

which describes a circle centred on $Z' = R_{SO} + R_{CT}/2$ with radius $R_{CT}/2$. This case corresponds to a kinetic control. Once R_{CT} has been calculated, C_{dl} can be determined from the value of ω at the maximum value of Z'', at this value $\omega_{max} = 1/R_{CT}C_{dl}$.

(2): $\omega \rightarrow 0$: The two expressions for an RC parallel are:

$$Z' = R_{SO} + R_{CT} + \sigma\omega^{-1/2} \qquad \textbf{(Eq 2.16 a)}$$

$$Z'' = -\sigma\omega^{-1/2} - 2\sigma^2 Cd \qquad \textbf{(Eq 2.16 b)}$$

which is a straight line of unit slope, which extrapolated on the real axis gives an intercept of $(R_{SO} + R_{CT} - 2\sigma^2 C_{dl})$. This case corresponds to diffusion control. The Warburg coefficient, σ, depends on the diffusion coefficients and concentrations.

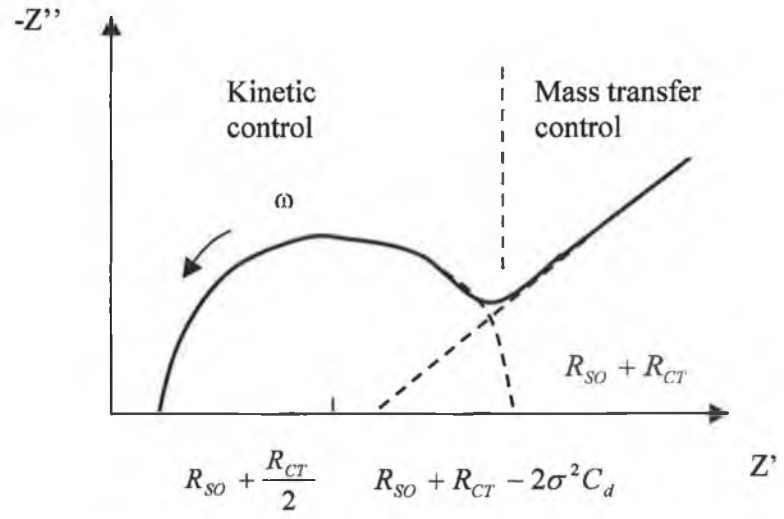

Figure 2.9: Plot of impedance in the complex plane for Randles Equivalent Circuit.

To summarise, the circuit at low frequencies behaves like a pure capacitor but at high frequencies it behaves like a pure resistor [13].

2.2.3 Applications of impedance for milk analysis

The electrical impedance method and its derivative applications such as admittance measurements have been successfully applied in a wide variety of biomedical, chemical and microbiology studies as a means to detect, quantify and even identify bacteria of certain media particularly milk [14-16], to study the water and fat content of milk [17-20] or assess the souring effect due to milk spoilage [17, 21]. Indeed, the impedance method has numerous advantages for studying milk as it is a technique that does not require any additional preparation of the samples and it avoids spurious measurements arising from electrode polarisation by operating at sufficiently high frequencies.

Nevertheless, even though milk conductive properties have been established for a long time, accurate measurements of the conductance of untreated milk samples remain a major problem for researchers. Prentice, on this subject, has stated that the conductivity of milk is significantly influenced by the amount and distribution of fat. The milk fats tend to form a non-conductive layer on any electrode surface, which explains why the salts such as chlorides, phosphate, sodium or calcium are impeded from moving between electrodes, thus reducing the overall measured conductance [22].

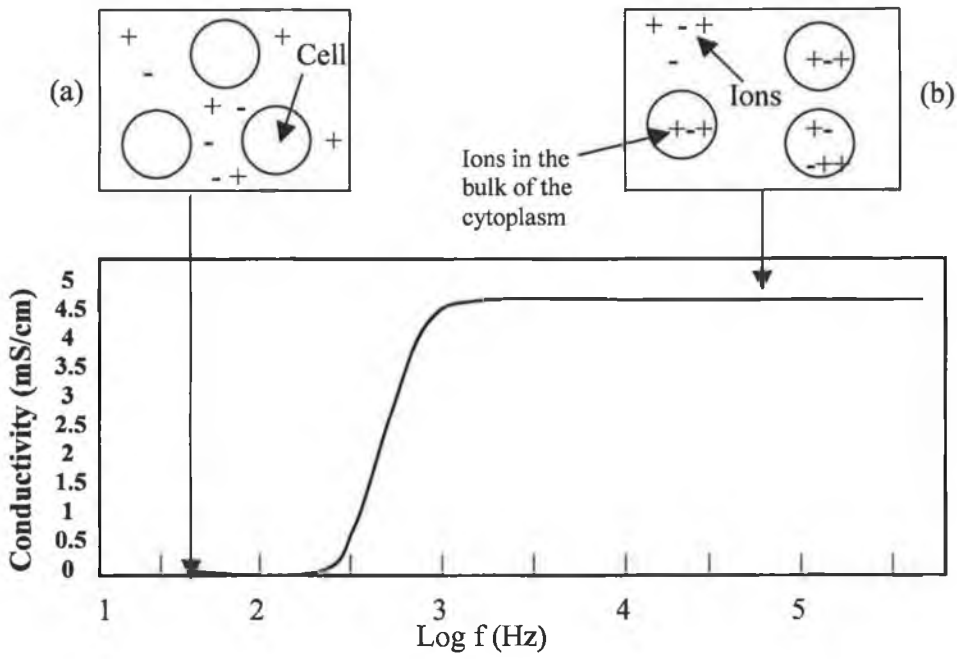

Figure 2.10: Graph representing the effect of frequency on normal milk conductivity. Electrical conductivity is measured at 27°C and 50 mV of sinusoidal excitation [23].

The frequency of the AC current applied to a colloidal type solution has major effect on its conductance. As shown in Figure 2.10, at low frequencies the plasma membrane of the cells stops the electric field extending into the cell cytoplasm and the cells appear as non-conductive elements. These non-conducting particles impede the electric field from considering the ions contained in these cells and thus reduces their concentration so the conductance is reduced in addition of the polarisation effect. At higher frequencies (of the order of a few decades of kilohertz), the electric field is able to cross the cytoplasm membrane of proteins and the medium is considered as a combination of ionic cytoplasm in an ionic suspending electrolyte [23, 24].

This conductivity then reaches a plateau at higher frequencies and stays constant.

2.3 Conductivity measurements

This section discusses the theory of conductivity which was used was a basis for developing the sensor and analysing the data obtained by AC conductivity measurement in the experimental section.

2.3.1 Electrolytic conductivity

2.3.1.1 Introduction

Matter can conduct electricity via two types of carriers. The first is through the electrons, which are called the electronic conductors and found in metals or semi-conductors. The second type of carriers is the ions, which are called the electrolytic conductors and found in liquids or molten solids [25].

The conduction properties in electrolytes were first established by F. Kohlrausch (1840-1910), who with a few instruments derived number of laws and also determined values of the conductivity of standard solutions for calibration [26]. These standard calibrations are still used in conductivity measurement nowadays [4].

It has been stated particularly that solutions of electrolytes conduct the current by migration of ions under an applied electrical voltage. Indeed, when an electrical field circulates between the anode (positively charged electrode) and the cathode (negatively charged electrode) the ions flow to the oppositely charged electrode. They follow Ohm's Law like metals. Alternating current (AC) is generally used to prevent substance changes and the formation of diffusion boundaries layers at the electrode.

With each AC cycle the polarity of the electrodes is reversed which in turn reverses the direction of ion flow. This will prevent electrolysis and polarisation from occurring.

2.3.1.2 Fundamentals

The conductivity of a solution is non-specific and it depends on the overall ionic concentration, type of ions, temperature and the geometry of the area in which current is carried. The electrical conductance G (in Mho) of an electrolyte solution is defined as the reciprocal of the resistance R (in Ohm) of 1 cm^3 of a liquid at standard conditions [27].

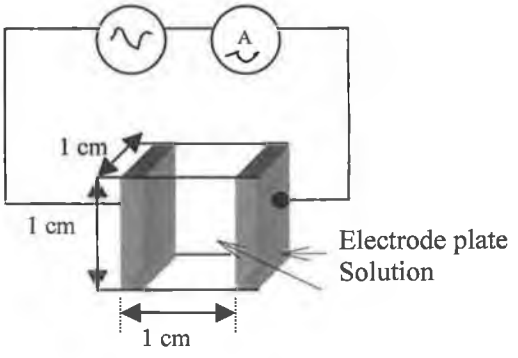

Figure 2.11: Principle of a conductivity cell with contacting electrodes and recommended cell constant $1cm^{-1}$

The ohmic resistance of a solution also depends of the geometry of the filled gap:

$$R = \rho\left(\frac{d}{A}\right) \qquad \textbf{(Eq 2.17)}$$

Where R is the electrical resistance in ohms

ρ is the specific resistance or resistivity of the solution in Ohm/cm.

d is the distance between two metals conductors plates in cm

A is the cross-section area between two electrodes in cm^2

Thus the conductance is given by:

$$G = \sigma \cdot \frac{A}{d} \qquad \textbf{(Eq 2.18)}$$

Where *G* is the Conductance in Siemens

σ is the Conductivity in Siemens per cm

A is the electrode surface area in cm^2 perpendicular to the flow current

d is the separation distance

In theory, a conductivity-measuring cell is formed by two-1cm square surfaces spaced 1 cm apart. Practically, the conductivity cell may adopt different configurations or geometries depending on the desired application. These diverse configurations are accounted for by introducing the cell constant *K* (in cm^{-1}), which is defined by the ratio between the distance *d* between the two electrodes and the area of the electrodes surface *A,* or simply *d/A*. This however does not consider the existence of a fringe field effect, which affects the electrode area by the amount AR. Therefore K, becomes d/(A+AR) because it is normally impossible to measure the fringe field effect and the amount AR to calculate the real cell constant.

In general, the smaller the cell constant the higher the signal that will be returned to the meter. Low conductivity solutions use a small cell constant and high conductivity solutions will use a larger cell constant sensor. Ultra pure water analysis with at conductivity of 0.055μS/cm would use a cell constant of 0,01 cm^{-1}.

The conductivity of a pure solution is given by:

$$\sigma = 10^{-3} \sum_{i} \left(Z_i\, C_i\, \lambda_i\right) \qquad \textbf{(Eq 2.19)}$$

Where Z_i represents the charge of the i^{th} ion

C_i is its equivalent concentration

λ_i is its ionic conductivity

The ionic conductance λ_i gives quantative information about the contribution of each ionic species to the conductance. Its value is somewhat dependent on the total ionic concentration of the solution and increases with increasing dilution. Some values of equivalent conductance are presented by MacInnes [28].

2.3.1.3 Measurement units

The SI unit of electrical conductance is S that stands for Siemens. It is equal to the conductance of a circuit or element with resistance of 1 Ohm.

That is, $1S=1Ohm^{-1}=1$ Mho.

To compensate for variations in electrode dimensions, the conductivity is expressed by a function of the specific conductivity κ. κ is defined as the conductance for a cell constant equal to 1 cm^{-1}. This occurs when the electrodes have an area of 1 cm^2 separated by a distance of 1 cm. The SI unit of conductivity is thus expressed in Siemens per meter ($S.m^{-1}$). The most common unit of κ or σ is $\mu S.cm^{-1}$. In literature, sub-units are usually mentioned and to simplify, the following conversions are usually given: **1 μS/cm=0.001 mS/cm=0.000001 S/cm=1 μmho/cm**

Examples of conductivity values:

Ultra Pure Water: 5.10^{-8} S/cm, Tap Water: 5.10^{-4} S/cm, Sea Water 5.10^{-2} S/cm

2.3.2 Instrumentation

Conductivity is fairly simple to measure, being directly proportional to the concentrations of ions in the solution. To measure conductivity the following instrumentation is required: a conductivity probe (contacting or toroidal), a temperature sensor and the supporting electronic [29]. The conductivity sensor then returns a current, which is dependent on the conductivity of the electrolyte in which the sensor is placed, to the conductivity sensor. The meter then uses the current from the difference in the drive current and the return current to determine the conductivity of the electrolyte. The temperature is also used to correct for any changes in conductivity due to temperature. The temperature device is usually integral to the conductivity sensor.

2.3.2.1 Types and characteristics of conductivity cells

Two main types of conductance measurement systems are in use, employing either contacting or toroidal (inductive or electrodeless) principles. The most widely used method utilises either two or more contacting electrodes immersed in the solution to be studied.

The contacting method uses a sensor with two or four electrodes. The two-electrode methodology uses two opposing electrodes; the anode is then used as a reference electrode. The two-electrode cell surface is however easily contaminated, adding resistance to the circuit. Owing to the increased tolerance for fouling coatings, four-electrode conductivity is becoming available and popular. The four-electrode methodology utilized the same two electrode-measuring scheme, but it also includes an additional two electrodes system to act as a reference point for the measuring circuit. This design provides a large linear measurement range without measuring errors associated with cable length, aging, and electrode fouling or polarization effects. The minimum range for this type of electrode is approximately 5000 μS/cm.

Conductivity cells typically hold metallic plates at a fixed distance using non-conductive spacers. The conductors are generally platinum, stainless steel, titanium or non-metal graphite but for certain applications silver, gold or tungsten may be employed. A patented feature utilizes a stainless steel electrode with frosted appearance to prevent polarization. Also, platinum electrodes which are coated with platinum black (called platinised) are commonly used. The main advantage of platinisation is the increased surface area and sensitivity of the probe when compared to the bright platinum. This coating is prepared by electrolysis in a chloroplatinic acid solution containing small amount of lead acetate [27]. The insulating material that holds them is usually glass or epoxy. The shape may vary from parallel flat plates, wires and rods, to rings of metals or graphite cast in the tube forming the body. Figure 2.12 shows a typical digital conductivity meter; in this design the sample is placed between the plates as the probe is dipped to a sufficient depth.

As mentioned previously an AC voltage is applied through the cell and the resulting AC current that flows between the electrode is used to determine the conductance G.

Figure 2.12: Digital conductivity meter and its graphite cell (Orion).

The second type of conductance measurement utilizes non-contacting sensors and depends on inductive or capacitive effects to measure conductance. Calvert et al. [30] and Salomon [31] were the first to propose the principle and the construction of inductive contactless cell. The method involves an AC signal, produced in an oscillator, which is sent to a toroidal coil perpendicular to the direction of liquid flow. The signal in the first toroid causes an induced signal in a second toroid called the pick-up toroid. The amount of current induced in the pick up toroid is proportional to the solution conductance as represented in Figure 2.13.

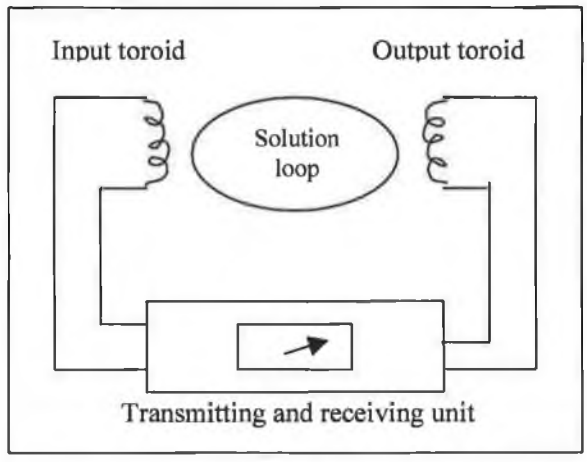

Figure 2.13: Representation of an electrodeless conductivity measuring circuit [32].

The major advantage is that the toroidal coils are not in contact with the solution. They are either encased in a polymeric material such as polypropylene and Teflon or are external to a flow through sensor. The toroidal conductivity is very useful in high conductivity applications. It also has a high resistance to coating of the cell and high temperatures. But, this method lacks the sensitivity of contacting measurement and these sensors are typically larger than contacting sensors.

2.3.2.2 Temperature compensation

Temperature variations have a great influence on conductivity results. Indeed, temperature has an influence on the viscosity of the solution and therefore on the mobility of the ions. As the temperature increases the viscosity decreases and the ions can move faster leading to an increase in conductivity. Therefore, it is important to carry out measurements at a constant temperature or to compensate for changes in temperature to be able to compare the conductivity of sample solutions without bringing them all to exactly the same temperature.

The temperature compensation is to counteract real changes in the conductivity of solution with temperature.

An algorithm determined by experiment is commonly used for temperature compensation:

$$C_T = C_o \times [(1+K\,(T-T_o)] \qquad \textbf{(Eq 2.20)}$$

Where C_T: Conductivity at temperature T in degree Celsius (°C)

C_o: Conductivity at T_o °C (T_o is the reference temperature (usually 25°C))

K: Temperature compensation coefficient in $°C^{-1}$

T: Measurement temperature, °C

T_o: Reference temperature, °C

For a given solution, the value of, the temperature coefficient, is relatively constant over a wide range of concentration, as the following Table 2.1 for potassium chloride solutions shows:

Standard(M KCl)	C_o (Scm^{-1})	(°C-1)
1	102050	**0.01900**
0.1	11670	**0.02055**
0.01	1278	**0.02083**

Table 2.1: Temperature coefficient for potassium chloride solutions at a reference temperature of 20°C [33].

The conductivity electrode incorporates an in-built temperature sensor that is used to compensate for changes in the conductivity of solutions with temperature.

2.3.2.3 Conductivity Calibration

As stated in the definition, the conductance is expressed as the product of a cell constant multiplied by the specific conductivity. The cell constant is determined experimentally using a solution of known specific conductivity during standardisation

and calibration. Indeed, a conductivity cell is calibrated using a standard known solution at a standard (20 or 25°C) or at a known temperature. Using some standard solutions that have well defined parameters allow the calibration to be performed.
Kolhraush [26] worked with potassium chloride solutions as conductivity standards and Jones and Bradshaw [34] showed that the values are still valid today. The most commonly used is a 0.01M potassium chloride solution. This may be obtained by dissolving 0.7440g of pure dry potassium chloride in distilled water and diluting to 1000 mL at 20°C. The general rule is to choose a standard that is closest to the conductivity of the solution to be measured. The calibration is necessary to determine the cell constant (avoiding the problem of geometry configuration or in case of a treated micro surface such as platinisation) and also to take in account the inhomogeneity of the electrical field at the edge of the electrode or fringe field effect (see section 2.1).

2.3.2.4 Circuitry

The measurement technique for electrolytic conductance by a conductivity sensor has remained basically the same over the years. Conductivity instrumentation consists of the conductivity sensor in the sample system, a temperature compensation sensor and the associated electronic. The conductivity of a cell can be measured by Wheastone type bridge methods (see Figure 2.14) where the electrochemical cell forms one of the resistance arms of the bridge or by direct measurement of the current through the cell when a fixed potential is applied.

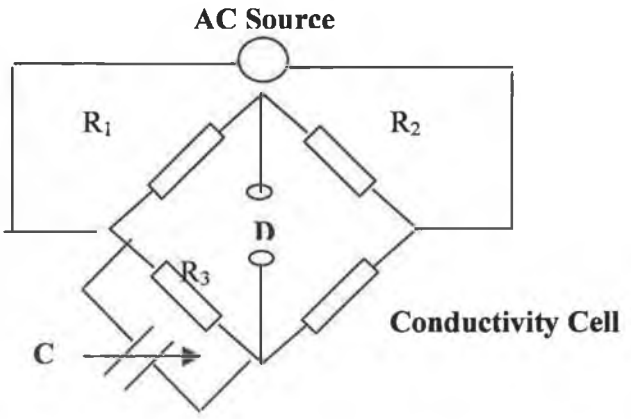

Figure 2.14: Schematic of an AC bridge circuit for conductivity determination: R_1 and R_2 are resistances, R_3 is a variable resistance, C is a variable capacitance (or condenser) and D is a detector (oscilloscope).

The variable capacitor C connected in parallel with the variable resistance R_3 serves to balance the capacity effects of the conductance cell. The value of C and R_3 are varied until the detector indicates zero voltage difference between the opposite junctions. In this condition of bridge balance, the resistance of the sample solution (R_{SO}) may be found from the expression:

$$\frac{R_1}{R_2} = \frac{R_3}{R_{SO}} \qquad \textbf{(Eq 2.21)}$$

2.3.3 Sources of errors in the measurement

The conductivity measurement of an electrolyte is often complicated by the polarization of the electrode at the operating voltage (see section 2.1). Faradaic or charge transfer processes can occur at the electrode surfaces. It is therefore desirable to operate a conductivity sensor cell at a low voltage where no faradaic process could occur and take into consideration the formation of a double layer adjacent to each of the electrodes when a potential is imposed on the cell.

2.3.3.1 Condition of the probe

Performing proper measurements as part of any electrochemical method requires rigourous cleaning of the cell. Probes tend to become inaccurate when they become coated with interfering substances as an extra resistance created by the fouling substances is added. Between each measurement, the probe needs to be washed thoroughly with laboratory grade water to minimise the build-up of the fouling substances. Cells in platinised platinum (black platinum) need replatinisation to refresh the cell plates and bring them back to the original cell constant.

2.3.3.2 Calibrations and Temperature compensation

Many spurious measurements in conductivity are because of improper calibration. The cell constant needs to be accurately determined and also the temperature sensor needs to be verified to avoid temperature compensation errors. The coefficient used in commercially available conductivity meters is usually set up at 2.1%/°C which is the normal value used for liquid solutions such as water but it needs to be changed if conductivity measurements of higher viscosity solutions (polymers, slimes) is required.

2.3.3.3 Polarisation effect

Polarisation effects are a highly undesirable side effect in conductivity measurements [3]. In most commercial conductivity meters, the operating frequency is in the range 400 to 2 kHz. The polarization effects and faradaic processes at the electrodes surface can be avoided by increasing the macroscopic surface of the electrode by platinisation or by operating at sufficient high frequency (above 50 kHz depending of the cell). In the case of a stainless steel electrode, it is given a satinised finish in order to increase the effective area [35]. It is assumed that if the polarization effect is diminished on the surface it will be the same for the bulk solution (see section 2.1).

2.3.3.4 Faradaic processes

For conductimetric measurements, the current used is an AC current. This is required to avoid reactions on the electrodes surfaces known as faradaic processes (see section 2.1). By maintaining the high cell constant (d/A) of the sensor, faradaic processes are minimised. It is important therefore to use a small electrode surface area and large inter-electrode distance so that the cell resistance lies in the range of 1 to 50 kΩ. This however reduces the sensitivity of the Wheatstone bridge. It is observed that by performing the analysis at high frequencies and using lower amplitude for the AC current that both double layer and faradaic effects can be minimised [35].

2.4 References

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Chapter III

Conductivity and AC Impedance Measurements

This chapter discusses the feasibility of using metal electrodes to analyse milk and the feasibility of developing a sensor based on impedance/admittance measurements to detect mastitis in cow's milk.

3.1 Introduction

Milk is a particularly difficult solution to analyse. It contains fats and macromolecules such as proteins that tend to block any microinjection system or to poison the surface of electrodes. Nevertheless, many studies are still aimed at analysing milk properties especially for the purpose of food quality control. For instance, methods such as inductively coupled plasma mass spectrometry (ICP-MS) can determine the elemental concentration in untreated milk [1]. This is useful for detecting heavy metals harmful to for humans present in contaminated milk samples. Chromatography techniques can also determine accurately the ionic concentration of milk previously centrifuged or diluted [2]. For determining the ion content, a few methods are used: ion selective electrodes (ISEs), AAS (Atomic Absorption Spectroscopy) and titration techniques. Chloride concentration is also often determined because of its direct relationship with mastitis disease. It can be measured using the classic analytical titration method [3] with silver nitrate reactant using potentiometric or indicator end-point detection, using a chloride ion selective electrode. It can also be determined using other recent techniques such as the sequential injection automated conductimetric method [4]. Other methods can be used to assess the quality of the milk such as fluorescent techniques on treated or untreated milk samples [5], by cryoscopic method [6], or by method based on changes in refraction [7]. Overall, there are many analytical methods used to investigate milk characteristics such as ionic concentration, fat content, etc. However, most of these techniques are laboratory-based techniques that require the use of large, expensive and fragile instrumentation (HPLC, AAS, etc…) and /or a prior treatment or dilution of the milk samples like centrifugation or solid phase extraction. Both of these treatments are time-consuming procedures and make these techniques impossible or difficult to use in a low-cost on-line system. For the purpose of this study, which is to develop a low-cost on-line mastitis detector, electrical methods remain the most suitable. Conductivity-based methods seem to offer a solution for detecting on-line mastitis disease in a milking parlour. Hamman stated that current available conductivity meters or milk checkers and analysers are not good enough for detecting mastitic milk samples and that further developments of the sensor are required [8].

In order to develop an accurate mastitis sensor based on conductivity or impedance measurement, milk samples have been probed by different electrochemical or analytical methods. Milk properties such as dielectric properties, conductivity and oxidation have been investigated using various electrochemical techniques. Cyclic voltammetry (CV) is used to roughly characterise the milk while Electrochemical Impedance Spectroscopy (EIS) is used to establish the specific electrical properties of milk and Basic Conductivity measurements are used to calculate the overall conductivity of milk. Cyclic voltammetry is indeed a very suitable technique and has been used to study various electrochemical systems [9]. This method has been recently used to analyse untreated milk samples in order to measure their acidity [10,11,12]. It is thus interesting to probe real milk samples exploiting electrochemical processes of species present in milk. Impedance spectroscopy methods are also powerful techniques for investigating milk [13,14,15,16]. As described in section 2.2.4, EIS measurements enable one to determine accurate values of conductance. The broad spectrum of frequency available for these measurements allows one to choose the best operating frequency for the instrumentation in order to minimise polarisation effect and also proves useful for choosing the best material for the sensor electrode [13].

3.2 Experimental

A brief description of the instrumentation is given followed by details of the methods and procedures of the analytical techniques and the electrodes used.

3.2.1 Materials and Electrodes

3.2.1.1 Samples and Chemicals

All Chemicals used for preparation of the standard calibration solutions or other preliminary experiments were of analytical reagent grade. Ultra pure water (resistivity≥18 MΩ cm), obtained from a Millipore Milli-Q-filtering system was used.

