Estimation of radiation dose rates’ changes on Hb using dielectric parameters

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ABSTRACT

In this paper we will introduce several physical parameters that are very promising to use as indicators to diagnose and prognose tissue, cellular and molecular status. Relaxation time, DC conductivity, activation energy and enthalpy of the haemoglobin (Hb) molecule are introduced as key parameters to detect changes in the polarizability of Hb relating this change to the state of the molecule. Using dielectric and impedance measurements, we fitted the relaxation peaks found within the frequency and temperature windows to the proper equations to get our pre-mentioned parameters. Activation energy, Ea, and enthalpy change, $\Delta H$, are also calculated. Results of higher dose rate (HDR) and lower dose rate (LDR) had different patterns for most parameters. Relaxation time and dc conductivity are sensitive to the electrical homogeneity of the Hb. Activation, E, and enthalpy change are sensitive to the damage of the Hb molecule. Early diagnosis and prognosis differentiating between higher and lower dose rates used is possible. Dielectric parameters represent promising bio-physical markers for diagnosis and prognosis of radiation exposure.

1. Introduction

Haemoglobin (Hb) is a metalloprotein molecule. Hb is composed of globin representing the protein part consisting of two chain types; $\alpha$ helices and $\beta$ plated sheets, in addition the haem group represents the other part of the molecule which is the metallo-organic part made up of four porphyrin rings that form a circumference around a ferrous, Fe$^{2+}$, atom covalently bonded to them through the nitrogen atoms. Each protein chain surrounds one haem part and each two Hb molecules combine forming a haem tetramer (Hill, Konigsberg, Guidotti, & Craig, 1962; Murray, Granner, Mayes, & Rodwell, 1993). The known functions of Hb are transporting oxygen from the lungs to the tissues and vice versa for carbon dioxide, in addition to acting as a buffer carrying hydrogen as Hb$^{2+}$. Our interest in this paper series is focused on rat Hb, which is different from human Hb in that it has at least six parts: two alphas, $\alpha$1 is the major, and three betas with $\beta$2 being the dominant one. The difference between the rat and human Hb lies in the amino acid residues and the important alterations occur in the haem pocket and the contact points between the $\alpha$ and $\beta$ chains (John, 1982).

The use of ionizing radiation such as gamma rays causes energy deposition within the system under assessment. Energy deposition occurs via several ways depending on both the energy of the gamma rays and the nature of the investigated system and its composition, the atomic weight of its constituting atoms, for instance. Starting from higher to lower energy, energy is transferred from radiation to systems by means of pair production, Compton scattering and photoelectric effect. Mostly what really happens is that the radiation rays or particles eject an electron from its hosting atom, causing ionization. The resultant damage depends on the site occupied by the ejected electron; if it constitutes an important bond, like a backbone of a protein, a DNA site that is related either to the cell function or to the proliferation cycle or RNA, it causes greater damage to the biosystem and is difficult to repair. On the other hand, if it is situated in a part that is not related to the mentioned sites or a product molecule then it is easy or even not that important to repair. For more information about the mechanisms of radiation damage to biological matter the reader is referred to references about radiobiology and the effects of radiation (Dainiak, 1997; Harrellson, 2014; IAEA, 2010; Reisz, Bansal, Qian, Zhao, & Furdui, 2014).

Consequently, the main event we’re looking for here is ionization which may produce a bond breakage, a...
new bond formation, a free radical formation which initiates a polymerization reaction within the system. These latter biochemical events result in biological damage to the biosystem and this harm is translated later to histopathological damage to the affected organ and finally to elevation or depletion of some biomolecules that are used as biomarkers showing the injury of the specified organ.

The physical effect occurs within $10^{-15}$ s, as $10^{-18}$–$10^{-14}$ s is the time needed for an energized electron with high speed to cross a mammalian cell of 10 μm diameter. The passing electron, caused by ionizing radiation, causes about $10^5$ ionizations within the cell for each 1 Gy of absorbed dose (Jaroszyk & Marzec, 1994; Joiner, van der Kogel, & Steel, 2009). Physical action, ionization or excitation happens first followed by free radical formation which arises within $10^{-14}$–$10^{-5}$ s, then the biochemical reaction consuming too much time and the biological impairment affecting the biochemical markers, if any, i.e. a long process taking days to weeks to produce a late effect which is mostly unrepairable for severe doses (Joiner et al., 2009).

