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DETERMINATION OF DEATH TIME USING DIELECTRIC SPECTROSCOPY TECHNIQUE

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ABSTRACT
The dielectric constant, impedance and dissipation factor for the life rat liver was measured in-vivo as a function of frequency at room temperature. Also, same measurements were repeated just after death and after different times ranging from 10-120 mins in order to evaluate the death time using dielectric spectroscopy techniques. The broad dielectric dispersion suggests the widely distributed relaxation time. The ac impedance increased with increasing the time after death. The peak frequency (resonance frequency) shifts to lower values with passing from life to death and by increasing death time.

KEYWORDS: Death time- liver – post mortem- forensic medicine-dielectric spectroscopy-relaxation time-resonance frequency

INTRODUCTION

One of the responsibilities of the forensic pathologist is to estimate the time of death. An accurate assessment is of great importance to police in narrowing down the list of suspects. It can allow police to pinpoint the time during which they need to find out what the suspects were doing and allows them to eliminate people who have an alibi for that period from their enquiries. Liver mortis is also known as hypostasis [(1)]. It is a term used to describe the draining of the blood to lower portions of the body due to the influence of gravity. Most literatures consider the fall in the availability of adenosine triphosphate (ATP) as the possible cause of terminal muscle fiber contraction after death [(2)]. Other plausible explanation includes the influx of calcium ions after cessation of the sodium pump. Hemoglobin inhibition imparts a shiny reddish discoloration on the surface of the liver. The liver parenchyma close to the gall bladder may be stained yellowish or greenish because of bile inhibition. As time progresses, the liver may loss its turgidity, become soft and clay-like in colour and consistency. The presence of gas-distended bubbles on the parenchyma usually suggests a more advanced stage of post mortem decomposition [(3)]. Liver has a "swiss cheese" appearance due to the invasion and proliferation of gas-producing organisms. The liver is our concern in this study because it is easily accessible in-vivo and for the large delay time in organ transplant. The most remarkable difference between our experiments and the ones in the literature [(4)] was that in our work these experiments were performed *in vivo*. This fact led us to think that the organs could vary their properties soon after excision from the living body. The novelty of this study could be emphasized from one hand to precisely determine the death time using a non invasive quick and cheap method. On the other hand, the results could serve as database for liver transplantation from post died animal.

This study aims to determine accurately the exact time within the first 2 hrs following death using a simple, non-destructive and fast technique by applying dielectric and impedance measurements. This involves the use of an impedance analyzer (RLC meter) and is used for the first time by MSL(1) group.

MATERIALS AND METHODS

Animals

Four Wistar adult albino rats (130-150 g); obtained from Animal House, Kasr Elini, Cairo, were used for this study. Animals were kept at a good hygienic conditions and Food and water offered *ad libitum*.

Anesthesia

The rats were anesthetized using pentobarbital sodium 30 mg/kg diluted in distilled water and injected intraperitoneally. The anesthesia level was determined by observing the palpebral and respiratory reflexes and muscle tone. The animals were considered anesthetized when their respiration was regular and superficial and muscle tone was flaccid with absence of reflexes [(5)].

Experiment

The animals were subjected to laparotomy after a midline abdominal incision under sterile conditions. After the identification of the liver, a transverse incision was made. For the characterization of the electrical properties such as Dielectric permittivity, impedance and AC conductivity for the rat liver cells in-vivo, we measured the frequency dependence of the abovementioned properties at rat body temperature. The electrical properties were measured using 2 platinum parallel electrodes (0.6*1.2cm) inserted in the right lobe of the liver (Fig. (1.a); and the other end is connected to the RLC meter (Hioki-3532) with two shielded cables. The measurements were carried out in the frequency range of 45 - 5 $x10^6$ Hz using a labview program for data acquisition. The program collects the

average of 8 readings for each parameter at each single frequency.

At end of experiment, animals were euthanized according to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Vermont and the rest of experimental part times were measures [6].

