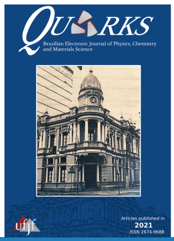
QUARKS: Brazilian Electronic Journal of Physics, Chemistry and Material Science
journal homepage: https://periodicos.ufjf.br/index.php/quarks
e-ISSN: 2674-9688



| Vol 4 | | No. 1 | | 2022 |

Electrical characterization of milk samples by Electrical Impedance Spectroscopy (EIS)

Lárissa Kellen Ribeiro de Lima[a](larissakellenrl@gmail.com), Isabela Tavares Costa dos Santos[a] (isabelatcsantos@gmail.com), Beatriz Duarte Lauredo da Silva[a] (beatrizduartelauredo@hotmail.com), Virgílio de Carvalho do Anjos[b](virgilio.anjos@ufjf.edu.br), Maria José Valenzuela Bell[b](mjbell06@gmail.com), Wesley William Gonçalves Nascimento[a](wesley.nascimento@ufjf.edu.br)

[a]Department of Pharmacy, Federal University of Juiz de Fora, CEP 35010-177, Governador Valadares, Brazil.
[b]Department of Physics, Federal University of Juiz de Fora, CEP 36036-330, Juiz de Fora-MG, Brazil.

*Article history:*Received:; revised:; accepted:. Available online:. DOI:

Abstract

This work presents a study of electrical impedance spectroscopy (EIS) as an alternative to characterize milk, aiming to develop an analysis methodology. The behavior of the electrical impedance module, the real part and the imaginary part (reactance), and the derived quantities such as admittance, phase and diffusion coefficient (D) were also analyzed and will be discussed. Comparisons of the measured spectra with theoretical models of equivalent circuits were carried out in order to understand the electrical behavior of the samples. Understanding this electrical phenomenon will help in the development of future analysis methodologies and applications. The results indicated that milk is a solution with predominantly resistive behavior with resistance of approximately 500 ohms, capacitance of about of 0.5 microFarad (μF) and admittance of the order of miliSiemens (mS), presenting small storaged charge due to low viscosity. The best-fitting equivalent circuit was a mixed RC model consisting of a series resistor coupled with a resistor in parallel with a capacitor.

Keywords: Development of Methodology; Electrical Impedance Spectroscopy; Materials Characterization;Technologic Innovation.

1. Introduction

In the last decade, several analytical procedures have been proposed for selective confirmation of milk quality and authenticity. Liquid chromatography and gas chromatography (HPLC, GC) are conventionally used for highly selective determinations, especially coupled with mass spectrometry (MS) [1]. Spectroscopic techniques, including Near Infrared (NIR), Middle Infrared (MIR), Ultraviolet-Visible (UV-Vis), Raman Spectroscopy, Digital Image Analyses were used to detect milk adulteration and to evaluate authenticity and intrinsic quality parameters [2]. However, these methods are often laborious, slow and costly.

*Corresponding author. E-mail:wesley.nascimento@ufjf.edu.br mailto:

In this context, a technique based on electrical measurements in milk emerges as an alternative to be applied to small and medium industry. From the electrical point of view, milk can be understood as an electrolytic solution in which the charge carriers are originated from the dissociation of salts. Therefore, as stated in SADAT; MUSTAJAB; KHAN, 2005 apud DURANTE (2016), the electrical conductance of milk is mainly attributed to the presence of ions, in particular, Na^+, K^+ and Cl^- [3]. Adulterations, for example, cause a change in the electrical behavior of milk when subjected to an external field.

The Electrical Impedance Spectroscopy (EIS) can be applied in the characterization of biological samples, based on the electrical response of these samples in a frequency range. The technique has also been shown to be useful in investigating the electrical properties of the membrane. Some parameters related to electrical and electronic transfer properties in and through the membrane can be extracted through equivalent electrical circuit analysis [4]. The technique can be used to differentiate normal cells from cancer cells [5]. There are other applications of the technique such as an alternative to differentiate biodiesel fuels prepared from vegetable oils [6].

In the literature, there are works using electrical techniques for the characterization of ultra-pasteurized milk [7] and in quality control, to determine the content of additives in liquid food products [8]. In milk are used for verification of the authenticity and safety, essential for industries [9] [10]. It allows exploring changes in the innate properties of milk, such as electrical conductivity and pH, after the addition of adulterants [11].