For some cases, the survival ratio can be improved by the early detection of the problem. Hereby, we thought of putting forward some physical parameters as early indicators for damage occurring to the biosystem on the molecular scale would do the job, i.e. a physical effect, or even a chemical one, can be detected almost simultaneously by a physical parameter. Thinking about the proper physical parameter which must be influenced by the main effector, ionization, we found that dielectric spectroscopy is a good tool for use in this area of science. It can detect changes in polarization within the molecule, cell or biosystem accurately (Elnasharty et al., 2018; Elwann et al., 2018).

The first scientist who approached this field was Debye in 1928, who was awarded the Nobel prize for his success linking changes in polarization occurring in the microscopic scale, atoms and molecules to the macroscopic bulk scale measurements of the permittivity equation (Swiergiel, Plowas, Grembowski, & Jadzyn, 2015; Swiergiel & Jadzyn, 2012). His theory was later modified to reach to a final general form by Havriliak and Negami (1966); the impedance form of the equation is:

$$Z^*(\omega, T) = \frac{R_{DC}(T)}{(1 + (i\omega\tau_\sigma(T))^\beta)}$$  \( (1) \)

where $Z^*$ is the complex impedance, $R_{DC}$ is the direct current resistance, $i$ is the imaginary number $\sqrt{-1}$, $\omega$ is the angular frequency, which equals $2\pi\nu$, and the latter is frequency, $T$ is the temperature, $\tau_\sigma$ is the conductivity relaxation time (time consumed until the total induced polarization declines to 1/e of its value upon the removal of electric field, Charles P. Smyth (1955)) and $0 < \alpha \leq 1$ and $0 < \beta \leq 1$ are shape parameters. Important results, relaxation time and direct current conductivity, are extracted from fitting the measured impedance to Equation (1). The extracted results would give us enormous information about the sample, molecule, cell or tissue, under test as the activation energy and enthalpy. All together contribute to the knowledge of the state of the investigated sample, integrity, damage repair.

Activation energy, minimum energy needed to perform certain process, is estimated from Equation (2) for DC conductivity, SDCc, and a similar one using the relaxation time instead of SDC.

$$\sigma = \sigma_0 \exp\left(\frac{-E_a}{RT}\right)$$  \( (2) \)

where $\sigma$ is dc conductivity at any absolute temperature, $T$, $\sigma_0$ is a pre-exponential factor and $R$ is the gas constant. The use of natural log for both sides will result in a linear equation enabling us from easy determination of activation energy (Idris, Majid, Khiar, Hassan, & Arof, 2005; Plowas, Swiergiel, & Jadzyn, 2014; Shamsuddin, Ahmad, Hassan, & Kaddami, 2015).

The enthalpy, $\Delta H$, is a state function expresses the energy absorbed by the system at constant pressure. It means “warm in” and was made up by Rudolf Julius Emanuel Clausius about 1850 (Haynie, 2008). In other words, enthalpy is the energy transferred to the system from the surroundings, or vice versa (Grant, South, Takashima, & Ichimura, 1971; Haynie, 2008). Consequently, the change in enthalpy when investigated provides valuable information about the system. Interestingly, the enthalpy change can be approached by detecting the system-surrounding communications from the following relation

$$\Delta H = R. \frac{\partial(\ln\sigma)}{\partial(\frac{1}{T})} - RT$$  \( (3) \)

where $\tau$ is the relaxation time in the general term and in this work represents $\tau_\sigma$.

In this work we’ll truncate our focus to the general behaviour of the resulting change in enthalpy, as a result of change in radiation dose rate with time, until a clear picture of the change in enthalpy is seen.

2. Material and method

2.1. Animals

Wistar albino female rats, 50 (120–150 g), were bought from the laboratory of the animal house, National Research Centre, NRC, and divided into two groups, control group (10 rats) and irradiated group...
with higher dose rate (HDR) of 533.35 mGy/min, 40 rats. After the end of this part another set of 40 rats of the same conditions were brought and divided into two groups, control group, 10 rats and irradiated group with lower dose rate (LDR) of 325.89 mGy/min, 30 rats.