For preliminary experiments, fresh milk samples (skimmed milk and low fat milk) were purchased in retail stores. This milk was already pasteurised and homogenised and it is referred to in this study as **normal or pasteurised milk**. The other types of milk samples used were bovine foremilk samples (first stream taken directly from the cow's teat) were provided by Teagasc (Moorepark, Ireland).

They did not receive any treatment and they will be referred to as **crude milk.** Depending on their SCC results, the crude milk samples are classified as negative, subclinical or positive samples. Table 1.1 shows the detail of the classification for crude samples.

	SCC (cells/mL)	*Diagnosis*
Negative milk	<200,000	Healthy
Subclinical milk	200,000>SCC>1,000,000	Subclinical
Positive milk	>1,000,000	Infected clinical

Table 3.1: Details for classification of crude milk samples.

Positives samples usually arise because of mastitis infection and will be referred to as infected or mastitic samples throughout the discussion. About 30 mL of fresh foremilk from each teat was collected in a sterilised test tube and analysed to determine their somatic cell count (SCC) using a Bentley Somacount 300 bench instrument. The accuracy of the SCC is typically less than 10 % covering from 100,000 to 1,500,000 cells per millilitre. The milk samples were kept refrigerated (in a polystyrene box) until analysis and were received and identified by trial number (see Appendix B). Depending on the trial, samples were given a trial number that identified both the quarter and the cow which from which the sample originated. Each cow was identified by a number, and each quarter by its position: RF standing for Right Front, RH for Right Hind, LF for Left Front and LH for Left Hind. This notation was useful for calculation of statistical data and differential threshold, or simply to observe how SCC rises in each of the teats of the cow when infected. The samples were analysed using different electrochemical methods and were untreated and undiluted during measurements.

3.2.1.2 Electrodes

Various two - and three-electrode cells were tested to find the most suitable electrochemical cell to be used and to determine the optimum material for performing the analysis (platinum, gold, nickel, stainless steel…).

Three different cell configurations illustrated in Figure 3.1 were designed and tested:

- A conventional 'three-electrode cell' with a platinum disk macroelectrode as the working electrode
- A homemade cell with 2 Pt electrodes
- Two prototype cells in Nickel and Stainless Steel.

The '**three-electrode cell**' consisted of a platinum disk electrode, a platinum wire as counter/auxiliary electrode and an Ag/AgCl reference electrode. The cells were positioned in a grounded Faraday cage. Platinum disk electrodes 2.0 mm in diameter were used.

The **homemade cell** consisted of two electrodes, one flat plate of platinum as the working electrode and a wire as counter and reference electrode (Combined electrode).

The **prototype cell** consisted of two plates of metal (10 x 2.5 x 0.2 mm) held 3.18 mm apart on a 8mm-nylon screw. Two prototypes were thus built: one with nickel coated brass plates and a second made using a pure grade of stainless steel 316. The electrodes were inserted into a plastic screw and glued to the screw body with a conducting polymer (type epoxy glue). The glue was then baked overnight at 80°C in an oven. The contact was fragile and so the cell was handled with care. This design enabled us to screw the cell into a pipe for testing flowing milk.

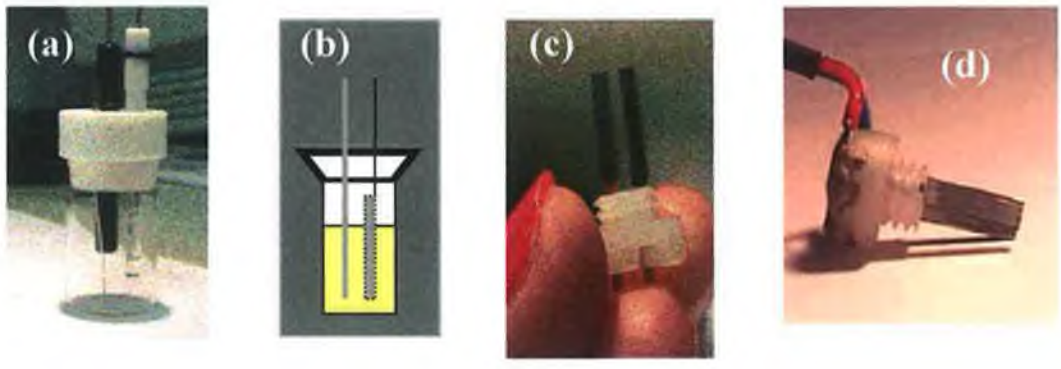

Figure 3.1: Photographs of the electrodes used for testing milk samples with the various analytical methods. **(a)** a conventional three electrode cell and glass cell and Teflon cap, **(b)** the cell described as a home-made cell, **(c)** the prototype cell made of nickel coated brass, and finally **(d)** the prototype in stainless steel.

3.2.2 Instrumentation

3.2.2.1 Conductivity instrument

An Orion Model 150 conductivity meter was used. The Orion cell includes an integrated temperature sensor. The material of the probe was non-metal graphite and the temperature sensor was made of stainless steel. The cell constant is 0.609 cm ± 1.5%. The probe used was a 4-electrode system to avoid any errors due to the polarisation effect at the electrodes. The four-electrode methodology utilized the same two electrode measuring scheme, but it also include an additional two electrode system to act as a reference point for the measuring circuit.

3.2.2.2 Cyclic voltammeter

A potentiostat was used for cyclic voltammetry experiments. Cyclic voltammograms (CVs) were obtained at room temperature (about 20°C) using a CH Instrument 660A potentiostat with the associated software. A conventional three-electrode cell was used. The figure and the details of this electrode are presented in section 3.2.1.

3.2.2.3 Impedance analyser

For Electrochemical Impedance Spectroscopy (EIS) measurements two instruments were used. The first instrument was the same computerised electrochemical workstation used for the cyclic voltammetry experiments (CH Instrument 660A,USA) with the associated impedance software. The instrument applies a fixed perturbation signal where only the direct current (DC) potential of the signal and the range of frequencies scanned can be changed. The signal is a mixture of multiple sine waves at different frequencies when the frequency is above 100 Hz. Below 100 Hz, the perturbation current adopted a sine wave form. The workstation measures and displays the real and imaginary part of the response signal of the cell to the applied perturbation, as well as the Bode plot parameters (impedance modulus and phase).

The second instrument used was the IM6d (Zahner electrick Gmbh) Frequency analyser with the software Thales®. Figures of the measurement system and the software Thales® are shown in Figure 3.2.

Figure 3.2: Photographs of the instrumentation for EIS measurement at high frequencies IM6 d and its software Thales®.

This machine was newly set up and needed many controls and calibrations run prior to experimentation. With this more powerful instrumentation, the frequency scan range is wider (from 1Hz to 5 MHz) and there are more options to choose from. For example, it was possible to perform a single frequency measurement, and also to monitor in real time the variation of impedance while the frequency of the perturbation signal changed.

3.2.3 Methods and Procedure

3.2.3.1 Cyclic voltammetry

Voltammetry is an Electro-analytical technique in which information about an analyte is derived from measurement of current as a function of applied potential under conditions that encourage polarisation of the working electrode. Cyclic voltammetry (CV) is a potential-controlled "reversal" electrochemical experiment. A cyclic potential sweep is imposed on an immersed stationary electrode and the faradaic current response is observed. The excitation signal is a linear potential scan with a triangular waveform, sweep or scan rate (ν, in V/s) which can range from a few millivolts per second to hundreds of volts per second. This triangular potential excitation signal sweeps the potential of the working electrode back and forth between two designated values called the switching potentials. The CV response is plotted as current versus potential. During the forward scan, the oxidised form is reduced, while on the reserve scan the reduced form is reoxidised. Chemical reactions coupled to the electrode processes can drastically affect the shape of the CV response. In addition to providing an estimate of the redox oxidation species occurrence and the value of the redox potential, it can also provide information about the thermodynamics and kinetics of electron transfer at the electrode-solution interface [9].

Procedure:

The cyclic voltammetry experiments were carried out in a glass cell and maintained in a Faraday cage used to avoid external noise interference. The samples were deaerated before measurement by bubbling nitrogen through them for few minutes. Prior to each measurement, the working disk electrode was held vertically and rubbed on the polish in a figure of eight pattern for few minutes; then it was rinsed carefully with Milli-Q-water. After polishing, it is still possible that some alumina particles remain, so therefore the electrode was left for 5 minutes in a beaker full of water in an ultrasonic bath. The surface of the electrode needed to be cleaned between each experiment, as if any materials are absorbed to the surface, then the current response would be modified.

In this case the proteins of the milk can stick to the working electrode and therefore it has to be lightly polished on a fine cloth using a fine polish of graded alumina powder (0.05 μm). Platinum electrode cleanliness or contamination of the reference electrode by milk was tested by reproducing the CV with a solution potassium chloride (0.1M) which would give a reproducible voltammogram.

3.2.3.2 Conductivity

A standard 4-electrode conductivity meter with automatic temperature compensation was used to measure the overall conductivity of milk or standard solutions. The procedure was straightforward with the milk being poured into a beaker and then homogenised using a magnetic stirrer. After stabilisation of the solution, the probe was placed into the beaker at a sufficient depth; the conductivity was read (compensated to 25°C if required). By changing the mode of the apparatus, the temperature could also be determined. Prior to each experiment, the conductivity was automatically calibrated using a NaCl standard solution and the probe was rinsed with ultra pure water and dried.

3.2.3.3 Electrochemical Impedance Spectroscopy

The EIS experiments were carried out by direct measurement which means that the electrode was directly immersed into the medium to analyse to collect data. The experimental conditions for all the samples were kept as similar as possible in order to better compare the results. A digital thermometer was used to measure and record the temperatures after stabilisation. Careful control of the temperature was necessary as the conductivity is sensitive to temperature changes. Before each experiment, the sensor was washed carefully with ultra pure water and dried by exposure to a flow of nitrogen or naturally in air. The milk sample (a few millilitres) was then placed in a cell in glass covered by a plastic cap to avoid temperature dispersion. The cell schematic is shown in Figure 3.3.

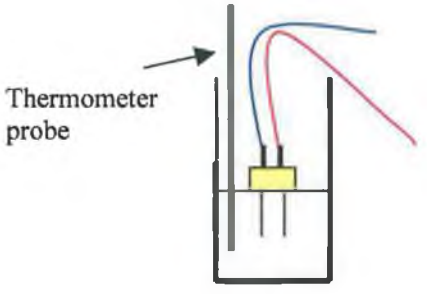

Figure 3.3: Schematic of the electrochemical cell in glass and the electrode simply dipped in the solution to analyse the sample.

The chosen electrode was then placed in the cell and connected to the impedance analyser with a preset configuration where available e.g. two electrodes with no buffer.

3.2.4 Experimental details

Cyclic voltammetry experiments

CV experiments were performed with the previously described three-electrode cell. CVs were recorded between 0 and 0.9 V versus the potential of the reference electrode (silver/silver chloride) with a scan rate of 0.05 V/s to 0.3 V/s). These parameters corresponded to the values which indicated that the voltammogram obtained was of good quality.

CVs for normal and low fat milk were carried out to assess the influence of the fat content on the shape of a voltammogram. CVs on mastitic milk were also performed and compared to see if the somatic cells count i.e. the amount of ionic content influenced the shape of the voltammogram or if it created or modified peak occurrence.

Electrochemical Impedance Spectroscopy experiments

Using the CH Instrument potentiostat, the influence of the frequency and potential of the perturbation signal response on the cell was observed. The real and imaginary parts of the current response were recorded, and the capacitance and reactance behaviour displayed using a diagram Bode plot. For the first instrument, the EIS measurements were performed as a function of the AC frequency from 1 to 100,000 Hz. The AC amplitude of the perturbation was fixed at a value of 5 mV and was defined as zero to peak.

These types of preliminary experiments were methodically performed using different cell designs and materials on different samples which included normal standard solutions of potassium chloride at different concentrations, normal pasteurised milk samples, positives (infected by mastitis disease and exhibiting a high value for the SCC) and negatives crude milk samples.

The experiments carried out on the standard solutions allowed the repeatability and the calibration of the instrument to be checked and also enabled calculation of the cell constant for a particular cell design. Experiments on normal milk allowed the most suitable material for the prototype used in the sensing system to be chosen.

The second series of experiments was carried out using a broader range of frequencies from 1 Hz to 2-3 MHz. This was possible by using the second impedance analyser available (the IM6d). Preliminary experiments were carried out and then repeated on pasteurised and crude milk samples.

3.3 Results and Discussion

This section summarises the results of the experiments performed in order to obtain the optimum parameters needed to design an instrument used for detecting mastitis.

3.3.1 Cyclic Voltammetry results

Cyclic voltammetry was done using normal and mastitic milk.

3.3.1.1 Normal milk

The cyclic voltammograms (CV) for pasteurised milk with 3.5% fat and a low fat (less than 1% fat) were compared with the CV obtained for a solution of potassium chloride at 0.1 M. The results are shown in Figure 3.4.

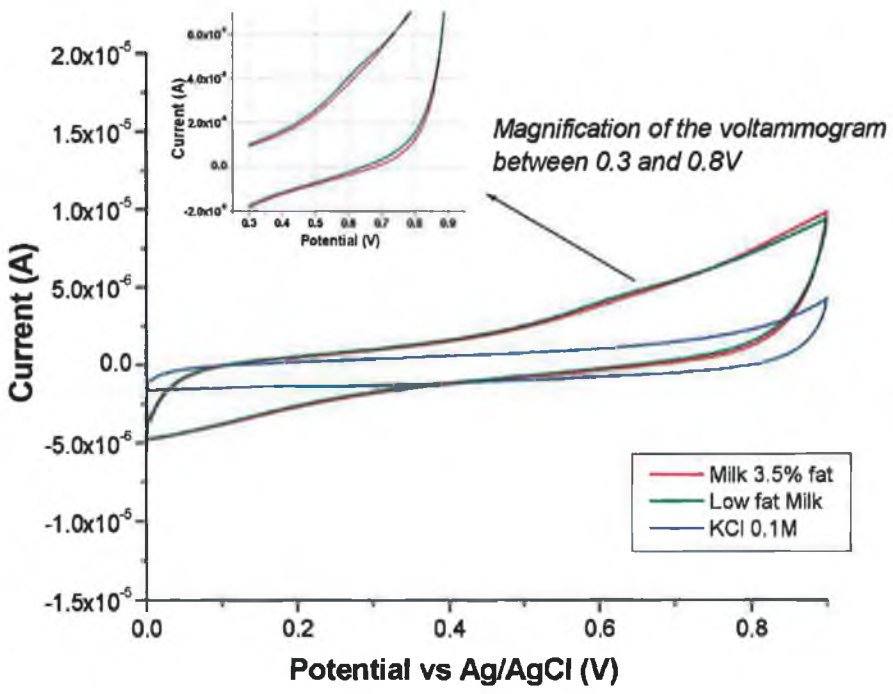

Figure 3.4: Cyclic voltammograms of pasteurised milk (3.5% fat and low fat milk) and KCl 0.1 M recorded at a 2 mm Platinum electrode. Scan rate 0.1 $V^{-1}s^{-1}$.

The cyclic voltammogram of milk is straightforward. The voltammograms show only one peak of oxidation at 600mV versus Ag/AgCl on the positive scan. Jawad et al. found that among the components susceptible to oxidation in milk, that uric acid corresponds best with this "wave" [12]. However, a different study attributed this peak to the hydrogen influence of acidic groups on the casein and the reduction of protons delivered from phosphates ions rather than uric acid alone [10,11]. The wave is due to the precipitation of $Ca_3(PO_4)_2$ and $CaHPO_4$ onto the electrode causing inhibition of the process resulting in this particular wave shape [11].

The cathodic peak (for both types of normal milk) appears at 0.6V versus Ag/AgCl reference electrode potential with a current of 4.10^{-6} A for a scan rate of $0.1Vs^{-1}$, where the standard potential of the reference electrode is 0.222V. The same value is found for both milk samples indicating that the amount of fat in milk does not interfere with the acidic concentration present in a fresh milk sample. The voltammogram of untreated milk is quite different to a normal aqueous solution studied with cyclic voltammetry. Compared to such an electrolyte, the milk shows lower conductivity. It is possible to add salt to increase the conductivity, thereby obtaining clearer voltammograms and better oxidation current, but in the case of milk, it can cause some denaturation and coagulation and so is not an option. Some researchers showed that using a platinum microelectrode of 25 μm could also improve the quality of the voltammogram [11]. A second problem occurring when using cyclic voltammetry is the absorption onto the electrode surface of the proteins and other macromolecules such as micelles of fat. A white residue constantly covered the tiny electrode surface of the platinum electrode after each experiment and careful control of its cleanliness was necessary. This absorption phenomenon tends to inhibit interfacial electron transfer processes. It is legitimate to consider whether some electrochemical processes would be completely inhibited. To verify the relative quality of the voltammogram shown in Figure 3.4, the CV obtained by Jawad et al. is illustrated in Figure 3.5 [12].

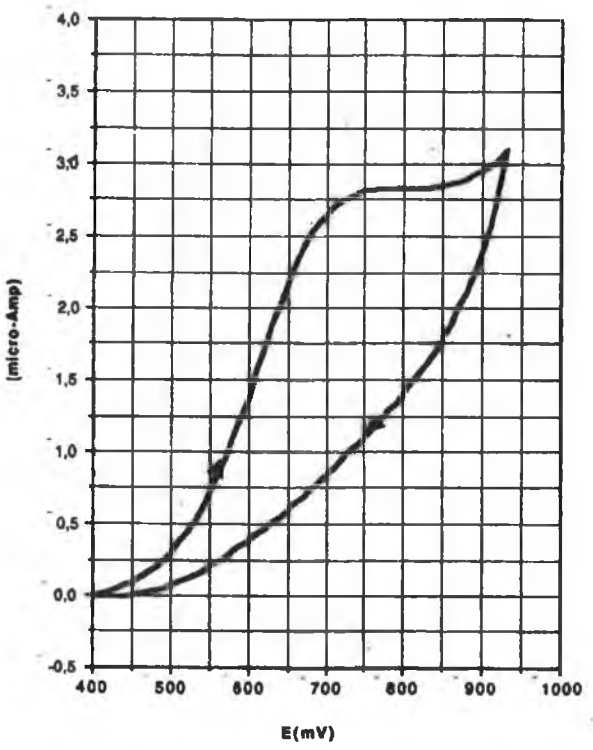

Figure 3.5: Cyclic voltammogram of UHT milk at carbon paste electrode, Electrode potential versus electrode reference (saturated calomel electrode). Scan rate 10 mVs^{-1} [taken from ref 12].

The voltammogram shape obtained with UHT (ultra high treatment) between 700-850 mV is similar as the one normal pasteurised milk and the peak of oxidation occurs at around 700 mV versus saturated calomel electrode. UHT treatment homogenises the milk and gives the milk a longer shelf life, as well as reducing the viscosity of the milk. This can explain why the current response is of better quality as such treated milk as a more watery consistence and would less tend to coat the electrode. Moreover, the electrode used is not a solid platinum disk electrode but a carbon paste electrode of larger surface area (0.2 cm^2 area instead of 0.12 cm^2 for the platinum disk). Few CVs of milk by direct measurement using solid electrodes are presented in the literature. However, those that exist show the occurrence of a single wave which is attributed to the acidic hydrogens contained in milk and not solely to urea protons, as mentioned previously [10]. Daniele et al. stated also that the CV of milk performed with a conventional-sized platinum electrode (3mm in diameter) gave a standard deviation of about 10% [10].

For detecting spoilage of milk, CV would be thus an interesting method but we expect that it would not be suitable for mastitis detection application as variations in the acidic hydrogens is not the main feature occurring when mastitis takes place.

3.3.1.2 Mastitic milk

The CV obtained for crude milk is shown in Figure 3.6. The wave of oxidation appears again at 0.6 V versus a silver/silver chloride electrode. The reduction scan does not show the occurrence of a new peak and the differentiation between the CV for milk of different values of SCC is not clear.

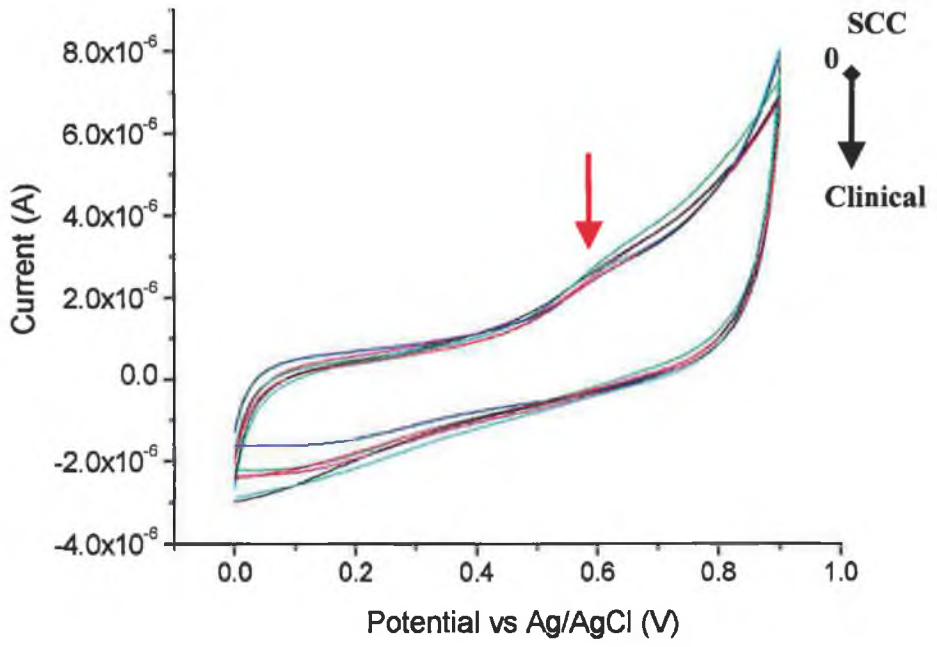

Figure 3.6: Cyclic voltammogram of negative, subclinical and positive milk samples recorded at 2 mm Platinum electrode. Scan rate 0.1 $V^{-1}s^{-1}$. The different colour lines correspond to different SCC milk samples.

The cyclic voltammograms do not differentiate between healthy or negative, subclinical and positive or clinical crude milk. The value of the ΔEp is small (≈0.5E-6 A) and is likely to be due to the difference in the electrode transfer phenomenon because different diffusion of ions in healthy and mastitic milk. Mastitic milk sometimes has clots that prevent the ions flowing freely in the solution. Moreover, the current responses in these kinds of measurement are very small (of the order of few μA) and they cannot be easily used for in an on-line situation considering the difficult conditions when operating in the milking parlour. Nevertheless, the cyclic voltammetry of normal milk and mastitic milk is interesting for determining the dc potential that needs to be applied to avoid any oxidation phenomenon when carrying out an EIS experiment for example.

From the CVs, it can be seen that no faradaic processes (absorption, oxidation, etc...) will occur between 0.1 and 0.5 V versus the Ag/AgCl reference electrode (E^{o}=0.222V), which means that no oxidation will take place if the instrument performs the measurement from 0V to 0.3 V. This was further checked with electrochemical impedance spectroscopy experiments later in this section.

3.3.2 Conductivity results

The conductivity of a solution tipically increases with temperature. This increase can be compensated for by using a linear equation involving a temperature coefficient, which is the percent increase in conductivity per degree (see Section 2.3.2). To compensate for the effect of temperature in the experiments performed throughout this project, the temperature coefficient was determined and compared with the values established earlier by Prentice [17].

For a variety of milk samples, the conductivity was measured without temperature compensation at different temperatures in the range 10 to 45°C. The milk was heated on a hot plate and stirred in order to homogenise the temperature in the milk solution. The slope of the straight line obtained was then calculated and the temperature coefficient K was deduced from equation 3.1:

$$C_T = C_{25}\left[(1 + K(T - 25)\right] \qquad \textbf{(Eq 3.1)}$$

To calculate the temperature coefficient, the slope was divided by the conductivity at 25°C (C_{25}) and multiplied by 100.

The results found for the temperature coefficient using different types of milk are shown in Table 3.2.

Milk SCC (x1000 cells/mL)	**Conductivity at 25°C** (mS/cm)	R^2	**Temperature coefficient** (%/ °C)
Pasteurised milk	3.914	0.99	**2.099**
196	4.586	0.99	**2.089**
246	4.867	0.99	**2.145**
486	5.101	0.99	**2.148**
1216	5.323	0.99	**2.150**
2751	4.182	0.99	**2.071**
3309	4.715	0.99	**2.118**

Table 3.2: Temperature coefficient calculated for different milk samples.

For healthy milk, the coefficient was found to be around 2.1%/°C. In the early 30's, researchers assumed that the temperature coefficient for milk at 25°C was not much different from that of 0.2 M solution of potassium chloride, for which it was given Schnorf's value of 2.0 %/°C [18]. Roeder performed further measurements in the range of 18-25 °C and obtained the value 2.3 %/°C [19].

The temperature coefficient for mastitic milk was found to be slightly higher than for normal milk. This could be due to different heat diffusion properties in the two media but it not due to the difference in ionic concentrations in the electrolyte. On this question, Prentice [17] did conclude that there is no major difference between the coefficient for negative and mastitic milk. For the rest of the project, the equation 3.1 was used for temperature compensation and the value of the coefficient was chosen as 2.1% which is in agreement with Prentice's results [17].

The background to electrochemical detection of mastitis by conductivity measurements is presented in detail in chapter 1. Linzell and Peaker stated that a relationship between SCC and conductivity exists and it can be used as method to detect mastitis [20]. The relationship between conductivity and SCC is examined in Figure 3.7. The conductivity is temperature compensated to 25°C and the temperature coefficient used is 2.1%/°C.

