



# Screening method for early detection of mastitis in cows



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## ABSTRACT

Mastitis in cows is an inflammation of the mammary gland usually caused by bacterial infection of udder tissues. This disease causes considerable damages to the cattlemen when reducing the quantity and the quality of the produced milk. An early detection and corrective action can lead to early cure. Although the universal method to measure mastitis levels is by determining the somatic cell counts per milliliter of milk, the electrical conductivity of milk could be a rapid test for checking the acceptability of milk to monitor the effects of udder infection. In this paper a low-cost circuit for estimating the quality of raw milk based on AC electrical conductivity measurements is proposed. It consists in the use of a modified Wheatstone bridge to minimize the parasitic effects. A conductivity cell consisting of two electrodes and a coaxial cable was also designed. The temperature of the milk is also measured and compensated its effect. This system can help the farmer to detect quickly and economically the state of health of their cows from a simple measurement of electrical conductivity performed on the dairy farm itself.

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## 1. Introduction

Mastitis in cows is an inflammation of the mammary gland and is usually caused by bacteria infection of the udder tissues. It is the most costly disease on the dairy farm today. According the statistical information total losses for the United States are in the range of 1.5–3 billion USD annually, or 11% of the total USA milk production. Mastitis can be clinical or sub-clinical. Clinical mastitis gives abnormal milk and swelling of the udder. Bacteria are present in the milk, and the composition of the milk is dramatically changed. Sub-clinical mastitis is more problematic because no visible changes appear in the milk or the udder, but milk production decreases, bacteria are in the milk, and the composition is altered. Mastitis has a very negative effect on product quality, due to increased enzymatic activity. This effect will reduce cheese yield, butter yield, change acid production in fermented products and lead to taste defects in all kinds of dairy products. To encourage farmers to lower the level of mastitis, many dairies offer

premium payment programs for milk free of mastitis and reduction in payment for milk with high mastitis level. A complete review about mastitis detection, current trends and future perspectives is presented in [1].

Different methods are used to assess milk quality. Some methods such as somatic cell count (SCC) are mandated. Other methods, while not mandated, are used to monitor milk quality and help diagnose potential on-farm problems associated with abnormally high counts and poor quality milk. One of these methods is the use of the electric conductivity of milk (ECM) [2–4]. Milk normally has an EC of between 4.0 and 6.0 mS/cm. However, milk from a cow affected by mastitis is a better conductor of electric current than that from a healthy cow due to increased  $\text{Na}^+$  and  $\text{Cl}^-$  ions and the reduction in the content of  $\text{K}^+$  ions and lactose [5]. Therefore, measurement of conductivity can therefore help in the early identification of mastitis.

There is a strong relation between the electrical conductivity of the foremilk and its lactose and salt content. Illness not related to the udder, problems in metabolism, changes in the diet, breed, stress and the stage of lactation influence the conductivity whilst having a lesser influence on somatic cell count. Studies of milk electrical properties

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and mastitis indicate there is no fixed point or number where mastitis is definitely present, or not present. Rather, there are increasing or decreasing degrees of infection as conductivity changes. When measured conductivity is in significantly extreme value (6.5–13.00 mS/cm) at 18 °C, an indication of mastitis is developed. The farmer can use the results to fight sub-clinical mastitis and by that optimize milk production both concerning the amount and the quality of the milk.

Application of electrical admittance measurements to quality control of milk is presented in [6,7]. In [8] the measurement of the ECM is performed by applying an extremely stable AC current source to the electrodes and measuring accurately the voltage in them. Based on measuring the EC there are several portable instruments like MASTITRON LF 3000 [9], DRAMINSKY mastitis detector [10] or Milk Checker N-4L [11]. These devices are used for detecting sub-clinical mastitis in its earliest, visually undetectable stage. Milking robots for on-line mastitis monitoring have increased significantly over the past years [12].

This paper presents the electronic design of a mastitis detector. The prototype can be connected to a computer, via USB interface, to monitoring the evolution of the mastitis in real-time. The quality of the milk is also tested by means of the lactose content.

## 2. Measurement of the electrical conductivity

The electrical conductivity is an important property of the ionic solutions used for analytical purposes. In the simplest form, EC of a solution can be calculated as:

$$\sigma = \frac{k_{\text{cell}}}{R_c} \quad (1)$$

where  $R_c$  ( $\Omega$ ) is the electrical resistance of the solution and  $k_{\text{cell}}$  ( $\text{cm}^{-1}$ ) is a characteristic geometric cell constant. For a cylindrical cell and electrodes with parallel surfaces in front of each other,  $k$  can be approximated as:

$$k_{\text{cell}} = \frac{d}{A} \quad (2)$$

where  $d$  (cm) is the distance between the cell electrodes and  $A$  ( $\text{cm}^2$ ) is the electrode effective surface area. The experimental effort in the determination of the electrical conductivity of a solution does consist then in the accurate evaluation of the cell constant  $k$  and of the resistance  $R_c$ .

