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Influence of Moringa Leaves Extract on the Response of Hb Molecule to Dose Rates' Changes: II. Relaxation Time and Its Thermodynamic Driven State Functions

Mohamed M. M. Elnasharty, Azhar M. Elwan

***Abstract*—**Irradiation deposits energy through ionisation changing the bio-system's net dipole, allowing the use of dielectric parameters and thermodynamic state functions related to these parameters as biophysical detectors to electrical inhomogeneity within the biosystem. This part is concerned with the effect of Moringa leaves extract, natural supplement, on the response of the biosystem to two different dose rates of irradiation. Having Hb molecule as a representative to the biosystem to be least invasive to the biosystem, dielectric measurements were used to extract the relaxation time of certain process found in the Hb spectrum within the indicated frequency window and the interrelated thermodynamic state functions were calculated from the deduced relaxation time. The results showed that relaxation time was decreased for both dose rates indicating a strong influence of Moringa on the response of biosystem and consequently Hb molecule. This influence was presented in the relaxation time and other parameters as well.

***Keywords*—**Activation energy, DC conductivity, dielectric relaxation, enthalpy change, moringa leaves extract, relaxation time.

I. Introduction

MORINGA is one of the useful medical plants and can be found in several countries. moringa oleifera is the preferred species of moringa [1]. All parts of this plant are nutritive, especially the leaves. *Moringa oleifera* contains several minerals such as magnesium, calcium, potassium, sulphur, zinc, copper and iron, amino acids, such as histidine, lysine, and methionine and generally, various antioxidants such as vitamin E, rutin, and quercetin [2], [3]. Therefore, moringa acts as anti-tumor, anti-oxidant, anti-inflammatory, antiulcer, antihypertensive, antispasmodic, hepato-protective, anti-fungal antibacterial [4]-[7], and additionally as a radioprotector [8]-[12].

Announcing dielectric parameters and thermodynamic state functions as potent estimators to diagnose, prognose and indicate problems affecting changes of electrical homogeneity of the biosystem [13], [14] initiates a new era of science participating to the biophysics, phys-med and clinical medicine. The appearance of a dipolar impedance relaxation during the impedance and/or dielectric investigation introduces a great opportunity to researchers investigating molecular dynamics. The latter is very important and delivers important information in such cases that the relaxation of interest is affected. In the other words, if the relaxation belongs to a molecule or a membrane and the effector of interest destroys the integrity, causing unfolding or flattening, of such a molecule or membrane which will definitely lead to disturbance of its net dipole moment and relaxation in response to the applied electric field, consequently, giving a response that relates to the strength of the effector. The impedance formula of Havriliak-Negami, (HN), represented in (1), would give us two important parameters namely relaxation time, τ, and direct current conductivity, σ_{dc}. These parameters help to obtain the activation energy of the biosystem under study in (2). Moreover, the enthalpy change, in (3), is attained from the relaxation time results as will be shown later. Equation (1) provides the practical fitting of the real world's dynamics for the investigated system where it can fit the relaxation even if it is broadening and/or has dissimilar distribution around the maximum frequency,

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

where z^* is the complex impedance, R_{DC} is the direct current resistance, i is the$\sqrt{-1}$, ω is the angular frequency equals 2πν and the latter is frequency, t is the temperature, τ is the relaxation time which is time required after switching off the electric field for most of the dipoles to return to its original random orientation state. Moreover, shape parameters are defined as $0 < \alpha \leq 1$ and $0 < \beta \leq 1$. On the other hand, activation energy was calculated from (2) using two routes; the first uses relaxation times and the second uses direct current conductivities. Both relaxation times and DC conductivities were obtained from fitting the measured dielectric data,

$$\tau = \tau_o \, exp[-\frac{Ea}{RT}] \quad (2)$$

where τ is relaxation time at any absolute temperature, t, τ_o is a pre-exponential factor and r is gas constant. Applying natural logarithm to both sides, a linear equation is obtained allowing activation energy determination [15]-[17].