Experimental design

Data obtained shortly before death, directly after death, then after 10, 20, 30, 40, 50, 60, 90 and 120 minutes from euthanized death.

Statistical evaluation: Mean of corresponding values for 4 rats were calculated from the obtained data using [7].

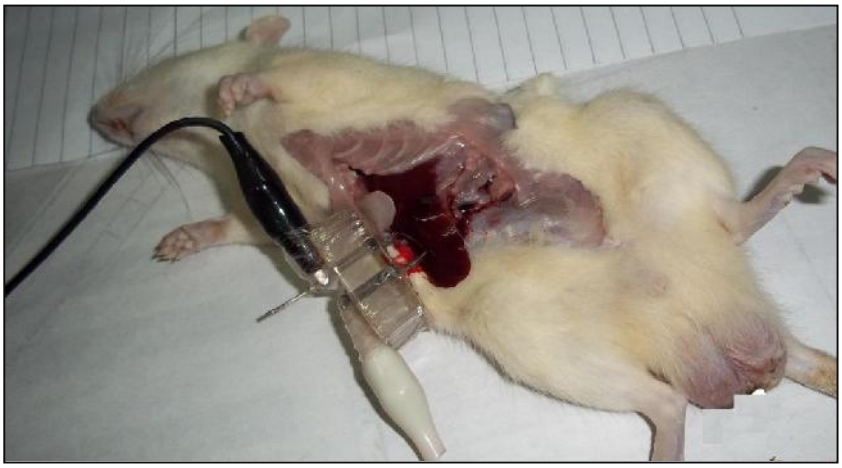

FIGURE 1a. The position of the 2 electrodes during measurements.

RESULTS AND DISCUSSION

Figure (1.b) illustrates the variation of the in-vivo impedance (Z) of the liver as a function of frequency. The impedance decreases with increasing frequency which is a general trend. The molecules of the fluid inside the liver tissue could not follow up the fast variation of the frequency accompanied by the external ac field. These results agree with that reported [8]. The spectrum in Fig. (1) could be divided into three regions; the low frequency region (I) in which the rate of decrease of Z is small Region (II) extends from 10^3 up to 10^5 Hz in which the rate of decreasing Z increases remarkably.