Unless non-polarized electrodes are used, conductivity measurements must be performed in a.c. regime. Under these conditions, we measure a complex impedance  $Z(f)$  dependent on frequency  $f$  and represented as:

$$Z(f) = R(f) + jX(f) \quad (3)$$

sum of the real resistive term,  $R(f)$ , and of the complex capacitive term,  $X(f)$ . It must be stressed that  $R_c$  is not necessarily coincident with the real part of  $Z(f)$  because of the several parasitic effects caused by the physical layout of the cell and by the connection wires to the measuring apparatus.

To avoid errors arising from electrode polarization effects, the electrical measurements need to be performed at high frequency [13]. Fig. 1 shows the conductivity probe constructed in the laboratory. It consists of two stainless steel electrodes with an effective area of about 100 mm<sup>2</sup> and 10 mm apart. Through the middle of the rings passes a coaxial cable whose mesh is welded to one of the rings and the inner cable to the other ring. Using the above values, from Eq. (2) the cell constant is  $k = 1 \text{ cm}^{-1}$ . Thus from Eq. (1) the conductivity is obtained by a resistance measurement, as long as the parasitic effects have been removed.

Using the above conductivity probe and the HP 4192A impedance analyzer measurements of ECM were made at different frequencies. Fig. 2 shows the frequency dependence of ECM for raw milk at 20 °C. The observed increases of conductivity with increasing frequency are characteristic features of a dielectric dispersion which, in this case, is associated with the phenomenon known as electrode polarization. This effect arises from the presence of ionic double layers at the electrode surface [14]. To eliminate the effect of electrode polarization, the ECM was measured at 10 kHz where the conductivity shows a stable value.

The conductivity of the milk is also temperature dependent. The reason is that when the temperature increases the mobility of the ions increases and so the conductivity rises. By definition, temperature compensated conductivity of a solution is the conductivity which that solution exhibits at the reference temperature, typically 25 °C. A useful algorithm for temperature compensation is:

$$\sigma_{25} = \frac{\sigma_T}{[1 + \alpha(T - 25)]} \quad (4)$$

where  $\sigma_{25}$  is the conductivity of the milk at 25 °C,  $\sigma_T$  is the measured conductivity of the milk at sample temperature,  $\alpha$  is the temperature coefficient (%/°C) and  $T$  the sample temperature (°C). Raising the temperature by 1 °C increases the solution conductivity by average of 2–3% [15]. The coefficient temperature of milk was experimentally obtained analyzing samples of milk at two temperatures and at different frequencies. In the range of 23–33 °C the average value of temperature coefficient is

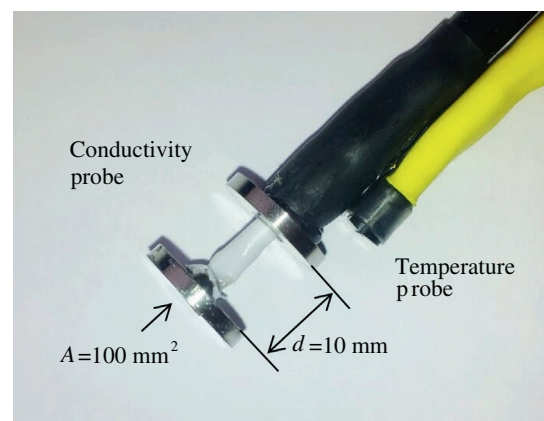
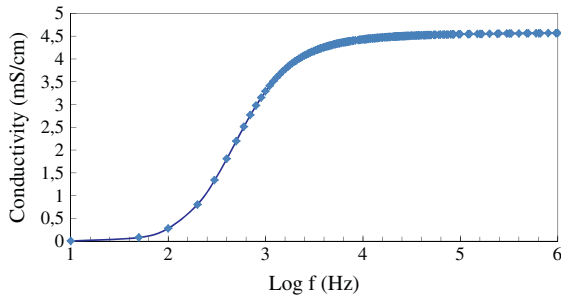


Fig. 1. Conductivity and temperature probes.