Mohamed M. M. Elnasharty is with the Department of Microwave Physics and Dielectrics, National Research Centre (NRC)-33 El Bohouth st., Dokki, Giza, Egypt, P.O.12622 (e-mail: mohamed.elnasharty@gmail.com).

Azhar M. Elwan is with the Department of Biochemistry, National Research Centre (NRC)-33 El Bohouth st., Dokki, Giza, Egypt, P.O.12622 (e-mail: z.mahmoud2006@gmail.com).

Activation energy means the minimum amount of energy required to perform certain process by the system. Shedding the light on this definition would enable the researcher from tracking the relaxation process of interest during different stages of his experiment, thus allowing the knowledge of the system status.

Enthalpy change, ΔH, is a state function and can be estimated from the relaxation time of the molecule and/or biosystem under study,

$$\Delta H = R.\frac{\partial(Ln\tau)}{\partial(\frac{1}{T})} - RT \quad (3)$$

where, ΔH is the change in enthalpy. Enthalpy means to warm, i.e. estimation of heat and/or work transfer between the studied system and its surrounding [18], [19].

Dipolar relaxation is a kinetic process and responds to heat exchange, consequently investigating the dipolar relaxation at different temperatures would provide information about the status of the biosystem under study including its response to various effectors. Thus, the use of dipolar relaxation can give an indication about both damages caused by the effector and healing processes performed by the system regardless the path taken in either the harm or the curing processes.

II. Material and Method

A. Plant Material

Ethanolic Moringa oleifera leaves extract (MOLE) was bought from the Association of Moring, Agricultural Division, National Research Centre (NRC). MOLE was prepared according to the method of Ugwu et al. [20]. The dose of extract used in this work was 1g/ kg/ day.

B. Experimental Animals

Wistar female albino rats (120-150 g) were bought from Animal House Lab. at National Research Centre. They were kept in rat polypropylene cages in ambient temperature (25± 3 °C).

C. Experimental Design

The adult female Wistar albino rats (86) were divided into four groups. 40 rats were used in the first group, for expected mortality. They were irradiated by acute dose (7 Gy) with high dose rate, HDR, of 533.350 mGy/min. Then, they were administered with Moringa extract (1 g/kg) for 15 days after irradiation. Second group, 30 rats, was irradiated by the same dose with lower dose rate, LDR of 325.89 mGy/min and also administered with *Moringa* extract by the same way. Both of groups were followed for four weeks post irradiation. The third and fourth groups were control for each irradiated and treated group (eight rats for each).

D. Irradiation Conditions

The irradiation process carried out in secondary standard ionizing metrology Lab., National Institute of Standards (NIS). Rats were irradiated from a ^{60}Co gamma radiation source built in Thermatron system, at 20 °C, under atmosphere of air. The dose rate was measured using secondary standard dosimetry system of 0.6 cc ionization chamber coupled with electrometer calibrated at BIPM, 2012. The dose rate was 325.89 mGy/min of total dose 7 Gy at 1 m from the source.

E. Sample Preparation

The withdrawn blood is injected into a heparinized tube. Sample was centrifuged at 3000 rpm and plasma was removed. Packed RBCs was washed 3-5 times with 0.9% NaCl saline solution. Then a similar volume of cold distilled water was added to the washed packed RBCs, shaked vigorously and centrifuged for 10 min at 10,000 rpm at 4.0 °C. The Hb, supernatant, was removed for analysis.

F. Instruments and Measurements

The dielectric investigation was carried out in the frequency range 106 Hz – 3x109 Hz using an impedance analyser (E4991B) from KeySight Co., USA. A homemade compartment and a measuring cell composed of two brass electrodes diameter 8 mm and a Teflon cylinder, the compartment is designed to hold measuring cell and maintain the cell in the desired temperature during measurement. A homemade compartment is fired to a patent no. (28776/2018).