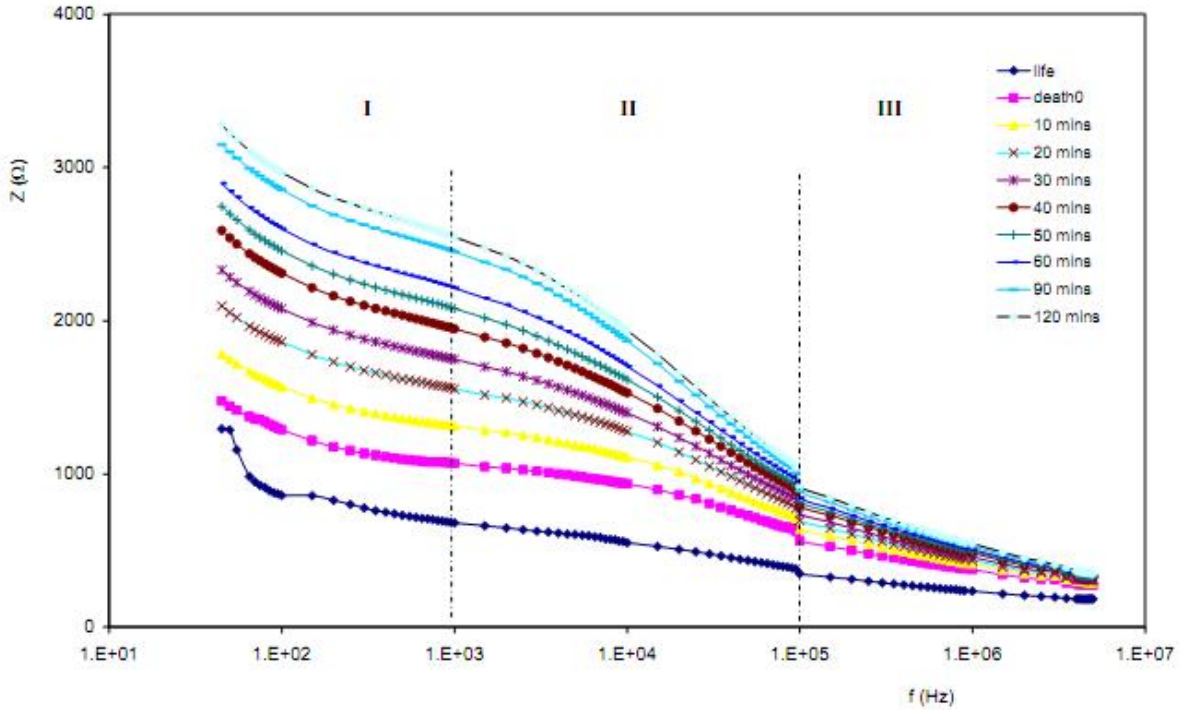

FIGURE 1b. Variation of the in vivo impedance of the liver with applied frequency at different death time

The last region (III) which is the high frequency one shows the largest decrease in the rate with less dispersion. The low frequency region (region I) informs about extracellular events whereas the high frequency (region III) informs mostly about intracellular events. Region II where an impedance drop is observed corresponds to the membrane capacitor shortening. The obtained data traced a broad dielectric [9-11] dispersion curve covering the measurable range of frequency. This suggests the widely distributed relaxation time. The increase in time after death leads to a monotonic increase in the impedance of the liver tissues. This could be reasonable and is attributed to the decrease in the electrolyte mobility. This is a direct result of the increase in the liver fluid viscosity. Also, the observed decrease in the degree of έ and times is explained on the basis of changing the viscosity of the liquid contained in the liver tissues as well as the variation of the valance of the cations exist in the liquid. From a hydrodynamic point of view, the rate of flow of liver fluids decreases after death and with increasing time which offered on the measured values. In our case, the in-vivo measurement involves the deal with the liver as a part of the body organs which was not separated. Consequently, this plays a significant role in the increase in the ac impedance. The change in the liver color from bright to dark to brownish red from the beginning to the end of the period under investigation (120 min) as observed during the experiment is expected to be due to the change in the oxidation state of the metal ions responsible for the conduction in this tissue. Accordingly, this contributes to the increase in the impedance with the death time at any frequency. The organs impedance, observed after death, could reflect deleterious changes originated by the interruption of the blood flow: in ischemia, anoxic injury starts with a decrease in mitochondrial energy production; in parallel, cellular ion homeostasis becomes impaired resulting in increased cytosolic calcium and sodium concentrations, which may activate lytic enzymes, cause osmotic swelling, disruption of plasma membrane and, ultimately, cell death [12].

Figure (2) illustrates the variation of the tangent of the dielectric loss angle with frequency as a function of death time. The plot shows a spectrum characterized by a broad relaxation peak; from which the relaxation time can be calculated. The dielectric loss is found to increase from life to reach maximum just after death (death time 0) and then decreased with increasing death time which is clear in the inset (a) of Fig. (2). As it is well known that, at the death time the electric impulses increased suddenly followed by a sharp drop in the pulse rate. Consequently, the rate of oxidation process was increased helping in increasing the viscosity as well as the friction between the dipoles. This will result in increasing the amount of energy dissipation and the increase in tanδ. The peak frequency (resonance frequency) shifts to lower values with passing from life to death and by increasing death time. This was an expected result because at the moment of death and post, the frequency of the vibrating atoms and molecules in liver tissues will be decreased. Thereby, this gives more relaxation [13, 14] as a direct result of weakening the bond strength.