This work presents a study of EIS in milk samples. And it aims to understand the electrical characteristics of milk and compare them with equivalent electrical circuits. To characterize samples by EIS it is important to know the main spectral profiles of different configurations of standardized circuits. This is essential for making comparisons between these profiles and the profiles of the analyzed sample. This comparison is important for the development of future applications of the technique. The impedance module, real and imaginary part of the impedance, admittance, phase and diffusion coefficient (D) were also studied in samples of genuine milk, in the frequency range of 1 kHz to 5 MHz and the results will be discussed.

2. Material and Methods

The electrical impedance measurements were performed in a HIOKI analyzer, model 3170, stimulated by an alternating external electric field, of the sine wave (AC) type, with 1 V peak to peak, in a frequency range from 1 kHz to 5 MHz. Two platinum plate electrodes in parallel (geometric constant K = 1 cm^{-1}) were adapted to the instrument (Figure1). In all experiments, the temperature (20 °C) was kept constant.

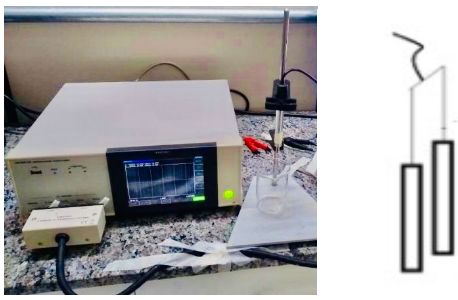


Figure 1: two platinum plate electrodes in parallel adapted to the impedance analyzer and simplified geometry of a cell for measurements in liquids.

Experiments were performed on samples of genuine raw milk collected from industries in the Governador Valadares region of Minas Gerais, which have activities focused on the milk production chain. All raw milk samples were in accordance with Brazilian legislation as shown in Brazil (2018), that is, with the following indices: acidity 14-18°Dornic, density of 1,028 g mL^{-1} to 1,032 g mL^{-1} and maximum cryoscopic index of -0.530 °H [12].

The measured quantities were: impedance module, real and imaginary impedance parts, admittance, phase and diffusion coefficient (D). The spectra were generated from OriginPro 9.0.0 (64-bit) SR2 b87 Data Analysis and Graphing Software.

3. Results and Discussion

3.1 Theoretical reference

The EIS is a very useful technique to characterize the electrical behavior of materials [13]. It consists of analyzing the material sample by applying an external electrical stimulus measuring the response of the system. Electrical quantities derive from the mobility of the ionic species in solution that depend on several factors related to the material.

The most common stimulus is to use an alternating voltage of the sinusoidal type, and to measure the real and imaginary parts of the complex impedance as a function of frequency, which generate characteristic spectra of each material. The parameters derived from a frequency spectrum are generally in two categories: those pertinent to the material itself, such as conductivity, dielectric constant, charge mobility, charge equilibrium concentration; and those pertinent to an interface between the material and the electrode, such as interfacial region capacitance [14]. In this work, the main interest will be to analyze the sample itself and the correlated quantities, therefore, the interface will be standardized for all measures, focusing on studying the influence of the medium.

3.1.1 Equivalent circuits

When switching from a DC circuit to an AC circuit, the concept of resistance as a parameter, which is usually attributed to resistors, needs to be extended because, in addition to resistors, capacitors and inductors, also provide resistance to the passage of the current. The resistances that these elements oppose to the alternating current are called reactive resistors or reactances. From the point of view of dissipated energy, the difference between resistance and reactance is that in the resistance, the energy is dissipated only in the form of heat, whereas in a reactance the energy is stored periodically in electric or magnetic fields without losses by heat. The joint action of resistance and reactance is defined as impedance [15].

The equation 1 is formally identical to the law of ohm, with impedance Z performing the same function of the equivalent resistance, but in an AC circuit. Therefore, the radical Z (equation 2), represented the impedance of the 3 elements, R, L and C. In impedance studies an approach with complex variances is required. In a series circuit, the real part of the impedance (Re[Z]) can be counted as resistance (R) and the imaginary part of the impedance (Im[Z]) can be associated with the reactance (X). Capacitive and inductive reactances are represented, respectively, by X_c and X_L, where ω is the angular frequency, C is capacitance and L is inductance. The inverse of the impedance is exactly the admittance (Y).The phase is represented by θ and the diffusion coefficient by D.