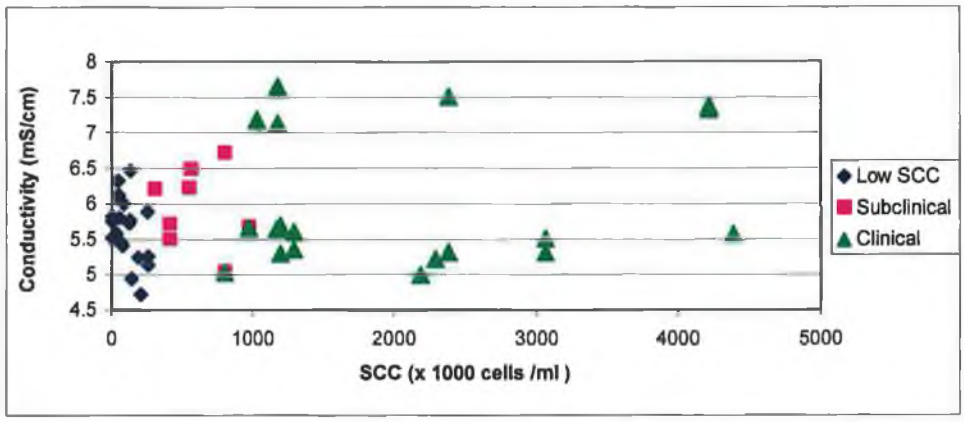

Figure 3.7: Influence of SCC on milk conductivity. Data are temperature compensated to 25°C.

The results show no clear differentiation between positive and negative samples. The interpretation of these results is not straightforward. Some samples with high SCC have low conductivity, and some samples with low SCC have high conductivity.

A possible explanation for this is that the clots and flakes present in clinical mastitic milk prevent ions from flowing and thus increase resistance and cause lower conductivity readings. It is possible that the conductivity probe is blocked by milk fat and that this layer causes spurious measurements. This phenomenon is illustrated in Figure 3.8.

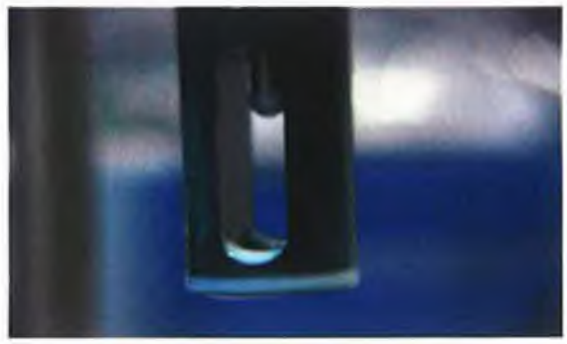

Figure 3.8: Conductivity cell after measurement; milk residue remains in the temperature probe, the graphite electrode and on the plastic body.

Also, it is likely that some of the SCC readings are incorrect as the accuracy of the counting bench used is 10% in the common range of SCC values measured. These results show that EC alone is not accurate enough to detect mastitis on-line. Other parameters such as temperature, concentration of chloride or calcium must be monitored along with EC. A similar conclusion was reached by Hamman i.e. the application of a single threshold conductivity value is not very useful for mastitis diagnosis [8].

3.3.3 Electrochemical Impedance Spectroscopy results

EIS experiments enable the determination of which material to use for the electrode. EIS shows the effect the effect of frequency on normal and mastitic milk, giving the best range of parameters to use for analysing milk while minimizing the polarisation effect.

3.3.3.1 Choice of the electrode

For all the experiments carried out with milk, the impedance shows a rapid variation with frequency below a certain value that turns out to be different for each material used as the electrodes plates in the cell probe. As previously explained in section 2.2.3, this increasing of conductance with the frequency is characteristic of the electrode polarisation effect in milk. The peak shape of the susceptance (B) is also a feature of polarisation. The frequency of relaxation of polarisation can be determined for the frequency where the susceptance reaches a maximum value [13]. For this body of work, the influence of the frequency was studied for platinum, Nickel coated brass and stainless steel grade 316 two-plate electrodes, and also for in the conventional cell described earlier. The results for the impedance and the susceptance are shown in Figure 3.9 and Figure 3.10.

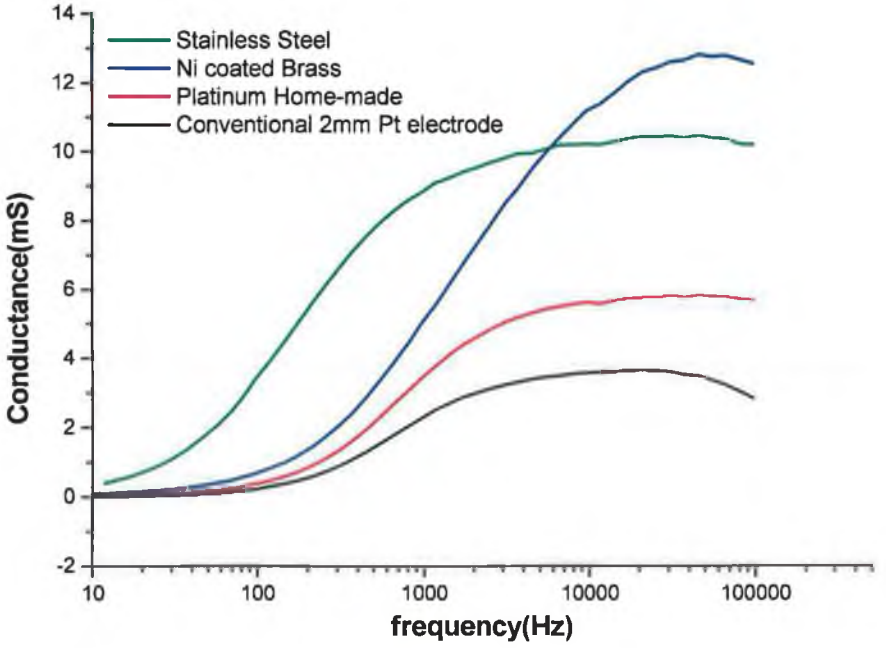

Figure 3.9: The variation of the conductance of normal milk at different frequencies using Stainless Steel (SS), Nickel coated Brass (Ni), Platinum home made and

conventional three electrodes platinum cell at 24.7° C for a perturbation signal E=0V and p= 5mV.

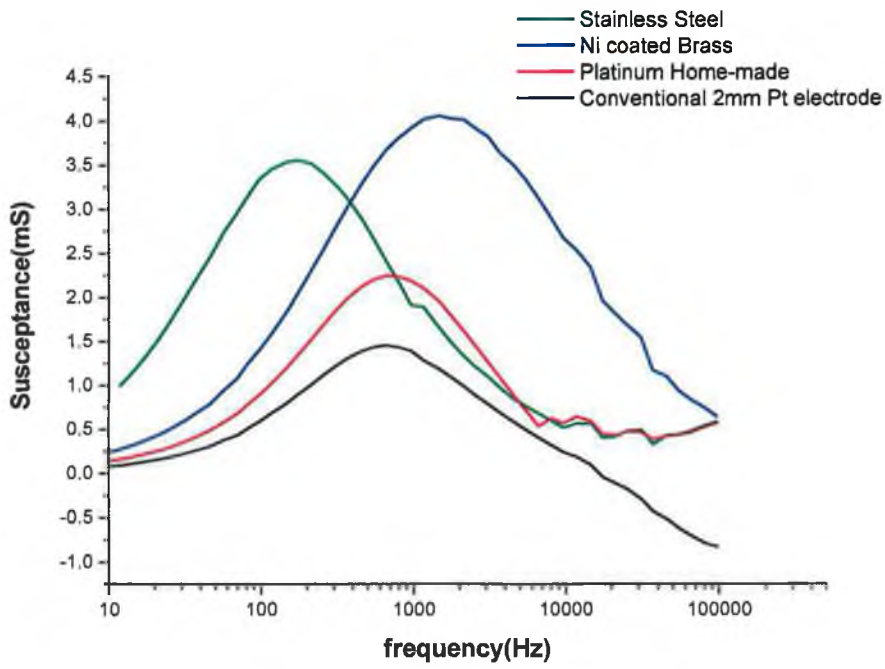

Figure 3.10: The variation of the susceptance of normal milk at different frequencies using Stainless Steel (SS), Nickel coated Brass (Ni), Platinum home made and conventional three electrode platinum cell at 24.7° C for a perturbation signal E=0V and p= 5mV.

From the curves of susceptance, it can be found that the polarisation effect starts at 100 Hz for Stainless Steel electrode, at 900 Hz for the two platinum electrodes and finally at 2 kHz for the electrode made of nickel plate coated with Brass. The variation of impedance with frequency shows clearly that the material with the best range of frequencies to operate is Stainless steel. The polarisation effect of the stainless steel seems to finish at 10 kHz which corresponds to the point at which the conductance reaches saturation. The conductance measurement of electrolyte is often complicated by the phenomenon of polarisation effects at the operating voltage. To eliminate the effect of electrode polarisation, the conductance of the milk should be measured at high frequencies where saturation exits. Lawton et al. also compared a stainless steel electrode against a platinum-bright electrode for determining the fat content of milk by EIS measurement [13]. He found that the polarisation for the stainless steel electrode ended at lower frequencies than for platinum. He recommended operation at 100 kHz to avoid these polarisation effects.

Hence, the impedance and susceptance results show that Stainless Steel should be the material of the choice for electrode for the prototype to be used for the milk sensing system. Stainless steel also offers other advantages for this application as it is a well-known metal, with good mechanical properties (rigidity, hardness...) and it is easy to manipulate and clean. Compared to noble metals, which are commonly used as electrodes, stainless steel combines good electronic conductivity and high corrosion resistance. The grade of stainless steel used is SS 316. SS 316 corresponds to a pure austenitic grade and has the following composition: Chrome 18%, Nickel 10%, Molybdenum 3% in addition to the steel (alloy made of iron and carbon). The inclusion of molybdenum increases its corrosion resistance. This type of steel fulfils all the requirements for the required mastitis sensor especially in terms of durability in milk, chemical resistance to salts and acids, bio-friendly aspects, easy cleaning and cost effectiveness.

3.3.3.2 Experiments on crude milk

Determining the material of the electrode and the operating frequency for the EIS is not enough to guarantee that the sensing system for mastitis will effectively distinguish healthy milk from infected samples in an on-line system. The influence of the SCC in milk on electric properties such as impedance, susceptance and other related parameters therefore must also be investigated. For the analysis, it is important to have in mind the degree of infection of milk corresponding to the SCC value as shown in Table 3.1.

Data obtained with the platinum three-electrode cell were not good enough due to poor reproducibility and multiple electronic artefacts. The EIS measurement is not suitable for a three-electrode cell like the one used in cyclic voltammetry. The results obtained from the homemade platinum cell gave less artefacts and relationship between the SCC and conductance is now apparent. As expected the impedance values decreases when the SCC increase i.e. when the mastitis infection spreads and the overall content of ionic species increases. The Nyquist plot obtained using the platinum homemade electrode is presented in Figure 3.11.

The Nickel electrode gives also interesting results and when plotting the susceptance against SCC, a good relationship between susceptance and frequency is noted. Figure 3.12 is plotted with four types of milk ranging from healthy to clinical.

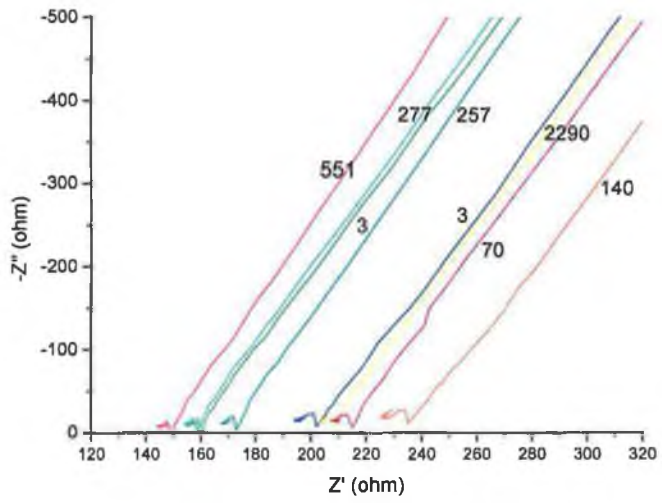

Figure 3.11: Nyquist plot of milk using homemade platinum electrode at temperature compensate at 20°C, E=0.3V and p=5mV. The different colour lines correspond to different SCC milk samples and the SCC is indicated in black numbers next to each of them.

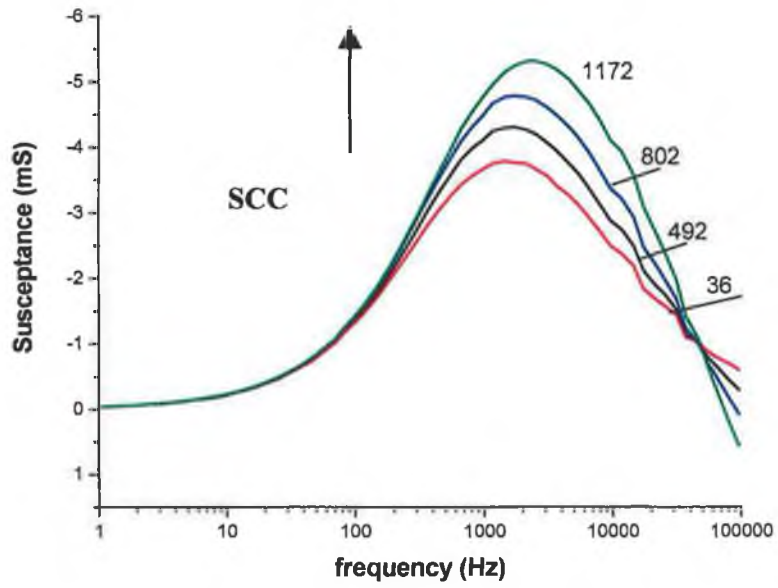

Figure 3.12: The variation of the susceptance with frequency for different milk samples using the two plates prototype cell in Nickel. The parameters are E=0V and p=5mV (amplitude of the perturbation), the results are compensated to 20°C. The different colour lines correspond to different SCC milk samples and the SCC is indicated in black numbers next to each of them.

The susceptance increases with decreasing SCC, but the frequency relaxation value remains the same at approximately 2 kHz which shows that the polarisation electrode effect is not affected by a higher content of ionic species when the milk is infected.

3.3.3.3 Experiment on crude milk samples using a Stainless Steel prototype

The stainless steel electrode was thoroughly tested, as it appeared to be the best choice of material for the electrode for this project. The relaxation frequency of polarisation effect for stainless steel is 200Hz, the saturation value is reached at about 10 kHz, and the plateau is reached at about 50kHz. Thus, the proposed operating frequency should be above 70kHz to be sure to avoid polarisation. To prove this, the impedance results at a single frequency and also to scan at higher frequencies, the stainless steel prototype was tested using a larger range of frequencies up to 2-3 MHz.

The high frequency machine was tested with standard solutions and pasteurised fresh milk. The electrochemical system gave good agreement with an ideal circuitry RC (Resistance-Capacitor in series) and with the KCl Bode and Nyquist plot obtained for KCl solutions with the CH 660A workstation.

The results obtained on the frequency analyser are clearer especially at high frequencies (above 80 kHz) where the workstation attained the working limit of the apparatus. The Bode plot for higher frequencies is shown in Figure 3.13.

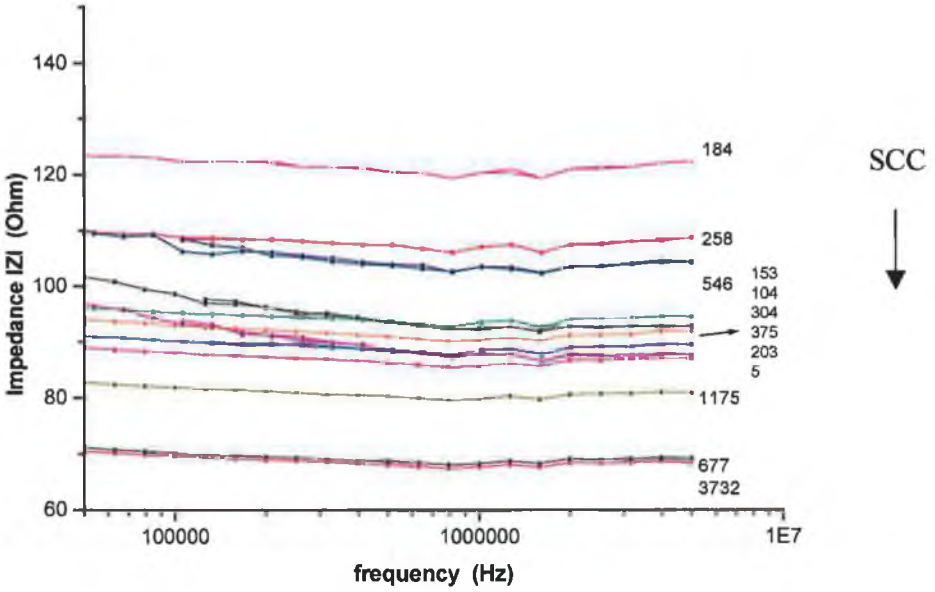

Figure 3.13: Bode plot of milk for different SCC values using a stainless steel electrode with temperature compensation to 20°C, E=0V and p=5mV. The plots correspond to an average of three EIS measurement with the same parameters RSD: ± 0.08.

The Bode plot in Figure 3.14 is the same Figure 3.13 but corrected to eliminate any spurious measurements.

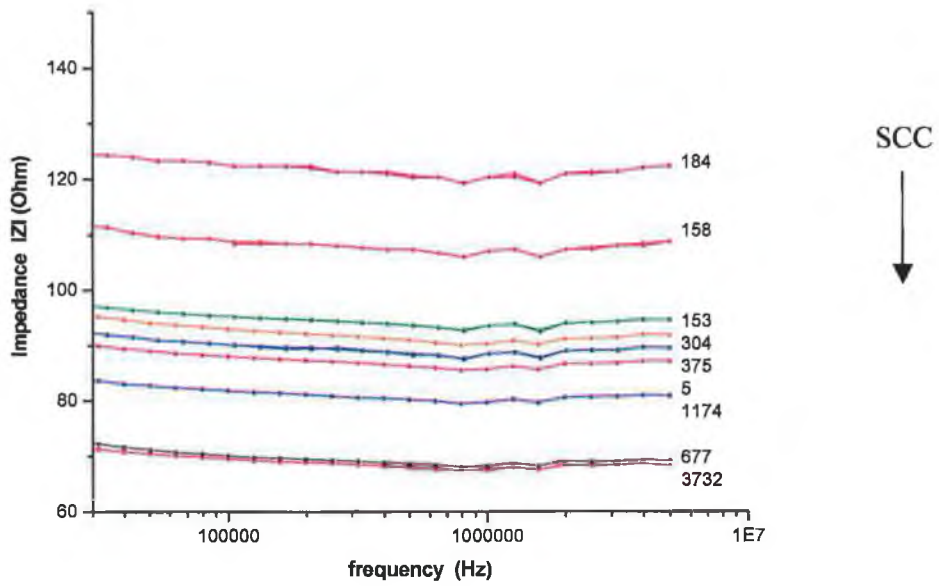

Figure 3.14: Improved bode plot of milk for different SCC values using a stainless steel electrode with temperature compensation to 20°C, E=0V and p=5mV.The spurious measurements were removed following a slope correction.

The results of the high frequency EIS measurements were very interesting. It appeared that using the EIS measurement; the SCC does have a direct relationship with milk impedance (or conductance) as already stated by many researchers [8]. EIS measurements demonstrate that the conductivity of the milk increases with increasing Somatic cell count (SCC). The plot of the impedance at 100kHz against the SCC values is shown in Figure 3.15.

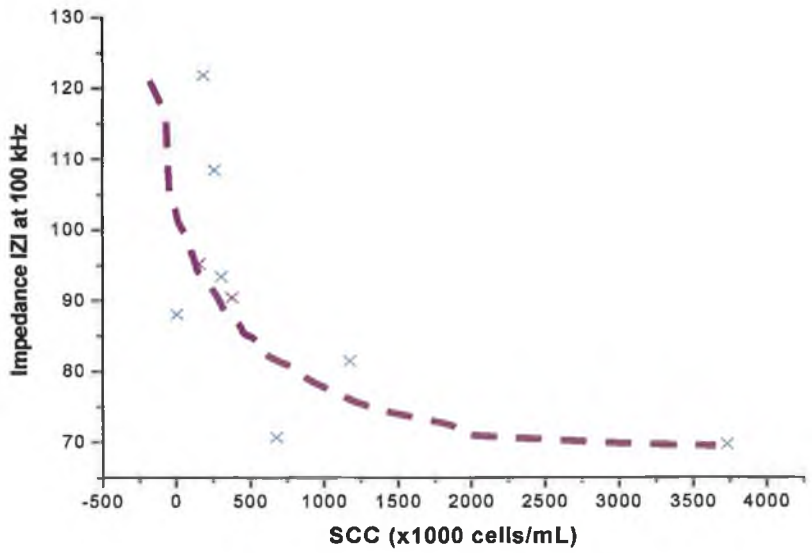

Figure 3.15: Relationship between the impedance (see Figure 3.14) at a single frequency of 100 kHz against the SCC.

There were still some false positive results (e.g. sample for SCC: 5) but it was hoped that the single frequency measurement (at 150 kHz) carried out by the new portable instrument would improve the quality of the results and increase the sensitivity to enable the calculation of limits to distinguish mastitic milk from healthy milk.

3.3.4 Specifications for the instrumentation of the portable sensing device

The previous experiments enabled the selection of suitable parameters for developing the device. These parameters included the characteristics of the applied signal in impedance measurements (see section 2.3), the direct current (DC) potential, the amplitude of the AC current applied as perturbation and the operating frequency. The instrumentation of the sensing system was expected to deliver similar results to those obtained by the bench laboratory machines such as the workstation potentiostat or the impedance frequency analyzer. Hence, the parameters of the circuitry to be built were chosen based on obtaining the most sensitive detection of mastitic milk samples.

The choice for the DC current value reflected the conclusions found after running the cyclic voltammetry experiments. This was important, as it is not possible to work at values where reduction or oxidation occurs. The first option involves the circuitry delivering a 0 V potential. This would be easier to design and no potentiostat would be required. In this case, a "simple" Wheastone bridge design could be used as circuitry. In the second option, a 0.2 to 0.3 V direct potential would be applied; this would assume that potentiostat circuitry is included in the circuit. These two possible options for the DC current where tested with impedance experiments which were performed at 0 V, 0.1 V, and 0.3V (see Appendix E). With so little difference observed between the spectra, it was concluded that any of the values of direct potential would be fine.

In addition to the DC current, a signal perturbation is applied. The value proposed for the rms (see section 2.1.2) is in the range from 10 mV (peak to peak) to 30 mV. By convention, the amplitude used for EIS measurements is chosen at 5 mV, as using higher AC amplitude could cause the measurement to deviate from linearity and also increase the polarisation effect (see section 2.2).

In the literature, the operating voltage for such applications is rarely mentioned. A French patent [21] described a system for mastitis detection in milk using a two-electrode system operating at an AC current amplitude of 1 V with a frequency of 100 kHz.

No detail of the shape of the signal was given. Mabrook and Petty used an RMS amplitude of 0.7V for testing fat content of milk, but added that no significant changes in the sensing properties were observed at lower voltages [14].

Finally, the frequency of the AC signal has an important role in the measurement of conductance/impedance. The polarisation effect has been observed to be lowered around 100 kHz depending on the material electrodes [13,14,15,16]. For the mastitis sensor prototype, the polarisation effect is minimised at 80 to 100 kHz.
For the device, it was decided to take measurements at 100 kHz. Since a sine wave AC current is not easy to generate, a square wave AC current was used instead.

3.4 Conclusion

The results of the EIS measurements showed that measurements should be taken above a certain operating frequency to avoid polarisation effects and to improve the sensitivity of the conductivity sensor. However, the identification of positive and negative samples was not very clear-cut within the range of frequencies examined up to 50kHz. Analysis at higher frequencies showed that the threshold operating frequency should be between 80 and 150 kHz. Above this frequency range, a correlation between Somatic cell count (SCC) and milk impedance readings was found but the sensitivity of the detection method is still being improved. Thus, the various techniques used enabled the choice of suitable parameters for the instrumentation-sensing device. The next step was to build the electronic device and use it to assess positive samples to check the efficiency of the impedance-based method and also to establish threshold limits for the detection of mastitic and healthy milk.

3.5 References

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Chapter IV

Design and Fabrication of the Prototype Impedance Device

4.1 Introduction

Devices used for detection of mastitis by electrochemical means are often based on conductivity measurements [1-5]. Different prototypes for on-line detection are described in the literature, however only a few studies give details on the instrumentation used to "predict" positive and negative milk samples. A French study proposed a prototype for measuring the biochemical quality of milk on-line. The system consisted of a claw equipped with electrodes and software for data collection. A sinusoidal potential of 1V at a frequency of 100 kHz was applied by an electronic card located between the claw and the computer but no information was given on the circuitry or even the location of the electronic card [4]. A Spanish project described possible instrumentation for an electric hand-held meter for mastitis detection [5]. The AC conductivity was measured using an analog multiplexer which resulted in a 100 μA square wave current with a frequency above 10 kHz. The study mentioned that it was possible to distinguish mastitic samples at a clinical or subclinical stage from healthy samples but only a basic flowchart of the mastitis detector was shown. The detector used a single absolute threshold of conductivity values and the conclusion on the efficiency contrasts with the conclusion of Hamman [6] who declared that the use of single threshold conductivity based detection is unlikely to give good sensitivity.

Our study aimed to integrate the whole detection system into the milking lines to avoid spurious electrical resistance due to long wiring and connection cables. After determining the optimum parameters for analyzing milk electrical properties, the next step was to build specific circuitry, instrumentation and required software to operate the sensing system. The requirements for this instrumentation were numerous: it must be a low cost device and small, it must have a fast response, and be a low powered instrument (in order to present no danger for the animal) in order to perform quality impedance measurements.