**Fig. 2.** Frequency dependence of conductivity using stainless steel electrodes for raw milk at 20 °C.

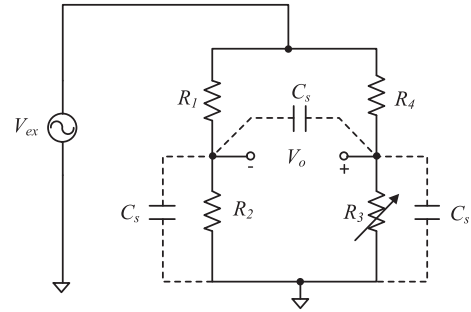
1%/°C [8]. The temperature of the milk is measured using the LM35 precision centigrade temperature sensor [16], which provides a linear +10.0 mV/°C scale factor and 0.5 °C of accuracy at +25 °C.

### 3. Circuit description

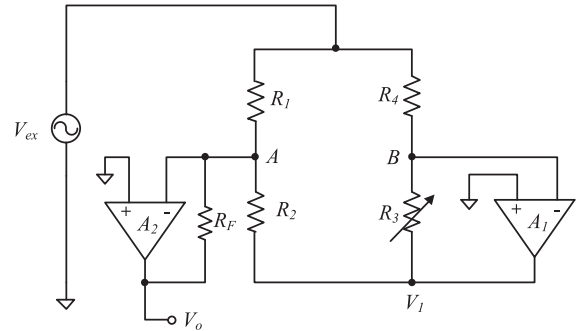
**Fig. 3** shows the block diagram of conductivity measurement set up. The conductivity cell is connected to one arm of a modified Wheatstone bridge. The output voltage of the bridge circuit depends upon the conductivity of ionic solution. The output of the bridge is rectified using a precision rectifier and a lowpass filtered to remove the high and low frequency noise. The output is given to a 10-bit analog to digital converter (ADC), included in the microcontroller block. The ADC is used to convert the analog conductivity and temperature into digital values. The microcontroller block is a PIC16F876 from Microchip Technology, a low power, high performance CMOS 8-bit. It is used for compensation, calibration and linearization purposes.

One of most accurate methods of measurement of unknown impedance are the bridge techniques [17], whose accuracy is limited essentially by the accuracy of the known values of the elements that constitute the bridge. The conventional AC Wheatstone bridge is shown in **Fig. 4**, where:  $C_s$  represent the stray capacitance that will exist between the bridge output lead wires and also between any output lead wire and the ground.  $V_{ex}$  is the AC bridge excitation voltage.  $R_1$ ,  $R_2$ ,  $R_4$  are known resistances and  $R_3$  represent the unknown electrical resistance of the milk.

Several modifications of the AC Wheatstone bridge have been proposed to achieve high accuracy in measurement [18–21]. In order to minimize the stray capacitances, the bridge is modified as shown in **Fig. 5**, where  $A_1$  and  $A_2$



**Fig. 4.** Conventional AC Wheatstone bridge.



**Fig. 5.** Modified AC Wheatstone bridge.

are two high-gain operational amplifiers with their non-inverting terminals connected to the common terminal of the circuit. Thus, the nodes  $A$  and  $B$  of the bridge are at the same potential with respect to the ground, and the effect of stray capacitance between the output lead wires and ground may be considered negligible.

The output voltage of the bridge  $V_o$  can be easily obtained applying the superposition theorem:

$$V_1 = -\frac{R_3}{R_1} V_{ex} \quad (5)$$

$$V_o = -\left( V_{ex} \frac{R_F}{R_2} + V_1 \frac{R_F}{R_4} \right) \quad (6)$$

$$V_o = \frac{R_F}{R_1 R_2 R_4} (R_3 R_2 - R_1 R_4) V_{ex} \quad (7)$$

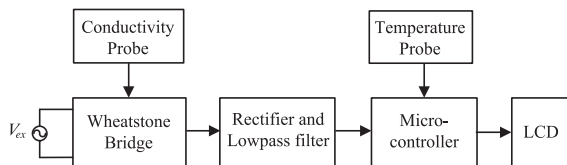
At the balance condition of the bridge,  $V_o = 0$ , which is identical with the conventional bridge network. From Eq. (7) the electrical resistance of milk is giving by:

$$R_3 = \left( \frac{V_o R_1 R_4}{V_{ex} R_F} + \frac{R_1 R_4}{R_2} \right) \quad (8)$$