.

$$V = ZI \qquad \text{(Equation 1)}$$

Where,

$Z = \sqrt{R^2 + (X_L - X_C)^2}$(Equation 2)

$X_C = \frac{1}{\omega C}$ (Equation 3)

$X_L = \omega L$ (Equation 4)

$Y = \frac{1}{Z}$ (Equation 5)

$\theta = \mathrm{arctg}\left(\frac{\mathrm{Im}(Z)}{\mathrm{Re}(Z)}\right)$ (Equation 6)

$D = \frac{\mathrm{Re}(Z)}{\mathrm{Im}(Z)}$ (Equation 7)

Equation 2 provides the impedance only for elements in series, however, can be deduce similar equations for the impedance Z in circuits with more complex configurations, generalizing the concept of resistance to the real part of the impedance. In this way, it is more convenient to treat the impedance in terms of the real part (Re[Z]) and imaginary (Im[Z]), as shown in equation 8:

$Z = \sqrt{[Re(Z)]^2 + [Im(Z)]^2}$(Equation 8),

Where,

$[Im(Z)] = X_L - X_c$ (Equation 9)

The real and imaginary parts of the impedance are associated respectively to the energies, dissipated and stored (in the form of electromagnetic field) in the system.

Compare the profiles of spectra measured in samples of interest to theoretical models of circuit in traditional configurations, helps to understand the behavior and nature of the studied material. Due to the nature of the milk, inductive effects are neglected and we can restrict the study to RC models, with different configurations. According to Delgado et al. (2003) and Chinaglia et al. (2009) it is possible to demonstrate that the series, parallel and mixed RC circuits (Fig. 2a, 3a e 4a) can be described respectively by the following Eqs. 10, 11 and 12, where the real part and the imaginary part are separated and have specific graphic behaviors [16][15].

It is possible to observe, in the equations, that for the case of series circuits, only the imaginary part is a function of the frequency, while for the parallel and mixed circuits both the real and the imaginary part demonstrate dependence on the excitation frequency of the system. Figures 2, 3 and 4shows theoretical spectra and example of diagrams, from Eqs. 10, 11 and 12, respectively. The numerical values in the spectra are illustrative.

Series RC circuit:

$$Z^* = R - \left(\frac{1}{\omega C}\right) j \quad \text{(Equation 10)}$$

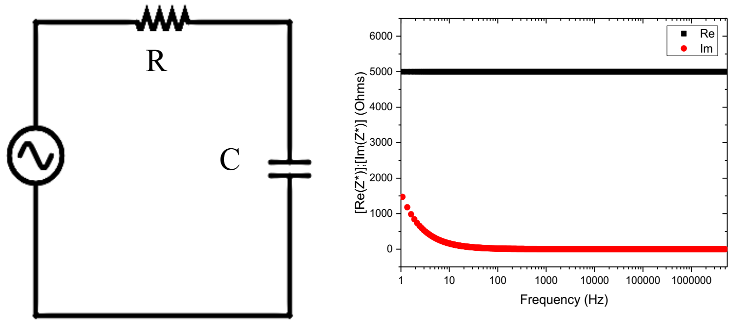


Figure 2: a)a capacitor and a resistor in series; b)spectrum of impedance for a capacitor and a resistor in series.

Parallel RC circuit:

$$Z^* = \frac{R(1-j\omega RC)}{1+(\omega RC)^2} = \frac{R}{1+(\omega RC)^2} - \frac{\omega R^2 C}{1+(\omega RC)^2} j \quad \text{(Equation 11)}$$

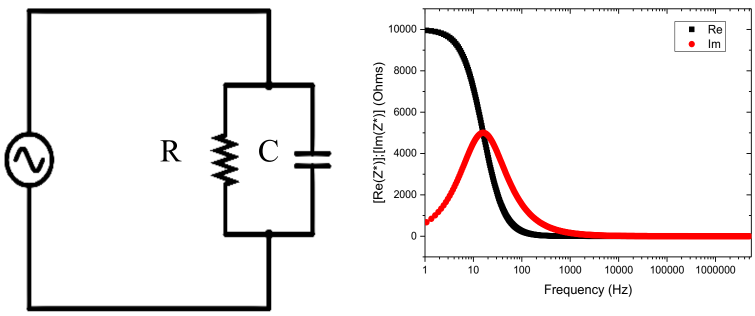


Figure 3: a)a capacitor and a resistor in parallel; b)spectrum of impedance for a capacitor and a resistor in parallel.