Hb is prepared by withdrawing the animal’s blood in a heparinized tube and shaking gently. The next step is to centrifuge the blood at 3000 rpm for 5 min and removing the plasma, supernatant. The remaining red blood cells (RBCs) were washed 3–5 times with 0.9% NaCl saline solution and centrifuged at 3000 rpm for 5 min to remove the supernatant, and the last step was repeated 3–5 times. Finally, a similar volume of cold, distilled water is added to the remaining RBCs. The sample is shaken vigorously and centrifuged at 10,000 rpm for 10 min the supernatant is the Hb solution, which is now ready to measure using our setup in the instruments section.

The use of laboratory animals and experimental protocol were approved by medical research ethics committee according to MREC 2003.

2.2. Irradiation

The animals were subjected to total body irradiation dose of 7.0 ± 0.03 Gy from a Co60 source with two different dose rates. The HDR group was subjected to 556 mGy/min and the LDR group was exposed to a dose rate of 325.89 mGy/min. Irradiation process was carried out in a secondary standard laboratory at 20°C at atmospheric air condition. The dose rate was measured using secondary standard dosimetry system of 0.6 cm³ ionization chamber coupled with an electrometer calibrated at International Bureau of Weights and Measures (BIPM), France, 2012 (Elwanm et al. 2018; Elnasharty et al., 2018a; Elnasharty et al., 2018b; Elnasharty & Elwan, 2018).

2.3. Instruments

Impedance analyser 4991B, KeySight Co., USA, frequency range 1 MHz–3 GHz. Home-made measuring cell of two brass electrodes and a Teflon cylinder allowing different thicknesses from 0.15 to 1.2 mm and a fixed diameter of 8 mm. Other home-made equipment was prepared, providing enough space for the newly made measuring cell and allowing temperature control for the whole system to allow the use of temperature measurement (patent no. 2065/2015, certified on 27/2/2018).

3. Results and discussion

As seen in Figure 1, the impedance spectrum of the Hb molecule exhibits a relaxation peak in the imaginary part of impedance as a function of frequency (Figure 1A) within the measured temperature window. The lower part of Figure 1B exemplifies the fitting of impedance dispersion at 15°C for clarification. All the following data are extracted and calculated from the fitting process, i.e. the relaxation time, direct current conductivity, SDC, etc.

Figure 2 demonstrates the effect of HDR and LDR on the Hb relaxation time, where Cntrl means Control and W1, … W4 represent weeks 1 to 4, respectively. The values of relaxation time of the Hb molecule at different temperatures were decreased in the first week after exposure to radiation and increased gradually during the following weeks, failing to retain their control values under the effect of LDR, giving the impression that the biosystem needs more time to recover from the dose rate. Upon exposure to HDR, the relaxation time of the Hb molecule was increased to about twice its values in the first week then decreased gradually to retain its control-like values in the fourth week.

Therefore, the LDR result is somewhat unexpected, because it indicates the need for a longer period for following up the results to give a clue about how would the HDR recover faster, as the data indicate, than the LDR. Another point worth mentioning here is that we don’t know if there a mechanism for repairing the incapacitated Hb molecule or if the biosystem starts destroying the damaged molecules by extinguishing the RBCs containing defective Hb and other cells with well-functioning Hb molecules are created. We think LDR gave energy to Hb to move faster so the relaxation time decreased. Also, the existence of Hb inside the RBCs with a powerful antioxidant, reduced glutathione, partially protected Hb molecule from LDR radiation damage.

On one hand, HDR group, relaxation time compared to control increased more than twofold in the first week and slightly more than 1.5-fold in the second week, while in the third week the mean
increase was only 15.3% then reached more control-like values in the fourth week after irradiation. On the other hand, the LDR irradiated group had its relaxation time decreased by about one-third in the first week and started to increase in the second, third and the fourth week trying to retain its normal relaxation time.

Moving to another interesting parameter, the DC conductivity, the region where frequency change does not affect the response of the sample as the applied frequency is still low enough providing sufficient time for the molecules to respond to the field adequately. SDC behaviour is illustrated in Figure 3 at LDR and HDR, where SDC exhibits the exact reverse of the relaxation time with respect to dose rate. Regarding LDR, SDC values increase to more than twofold in W1, and then fall down trying to approach the control values in the following weeks, but fail to reach it even in the fourth week, while that of HDR declines to more than half values of its control then rises back in the following weeks, reaching the control values in the fourth week.