Substituting in Eq. (1), the ECM is determined by:

$$\sigma_{milk} = \frac{k_{cell}}{R_3} = k_{cell} \left[ \frac{V_o R_1 R_4}{V_{ex} R_F} + \frac{R_1 R_4}{R_2} \right]^{-1} \quad (9)$$

**Fig. 6** shows the schematic of electronic circuit. Practical considerations must be taken in order to design the Wheatstone bridge. The bridge output voltage is



**Fig. 3.** Block diagram of the EC measurement system.

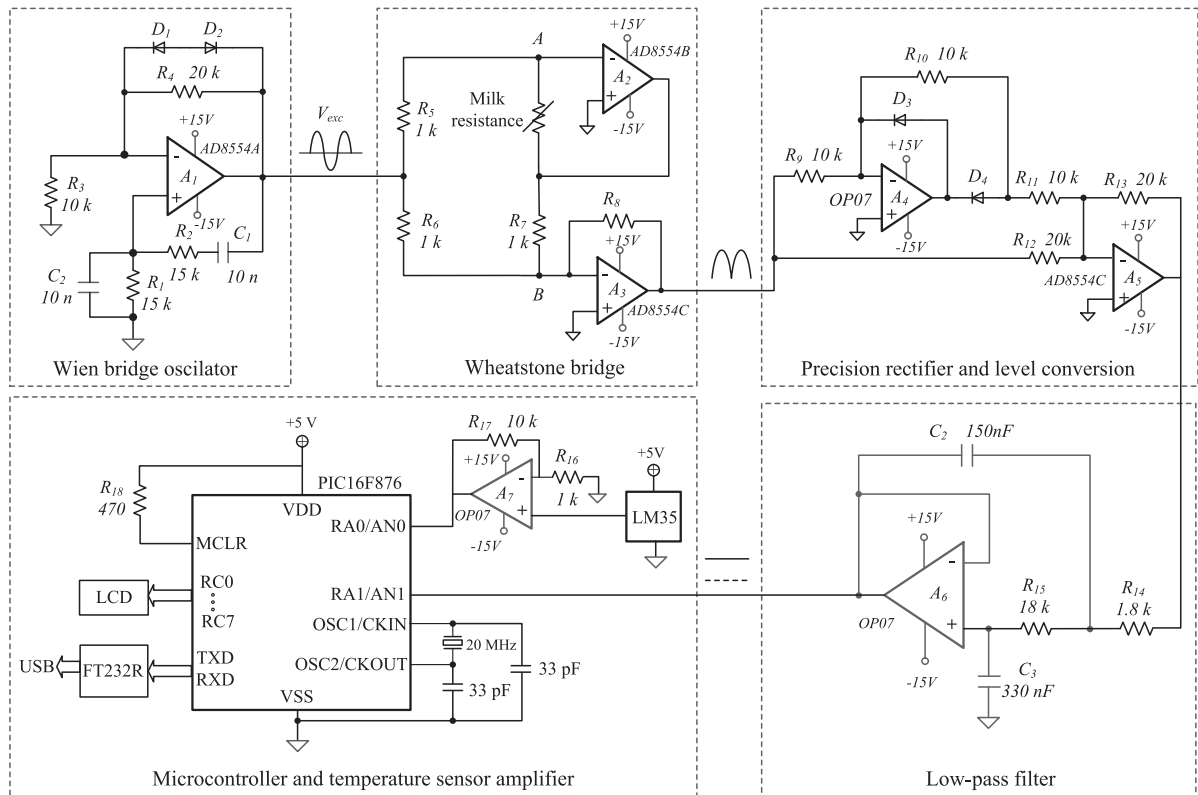


Fig. 6. Electronic circuit of the conductivity measurement system.

proportional voltage excitation as shown Eq. (7). Therefore, the bridge requires an extremely stable voltage source because any change in the voltage will be misinterpreted as a resistance change. The voltage excitation was implemented by means of a using a Wien bridge oscillator of 1 V of amplitude and 10 kHz frequency. The accuracy and stability of the oscillation are affected by the quality of the passive components as well as operational amplifier characteristics. Good choices for the elements in the positive-feedback network are polycarbonate capacitors and 1% thin-film resistors. In order not to unbalance the bridge precision resistor with low tolerance and low temperature coefficient must be used. Operational amplifiers with low offset, low input bias current and low long-term drift minimize or eliminate recalibration requirements. A good choice for oscillator circuit, Wheatstone bridge and precision rectifier circuit is to use an auto-zero amplifier, such as the Analog Devices AD8554, which has ultralow offset, drift, and bias current. For the low-pass filter and the temperature sensor amplifier it is possible to use a cheaper operational amplifier, like the Analog Devices OP07. It presents good features and it is 10 times cheaper than AD8554.