Mixed RC circuit:

$$Z^* = R_2 + \frac{R_1}{1+(\omega R_1 C)^2} - \frac{\omega {R_1}^2 C}{1+(\omega R_1 C)^2} j \quad \text{(Equation 12)}$$

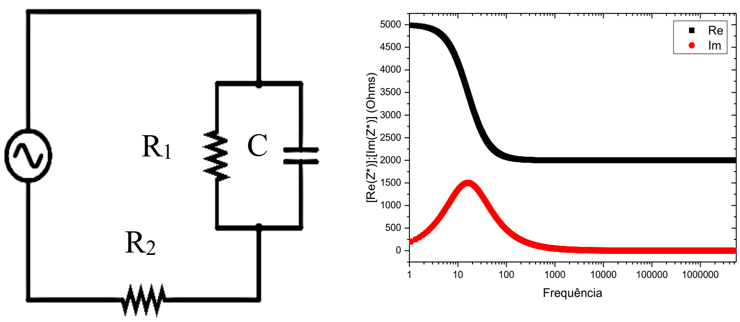


Figure 4: a) a resistor (R2) in series with an association of a resistor (R1) in parallel with a capacitor; b) spectrum of impedance for a resistor in series with an association of a resistor in parallel with a capacitor.

Compare impedance spectra measured with known profiles, such as those shown in Figs. 2, 3 and 4, helps to identify the electrical characteristics of the samples. The equivalent electrical circuits allow to reproduce the electrical characteristics of the sample. This work brings the comparison between impedance spectra measured in milk with known profiles, in order to identify the electrical characteristics of this sample.

3.2 Measures of impedance module, real and imaginary part, admittance, phase and diffusion coefficient

The experiments were carried out at frequencies above 1kHz to avoid adsorption effects characteristic of low frequencieswhen platinum electrodes are used. According to Lenzi et al. (2013), if considered the phenomenon of adsorption, it gives rise to an increase of the real part of the impedance, Re[Z], at the limit of $\omega \rightarrow 0$ (below 1kHz), compared to the predicted theoretical values[17]. The interest of the work being the analysis of the material itself and not specifically the region and the interface effects between the electrode and the sample in the system.The spectra measured in the samples of raw milk in this frequency range are shown in figures 5 to7.

The electrical impedance module showed a dependence with the frequency (figure 5)in which it decreases with increasing frequency. The electrical impedance module is a measure of the energies dissipated and stored by the system and has a concept associated with the opposition to conduction of electric current, similar to resistance, but in an alternating system (AC). These results are in accordance with the expressions described in equations 10, 11 and 12.The effects related to impedance module are more intense at low frequencies. In terms of the electrochemical system, we can associate this observed spectrum profile to the fact that, with increasing frequency, there is a reduction in the number of ions oriented by the external excitation field due to their masses and interaction effects, it reduction in the number of load carriers moving and thus there is a reduction in the energy dissipated and stored in the system and a reduction in signal the impedance module measured.The electrical impedance moduledepends on each material, demonstrating that this quantity has the potential to be used in the characterization of samples.

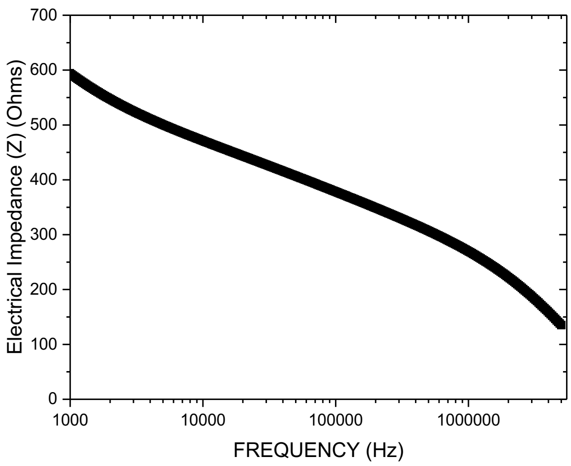


Figure 5: spectrum of impedance module in raw milk.

The real and imaginary part of the impedance are discussed in Fig. 6a. The analyzed samples present resistance value measured at approximately 500 ohms, which can be measured by this approach from the low frequency spectrum (plateau) trend value (figure 6a). This resistance value can be considered low, due the milk isa conductive electrolyte. This is due to a significant concentration of free ions, especially chloride, sodium and potassium.