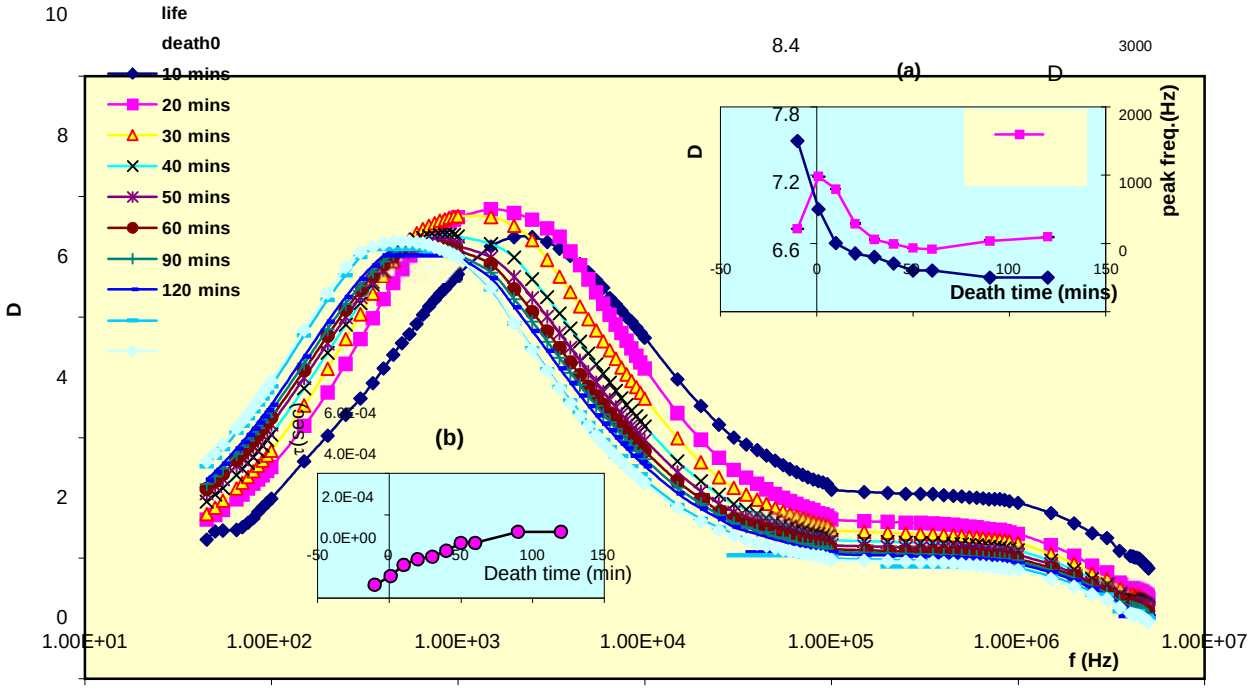

FIGURE 2. Variation of the in-vivo tangent of the dielectric loss angle of the liver with the applied frequency
Inset a: the peak value of D and the peak frequency as a function of death time
Inset b: the calculated values of the relaxation time in seconds as a function of death time

Accordingly, the resonance frequency decreased accompanied by a slight increase in the relaxation time (inset b) until reaching stable values after 60 minutes. Cell death, such as apoptosis or necrosis, is usually accompanied by a diminished relaxation due to a cell membrane that is not able to separate the cell intra- from the cell extracellular environment. In the 1st few minutes after death and in-vivo measurements we could expect that the blood flow could not be stopped suddenly but decreases gradually from the heart to other body organ. Therefore, one argue that complete necrosis could not be reached just after death but after a certain time depending on the organ and on the arterial feed to this organ; liver; in our case. The observed stability in the relaxation time and resonance frequency after 60 minutes means that no more motion of the molecules could be affected by the electric field. Also, the ATP enzyme was ceased and no more production of ADP [15]. The reaction {ATP↔ADP} will be stopped. One expected that after 120 minutes, the relaxation time decreased due to rigidity resulting from post mortem changes. Some researchers [16] concluded that no observable changes in the relaxation time of the liver within the 1st 10 hrs following death.