A first prototype was developed with a U.K. partner (Whistonbrook Technologies) according to the recommendations given by us for voltage, perturbation potential and frequency range. This instrumentation constituted a major step forward in the project where the final aim was on-line detection.

4.2 Experimental

4.2.1 Samples and Chemicals

All chemicals used for preparation of the standard calibration solutions or other titration experiments were of analytical reagent grade. Ultra pure water (resistivity≥18 MΩ cm), obtained from a Millipore Milli-Q-filtering system was used.

Some analytical test strips were used to measure chloride concentration from 500 to 3000 ppm (or mg/L) at an interval of 500 ppm. Mercoquant ® chloride strips were purchased from Merck .

The milk samples used were similar to those described in the experimental section of chapter 3. Fresh milk samples (normal pasteurised milk) were purchased in retail stores. Bovine foremilk samples (crude milk) were provided by Teagasc (Moorepark, Ireland). The milk samples were kept cooled (in a polystyrene box) until analysis. The milk samples were received and identified by trial number (see Appendix B). The samples were analysed untreated and undiluted.

4.2.2 Device

An instrumentation was built in collaboration with a U.K. partner (Whistonbrook Technologies) following the technical specifications recommended in section 3.4. The advantages of this device were many. It was of small size and easy to use and overall it was going to be similar to the design for integration into the milking parlour system.

It included a PC interface, output device and sensing unit (cell and temperature sensor). A wall-mounted box (180 x 90 x 50 mm) contained a transformer working off mains electricity to power all the other parts of the device. It also contains circuitry for transmitting data to the parlour based PC spaces.A programmable integrated circuit (PIC) served to send data to the output device and the PC interface device. These new chip devices could be programmed to output signals at a range of frequencies and amplitudes using a small number of external components and read in data from eight or more sensors at one time. They could also perform calculations using this data and communicate with, or control an external device. This current prototype was referred to as the "**prototype impedance device**". It included one measuring channel and a temperature sensor that could be dipped in the milk sample during measurement.

The temperature sensor used in this application consisted of a thermistor (see Appendix C for datasheet). It was robust and sensitive enough for this application. By measuring the freezing temperature of normal tap water, the accuracy of the temperature sensor was verified. The probe was submerged in crushed ice and water and the temperature found (0.71°C) allowed us to assume that the accuracy of the temperature sensor was about ± 0.5°C as the temperature of crushed ice and water is slightly above 0°C.

The device measured impedance and temperature at a single frequency of about 150 kHz using a small perturbation of 10 mV (peak to peak). The shape of this signal was a square form because sine waves are difficult to obtain at such frequencies with small circuitry. A very low direct current was applied from a potentiostat which was necessary for operating the entire circuitry (temperature sensor, PC interface…). Measurements were automatically recorded every two seconds. The impedance, conductivity and temperature readings were displayed via a communication terminal (hyper-terminal: an MS communication tool) and then saved for further analysis.

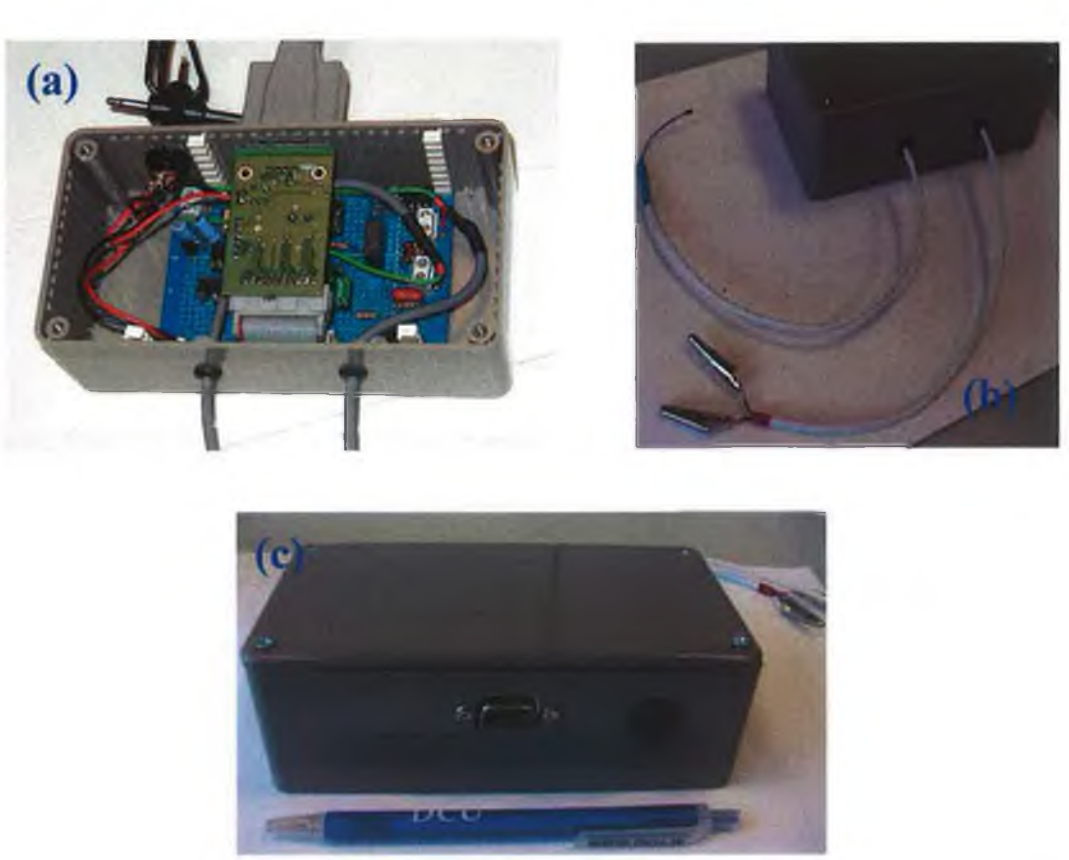

Figure 4.1: Photographs of the prototype impedance device showing: (**a**) a view of the circuitry, (**b**) the connections including one channel measurement and the temperature sensor, (**c**) the box and its interfaces (PC connection and Power).

4.2.3 Procedure for Impedance measurement

The measurements on the prototype impedance device were performed in a similar manner to the one described for EIS experiment in section 3.2. A plastic cell was built to carry out the measurement in similar conditions, particularly to keep the electrode immersed at the same level. A stainless steel electrode described in section 3.3 is used for collecting the data. The design is illustrated in Figure 4.2.

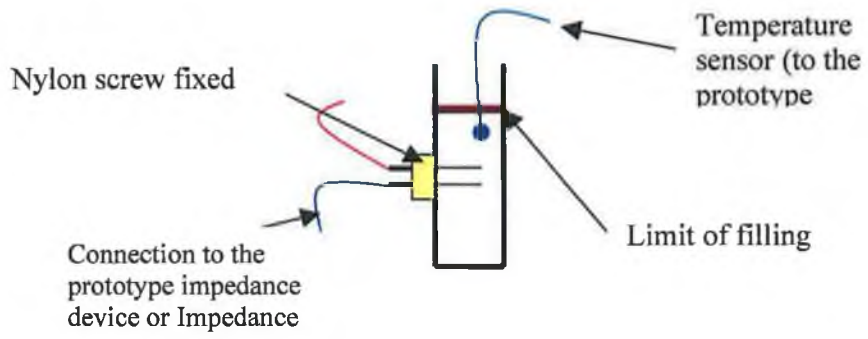

Figure 4.2: The plastic cell and the Stainless steel electrode screwed into the side of the tube standing vertically.

The electrode was screwed into the plastic tube closed and tied at its extremities by a waterproof cap. The cell with the electrode in place was then filled with few millilitres of milk until it reached the limit level. The electrode was then connected to the instrument using crocodile clips and the temperature sensor was dipped into the milk. The milk was then discarded and the electrode was rinsed with water and dried between each measurement. The experiment was performed with the various samples and repeated three times. The temperature was controlled at all times and measured to compensate eventual temperature variations.

The experiment using electrochemical impedance measurement followed the same procedure described in section 3.3.

4.2.4 Procedure used for chloride analysis

The method used was a simple titration by chloride analytical tests strips. The Merckoquant ® strips were dipped into the sample until the entire testing zone was immersed. It was left for 2 to 5 seconds and then removed. The results were read on the strips after 10 seconds. This time interval of 10 seconds was adhered to in all cases.

4.2.5 Experimental details

The prototype impedance device was tested using different real liquid samples and the results were compared with the results obtained from the frequency analyser BAS Zahner IM6.

The results obtained from the frequency analyser BAS were calculated by averaging the data in the frequency from 50 to 300 kHz instead of taking single data points at 150 kHz. The purpose of this comparison study was to see the effect of square wave signals instead of sine waves on high frequencies and also to verify if the operating parameters decided on in section 3.4 were proving to be optimal.

In order to assess the sensitivity of the prototype impedance device, crude milk samples were analysed. The purpose was to see if the method was suitable for predicting positive mastitic milk and also to decide on a possible threshold. The data obtained with the prototype impedance device for each samples were from an average of three sets of measurements with 15 measures (equivalent to about 30 s) in each set.

Meanwhile, titrations of chloride in the milk were carried out to investigate whether the concentration of chloride could help us to differentiate between false positive and false negative results.

4.3 Results and Discussion

The prototype impedance device was studied. A comparison between the results of BAS Zahner and the prototype impedance device was carried out and a possible threshold for the mastitis-sensing device was determined.

4.3.1 Calibration and Comparison

The prototype impedance device was calibrated and tested with standard solutions and various resistors (error less than 3%) and then assessed on real liquid samples. The resistance of real samples such as KCl standard solutions and pasteurised milk samples were also measured. It was shown that the results correlated very well with the preliminary high frequency analyser IM6 results (see Appendix F). Same samples were run on both instruments and the results already showed a relationship between SCC and impedance which will be discussed in section 4.3.2. The performance of the two instruments is compared for similar perturbation potential in Figure 4.3.

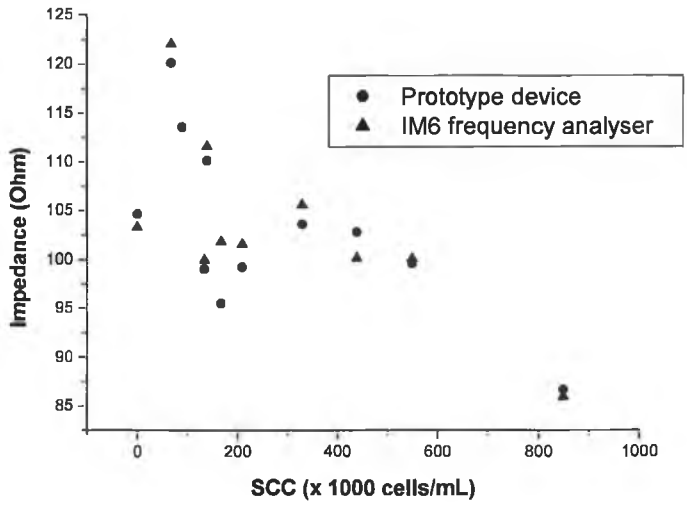

Figure 4.3: Influence of mastitis infection on the impedance of crude milk samples measured using both the prototype impedance device and IM6 frequency analyser. Temperature was compensated by one or two degrees to 15°C.

The data shown for the IM6 corresponds to the average impedance measured between 50 kHz up to 300 kHz for an applied direct current of 0 V and a superposition of sinusoidal perturbation of 5 mV. The profiles of the results for the two instruments were very similar although the two instruments did not give exactly the same results. This difference is due to the differences in the parameters values used for the measurements such as signal perturbation shape and electric filters presents in the frequency analyser.

Overall, the correlation between the two types of impedance measurement (using the prototype impedance device and standard frequency analyser) was good. The quality of the results obtained attested to the accuracy of the prototype impedance device.

4.3.2 Prototype impedance device Evaluation on crude milk samples

The 150 kHz frequency was fixed on the prototype impedance device and this was found suitable for our application. The results were clearer above 80 kHz as the polarisation effects were reduced.

4.3.2.1 Effect of preservatives on impedance of crude milk samples

The data shown in Figure 4.4 were collected by the prototype impedance device and correspond to the impedance at 150 kHz and a square signal perturbation of 10 mV.

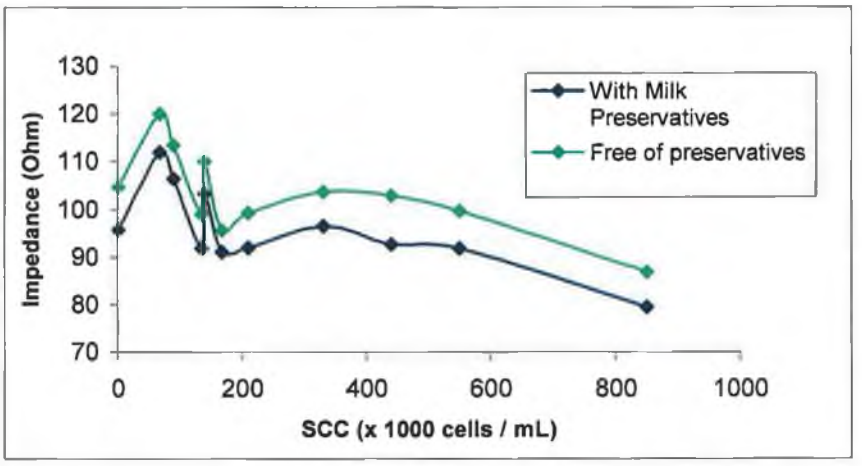

Figure 4.4: Influence of preservative on impedance of crude milk samples.
Data are obtained by averaging 15 measurements (each measurement was taken every 2 seconds). Temperature has been compensated to 15°C.

As we can see, in Figure 4.4 impedance values for milk samples free of preservatives are greater than those for milk samples with preservatives because the preservatives bring to the solution extra ionic content such as chloride. The first point in every case is significantly lower than the trend of the results for lower SCC. This fact might be due to an 'exposure' issue with the sensor .To check if any exposure is encountered during the first measurements with milk samples, a random approach was adopted for the later experiments carried out but it seemed that the initial results (SCC=1000 cells/mL) still remained. The other possible for this difference could be the fact that the SCC results are not accurate (accuracy is approximately 10% in the normal range of use). Finally, the impedance of milk with preservatives does not seem to have any different effect on the mastitis infection as the SCC increases.

4.3.2.2 Effect of SCC on impedance of crude milk samples

The results for the eleven milk samples (without preservatives) show a decreasing trend between the SCC and impedance value for the pre-set parameters.

The impedance decreases as the SCC increases in Figure 4.5.

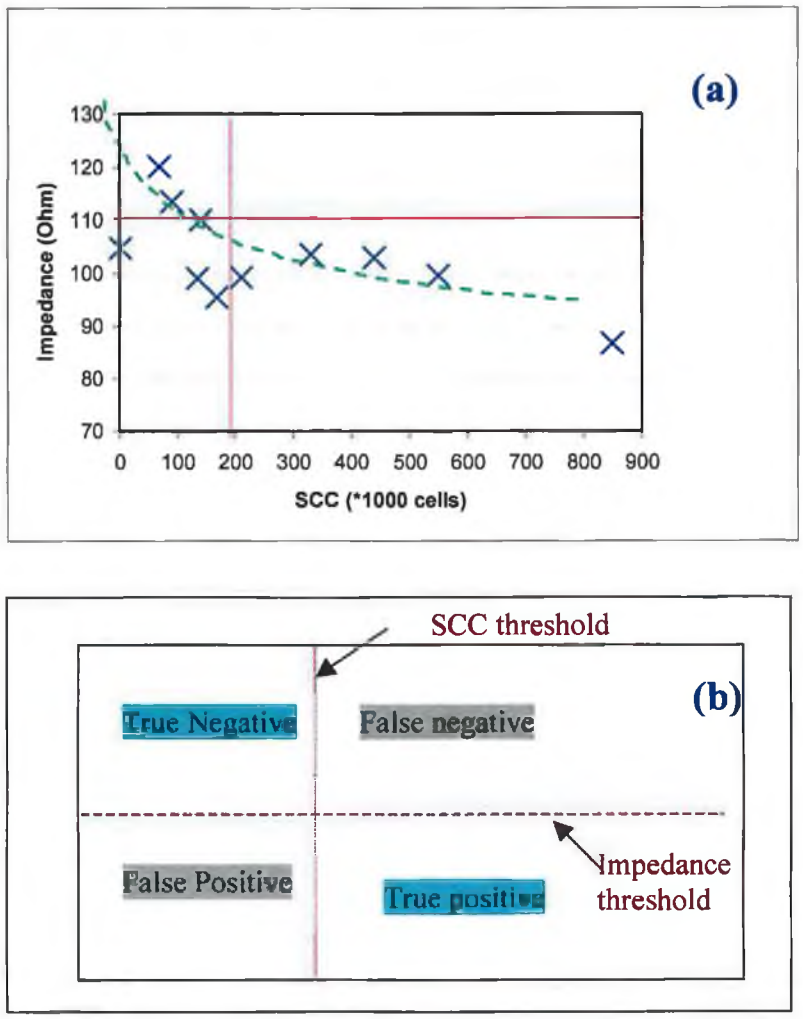

Figure 4.5: (a) Preliminary relationship between SCC and Impedance values for milk samples without any preservatives. Temperature has been compensated to 15°C. (b) Diagram showing diagnosis zones and threshold lines.

To verify this assessment, the standard deviation of the data collected by the prototype impedance device was calculated. For experimental convenience, the standard deviation is calculated for milk with preservatives and shown in Figure 4.6.

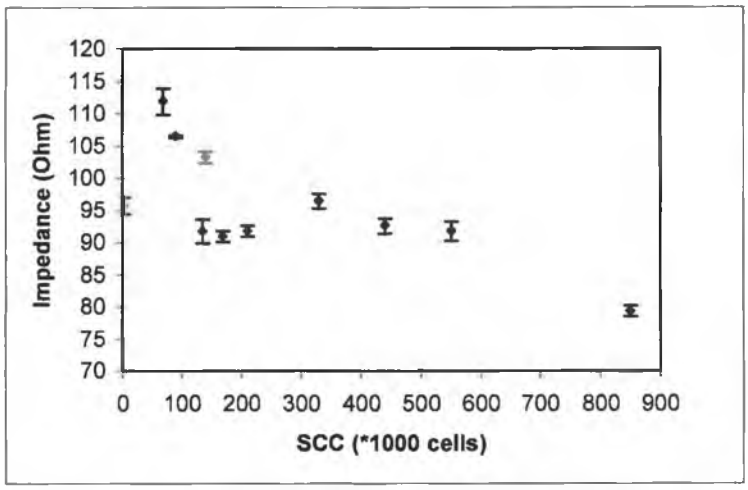

Figure 4.6: Preliminary relationship between SCC and Impedance value for milk samples with preservatives. Temperature has been compensated to 15°C. The error bars represent the relative standard deviation in %.

The results from the prototype impedance device were reproducible with a standard deviation (for N=3) of less than 2.5% as can be seen from Figure 4.7.

The prototype impedance device developed by Whistonbrook (using the parameters indicated by analytical results and experiments carried out in DCU using different electrochemical methods) was found to work properly and deliver rapid results. The sensitivity of the instrument was good and it allowed already to 'predict' positive and negative samples from crude milk samples. No false negatives were detected and three negative samples according to their SCC (i.e. SCC< 200,000 cells/mL) exhibited low impedance values (false positives).

4.3.2.3 Effect of souring effect on impedance of crude milk

Further experiments showed a lot more false positive results. The relationship expected with SCC was not evident and it is likely that other effects affected the milk samples (see Figure 4.7). For the samples suspected of exhibiting false results with respect to their SCC, chloride titrations were carried out. Some of them showed high impedance and a normal chloride level as expected for such an impedance value. For example, the sample with 716 000 cells/mL shows high impedance. The other with SCC =214,000 cells/mL gave low impedance. These results cannot easily be explained, except by errors in the SCC measurements.

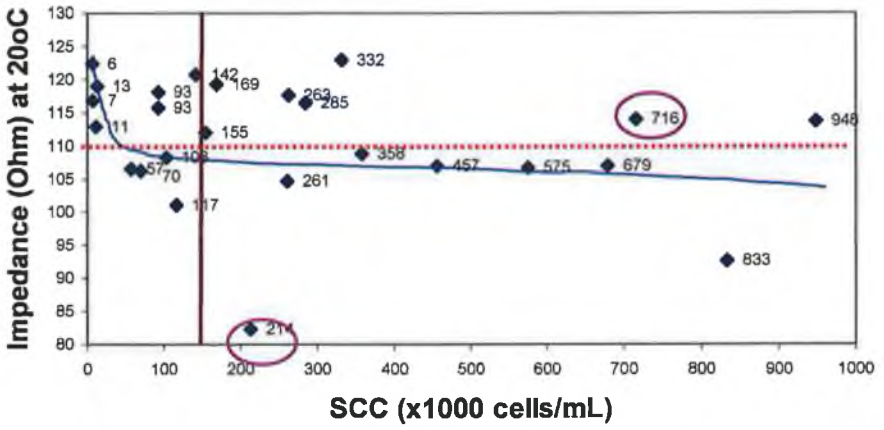

Figure 4.7: Influence of the SCC on the impedance for absolute values of crude milk samples using the prototype impedance device. Impedance readings compensated to 20°C. (Day one).

A complementary chloride titration showed that the "strange results" are due to the somatic cell count and not the degree of infection of the samples (as the sample gave a normal chloride content of less than 1500 ppm of chloride). The effect of souring effect on few samples when transported was questioned. To check this, the effect of spoilage on milk impedance was tested. The experiment was repeated on day five (milk refrigerated during day 1 to 5) and the results are shown in Figure 4.8.

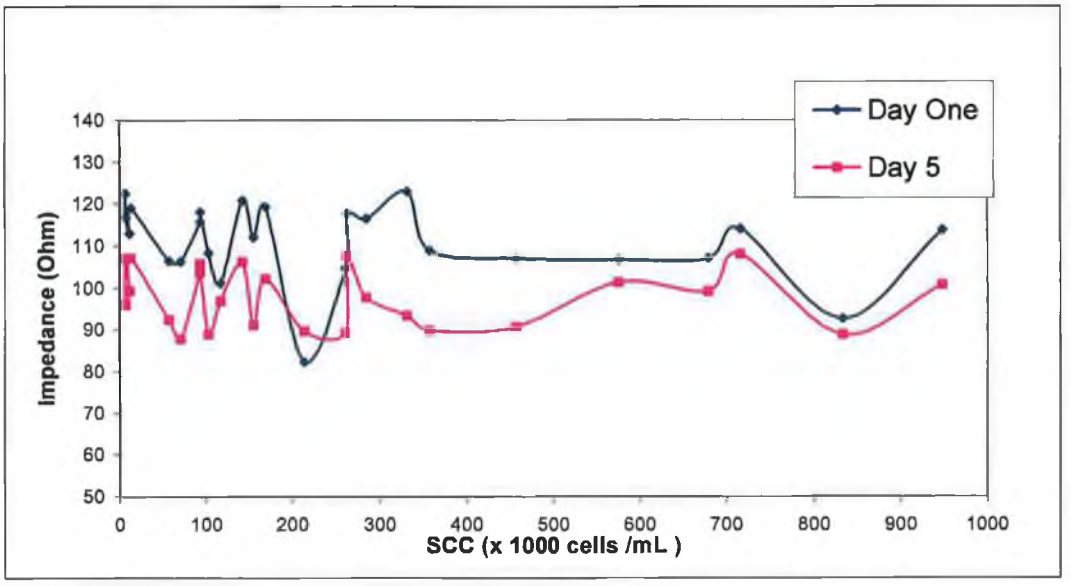

Figure 4.8: Influence of the SCC on the impedance at day one and five for absolute values of crude milk samples using the prototype impedance device. Impedance readings compensated to 20°C.

It was clear that the impedance decreases when the milk become naturally spoiled. This effect has already been shown in reference [7]. Some results are different from day 1and it could be due to the experimental procedure or a faster souring effect in these particular samples. Measuring the impedance in-line will prevent this error.

4.3.2.4 Effect of mastitis infection on impedance of crude milk

Further experiments with larger trials showed again a relationship between SCC and impedance for milk samples but with some false negative results were apparent (see Figure 4.9) although precautions were taken to keep the milk fresh.

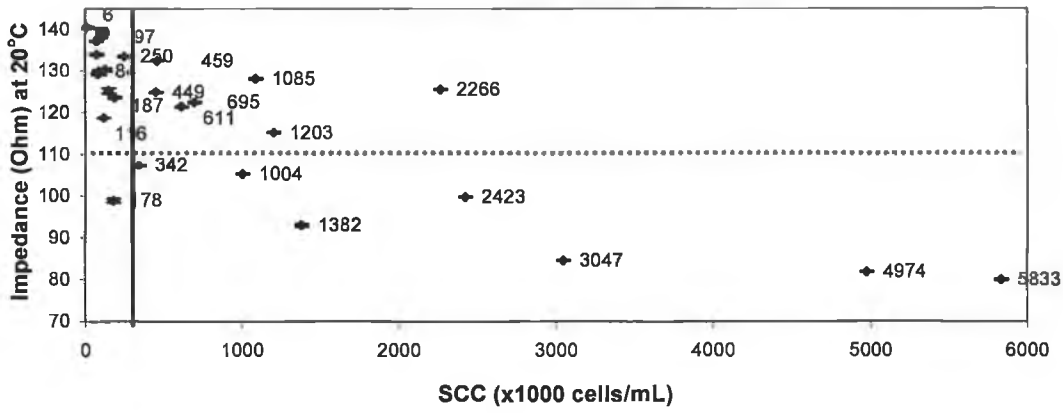

Figure 4.9: Relationship between SCC and Impedance for absolute values of 58 samples using the portable prototype impedance device. Impedance readings compensated to 20°C.
The red line represents a possible impedance detection threshold (110 Ohm at 20°C), below which a sample was deemed 'positive'. As shown in Figure 4.9, the impedance decreases when the SCC values are high but there was still some occurrence of false positives or negatives and it seemed to increase with the number of samples tested. In the literature, it has been observed that detection of mastitis using only absolute values of conductivity/impedance is difficult. A combined threshold (absolute value and lowest quarter value in conductivity) has been recommended to improve the sensitivity and specificity of the detection system [8,9].