The LM35 temperature sensor has a sensitivity of 10 mV/°C. To adapt the output voltage of the sensor to the input range of the ADC (embedded in microcontroller) an amplification of 10 V/V has been implemented. Without amplification the ADC should have more bits to obtain the same temperature resolution.

The microcontroller is connected to the computer via the FT232R USB serial interface. This feature permits to show the EC in real-time, without increase significantly the price of the prototype. In this way the farmer could have a more complete information about the time evolution of the mastitis.

The microcontroller programming is developed in C language using the CCS compiler. Fig. 7 shows flow chart for performing conductivity measurements and mastitis detection. Firstly, the ports, the ADC and the LCD are initialized. Then, the temperature channel is selected and the A/D conversion is launched, keeping the result in a variable which is subsequently dealt with to convert the result in °C. Next, the channel conductivity is selected and a new conversion is launched. Conversion voltage to conductivity is done using the obtained calibration curve. An external 20 quartz crystal is used for the microcontroller. The reference voltage of the ADC was VDD equal to 5 V. This voltage was selected by software by means of bits PCFG3:PDFG0 of the ADCON1 register of the microcontroller.

A cow suffering from mastitis may not always show an increased ECM from the infected quarter, but the within-milking variation in EC from an infected quarter may be larger than variation in EC of milk from healthy quarters. Therefore, a combination of the absolute conductivity values and the differential conductivity values between quarters is used to detect abnormal milk as shown in Fig. 7. Typical thresholds values of conductivity are:  $\sigma_{th} = 6.2 \text{ mS/cm}^{-1}$ ,  $\Delta\sigma_{th} = 0.5 \text{ mS/cm}^{-1}$  [8].

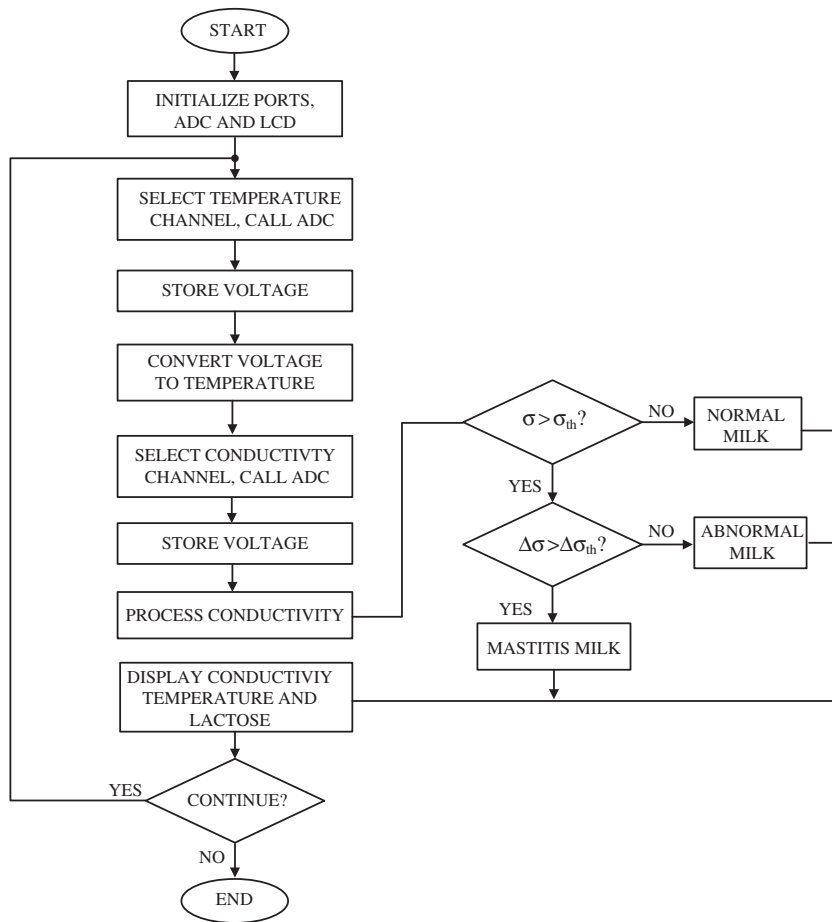


Fig. 7. Flow chart for performing conductivity measurement and mastitis detection.