Comparing the spectrum, the imaginary part with theoretical models of circuits (Eqs. 10, 11 and 12), it appears that milk presents a behavior similar to that of a mixed RC circuit (represented by Eq. 12, and Fig. 4). Although the imaginary part has low values and close to zero, this spectrum has a small band, positioned at the megahertz (MHz) frequency, which indicates a poorly selective circuit. This is explained because milk is a disorganized system with too many ions available for conduction. The real part showed a slight decrease. This is because milk has a low capacitive profile and is predominantly resistive across the spectrum, due to its low viscosity, with little charge accumulation, having the greatest contribution by the effects of conduction and energy dissipation, similar to a purely resistive circuit (R), where Im[Z] is zero and Re [Z] is constant, being equivalent to resistance.

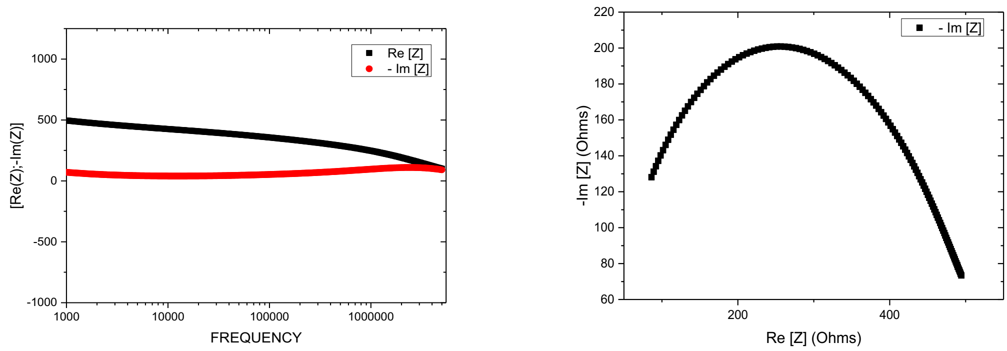


Figure 6: a) spectrum of the real part and the imaginary part of the impedance of raw milk; b) imaginary part of the impedance as a function of the real part.

In EIS studies, an approach can be taken by plotting the imaginary part of the impedance as a function of the real part. Figure 6b shows the resulting graph measured for a frequency range from 1 kHz to 5 MHz. The figure is a semicircle where it is deduced, as in Souza et al. (2013), that the diameter of an impedance semicircle is equivalent to the resistance of the analyzed material [18]. Again, the results indicated a sample resistance around 500 ohms. Analyzing this same Re[Z] spectrum at the frequency of 1kHz and using equation 3, it is possible to estimate the capacitance of milk around 0.5 microFarad(0.5μF).

There are other measured or derived quantities that are related to impedance [14]. Some of these quantities were evaluated and presented in Figs. 7a, b and c. The electrical admittance (Fig. 7a) is described as the inverse of the impedance, which can be seen by Eq. 5 and, therefore, has a spectrum opposite to the impedance. The admittance measures the conduction capacity of the milk in an alternating current circuit. In the spectrum of figure 7a it is possible to observe that the admittance tends to increase with frequency, having an order of magnitude in millisiemens, which is relatively high, compared to water, for example, due to the amount of charge carriers present in the milk.

The ion orientation effects result in a delay in the electrical response of the system in relation to the excitation electrical field and this delay (phase) is shown in Fig. 7b.The phase angle, mathematically, can be indicated as the tangent arc of the ratio Xc to R [19], that is, in terms of complex variance, the tangent arc of the imaginary part of the impedance to the real part of the impedance (Eq. 6).In a vector approach, it is the angle formed between the impedance vector Z and the real part Re[z].It may be noted that the electrochemical system has a resonance region where the delay (phase) is minimal approximate zero and therefore it is the frequency at which the system has the greatest possible orientation of the charge carriers. For system purely resistive, the phase is zero [14], as milk has a predominantly resistive behavior, the phase approaches zero for a most the frequencies. Only for high frequencies the phase increases tending to a maximum lag of 90º that occurs in the megahertz region, when the charge carriers are disoriented.