4.3.3 Chloride titration

Chloride concentration determination was used to double check data or to investigate false positives samples that exhibited low impedance for a high somatic cell count, as was the case for few measurements in section 4.3.3. Two ways of measuring chloride are proposed, a classic potentiometric titration (see details Appendix G) and a quick titration using chloride analytical test strips.
The analytical strips were initially tested with fresh pasteurised samples that gave a normal range of chloride concentrations from 800ppm to 1400 ppm.

The results of chloride titration versus the data obtained for impedance for the same samples in Figure 4.6 are shown in Figure 4.10.

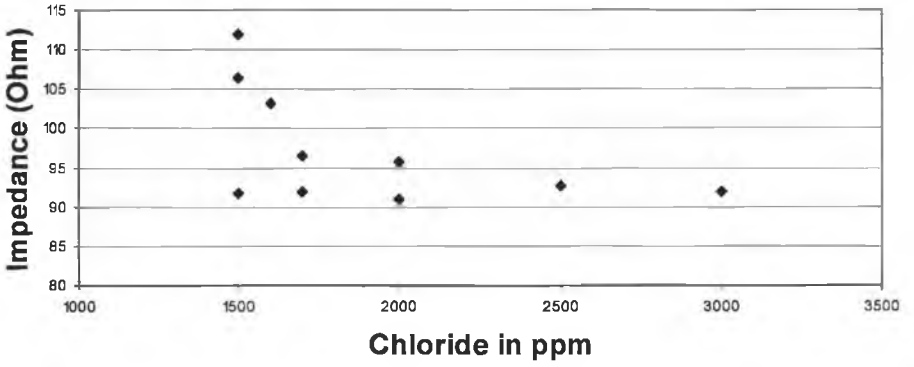

Figure 4.10: 2D Graph Impedance versus Chloride determine by analytical strips for crude milk samples with preservatives.

The chloride content was higher than for normal milk as the preservatives contain some chloride but it was interesting to see the relationship between chloride and impedance. From Figure 4.11, it was shown that chloride is a good indicator of degree of mastitic infection. The two false positive results from Figure 4.5 and 4.6 were explained using the chloride concentration (which gives an idea of the damage of the membrane between the milk and blood system of the cow). They were found to be abnormal (above 1500 ppm) although the SCC displayed a healthy result (under 200,000 cells/mL). It was strongly suggested that some SCC data could be inaccurate, as even spoilage could not increase chloride to those levels.

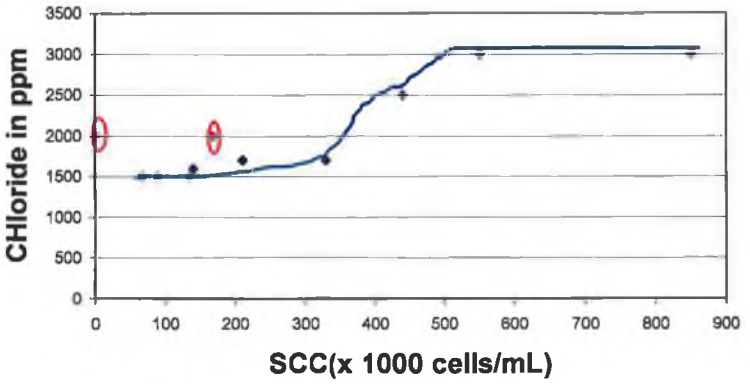

Figure 4.11: 2D Graph of chloride concentration plotted against SCC (Same milk samples as those presented in Figure 4.6 and Figure 4.10). The two red-circled data correspond to the false positive samples.

A 3D plot is shown in Figure 4.12 which corresponds to the same results shown in Figure 4.10 and 4.11 but it presents the results of the chloride titration in a clearer way.

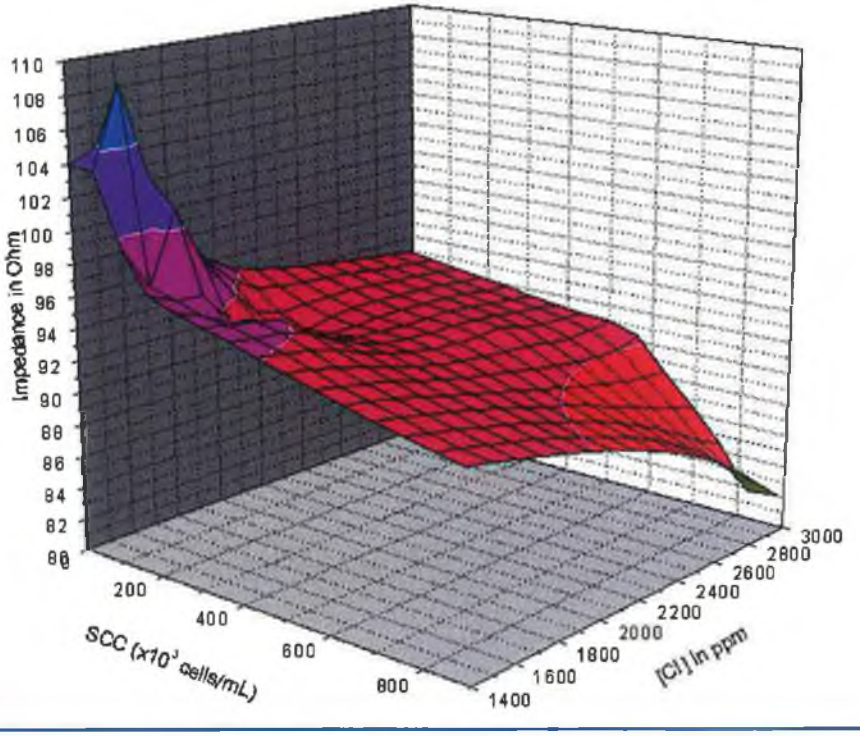

Figure 4.13: 3D Graph showing the relationship between SCC, Impedance and Chloride concentration. Data and samples are identical to those plotted in Figures 4.11 and 4.12.

The mesh was constructed using a gridding correlation method using Origin 5.0 Plot 3D. On the edge of the graph the two curves are the same as those plotted in Figures 4.10 and 4.11.The graph shows the relationship between the three parameters used in our study to detect mastitis. Of particular interest is the relationship between the

impedance and the chloride concentration. As expected, the impedance decreases when the ionic concentration increases confirming that the prototype impedance device is working properly.

The relationship (Eq 1.2) between the chloride content and the milk conductivity has been discussed by Puri and Parshak [10]. It has been found to be useful to double-check false positive results in the laboratory but it is too difficult to set up the method in the milking parlour. This is because the milk proteins lead to many spurious chloride measurements because of the fouling electrode effect in the case of ion selective electrodes.

4.3.4 Threshold Limits

To evaluate results, a threshold value needed to be defined. In the literature, it has been observed that detection of mastitis using only absolute values of conductivity is difficult (single threshold) and that thresholds based on the difference between the highest and the lowest quarter conductivity values are not reliable. Other methods for classifying results had to therefore be investigated. Values such as differential threshold or combined threshold were investigated.

4.3.4.1 Differential threshold

The first threshold evaluated is the differential threshold or inter quarter ratio (IQR) threshold which is defined as the ratio between the quarter with the lowest conductivity and the other quarters of the same cow (see section 1.3). A value of 16% compared to the lowest quarter has been suggested by Linzell and Peaker [8] and this is a good reference to assess the method sensitivity and the sensor performance. This threshold stated that infected quarters can be detected if one or more is 16% or above the results of the inter ratio quarter (IRQ referenced to the lower conductivity quarter) for each quarter. In this study, as impedance was used instead of conductivity, the highest quarter was chosen (see Figure 4.13).

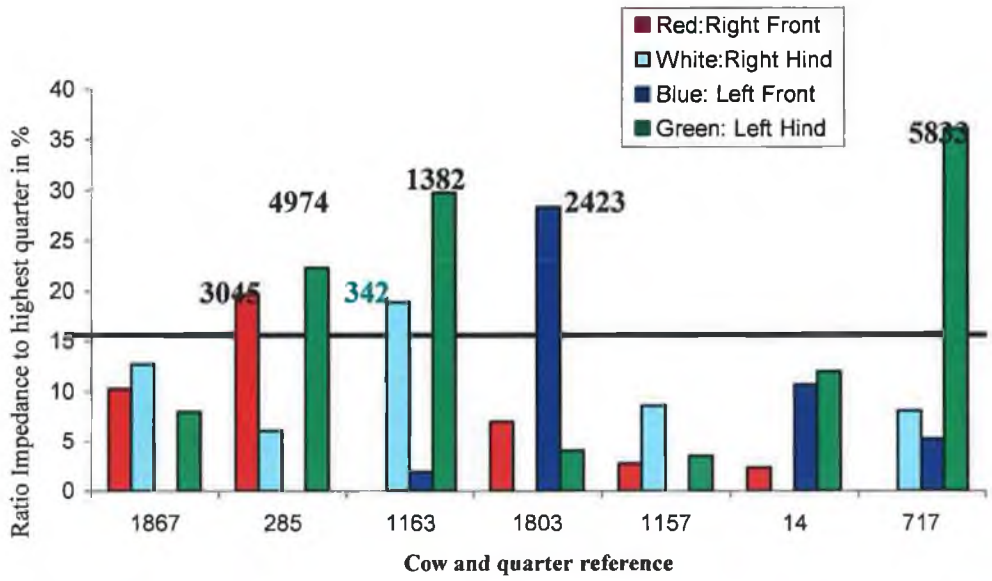

Figure 4.13: Inter Ratio quarter calculated to the highest quarter in impedance. The number above the black bar (representing the threshold of 16 % calculated in ref [8]) corresponds to the SCC in thousands of cells per ml. (Trial n°10).

The differential threshold calculated by Linzell and Peaker [8] allows the detection of six positive samples (above 200,000 cells/ml) and it does not permit assessment of the degree of infection. For this trial of 28 samples, according to the SCC data there should have been nine clinical samples (SCC above 1,000, 000 cells/mL), six subclinical samples (SCC between 200 and 1200 thousands of cells per ml) and thirteen healthy samples (SCC under 200,000 cells/mL). The method based on impedance with the prototype impedance device appeared to be quite efficient but it was expected that using a different threshold would increase the diagnosis sensitivity.

4.3.4.2 Combined threshold

The more sophisticated approach is to use a combined threshold which is a combination of IRQ and absolute values. Indeed, impedance data combined with the ratio-metric method described above was likely to yield valuable information. For example, it will help in identifying which particular quarter is infected and also to detect cases where all four quarters are infected and with a differential threshold will show a healthy status. Table 4.1 shows the results of impedance and the ratio to the highest quarter as well as the chloride titration for evaluating the quality of the SCC data provided. The colour codes used to identify clearly the degree of infection of the samples are explained in the caption of Table 4.1.

	1867_RF	1867_RH	1867_LF	1867_LH
SCC	**449**	**611**	**97**	**1085**
Impedance	124.84	121.42	139.41	127.48
Impedance Ratio	**10.25**	**12.71**	**0.00**	**7.99**
[Cl-] ppm	1000	1000	1000-1500	1000-1500
	285_RF	285_RH	285_LF	285_LH
SCC	**3047**	**178**	**1004**	**4974**
Impedance	84.56	98.85	105.26	81.74
Impedance Ratio	**19.67**	**6.09**	**0.00**	**22.35**
[Cl-] ppm	2800	2000	2500	3000
	1163_RF	1163_RH	1163_LF	1163_LH
SCC	**459**	**342**	**84**	**1382**
Impedance	132.38	107.32	129.87	92.94
Impedance Ratio	**0.00**	**18.93**	**1.90**	**29.80**
[Cl-] ppm	1000	2000	1000	2500
	1803_RF	1803_RH	1803_LF	1803_LH
SCC	**91**	**109**	**2423**	**250**
Impedance	129.51	139.22	99.72	133.52
Impedance Ratio	**6.97**	**0.00**	**28.37**	**4.09**
[Cl-] ppm	1000	800	2000	1500
	1157_RF	1157_RH	1157_LF	1157_LH
SCC	**124**	**695**	**69**	**78**
Impedance	130.3	122.44	133.98	129.22
Impedance Ratio	**2.75**	**8.61**	**0.00**	**3.55**
[Cl-] ppm	1000	1000	800	800
	14_RF	14_RH	14_LF	14_LH
SCC	**71**	**6**	**2266**	**187**
Impedance	137.16	140.49	125.45	123.65
Impedance Ratio	**2.37**	**0.00**	**10.70**	**11.99**
[Cl-] ppm	1500	1000	1000	1500
	717_RF	717_RH	717_LF	717_LH
SCC	**147**	**1203**	**116**	**5833**
Impedance	125.21	115.16	118.68	79.99
Impedance Ratio	**0.00**	**8.03**	**5.22**	**36.12**
[Cl-] ppm	1400	1000	1500	3000

Table 4.1: Detail of the results of trial n°10 where the impedance of crude milk samples (in Ohm) was measured following the procedure used for the prototype impedance device experiment (see section 4.2) with the stainless steel electrode. All the data was compensated to 20°C. The ratio was calculated to the highest quarter in impedance. The chloride content is obtained by the quick titration procedure.
The red font correspond to clinical samples and the green font to the subclinical ones according to their SCC data.

Three classifications were determined from the results of Table 4.1 which are given in Table 4.2. These groups were defined only for the particular conditions of these experiments (see section 4.2 and section 3.4) as the threshold for impedance would change if the electrode size was modified or if there was a change in the material, the temperature sensor or the perturbation signal used.

Group I: Impedance <110 Ohm.

285_RF	285_RH	285_LF	285_LH
3047	**178**	**1004**	**4974**
1163_RH	1163_LH	1803_LF	717_LH
342	**1382**	**2423**	**5833**

Group II: Impedance >110 Ohm and Ratio >10

1867_RF	1867_RH	14_LF	14_LH
449	**611**	**2266**	**187**

Group III: Impedance >110 Ohm and Ratio >7

1867_LH	1157_RH	717_RH
1085	**695**	**1203**

False negative: Positive samples not detected by combined threshold *:

	1163_RF	1803_LH
SCC	**459**	**250**
Impedance	132.38	133.52
Impedance Ratio	**0.00**	**4.09**
[Cl-] ppm	1000	1500

False positive: Negative (Healthy) samples tested positive by combined threshold*

	285_RH	14_LH
SCC	**178**	**187**
Impedance	98.85	123.65
Impedance Ratio	**6.09**	**11.99**
[Cl-] ppm	2000	1500

**Positive /Negative sample according to Somatic Cell Count (see section 3.2).*

Table 4.2: Possible Groups of classification to be used for combined threshold values for detecting mastitic samples. Data from Table 4.1.

The threshold of 110 Ohm was the same threshold used for the previous trial (see Figures 4.7 and 4.9) tested under similar conditions of temperature (compensation at 20°C). The false negatives (1163_RF and 1803_LH) mentioned in Table 4.2 were reclassified as healthy milk samples when the chloride content was considered and indeed their impedance was well above the absolute threshold of 110 Ohm, as well as two false positives (285_RH and 14_LH) that exhibit a higher chloride content than normal value were reclassified as true positive. For the calculation in Table 4.3 of the statistical detection or probability of the sample [6], these four samples were still considered to be negatives, respectively positives.

Cow No	SCC (RF)		SCC (RH)		SCC (LF)		SCC (LH)	
1867	449	TP	611	TP	97	TN	1085	TP
285	3047	TP	178	FP	1004	TP	4974	TP
1163	459	FN	342	TP	84	TN	1382	TP
1803	91	TN	109	TN	2423	TP	250	FN
1157	124	TN	695	TP	69	TN	78	TN
14	71	TN	6	TN	2266	TP	187	FP
717	147	TN	1203	TP	116	TN	5833	TP

TN: *True negative*, **TP**: *True positive*, **FN**: *False negative*, **FP**: *False positive (see Table 1.4)*

	Formula	**Differential threshold** TP: 5, FN: 10, FP: 1, TN: 12	**Combine threshold** TP: 13, FN: 2 FP: 2, TN: 11
Sensitivity	TP/(TP+FN)	**33.3**	**86.6**
Specificity	TN/(FP+TN)	**92.3**	**84.6**
Positive predictive value	TP/(TP+FP)	**83.3**	**86.6**
Negative predictive value	TN/(TN+FN)	**54.5**	**84.6**
Prevalence	TP+FN/(TP+ TN+FN+FP)	**53.6**	**46.42**

Table 4.3: Classification for data analysis for the sample compared by combined threshold results. Determination of the probability of the samples (see Table 1.4).

The combined threshold appeared to improve the detection sensitivity with a sensitivity of 86.6% compared to 33.3% for differential threshold. It is noticed that if we consider the above remark regarding the diagnosis based on solely impedance and chloride results the sensitivity and specificity will become 100% as there will not be any false positive or negative. This fact is very important as it proves that the diagnosis based on impedance is more powerful than the SCC results and suggests that the SCC data might be inaccurate in certain samples.

The "rate of detection" should be greater than 85 % as a good sensor should have a high specificity and high sensitivity. The choice of the threshold was therefore extremely important and the combined threshold was necessary.

The principle of the sensing system was efficient but it was still not clear how to correctly differentiate between positive and false positives. When the cow and quarter were known, it was easier to detect true positive samples and it was even possible to further classify into: **Group I**: Blue light (milk to be discarded), **Group II**: Red light (Alert), **Group III** Green (Need to check the quarter but Impedance value still normal and the electrolyte concentrations are still within range).

4.4 Conclusion

The small prototype impedance device built by Whistonbrook Technologies gave comparable results to the large bench top frequency impedance analyser. The results were found to be reproducible and the standard deviation was acceptable (2-3%). The results of frequency measurements gave a good relationship between SCC (Somatic Cell Count) and impedance value. The chloride concentration was also found to be a good indicator of infection of the cow but it was not feasible to monitor chloride on-line in an easy way. The results also allowed prediction of positive or negative mastitic samples with absolute values of impedance. The sensitivity of the prototype impedance device was excellent using the combined threshold. Using this threshold improved significantly the sensitivity and specificity of the detection system and also it showed that the impedance method could detect cases not detected by SCC diagnosis.

4.5 References

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Chapter V

Integration of the sensor into the milking claw in the parlour

This chapter deals with the problem of on-line integration of the sensing device for mastitis detection. It includes the description of the possible design of the claw to be used for on-line measurements as well as details of the influence of dynamic measurement on the impedance results.

5.1 Introduction

The integration of a on-line monitoring in a milking parlour remains a major issue in the development of an on-line mastitis detection system. Many studies have dealt with on-line measurement based on electrical conductivity in milking systems [1-10]. Automated systems collect almost continuous electrical conductivity data but unfortunately in parlour systems, measurements are usually made for the whole udder milk and thus the detection of any mastitis occurrence at the individual quarter is immediately reduced [11]. Among the on-line systems studied in the literature (see section 1.3), a study by Puckett et al. described a milking claw equipped with sensor electrodes for each quarters. To minimise the influence of milk level in the sensor cell, which was proportional to milk flow, a flat sensor surface at the end of the electrode was adopted. The false positive and false negative results were avoided by integrating data from up to three milking procedures [3]. Jones developed another system where the conductivity cell consisted of a well containing accumulated milk, and a central stainless steel electrode. The data acquisition system collected milk conductivity readings at 6 s intervals (instead of 2 s which was previously used) to decrease the variability of the results. As milk flow increased, Jones noticed that the conductivity also increased but was quite erratic [8].

In this project, the design of an on-line integrated system was investigated. The work involved researching the problem, drawing up conceptual designs and some prototyping. A prototype claw for on-line measurement was developed in collaboration with Teagasc (Moorepark, Ireland) and Whistonbrook Technologies (Luton, England) and built for testing under static conditions before on-site testing. To assess the influence of milk flow on the impedance measurement (using prototype impedance device), two milk flow simulators were developed, one of which allowed control of the flow rate of the milk. These dynamic studies were designed to evaluate whether the readings were possible and reliable and to examine the response of the instrumentation in flowing milk. As such, it was an important step that would provide useful data for the design of the integrated sensing claw.

5.2 Experimental

5.2.1 Samples and Chemicals

All chemicals used for the preparation of the standard calibration solutions were of analytical reagent grade. Ultra-pure water (resistivity $\geq$ 18 MΩ cm), obtained from a Millipore Milli-Q-filtering system was used.

The milk samples used were similar to those described in the experimental part of chapter 3. Fresh milk samples (normal pasteurised milk) were purchased from retail stores. Bovine foremilk samples (crude milk) were provided by Teagasc (Moorepark, Ireland). The milk samples (about 250 mL used for dynamic measurements) were kept refrigerated (in a polystyrene box) prior to analysis.

5.2.2 Designs of the on-line system measurement

Two designs for the cell to be integrated in the on-line system for impedance measurement were investigated.

5.2.2.1 Cell design for direct flow measurement

The first cell to measure impedance in direct flow adopted the same design as the one discussed in sections 3.2 and 4.2. This two-electrode design is shown in Figure 5.1.

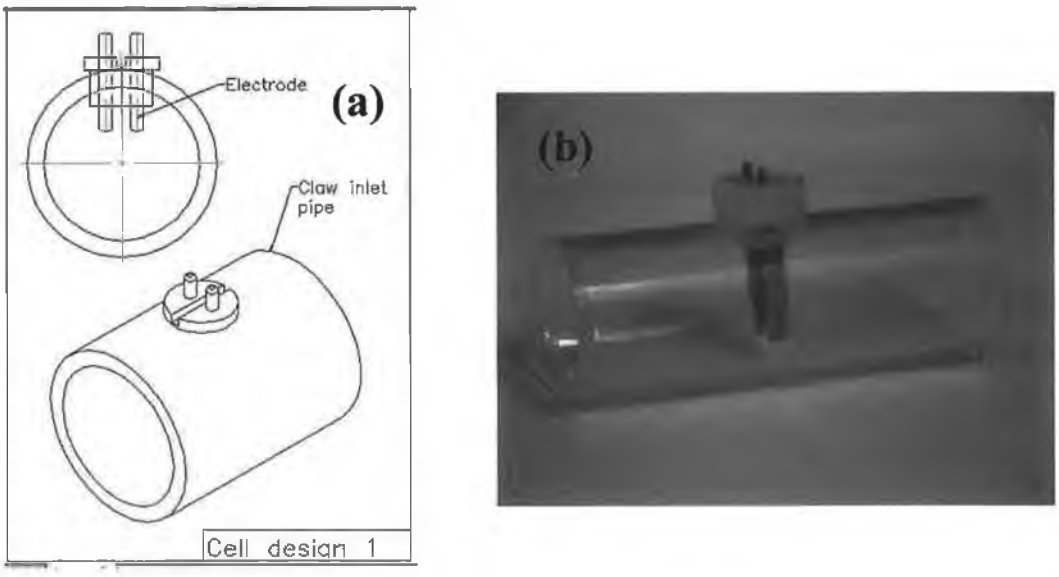

Figure 5.1: (a) A schematic drawing of the cell proposed for flowing milk measurements, (b) a photograph for the cell screwed into a plastic pipe.

The cell could be easily screwed into a hole drilled in the inlet pipe of the milking claw for example. The design however would not allow the incorporation of control electronics or temperature sensors.

5.2.2.2 Prototype claw

The second design consisted of a claw with integrated electrodes and it was designed and fabricated in collaboration with Teagasc (Moorepark, Ireland). This claw was to be linked with the detection instrumentation discussed in section 4.2. This prototype is referred to as the **claw prototype.**

The design consisted of an electrode that was composed of two steel screws integrated inside the claw. Four electrodes were positioned at the junction of the tube of a quarter and the collecting area for the milk. Figure 5.2 shows a drawing of the prototype claw.

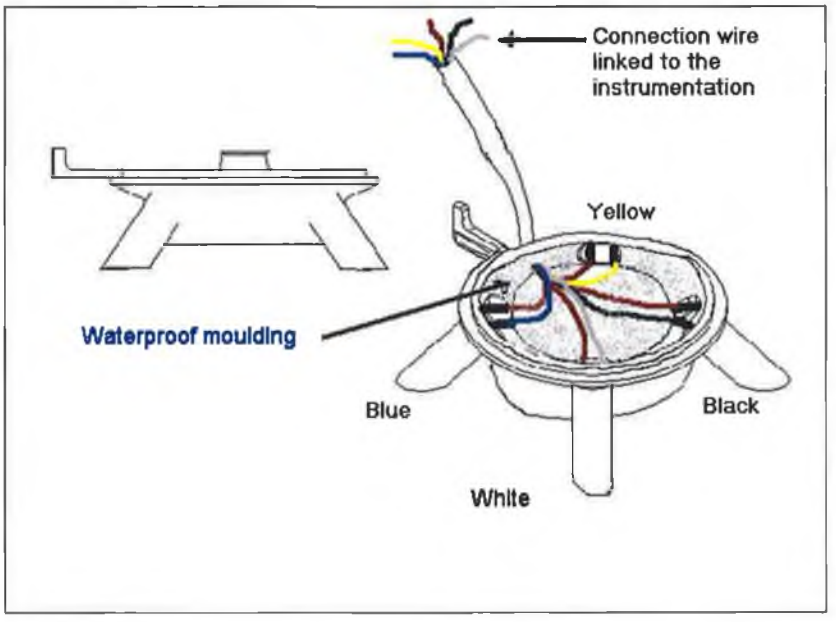

Figure 5.2: Schematic of the claw prototype with the coloured connections.

Two wires were used to connect the four electrodes to the prototype impedance device: a brown "common" wire linked to the four cells and a second coloured wire for each individual cell. The cell was identified by the colour of this second wire: yellow, black, white or blue. The connection wires were located inside the body of the claw with a protecting waterproof moulding. The moulding and the electrodes are illustrated in Figure 5.3.

Figure 5.3: Photographs of the prototype claw: (a) the claw with waterproof moulding and coloured wire connections, (b) a magnification of one of the embedded electrode.