The HDR caused a sharp decrease in DC conductivity that reached about 42% of the control values in the first week and increased gradually in the following weeks until it almost reached the control values in the fourth week. The LDR group showed a reverse effect to that of the HDR as the DC conductivity was doubled in the first week followed by a step down to 1.16-folds of the control in the second week. Another rise to about 1.3-folds and finally reaching about 1.2-folds of the control in the fourth week.

Looking at the activation energy curve, Figure 4, one can find interesting outcomes. HDR results show higher activation energy assigned than that of LDR, especially in the first week, indicating more damage to the HDR group as more energy is needed to perform the same function than its counterpart of the LDR group. Changes occurring later may be due to either normal repair, replacement or both processes done by the biosystem to overcome the damage of Hb molecule. Both LDR activation energies from DC conductivity and relaxation time have mostly the same behaviour and the same nearly applies for HDR as well. It is also noticed that the first week has mostly the major change, which is expected, apart from the activation energy estimated from DC conductivity of the HDR group which is a strange thing, and might be due to the formation of permanent damage. From Figures 3 and 4 we can deduce that the DC conductivity is more easily recovered than the relaxation time. This can be explained as the radiation causes destruction of the ternary and quaternary structure of molecule, causing unfolding of the macromolecule leading to consumption of more time for molecular relaxation due to molecular unfolding, flattening, which is very obvious in the case of HDR. This induces the biological system to either make a repair, replace the molecule or both. While this is a kind of expected behaviour for the biosystem, what is really strange is the behaviour of the relaxation time in the case of LDR, which decreased the relaxation time of the Hb molecule and failed to retain the control values even in four weeks, as shown in Figure 2(A, B). We don’t know what happened; has the LDR energized the molecule or has the biosystem given up one or more of the repair options thinking that there is nothing wrong with the Hb molecule, or is the damage minor, consequently causing a delay in repair time?

The enthalpy change, ΔH, illustrates that even after four weeks of irradiation the LDR group still has a long way to go, compared to the HDR group, to achieve repair and return to a normal state (Figure 5A). The group irradiated using the higher dose rate, Figure 5B, returned to control-like values in the fourth week; we don’t know if this is the final state or if it needs more time to reach a final normal-like state, because our experiment stopped after one month.
The behaviour is systematic for both dose rates; a jump in the enthalpy change of HDR occurs from the first to second and to the third weeks, then a drop occurs in the fourth week. However, in case of LDR, the jump occurring among weeks is higher than its corresponding one in the HDR, and the biosystem fails to reach the control-like values in the fourth week. In addition, the LDR group has a steep decline in enthalpy in the first week, control-like values in the second, values higher than control in the third week and a drop below control values in the fourth week. Unfortunately, we can’t say much about

Figure 3. $\Sigma_{\text{DC}}$ as a function of temperature for (A) lower dose rate, LDR, and (B) higher dose rate, HDR.

Figure 4. Activation energy, as a function of time in weeks, resulting from relaxation time and DC conductivity for both HDR and LDR.

Figure 5. Enthalpy change, $\Delta H$, for both LDR and HDR.
this situation except that the Hb molecule gains energy from its surroundings as its enthalpy change is positive. Further work needs to be done to investigate the type of damage occurred within the macro-molecule is there an unfolding, bond breakage or H bonds destruction.

4. Conclusion

In the current work, dielectric parameters proved to be useful in differentiating between Hb molecules exposed to different dose rates of total body irradiation in spite of all animals having the same total dose. This deliberate example has opened the door towards a new way of diagnosis and prognosis that makes use of the dielectric parameters and widens the scope of dielectric spectroscopy in the biology and life sciences in general. Relying on the theory of dielectrics and the way radiation deposits its energy within the biological systems, the dielectric parameters are able to make early diagnosis even after one hour of exposure which render biophysical markers superior to either biochemical or histopathological ones.

Disclosure statement

No potential conflict of interest was reported by the authors.

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