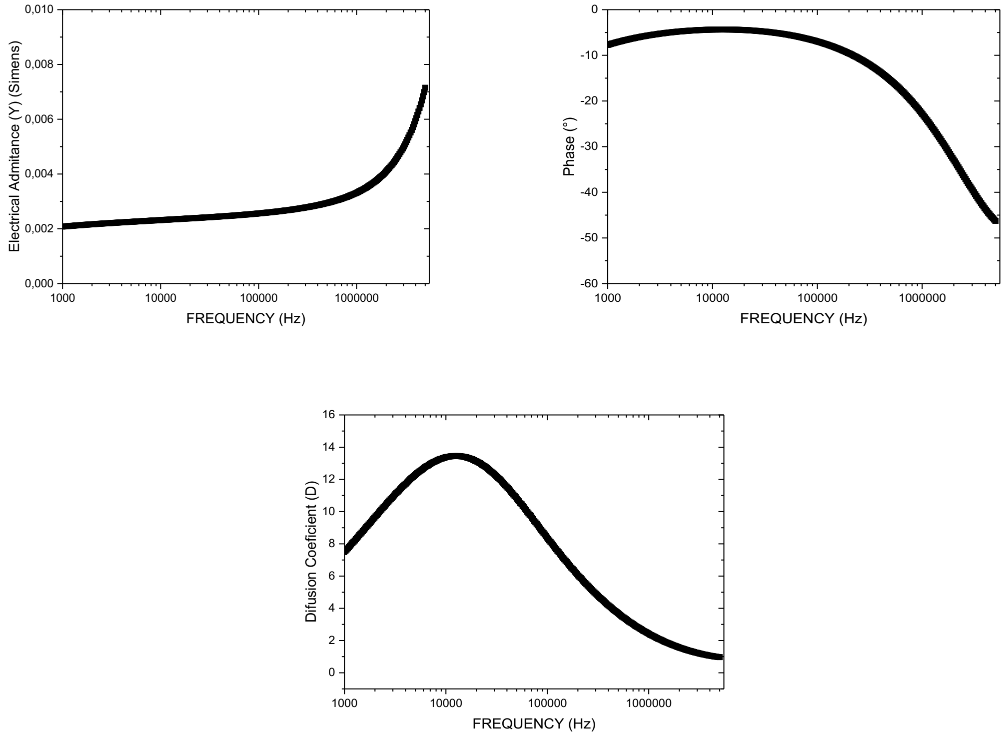


Figure 7: a) admittance spectrum of raw milk; b) phase spectrum of raw milk;c)spectrum of the diffusion coefficient of raw milk.

The diffusion coefficient (D) (Fig. 7c, Eq. 7) measures the ratio of energy dissipated at the system in and energy stored an electric field format, (in this case, RC circuit). The diffusion coefficient (D), presented a band with a peak around 10 kHz. This frequency point is highlighted is related to the resonance of the system(electrode + milk)seen also in the phase spectrum previously discussed and indicates a region greater alignment of charge carriers in relation to the applied electric field and is particular to each material. The position this point also have potential to be used for characterization of a sample, for vary depending on its composition. This effect becomes more pronounced in the spectrum of the diffusion coefficient in the case of milk, due to the fact that it is predominantly resistive and not capacitive.

5. Conclusions

This work presented an electrical impedance study measured in raw milk, evaluating spectral profiles of standardized equivalent circuits to characterize samples and comparing them with profiles obtained from milk samples. From the comparisons with the theoretical profiles of each equivalent circuit type and with the equations of electrochemical model fittings, it can be concluded that the milk can be adjusted by a mixed type RC circuit with equivalent resistance values of the 500 ohms, of capacitance around 0.5 microFarad(0.5μF) and admittance in the order of MilliSiemens (mS) measured at a temperature of 20 °C. The samples presented a predominantly resistive character and low capacitance, that is, the main effects observed were dissipative and the energy stored in the system due to the accumulation of charges was small. These effects were complemented with study of diffusion coefficient (D) that a peaked around 10 kHz. This point refers to the resonance point of the system where there is a smaller delay in relation to the external excitation fieldas was evidenced in the measured phase spectra. Therefore, electrical impedance spectroscopy (EIS) can be used in the electrical characterization of milk samples, helping to understand milk behavior and has the potential to be used as a basis for the development of analytical methodologies.

Acknowledgments

The authors would like to thank CAPES, CNPq, FAPEMIG and Propp / UFJF for research funding.

References and Notes

[1]Abernethy, G.; Higgs, K. *Journal of Chromatography.***2013**, 1288, 10.https://doi.org/10.1016/j.chroma.2013.02.022.