5.2.3 Designs of milk flow simulation system

Two designs for the milk flow simulator were developed.

5.2.3.1 First Milk flow simulator

A flow device was built to pump milk through the prototype cell (see section 3.2) in order to evaluate the design in a milk flow situation. The prototype cell was screwed into a plastic pipe as described in section 5.2.2.1. At the electrodes, the milk flow rate was 0.3 mL per second. The system required 200 mL of milk to operate properly. Figure 5.4 shows the first flow simulator system.

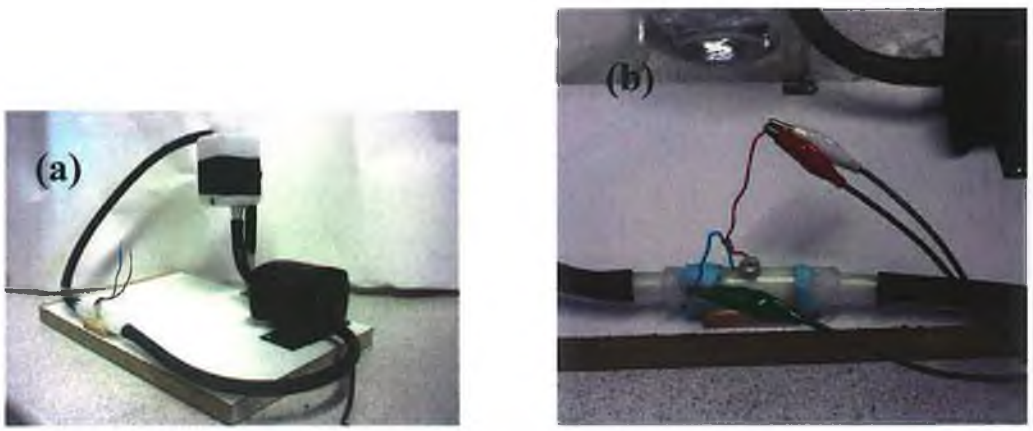

Figure 5.4: Photographs of the first milk flow system: (a) the whole system, (b) a magnification of the tubing

The pump used was a fish-tank pump and the tubes were made of black rubber. The pump and the cell were fixed on a wooden base. The temperature could be measured by dipping a digital thermometer into the milk reservoir.

5.2.3.2 Second Milk flow simulator

The second milk flow system design had a much smaller flow rate than the previous simulator. The main advantages of this system were that the flow rate could be controlled and changed accurately using a peristaltic pump and the motor of the pump did not significantly raise the temperature when the milk was flowing through the system unlike the fish-tank pump.

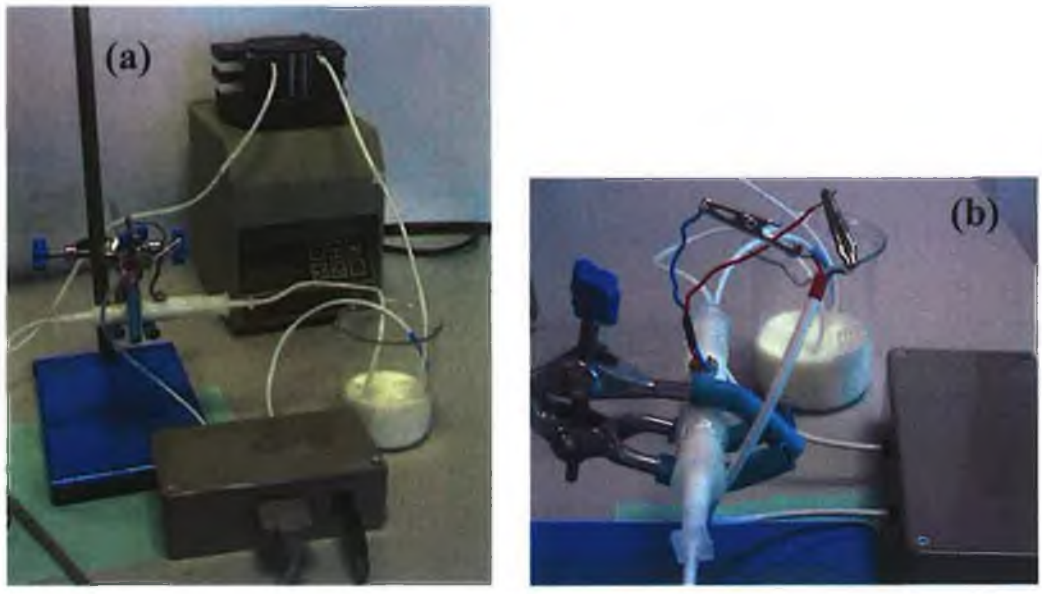

Figure 5.5: Photographs of the flow system 2: (a) Second Milk flow simulator, (b) Magnification of the cell and its connections.

As shown in Figure 5.5, the cell prototype was screwed into a horizontal similar plastic tube. The cell was then connected to the prototype impedance device. The milk is pumped through using a peristaltic pump Gilson Minipuls 3.0. The cell used consisted of a stainless steel 316 electrode and silicone tubing TYGON® R-3603 as the pump tubing. The flow rate was varied by changing the pump head speed expressed in rpm (revolutions per minute). The temperature was measured using the prototype impedance device by dipping the temperature probe into the beaker source. The results were then temperature compensated to 20 °C.

5.2.4 Experimental Details

The first prototype claw was built in order to evaluate what modifications would be needed for the next generation prototype design. It was tested with pasteurised milk samples. The performance of the claw prototype was assessed using the prototype impedance device.

The experiments on the first and second flow simulators were carried out to evaluate how the flow of milk past the electrodes affected conductivity measurements. The second simulator was particularly useful to see the influence of changing the flow rate on the impedance readings. Some of the problems encountered such as coating or poisoning of the electrode may be avoided during the milk flow. The experiments with the first flow simulator were carried out using the workstation 660A (CH instrument ®) following the EIS procedure (see section 3.2). The experiments on the second flow simulator were performed using the prototype impedance device.

5.3 Results and Discussion

A prototype claw with integrated electrodes and electronics was studied and tested. The influence of a direct milk flow on conductivity was examined. In order to see the influence of dynamic measurement on currently static measurements, some dynamic measurements were performed using the instrumentation used for the static ones.

5.3.1 Design of integration system

To test the performance of the claw design under static conditions, the influence of temperature on the impedance for each cell was determined (Yellow/Black/Blue/White). They exhibited different impedance values for the same sample (pasteurised milk or 0.1 M KCl) as can be seen in Figures. 5.6 and 5.7. The impedance data were higher compared with the previous experimental data obtained for pasteurised milk with a similar cell constant and working area electrode. This difference could be explained by the long wire (3 meters) that increased the total resistance measured (resistance of the milk and resistance of the wire). Nevertheless, this should not have affected the final results as the effect of the added resistance was encountered by all cells.

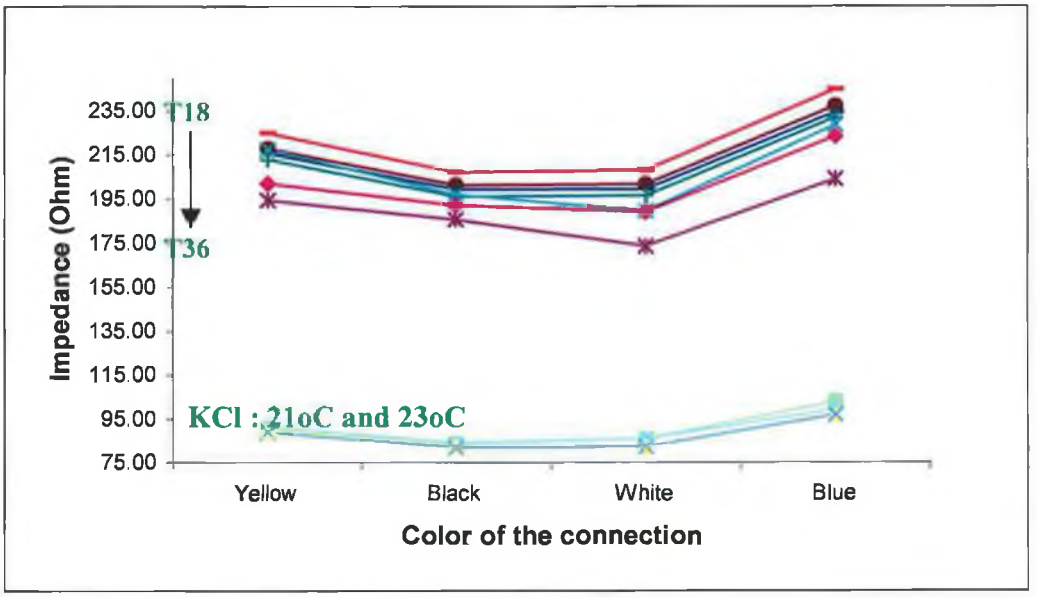

Figure 5.6: Influence of the temperature on the impedance of 0.1M KCl solution and fresh pasteurised milk samples measured with the prototype impedance device using the prototype claw.

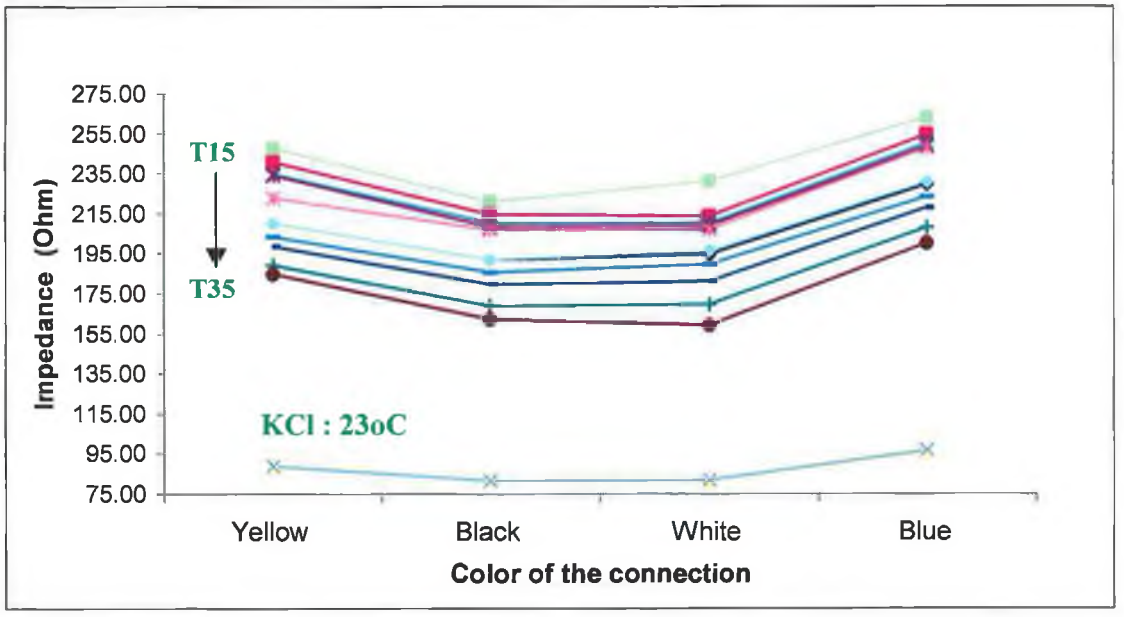

Figure 5.7: Repeat of experiment of Figure 5.6. Influence of the temperature on the impedance of 0.1M KCl solution and fresh pasteurised milk samples measured with the prototype impedance device using the prototype claw.

The two experiments shown in Figure 5.6 and Figure 5.7 are plotted on two separate graphs for clarity. The results for each cell are highly reproducible (same curve profiles and repeatability with KCl solution) and the influence of the milk temperature on impedance can be clearly seen .

The dependence of the impedance on temperature change in pasteurised milk is clear from Figure.5.8 when the data for the two experiments (Figure 5.6 and Figure 5.7) are plotted against temperature on the same graph.

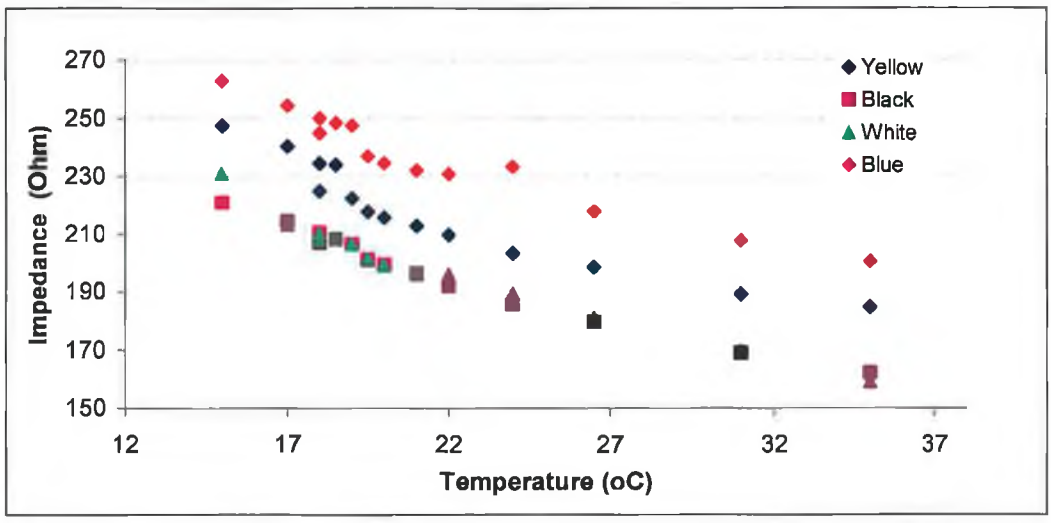

Figure 5.8: Impedance data (from Figures. 5.6 and 5.7) for each claw connection against temperature.

These experiments enabled proportionality parameters to be estimated for each cell, as well as the standard deviation on each cell for the same sample. To compare the connections within the cell, the standard deviation (called coefficient C_{colour}) for each connection was calculated using the following equation:

$$C_{colour} = \text{Average}\left(\sum_{i} \frac{\text{Im}\, colour\ i}{\text{Im}\, white\ i}\right) \text{ for Ti } (^{\circ}C)\ (i=1,2,3,4,5\ldots) \qquad \textbf{(Eq 5.1)}$$

The calculations produced the following results:

$C_{black} = 0.9937$
$C_{yellow} = 1.0968$
$C_{white} = 1$
$C_{blue} = 1.1933$

It was noticed that the impedance measurement was impossible to carry out when the cells were not completely immersed in the milk. When the milk is coming from the cow in a real milking situation, the milk may not completely fill the tube and therefore may not completely cover the electrodes which were on either side of the tube. This could result in spurious impedance readings. In order, to carry out repeatable and correct measurements the electrodes need to be in contact with the electrolyte to form an electrolytic bridge. This problem could be minimised if the distance between the electrodes would be reduced.

A second problem was identified with the current design. When the claw was slowly filled by the four quarters, the readings obtained represent the overall impedance of one or more of the quarters. By simulating flow milk in the claw, it was noticed that the cells were quite close to the bulk collecting area and the milk from each quarter got mixed before it was measured according to quarter. This problem could be solved by changing the electrodes' location in the inlet pipe, for instance placing the electrodes at the end of the tube.

A claw developed by a Netherlands research team proposed an integrating design with embedded electronics and quarter electrodes [12]. The drawing of the design (patented) is shown in Figure 5.9 where the flowing milk is trapped in a well.

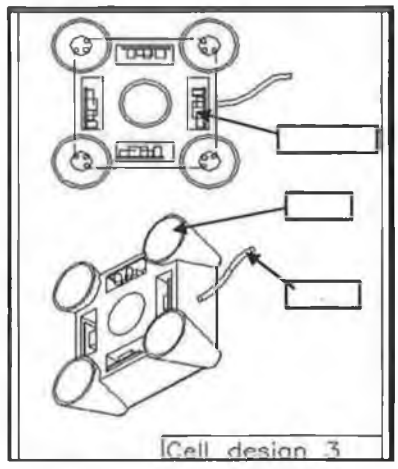

Figure 5.9: Schematic diagram of an other claw prototype with a well placed inside the claw body [adapted from ref 12].

The idea is similar to the claw prototype discussed in this thesis as the cells are integrated. However, instead of being integrated directly in the inlet tube of the claw, the electrodes and a temperature sensor are located in a well housed inside the claw body. The milk from the four inlets to the claw fills these wells and remains there until flushed out by the next plug of milk.

This design appears to be efficient, however after further analysis the central unit containing the well occupies the whole volume of the claw making it difficult to create a good vacuum inside the cluster. Therefore, for the next generation prototype of our claw, the idea of using a well inside the claw body was not considered.

To conclude, the current prototype claw needs optimisation to give more effective measurement of milk quarter impedance, especially when the milk was flowing. A new generation prototype design has been produced that will allow the mastitis detector system to be integrated on-line.

5.3.2 Influence of dynamic measurements on the impedance of milk

The dynamic impedance measurements with the first flow simulator showed an important shift in the conductance curve profiles, and so it seems that static measurements would be better for conductivity measurements in order to avoid diffusion effects.

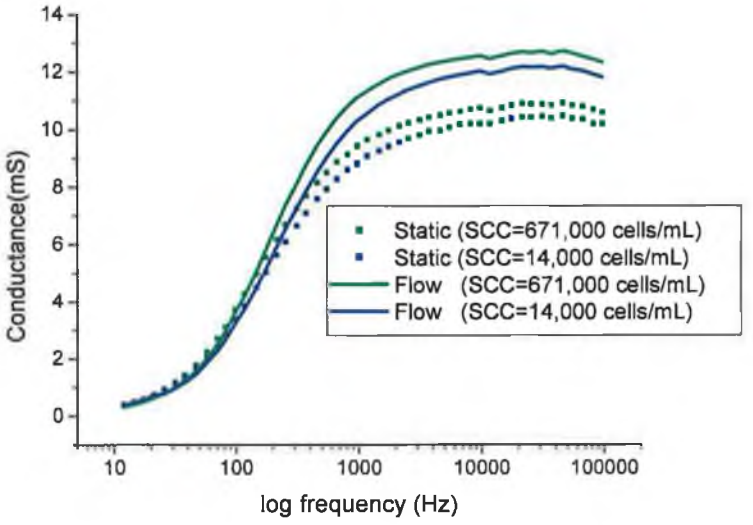

Figure 5.10: Variation of the conductance against the frequency in static and flowing milk conditions for two crude milks. The experiment was performed using the stainless steel electrode cell, at E=0V and p=5mV. The data were compensated to 24.7oC.

This important shift noticed in the impedance results is probably due to the fast changes of the temperature in the system. Using an EIS experiment, the flow does not cause too many spurious effects upon scanning the frequency. This is because that the flow in the tube of the first flow simulator is constant. The difference in conductance values between the two types of milk shown in Figure 5.10 remained the same and the shift was

likely to be due to the temperature effects which cannot be well controlled with the first design. Furthermore, the pump brought some heat into the system while operating. In the literature, dynamic flow measurements are considered to be too erratic for good accuracy [8]. A second design involving a peristaltic pump (which does not generate heat) was studied. It allowed comparison of the two flow simulators to examine the influence of the flow rate on the impedance results.

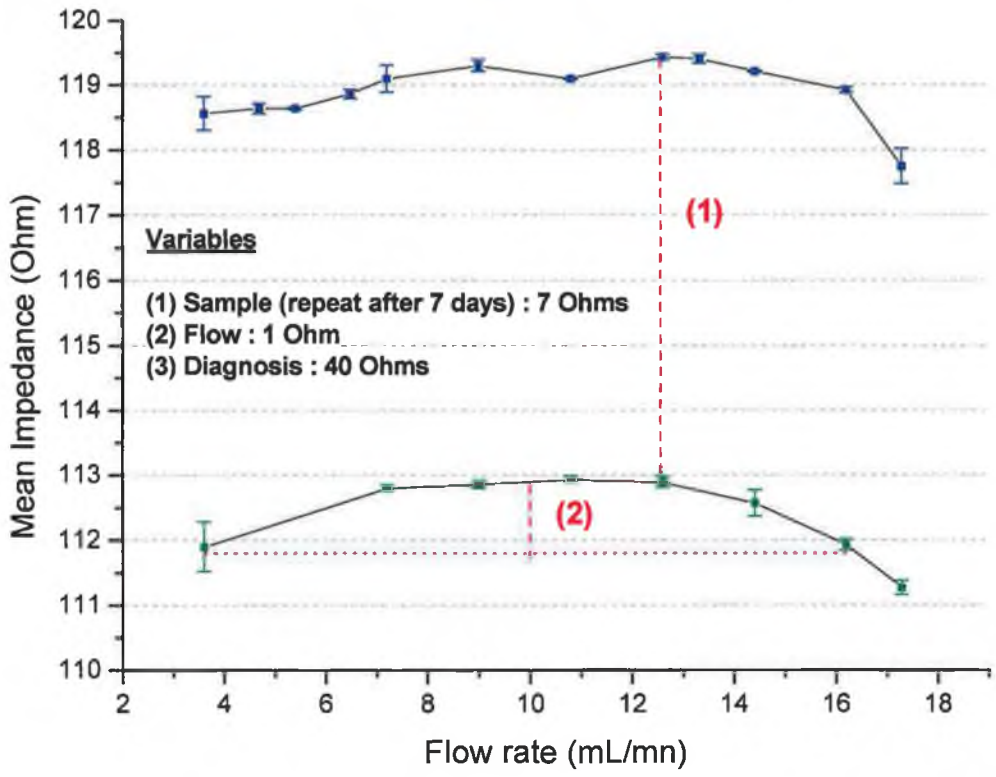

Figure 5.11: Influence of the flow rate on the impedance of pasteurised fresh milk and repeat after 7 days (Stainless steel electrode with prototype impedance device at 20°C).

These two plots (Figure 5.11) show that when the flow is changing the impedance remains the same in the flow rate range of 6 to 15 mL/min. At very low and high flow rates, it appears that the impedance readings are not that accurate represented by the increase in the standard deviation increases and the decrease of the impedance results.

A possible explanation could be that there are air bubbles forming at the surface of the electrodes when the flow is too low or too high which could influence the impedance readings. The use of the peristaltic pump improved the results, as the influence of the temperature was minimised (only 1°C shift in a few hours). It was also possible to see the effect of the flow on the impedance measurement and to understand better the phenomena involved in an on-line measurement.

5.3.3 Next generation prototype and instrumentation

Following the previous results, a new design has been developed in collaboration with Teagasc and a milking machine fabrication company called Dairymaster (Kerry, Ireland).This prototype will have smaller, flat rectangular electrodes embedded into the claw body. The electrodes were also placed further up the tube towards the teat, both on the same side as the milk flow so that the issue of spurious readings would be addressed.

A claw with only one embedded electrode was tested to date. The measurement was difficult as the milk could not cover the electrode completely when measuring the impedance. The current design of the electrode position still needed to be improved. Indeed, it is likely that when tested in the field, this prototype design may produce erroneous measurements due to the problem of milk bubbles forming on the flat surface of the electrodes. The solution would be to change the design of the electrodes or to place them in a small plastic well inside the inlet tube to reduce the flow when taking impedance measurements.

Finally, new improved instrument (see section 4.2) of reduced size was developed. Compared to the first prototype impedance device, the size of the box was reduced using smaller electronic components. Ultimately, the electronics (integrated circuitry) will be embedded in the free space of the claw. The connections were more robust and it should be easier now to perform simultaneous measurement due to a double channel switch to measure the quarter milk conductivity of each quarter individually. User-friendly software was also developed to run the device. The new instrumentation prototype is shown in Figure. 5.12.

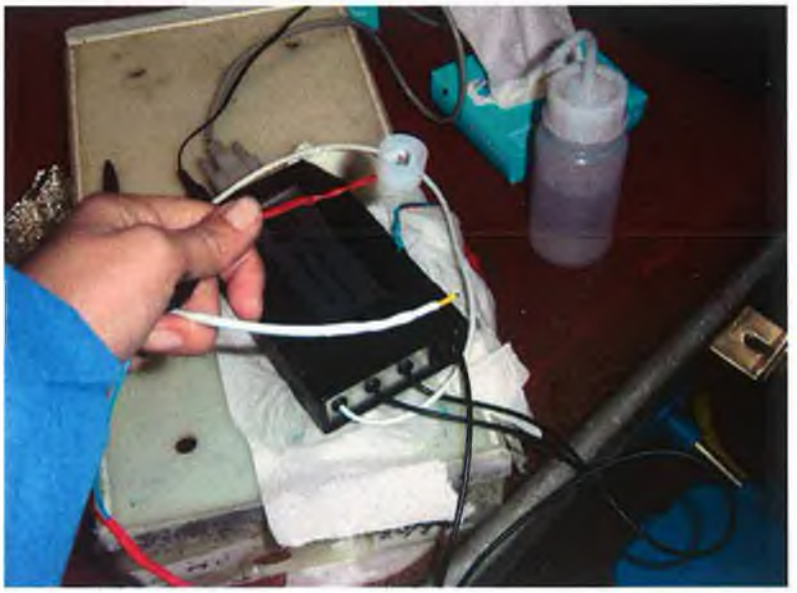

Figure 5.12: Photographs of the new prototype impedance device.

This software displays the impedance and the temperature as well as a graph of the impedance against the time for one channel.

5.4 Conclusion

The design of the prototype claw proved to be adequate for static measurements when the electrodes were completely immersed in the milk but inadequate for flowing milk. The dynamic experiment investigations revealed that flowing milk gave more erratic results particularly when the milk flow rate was too high or too low. Other fluctuations occurred in the contact region between milk and the electrodes due to phenomena such as turbulence in the milk or insufficient volume to completely cover the electrode. This point was taken into account into subsequent designs. The claw produced satisfactory measurements to be obtained when the electrodes were completely covered by milk, although the repeatability was less good than those obtained with the prototype stainless steel cell. Using other materials with a flat surface for the electrodes instead of the screws could easily solve this issue of repeatability. After making recommendations and optimising the design, a second-generation prototype claw was built. It shows that the measurements in the milking parlour will still be a challenge with the new design but it is improving all the time.