No more oxidation of the cations contained in the liver after 60 minutes of death. The resonance frequency is shifted towards lower values depending on death time. At resonance frequency the applied frequency= normal frequency of the liver molecules in another words passing from life to death, the viscosity of liver tissues increases due to dehydration i.e. the decrease of liver fluids. Accordingly, the degrees of freedom of the movable dipoles will be decreased and the relaxation time is increased monotonically with increasing death time until reaching saturation after 60 minutes. This is due to the localization and neutralization of the charge carriers [17, 18]. This is enhanced by the observed overlapping of tanδ vs f spectrum at 90 and 120 minutes Fig. (2).

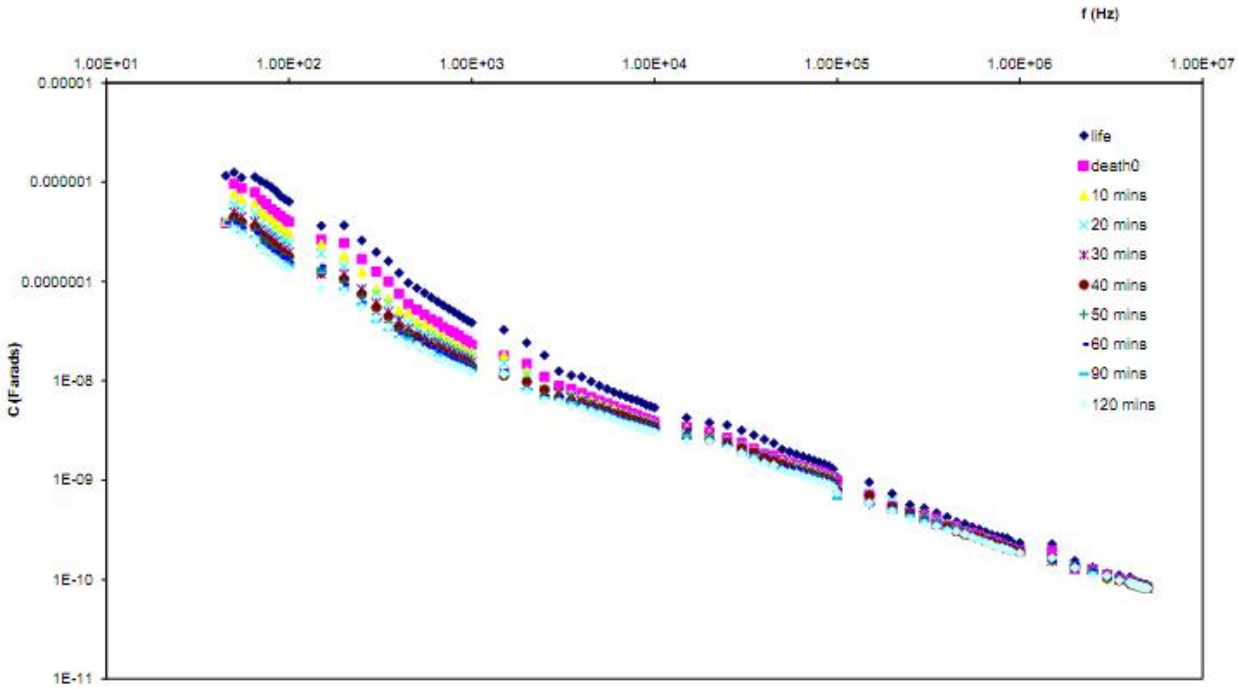

FIGURE 3. Variation of the in-vivo capacitance of the liver with the applied frequency

When comparing the variation of Z as a function of frequency with the plot of D times vs death time, one could easily find that the peak in the 2nd plot corresponds to the large dispersion occurred between life and just after death. From a closer look to (Z vs f), one can find that, with increasing the death time, the dispersion decreases which is an acceptable result as it agrees well with (C vs f) plot. Finally one can state that, the most important factors affecting on the dielectric properties are: The first one is the tissue surface condition where the surface can be dry or wet while the second one is the effective penetration of path of the electric field lines which is defined by the geometry of the prides characteristics.

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