#### 4. Experimental results

The calibration procedure is made using NaCl solutions with EC close to ECM. The EC of these solutions are measured using both a commercial conductivity meter (Testo 252) and the output voltage of the prototype, obtaining the calibration curve of Fig. 8.

For the range of EC values among which the milk is found it can be adjusted by a straight line using the method of least squares. The error of nonlinearity, referred to full scale is about 4.2%. The equation of the straight line is implemented in the microcontroller to obtain the ECM. The measurement system was tested with different samples to check the reproducibility.

A desktop application has been also developed in Python language to show the time evolution of EC, using the USB interface. Fig. 10 shows an example of EC profile of all four quarters from one subclinical infected cow. EC was measured in milk from each quarter during every milking at 2-s intervals.

On the other hand, lactose is one of the most important indicators of the quality of milk. In the incidence of mastitis, the lactose decreases in milk and consequently sodium and chloride ions levels increase in the milk in order to

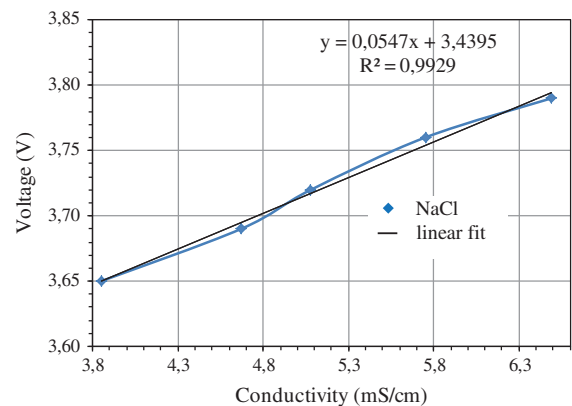


Fig. 8. Calibration curve for different values of EC.

maintain and adequate osmotic pressure [18], hence giving rise to a salty taste. There were analyzed milk samples from 20 cows in the first lactation obtaining the percentage of lactose. The samples are provided by The Dairy, Food and Agriculture Laboratory of Asturias, (Spain). Fig. 9 shows the relationship between the percentage of lactose

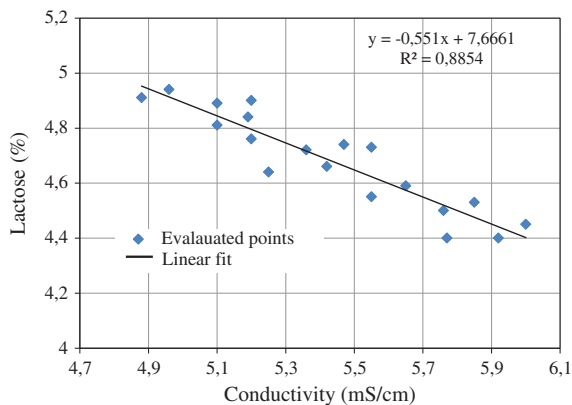


Fig. 9. Relationship between lactose and ECM.

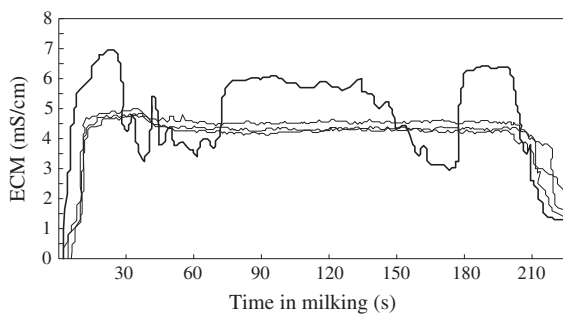


Fig. 10. EC time variation of 4 quarters of a cow with mastitis. The bold line shows the EC profile of the infected quarter.

and the ECM. The error of nonlinearity, referred to full scale, is about 8%.

## 5. Conclusions

Bovine mastitis is a significant disease of dairy herds, having a large adverse effect on farm economics, due to a reduction on milk production and treatment costs. There are a large number of methods of detection currently in use, in order to monitor udder health performance. Most of these methods are extremely accurate, however they are expensive and are not available for cow-side. This paper propose a low-cost electronic circuit for helping the farmer to detect quickly and economically the state of health and the milk quality of their cows is proposed. It is based on measure of electrical conductivity of milk on the dairy farm itself. Abnormal milk is detected based on absolute conductivity values and differential conductivity values between quarters of udder. The accuracy and repeatability of the instrument are compared to Testo conductivity meter and it is found that error in measurement

is less than 5%. This error may be acceptable is this kind of application. The prototype has high sensitivity, easy to use and its cost is around \$20.

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