5.5 References

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Conclusion and future work

Significant efforts have been directed towards the development of inexpensive and reliable sensors for monitoring ions in solution, gases in air, bacteria in food and for assessing and detecting diseases such as cystic fibrosis or diabetes in the human body and diseases such as mastitis in dairy cattle. The latter example represents one of today's major research challenges for the dairy industry. At this time, there is no suitable on-line mastitis detector available for use in milking parlours.

Mastitis is a complex disease that affects dairy herds and remains a major issue causing major economical losses due to reduced milk quality, lower yield and animal culling. Many ideas for developing an efficient detector have been published and reviewed, but effective diagnosis is still restricted to laboratory-based methods such as bacterial counts or somatic cell counting (SCC). Commercially available handheld meters can detect clinical cases but are largely ineffective for subclinical cases. Sub-clinical mastitis is indeed much more difficult to detect than clinical cases of the disease, as it cannot be detected by simple visual examination of milk from the infected cow and relies on detection of more subtle changes in the milk.

In response to this need, farmers would derive great benefit from an on-line sensor that can detect mastitis at a subclinical stage and that would allow them to take early remedial treatment and to intervene in good time, particularly in regard to identifying and discarding infected milk. This system should be integrated in the on-line milking system into the parlour and should not give false positives or negatives results.

Among the current solutions, it seems that the measurement of changes in milk conductivity is a reliable method for automatically detecting mastitis in cows that are milked robotically. Abnormal milk shows some changes in its composition in which the concentration of lactose (a non-conductor) decreases while the concentration of salts (conductors) increases. These changes in milk composition cause variations in its electrical characteristics, such as conductivity or capacitance. It has been observed that infected milk exhibits higher conductivity as well as higher SCC. EC conductivity measurements are very advantageous because it is a rapid, inexpensive tool, easy for farmers to use, as it is easy to interpret and to maintain. Additionally, changes in conductivity tend to occur prior to the development of visible clinical signs of mastitis, thus allowing early identification and treatment of mastitis, potentially improving bacteriological cure rate, reducing recurrence rates and, perhaps, overall antibiotic usage. However, despite the clear scientific principle behind conductivity-based measurements, there have been few reports of its practical application on farms. Alternating current (AC) impedance measurement techniques appear to be promising

for analysis in milk and may allow the development of a new generation of sensors. One of the main advantages of the AC impedance system we have developed is the possibility of integrating the sensing system on-line. Furthermore, the electrochemical cell used can be sterilised and the electrodes are inert (bio friendly).

The work presented in this thesis investigates whether impedance electrochemical spectroscopy (EIS) measurement would help in the design of a sensing system to be used as a diagnostic method for mastitis. The results clearly show that this is the case, and the development of online sensor for detection of mastitis in dairy cattle is possible. The EIS experimental results showed that measurements should be taken above a certain operating frequency to avoid polarisation effects and to improve the sensitivity of the conductivity sensor. The threshold operating frequency could range from 80 to 150 kHz. In this frequency range, a good correlation between SCC (direct indicator of the degree of mastitis disease) and milk impedance readings was found but the accuracy of the diagnosis still needs to be improved.

A first prototype portable EIS instrument for measuring impedance was developed following the specifications recommended during this project and it was found to correlate very well with the bench top instrument. The experiments performed with the instrument using a stainless steel cell showed that below a threshold of 110 Ω (in certain conditions, see section 4.3), samples could be deemed 'infected' with reasonable accuracy. A combined threshold involving the ratioing of the highest quarter gave more accurate results and a good sensitivity of 86.6% when diagnosis based on SCC and 100% for diagnosis based on impedance and chloride contents values. Thus, the method allowed detecting clinical and subclinical cases and it is also proving that the diagnosis based on impedance values using combined threshold is more powerful than the SCC results and it may suggest that the SCC data might be inaccurate in certain samples.

This prototype impedance device was then reduced in size by using smaller and more integrated components. A prototype claw in which the electronics would be embedded was then developed and has been since improved. At this stage, it seems that some work on the design of the claw especially around the position of the electrodes in the tubes remains to be done. This work will require the help of specialists in milking parlour equipment manufacturing and farming profession.

Future work should focus mainly on solving the problem of integration claw design. Ideally, the sensing system would consist of a small device with integrated circuitry built into the cluster of the milking machine. This device would contain four measuring cells to collect data on quarter impedance values. The presence of a temperature sensor is required, even though the milk next to the cow's body should not vary dramatically from 37°C. As impedance varies with temperature and in the cases of fever the temperature would rise in a similar way in the milk from all four quarters. All the sensors will be connected to a micro-controller or similar device, which may perform all the threshold calculations and temperature compensation (if needed). The problem of switching channels in the circuitry when measuring quarter conductivity should also be investigated as the electronics require ten seconds of relaxation time before to taking a new measurement when switching channels.

After developing a claw with integrated electrodes, the entire system must be evaluated in field studies. Following on-line integration of this simple impedance system, early detection of mastitis in cows would be possible, thus allowing fast intervention by the farmer to treat the cow and improve the quality of the bulk milk. This would represent a tremendous advance in mastitis detection and treatment, and would ultimately lead to major savings for the dairy industry.

APPENDICES

A. Milk properties tables

B. Description of the trials of milk samples

C. Datasheet temperature sensor for the prototype impedance device

D. Raw data for Chapter 4 figures

E. Additional Data for Chapter 3

F. Additional Data for Chapter 4

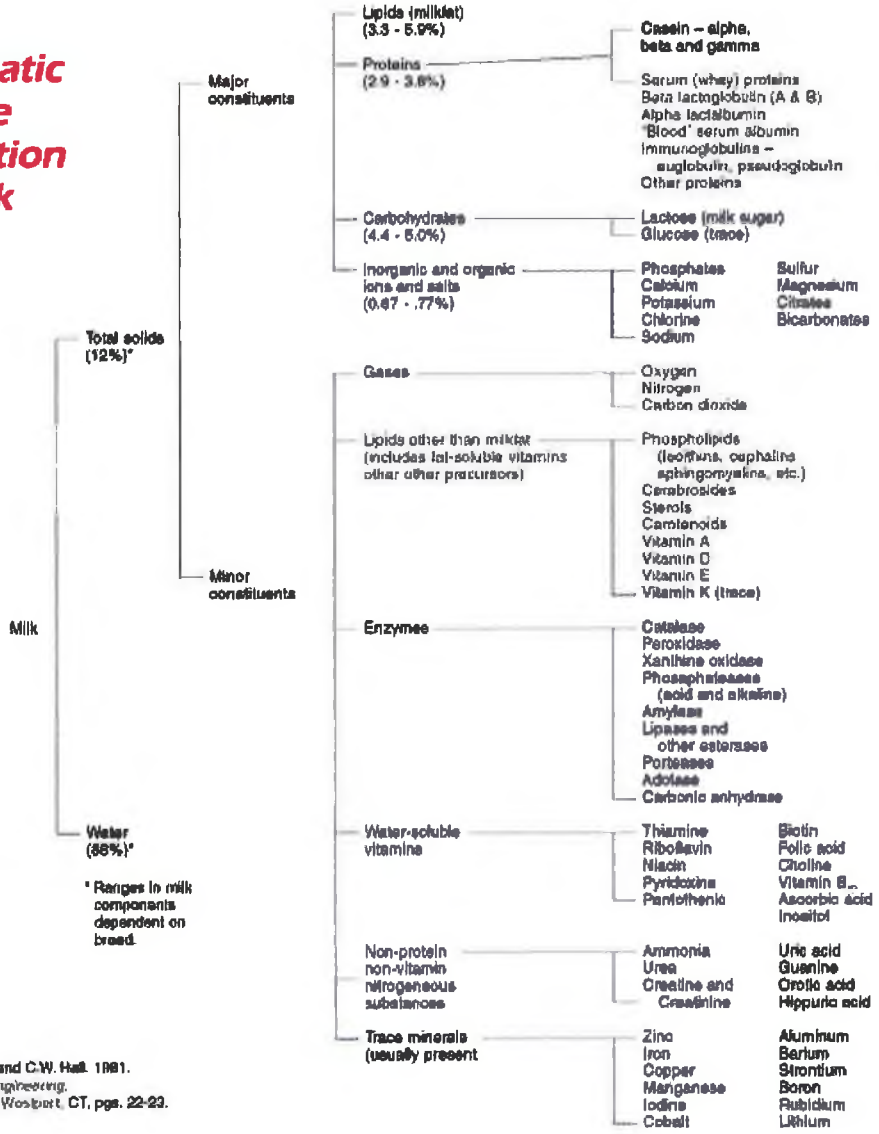
G. Chloride titration by potentiometric method

H. Additional Pictures

APPENDIX A

Milk properties tables

A Schematic on the Composition of Milk



Reference: Harper, W.J. and C.W. Hall. 1991. *Dairy Technology and Engineering.* AVI Publishing Co., Inc., Westport, CT, pgs. 22-23.

TABLE 14

General Physical Properties of Milk

Property	Value	Definition and Significance
Titratable acidity, % max	0.16	The total acidity or the amount of alkali required to neutralize the acidic constituents. Generally expressed as lactic acid. Used to determine bacterial growth in fermentations and compliance standards.
pH	6.6 ± 0.2 at 25° C	Fresh milk is slightly acid (pH of drinking water is 7.0-8.5). Generally the pH is lower (pH 6.0) in colostrum and higher (up to 7.5) during mastitis than in normal milk of mid-lactation.
Surface tension	50-52 dynes at 20° C	Normally, cow's milk's surface tension is about 70% of that of water. Involved in adsorption and formation and stability of emulsions. Important to creaming, functions of fat globule membranes, foaming, and emulsifier use.
Specific gravity	1.032 at 15° C	Ratio of the density of the product and the density of water at the same temperature. Many milk constituents have a specific gravity (sg) greater than that of water which has a sg of one. The more fat in milk, the lower the sg as fat has an sg less than one. Used to estimate solids not fat.
Freezing point	-0.540° C	Lower than that of pure water (0° C) due to dissolved substances in milk. Used to detect adulteration of milk with water.
Boiling point	100.17° C	Greater than that of pure water (100° C) due to dissolved substances in milk. Used to detect adulteration of milk with added water.
Specific heat at 0° C 15° C 40° C	 0.92 0.94 0.93	The specific heat of milk products depends on their composition and the temperature. Important in processing as the amount of heat or refrigeration required may be calculated from the weight and specific heat of the different products being pasteurized or cooled.
Coefficient of expansion at 10° C 15.6° C 21.1° C	 0.9975 0.9985 1.0000	The ratio of an increase in volume per unit increase in temperature. Milk expands when heated and contracts when cooled. Used for design of dairy equipment.
Viscosity	2.0-2.1 cp at 20° C	Refers to resistance to flow measured in centipoise (cp). Used to assess aggregation of protein micelles or fat globules. Also used for design of dairy equipment.
Electrical conductivity	$45\text{-}55 \times 10^{-4}$ mho	In milk, fat and colloidally dispersed substances decrease conductivity. Used to detect added neutralizers, follow fermentation, and monitor demineralization of whey.
Osmolality*	275 m Osm/kg	The osmolality of a solution is based on the number of particles in solution – the greater the number of particles, the higher the osmolality. Osmolality of foods is important in planning diets of low osmolality for certain patients. Since a solution of lower osmolality requires transfer of less water to the stomach and gastrointestinal tract to dilute it, it should be better tolerated than one of higher osmolality.

* Source: The Doyle Pharmaceutical Company, Minneapolis, Minn.

APPENDIX B

Description of the trials of milk samples

Trial 1 (28/02/02)

Sample	SCC (x 1000 cells/mL)
1	1
2	5
3	0
4	43
5	42
6	58
7	55
8	5
9	142
10	59
11	362
12	1187
13	8500
14	188
15	843
16	846
17	109
18	4257
19	328
20	141
21	141
22	1
23	2
24	3
25	Clinical
26	Clinical
27	Clinical

Trial 2 (22/03/02)

Sample	SCC (x 1000 cells/mL)
1	42
2	78
3	75
4	277
5	197
6	4568
7	133
8	492
9	36
10	1200
11	140
12	3
13	61

14	2189
15	159
16	102
17	305
18	799
19	3
20	4
21	86
22	374
23	130
24	124
25	54
26	191
27	1
28	4
29	70
30	205
31	254
32	129
33	47
34	2290
35	197
36	1
37	51
38	635
39	563
40	551
41	414
42	2381
43	257
44	802
45	4217
46	4388
47	1172
48	1179
49	3066
50	1293
51	4307
52	978
53	1033

Trial 3 (04/02)

Large samples:

Number	SCC (x1000 cells/mL)
1	2751
2	196
3	3309
4	246
5	486
6	1216

Normal samples:

	Red Cap	White Cap	Blue Cap	Green Cap
Cow N°	**Right Front**	**Right Hind**	**Left Front**	**Left Hind**
387	80	204	5440	134
3063	1429	343	2390	2099
230	917	358	769	198
44	147	813	110	1688

Trial 4 (05/02)

Sample	SCC (x 1000 cells/mL)
1	14
2	34
3	116
4	554
5	260
6	302
7	849
8	267
9	2231
10	142
11	405
12	2419
13	5332
14	671
15	406
16	653

Trial 5 (06/2002)

Sample	SCC (x 1000 cells/mL)
1	153
2	258
3	51
4	304
5	3732
6	117
7	203
8	104
9	677
10	546
11	1174
12	803
13	375
14	184
15	1941
16	5
17	5
18	49
19	82
20	283

Trial 6 (2002)

Sample	SCC (x 1000 cells/mL)
1	68
2	1
3	850
4	140
5	440
6	330
7	135
8	550
9	210
10	168
11	90

Trial 7 (2003)

Sample	SCC (x 1000 cells/mL)
1	73
2	89
3	86
4	68
5	103
6	74
7	87
8	204
9	191
10	157
11	230
12	761
13	1696
14	1489
15	309
16	508
17	577
18	785
19	1546
20	415
21	193
22	251
23	216
24	503
25	255
26	319
27	55
28	192

Trial 8 (02/03)

Sample	SCC (x 1000 cells/mL)
1	79
2	11
3	113
4	125
5	67
6	2
7	236
8	894
9	440
10	516
11	447
12	129
13	83
14	92

15	169
16	250
17	2332
18	557
19	281
20	1016

Trial 9 (03/03)

Sample	SCC (x 1000 cells/mL)
1	93
2	7
3	11
4	103
5	214
6	332
7	57
8	948
9	70
10	833
11	457
12	263
13	139
14	13
15	575
16	155
17	679
18	285
19	261
20	358
21	716
22	117
23	142
24	93
25	6

Trial 10 (04/03)

	Red Cap	White Cap	Blue Cap	Green Cap
Cow N°	**Right Front**	**Right Hind**	**Left Front**	**Left Hind**
1867	449	611	97	1085
285	3047	178	1004	4974
1163	459	342	84	1382
1803	91	109	2423	250
1157	124	695	69	78
14	71	6	2266	187
717	147	1203	116	5833

Trial 11 (05/03)

	Red Cap	White Cap	Blue Cap	Green Cap
Cow N°	**Right Front**	**Right Hind**	**Left Front**	**Left Hind**
1157	125	420	6	4
1563	703	82	58	2527
598	732	155	1257	81
1852	514	121	65	62
1663	68	54	10	1350
1658	876	clots	103	76

APPENDIX C

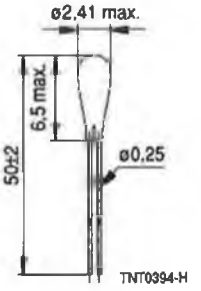
Datasheet temperature sensor for the prototype impedance device

Applications

- Heating and air conditioning systems
- Industrial electronics
- Automotive electronics

Features

- Fast response
- High measuring accuracy
- Different tolerances available
- Epoxy resin encapsulation
- Silver-plated nickel leads



Dimensions in mm
Approx. weight 60 mg

Options

Non-standard lead lengths

Delivery mode

Bulk

Climatic category (IEC 60068-1)		55/155/56	
Max. power at 25 °C	P_{25}	60	mW
Resistance tolerance	$\Delta R_N/R_N$	± 1 %, ± 3 %, ± 5 %	
Rated temperature	T_N	25	°C
B value tolerance	$\Delta B/B$	± 1 %	
Dissipation factor (in air)	δ_{th}	approx. 1,5	mW/K
Thermal cooling time constant (in air)	τ_c	approx. 12	s
Heat capacity	C_{th}	approx. 18	mJ/K

R_{25} Ω	No. of R/T characteristic	$B_{25/100}$ K	Ordering code
2 k	1008	3560	B57867S0202+140
3 k	8016	3988	B57867S0302+140
5 k	8016	3988	B57867S0502+140
10 k	8016	3988	B57867S0103+140
30 k	8018	3964	B57867S0303+140
50 k	2901	3760	B57867S0503+140
100 k	2014	4540	B57867S0104+140

+: F for $\Delta R_N/R_N = \pm 1$ %
H for $\Delta R_N/R_N = \pm 3$ %
J for $\Delta R_N/R_N = \pm 5$ %

APPENDIX D

Raw data for chapter 4 figures

Fig 4.3

Sample	SCC	Z_{Comp} BAS	Z_{Comp} WI
1	68	122	120.04
2	1	103.33	104.62
3	850	85.94	86.63
4	140	111.52	110.08
5	440	100.15	102.81
6	330	105.6	103.59
7	135	99.93	98.97
8	550	100.1	99.57
9	210	101.52	99.17
10	168	101.82	95.48
11	90	125.86	113.49

Z BAS value read on impedance curve at 100kHz.

Fig 4.4 and 4.5 (Without preservatives)

Sample	SCC	Z_{Comp}
1	68	120.04
2	1	104.62
3	850	86.63
4	140	110.08
5	440	102.81
6	330	103.59
7	135	98.97
8	550	99.57
9	210	99.17
10	168	95.48
11	90	113.49

Example Z data not compensate to 15°C

Milk01

1	119.70	15.06
2	119.58	15.15
3	119.45	15.16
4	119.43	15.18
5	119.43	15.25
6	119.42	15.26
7	119.41	15.26
8	119.41	15.27
9	119.40	15.35
10	119.30	15.35
11	119.23	15.35
12	119.16	15.35
13	119.11	15.37
Av	119.39	15.26

Fig 4.7 (Trial 9) Day 1: Blue, Day 5: Pink

Sample	SCC	Z $_{Comp}$(Ohm)	Cl^-[ppm]	Z $_{Comp}$ (Ohm)
1	93	115.31		103.69
2	7	116.79		95.94
3	11	112.86		99.18
4	103	108.21		88.90
5	214	82.28	2000	89.57
6	332	122.86		93.31
7	57	106.46		92.41
8	948	113.72		100.68
9	70	106.23		87.71
10	833	92.62		88.82
11	457	107		90.6
12	263	117.57		107.58
13	139	119.24		102.09
14	13	118.94		107.04
15	575	106.66		101.29
16	155	111.98		90.99
17	679	106.96	1500	99.13
18	285	116.45		97.60
19	261	104.67		89.14
20	358	108.81		89.76
21	716	113.96	1000	108.01
22	117	101.06		96.84
23	142	120.72		106.18
24	93	118.71		105.75
25	6	122.34		106.96

Example Z (15 data): Day 1

Milk1	Z	Z_{Comp}	Tp°C
1	119.44	115.72	18.51
2	119.44	115.71	18.51
3	119.43	115.71	18.51
4	119.44	115.72	18.51
5	119.43	115.71	18.51
6	119.43	115.71	18.51
7	119.43	115.71	18.51
8	119.43	115.70	18.51
9	119.45	115.72	18.51
10	119.44	115.72	18.51
11	119.43	115.71	18.51
12	119.43	115.71	18.52
13	119.43	115.71	18.51
14	119.44	115.72	18.52
15	119.44	115.72	18.52
Av	119.44	115.71	18.51

Example Z(15 data): Day 5

Milk1			
1	104.47	103.73	19.664
2	104.46	103.72	19.663
3	104.46	103.72	19.664
4	104.46	103.72	19.663
5	104.46	103.72	19.664
6	104.46	103.72	19.663
7	104.44	103.70	19.663
8	104.43	103.69	19.663
9	104.42	103.69	19.665
10	104.42	103.68	19.664
11	104.38	103.65	19.666
12	104.35	103.62	19.667
13	104.35	103.62	19.666
14	104.3	103.57	19.665
15	104.28	103.55	19.666
Av	104.43	103.69	19.66

Fig 4.9 and Fig 4.13 (Trial 10)

Sample	Z_1	Z_2	Z_3	Z	RSD	SCC	Cl^-[ppm]	pH
1867_Red	124.77	124.84	124.91	124.84	0.07	449	1000	6.62
1867_White	121.31	121.48	121.45	121.42	0.09	611	1000	6.6
1867_Blue	140.36	138.52	138.41	139.10	1.09	97	1500	6.65
1867_Green	128.11	127.94	127.90	127.98	0.11	1085	1500	6.64
285_Red	84.45	84.61	84.62	84.56	0.09	3047	2800	6.97
285_White	99.30	98.68	98.57	98.85	0.39	178	2000	6.87
285_Blue	105.24	105.18	105.38	105.26	0.10	1004	2500	6.84
285_Green	81.89	81.72	81.01	81.74	0.14	4974	3000	6.98
1163_Red	132.62	132.47	132.37	132.38	0.08	459	1000	6.74
1163_White	107.29	107.37	107.14	107.32	0.20	342	2000	6.81
1163_Blue	130.18	129.73	129.70	129.87	0.27	84	1000	6.78
1163_Green	93.23	92.9	92.69	92.94	0.27	1382	2500	6.97
1803_Red	129.25	129.25	130.03	129.51	0.45	91	1000	6.77
1803_White	139.57	139.20	138.88	139.22	0.34	109	800	6.77
1803_Blue	99.71	99.66	99.80	99.72	0.07	2423	2000	6.87
1803_Green	133.74	133.37	133.46	133.52	0.19	250	1500	6.78
1157_Red	130.70	130.13	130.08	130.30	0.34	124	1000	6.71
1157_White	122.33	122.44	122.55	122.44	0.11	695	1000	6.7
1157_Blue	134.23	134.03	133.69	133.98	0.27	69	800	6.71
1157_Green	129.45	129.15	129.06	129.22	0.20	78	800	6.77
14_Red	137.38	137.06	137.14	137.16	0.11	71	1500	6.66
14_White	140.40	140.38	140.69	140.49	0.17	6	1000	6.652
14_Blue	125.63	125.27	125.46	125.45	0.18	2266	1000	6.67
14_Green	123.50	123.04	123.80	123.65	0.15	187	1500	6.7
717_Red	125.85	125.16	124.62	125.21	0.62	147	1400	6.78
717_White	115.28	115.01	115.10	115.16	0.11	1203	1000	6.81
717_Blue	118.69	118.69	118.65	118.68	0.02	116	1500	6.77
717_Green	79.98	80	79.98	79.99	0.01	5833	3000	6.96

Example raw data excel 1867 _Blue

Imp1				Imp2				Imp3	
1	143.57	140.38	18.939	140.67	138.52	19.272	139.76	138.31	19.51
2	143.56	140.24	18.898	140.67	138.53	19.278	139.71	138.36	19.54
3	143.48	140.16	18.899	140.67	138.54	19.279	139.73	138.44	19.56
4	143.36	140.33	18.993	140.64	138.51	19.279	139.72	138.45	19.57
5	143.16	139.85	18.899	140.65	138.52	19.279	139.7	138.43	19.57
6	144.04	140.59	18.858	140.67	138.54	19.279	139.69	138.42	19.57
7	143.93	140.59	18.897	140.65	138.53	19.28	139.7	138.43	19.57
8	143.66	140.34	18.899	140.64	138.52	19.28	139.7	138.43	19.57
9	143.6	140.57	18.993	140.64	138.52	19.281	139.7	138.43	19.57
10	143.58	140.54	18.993	140.64	138.52	19.281	139.69	138.42	19.57
Av	143.60	140.36	18.93	140.65	138.52	19.28	139.71	138.41	19.56
Mean	**139.10**		**SD**	**1.09**					

Fig 4.4, 4.10, 4.11 and Fig 4.12 (With preservatives)

Sample	Z_1	Z_2	Z_3	Z	RSD	SCC	Cl^-[ppm]
1	113.58	112.47	109.62	111.89	2.05	68	1500
2	94.53	95.47	97.07	95.691	1.32	1	2000
3	78.85	78.87	80.36	79.36	0.87	850	3000
4	103.64	105.26	102.18	103.21	0.9	140	1600
5	93.23	93.28	91.24	92.58	1.17	440	2500
6	97.58	96.49	95.30	96.46	1.14	330	1700
7	92.85	92.66	89.30	91.7	1.82	135	1500
8	93.08	92.05	90.20	91.78	1.47	550	3000
9	91.81	90.98	92.65	91.81	0.83	210	1700
10	90.42	90.43	91.90	90.91	0.85	168	2000
11	106.50	106.49	106.23	106.44	0.20	90	1500