[2]Nascimento, C. F.; Santos, P. M.; Pereira-Filho, E. R.; Rocha, F. R. P.*Food Chemistry.***2017**, 221,1232.https://doi.org/10.1016/j.foodchem.2016.11.034.

[3]Durante, G.; Becari, W.; Lima, F. A. S.; Peres, H. E. M.*IEEE Sensor Journal.***2016**, 14,861. https://doi.org/10.1109/JSEN.2015.2494624.

[4] Zhang, M.; Zhai, Q.; Wan, L.; Chen, L.; Peng, Y.; Deng, C.; Xiang, J.; Yan, J.*Analytical Chemistry.***2018**, 90, 7422. https://doi.org/10.1021/acs.analchem.8b00884.

[5] Giana, F. E.; Bonetto, F. J.; Bellotti, M. I. *Physical Review e 97.***2018**, 97, 032410-1.https://doi.org/10.1103/PhysRevE.97.032410.

[6] M'Peko, J.-C.; Reis, D. L. S.; De Souza, J. E.; Caires, A. R. L.*International Journal of Hydrogen Energy*.**2013**, 38, 9355.https://doi.org/10.1016/j.ijhydene.2013.05.086.

[7] Lopes, A. M.;Machado, J. A. T; Ramalho, E.; Silva, V.*Food Analytical Methods.***2017**, 11**,**901. https://doi.org/10.1007/s12161-017-1054-4.

[8] Nakonieczna, A.;Paszkowski, B.; Wilczek, A.;Szypłowska, A.;Skierucha, W.*Food Control.***2016***,*66,116. https://doi.org/10.1016/j.foodcont.2016.01.044.

[9] Nascimento, W. W. G.; Oliveira, M. A. L.; Furtado, M. A. M.; Anjos, V. C.; Bell, M. J. V. *Journal of Food Science and Engineering.***2013**, 3, 363.https://dx.doi.org/10.17265/2159-5828/2013.07.004.

[10] Das, S.;Goswami, B.;Biswas, K.*Sensor Letters.***2016**, 14, 4. https://doi.org/10.1166/sl.2016.3580.

[11] Tripathy, S.; Ghole, A. R.; Deep, K.; Vanjari, S. R. K.; Singh, S. G.*Food Chemistry.***2016**,217, 756. http://dx.doi.org/10.1016/j.foodchem.2016.09.037.

[12] BRAZIL. Normative Instruction No. 76, of November 26, 2018. Approves the Technical Regulations that establish the identity and quality characteristics that must present refrigerated raw milk, pasteurized milk and pasteurized milk type A, in the form of this Normative Instruction and the Single Annex. Official Gazette of the Union: section 1, Brasília, DF, edition 230, p. 9, 30 Nov. **2018**.

[13] Grossi, M.; Riccò, B. *Journal of Sensors and Sensor Systems.***2017**, 6,303. https://doi.org/10.5194/jsss-6-303-2017.

[14] Macdonald, J. R.; Johnson, W. B. *Impedancespectroscopy: Theory, ExperimentandApplications.***2005**, 1.https://doi.org/10.1002/0471716243.ch1.

[15] Chinaglia, D. L.; Gozzi, G.; Alfaro, R. A. M.; Hessel, R.*Revista Brasileira de Ensino de Física.***2009**, 30, 4504.https://doi.org/10.1590/S1806-11172008000400013

[16] Delgado, A.; García-Sánchez, M. F.;M'Peko, J.-C.; Ruiz-Salvador, A. R.; Rodríguez-Gattorno, G.;Echevarría, Y.; Fernández-Gutierrez, F. *Journal of Chemical Education*. **2003**, 80,1062.https://doi.org/10.1021/ed080p1062.

[17] Lenzi, E. K.; Fernandes, P. R. G.; Petrucci, T.; Mukai, H.; Ribeiro, H. V.; Lenzi, M. K.; Gonçalves, G.*International Journal of Electrochemical Science.***2013**, 8,2849.

[18] De Souza, J. E.; Scherer, M. D.; Cáceres, J. A. S.; Caires, A. R. L.; M'Peko, J. -C.*Fuel.* **2013,** 105, 705. https://doi.org/10.1016/j.fuel.2012.09.032.

[19] Vicenzo, O. D.; Marra, M.; Scalfi, L.*JournaloftheInternational Society of Sports Nutrition.***2019,** 16. https://doi.org/10.1186/s12970-019-0319-2.