Example Raw data Milk 1

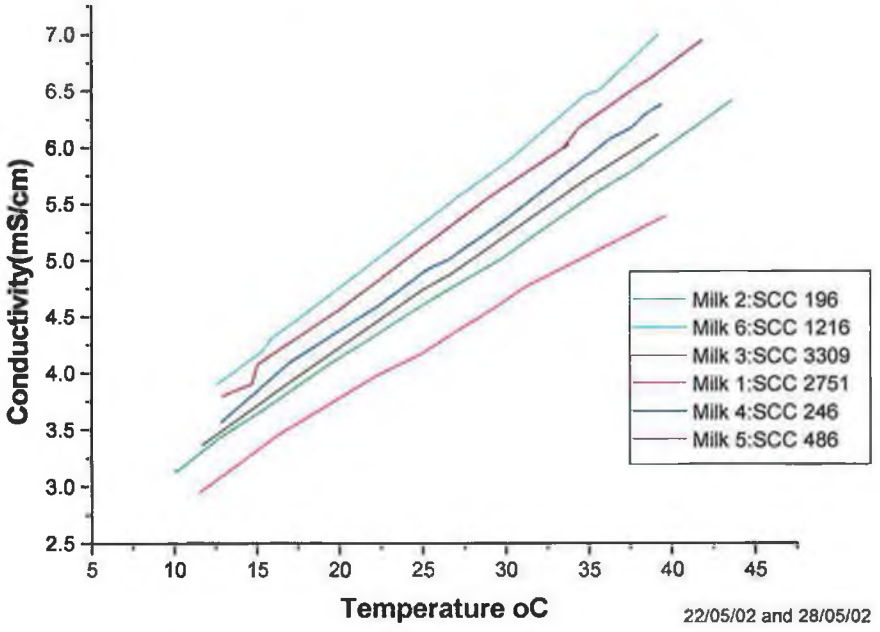
Milk01	1			2			3			STD
1	126.01	113.52	10.28	115.99	112.72	13.66	108.47	109.94	15.65	1.88
2	125.99	113.52	10.29	115.73	112.40	13.63	108.26	109.79	15.67	1.91
3	125.80	113.58	10.37	115.73	112.40	13.63	107.96	109.57	15.71	2.06
4	125.67	113.46	10.37	115.73	112.41	13.63	108.03	109.69	15.73	1.95
5	125.64	113.68	10.47	115.73	112.45	13.65	107.86	109.53	15.73	2.14
6	125.64	113.69	10.47	115.72	112.45	13.66	107.91	109.57	15.74	2.11
7	125.63	113.70	10.48	115.72	112.50	13.68	107.87	109.53	15.74	2.14
8	125.62	113.89	10.55	115.71	112.53	13.69	107.88	109.55	15.74	2.22
9	125.44	113.51	10.47	115.69	112.52	13.70	107.93	109.59	15.74	2.04
10	125.33	113.41	10.47	115.65	112.54	13.72	107.88	109.54	15.74	2.03
11	125.26	113.41	10.50	115.57	112.46	13.72	107.92	109.58	15.74	2.00
12	125.26	113.59	10.56	115.45	112.36	13.72	107.92	109.58	15.74	2.05
13	125.26	113.59	10.56	115.41	112.32	13.72	107.88	109.55	15.74	2.07
14	125.25	113.59	10.56	115.41	112.32	13.72	107.92	109.59	15.74	2.04
15	125.23	113.57	10.56	115.39	112.30	13.72	107.96	109.63	15.74	2.01
Av	125.58	113.58	10.45	115.68	112.47	13.68	107.98	109.62	15.72	2.05
			Av :	111.89						
			std	2.05						

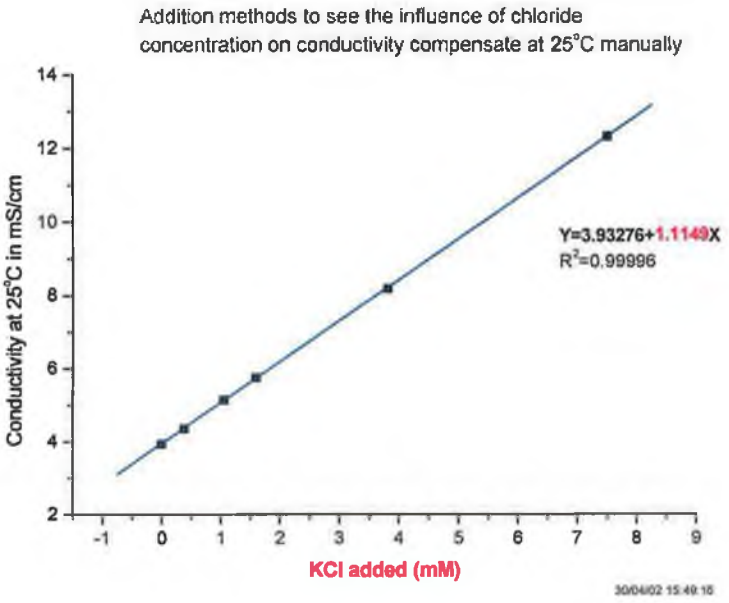
APPENDIX E

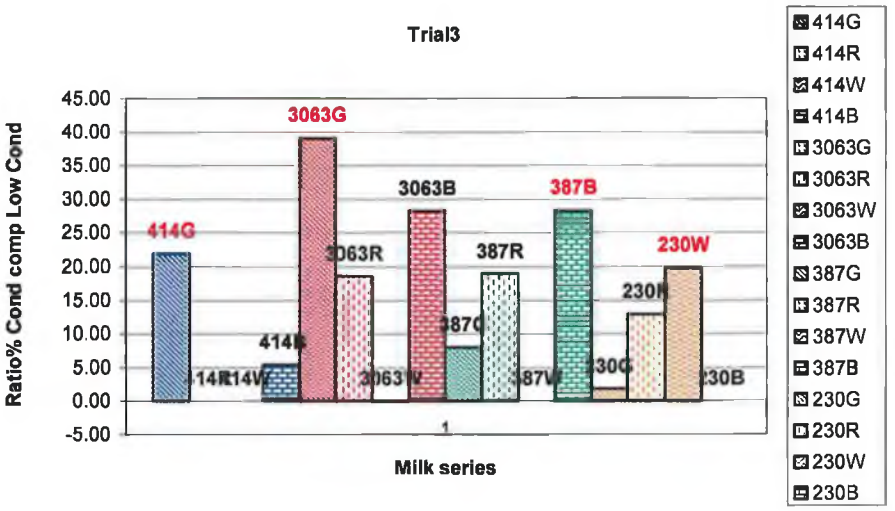
Additional Data for Chapter 3

Additional Data Conductivity measurements

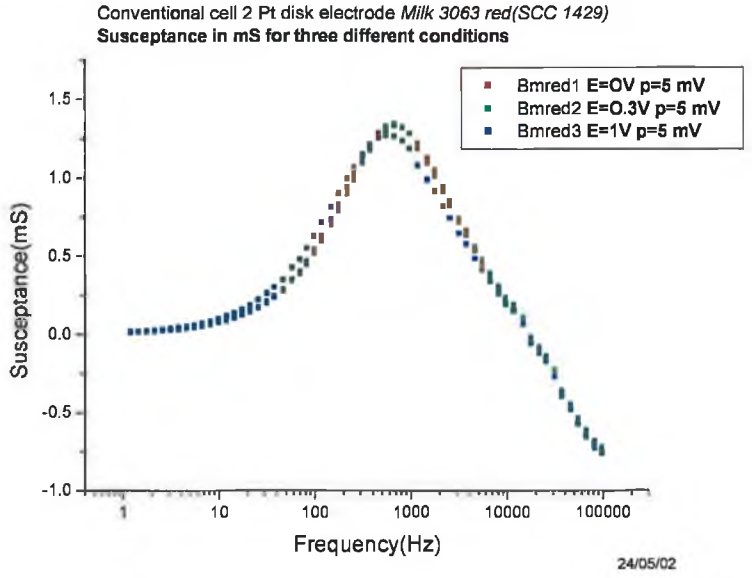
Conductivity variation against temperature for different infected milk samples

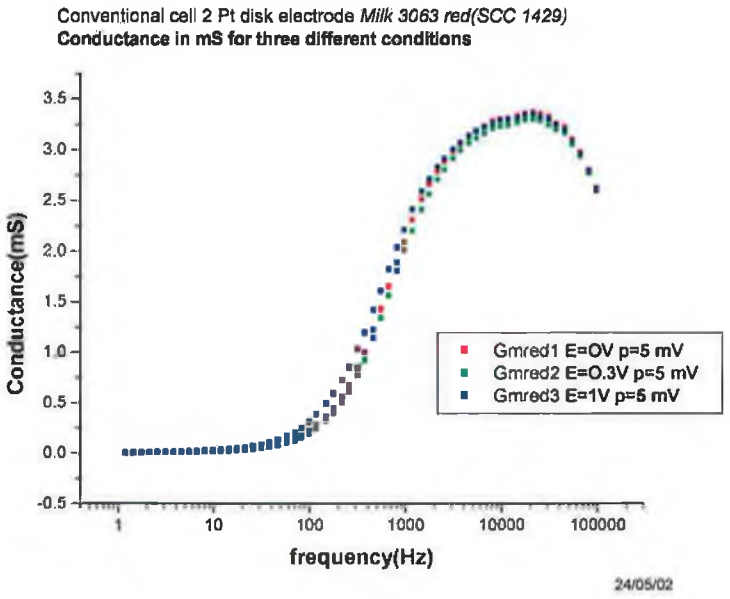




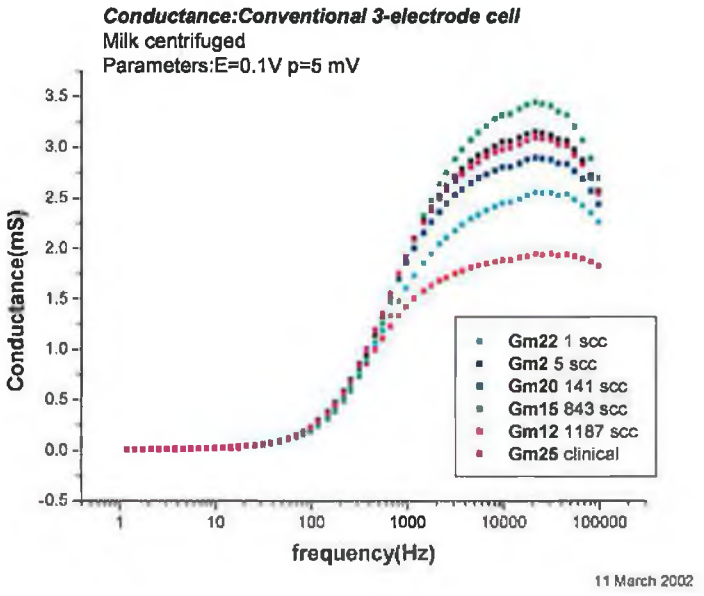


Impedance plot CH 660A at 0V, 0.1V, 0.3V. (See section 3.3.4)

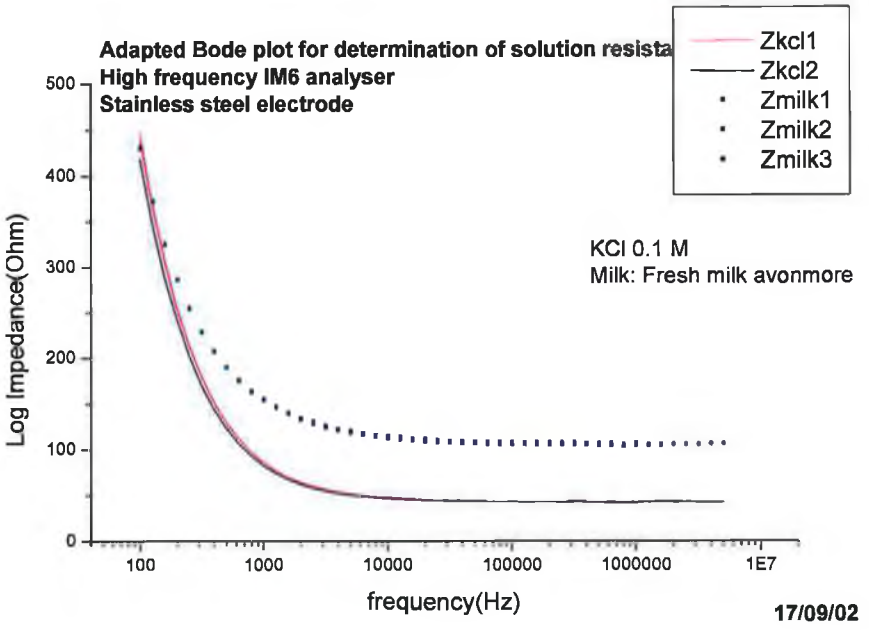




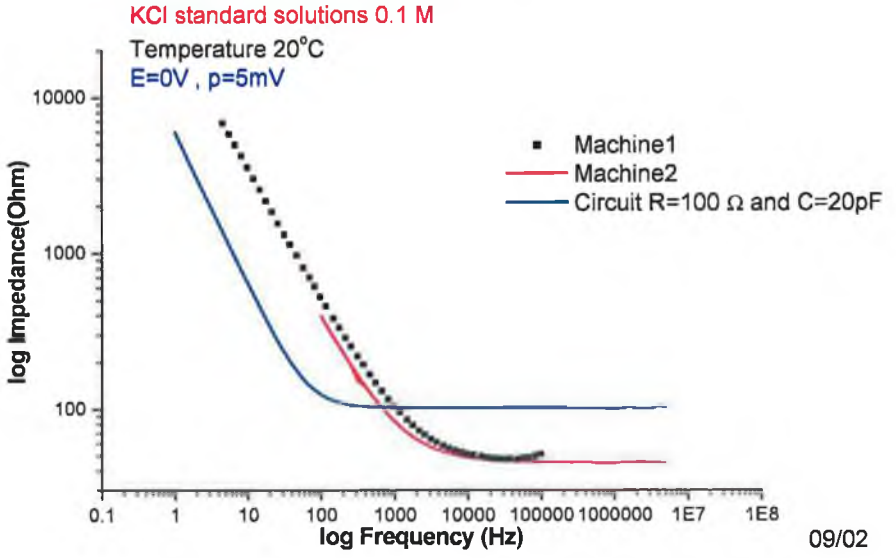
Impedance plot CH 660A Centrifuged crude milk



Impedance Plot FRA IM6 :



Comparison between 2 Machines for impedance measurement
Machine 1: Potentiostat CHInstrument 660 A
Machine 2: Frequency analyser IM6 BAS ZAhner



APPENDIX F

Additional Data for chapter 4

Preliminary tests on the prototype impedance device

Temperature sensor test in ice water (raw data):

temperature => 0.709508
temperature => 0.709508
temperature => 0.712381
temperature => 0.708553
temperature => 0.714300
temperature => 0.716219

Preliminary calibrations with resistor and real samples and comparison with IM6 instrument results.

Open circuit: impedance => -31221.059262 ohms
impedance => -31221.059262 ohms

Kcl solution 0.1M: impedance => 48.247298 ohms
conductivity => 20.726548 milli siemens
temperature => 23.303630

impedance => 48.247298 ohms
conductivity => 20.726548 milli siemens
temperature => 23.303630

Comparison IM6/Prototype device

	IM6	Device
Milk pasteurised (20°C)	112-115	118-121
Mastitic Milk (SCC: 3732)	70.1	72.5
KCl 0.1 M	52-53	55

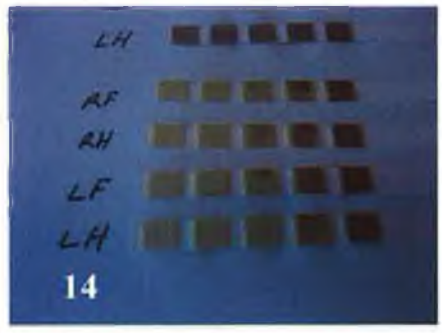
Resistors

- R 1kΩ : Z device → 821.002 Ω (Before calibrations we get accuracy 5% systematique)

Chloride titration by analytical strips

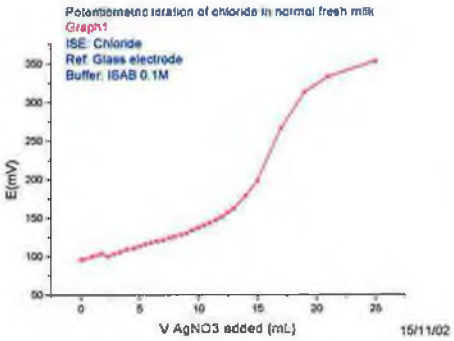
Mercockant strips ®:

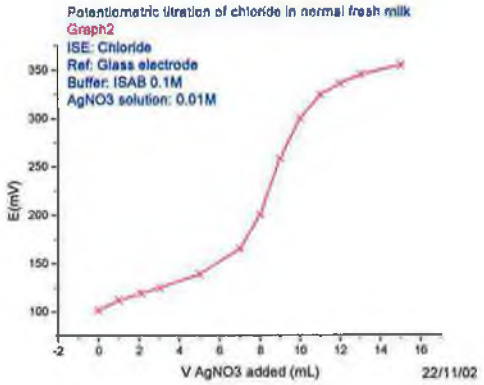
Example Data Table 4.1:

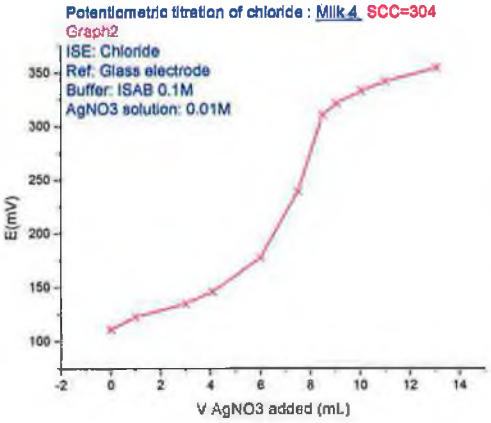


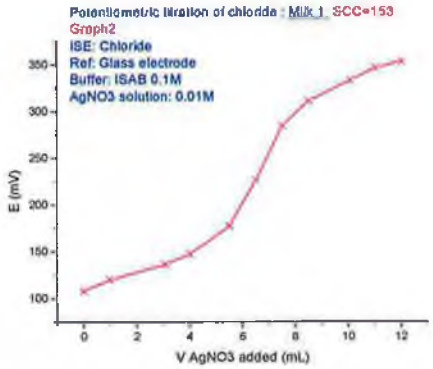
APPENDIX G

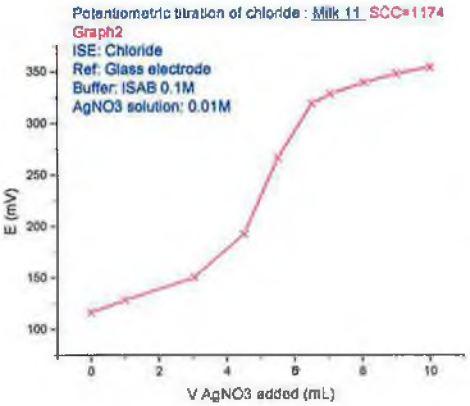
Chloride titration by potentiometric method

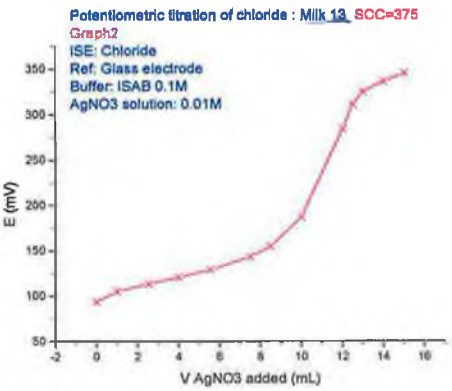

Potentiometric titration of chloride in normal fresh milk
Graph1
ISE: Chloride
Ref: Glass electrode
Buffer: ISAB 0.1M
E(mV)
V AgNO3 added (mL)
15/11/02

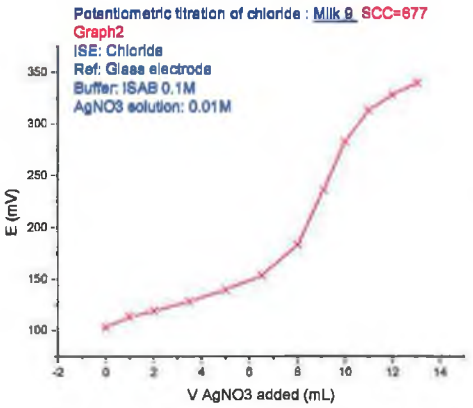

Potentiometric titration of chloride in normal fresh milk
Graph2
ISE: Chloride
Ref: Glass electrode
Buffer: ISAB 0.1M
AgNO3 solution: 0.01M
E(mV)
V AgNO3 added (mL)
22/11/02

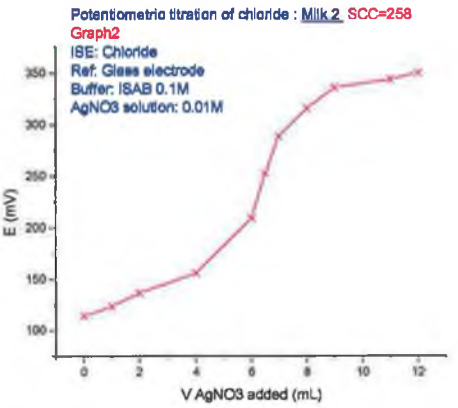

Potentiometric titration of chloride : Milk 4 SCC=304
Graph2
ISE: Chloride
Ref: Glass electrode
Buffer: ISAB 0.1M
AgNO3 solution: 0.01M
E(mV)
V AgNO3 added (mL)

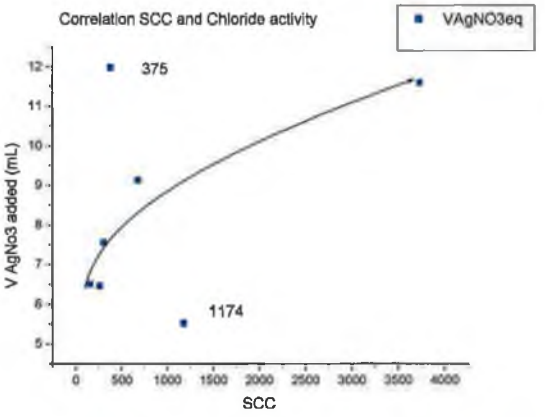

Potentiometric titration of chloride : Milk 1 SCC=153
Graph2
ISE: Chloride
Ref: Glass electrode
Buffer: ISAB 0.1M
AgNO3 solution: 0.01M
E (mV)
V AgNO3 added (mL)

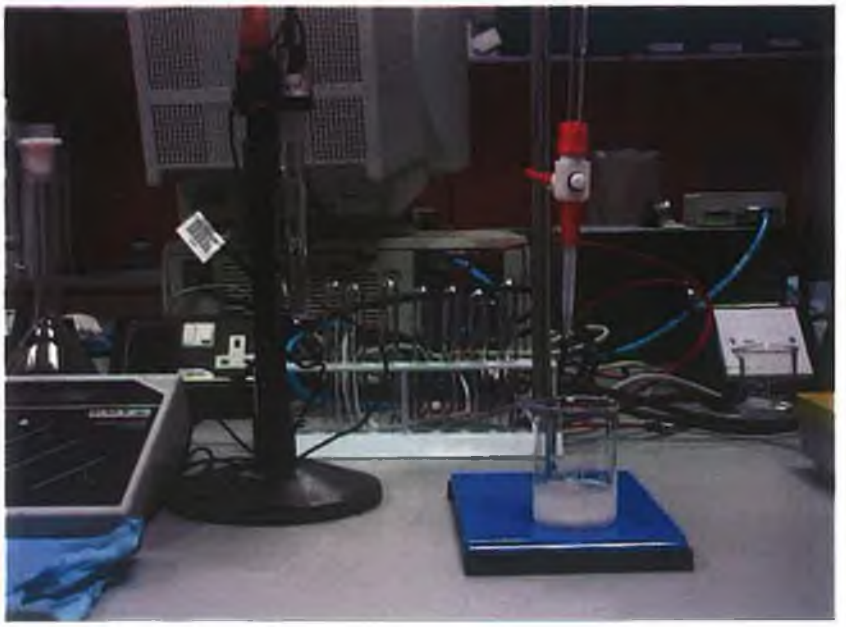

Potentiometric titration of chloride : Milk 11 SCC=1174
Graph2
ISE: Chloride
Ref: Glass electrode
Buffer: ISAB 0.1M
AgNO3 solution: 0.01M
E (mV)
V AgNO3 added (mL)


Potentiometric titration of chloride : Milk 13 SCC=375
Graph2
ISE: Chloride
Ref: Glass electrode
Buffer: ISAB 0.1M
AgNO3 solution: 0.01M
E (mV)
V AgNO3 added (mL)


Potentiometric titration of chloride : Milk 9 SCC=677
Graph2
ISE: Chloride
Ref: Glass electrode
Buffer: ISAB 0.1M
AgNO3 solution: 0.01M
E (mV)
V AgNO3 added (mL)

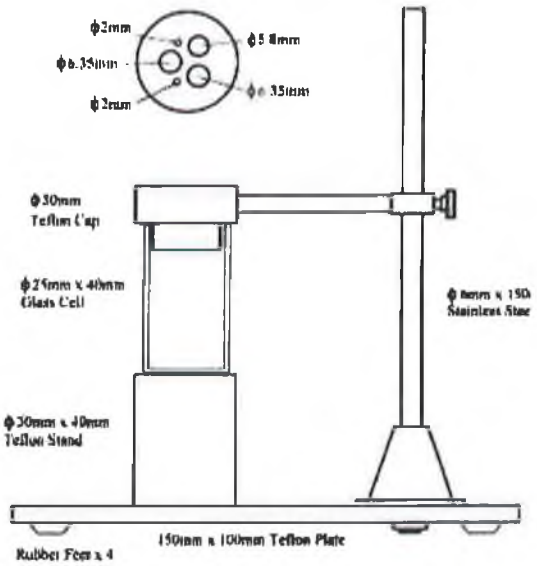

Potentiometric titration of chloride : Milk 2 SCC=258
Graph2
ISE: Chloride
Ref: Glass electrode
Buffer: ISAB 0.1M
AgNO3 solution: 0.01M
E (mV)
V AgNO3 added (mL)


Correlation SCC and Chloride activity
VAgNO3eq
375
1174
V AgNo3 added (mL)
SCC



APPENDIX H

Additional Pictures


ϕ2mm
ϕ5.8mm
ϕ6.35mm
ϕ6.35mm
ϕ2mm
ϕ30mm
Teflon Cap
ϕ25mm x 40mm
Glass Cell
ϕ8mm x 150
Stainless Stee
ϕ30mm x 40mm
Teflon Stand
150mm x 100mm Teflon Plate
Rubber Feet x 4