III. Results and Discussion

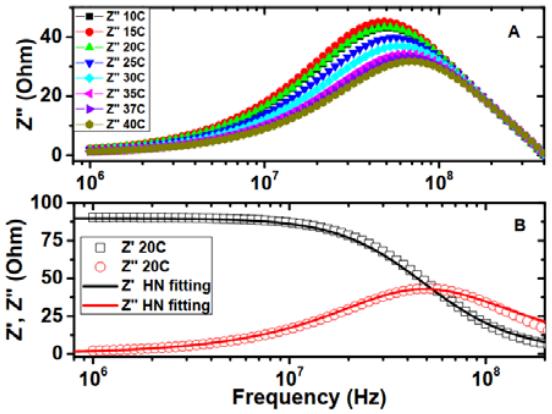

Fig. 1 Impedance relaxation Z'' as a function of frequency and temperature (A) and an example of fitting (B)

Dielectric measurements exhibit an impedance relaxation on the frequency scale from 10^6 Hz to 4 x 10^8 Hz as shown in Fig. 1 (A). Impedance relaxation is very important that one can estimate two parameters out of its fitting as shown in Fig. 1 (B), namely the relaxation time and direct current conductivity. The deduced parameters are used to compare the response of the animals to both irradiation and Moringa leaves extract effects. Then, both parameters are used to calculate activation energy, and relaxation time is used to calculate the enthalpy change.

The response of relaxation time, tau, in the LDR group, as shown in Fig. 2 (A), has decreased to minimum values in the 1st week then it increased gradually in the 2nd and 3rd weeks. Two weeks after stopping Moringa leaves extract administration, in the fourth week, tau increases steeply. In

case of HDR group shown in Fig. 2 (B), tau response was different stepping down in week one and further in the 3rd week. Then an increment occurred in the 4th week; however, relaxation time values were still below that of the 1st week.

There was a slight change in the Moringa treated LDR group where the relaxation time increased significantly in the 4th week. On the other hand, tau decreases in all measured weeks in contrast to the results of tau of the HDR group (data not shown) where we used the same dose rates and total dose without Moringa leaves extract treatment. These data point out that not only Moringa leaves extract has eradicated a major feature of HDR which is increasing the relaxation time but also reversed the effect causing decrement of the relaxation time. This may be due to the excess amounts of antioxidants, nutrients, metabolites and trace elements within the Moringa leaves extract [21]-[23] that are used by the biosystem to overcome and repair most of radiation damages such as oxidation, free radical formation and bond break.

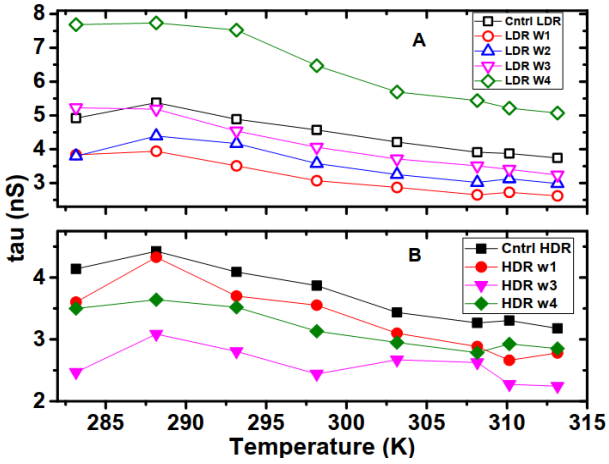

Fig. 2 Relaxation time as a function of temperature and time for LDR group (A) and HDR group (B)

In the LDR group, Fig. 3 (A), there is a general increase in the DC conductivity for three weeks and then decreases below control values. HDR group, Fig. 3 (B), showed a relative increase in the 1st and 4th weeks and a steep increment in the 3rd week. The influence of Moringa leaves extract increased the response of dc conductivity above the control level for all the experimental duration. In other words, the Moringa leaves extract enabled the bio-system to reverse the effect HDR radiation as seen in the DC conductivity which became higher than the control level in contrast to those exposed to the same dose rate but did not had Moringa leaves extract (data not shown).

Enthalpy change in the LDR group, Fig. 4 (A), had all values raised above the control, stepping up from the 1st week to the 3rd one and then decline in the 4th week just above the 2nd week. ΔH of HDR group, as shown in Fig. 4 (B), in the 1st week rises above control and then a steep decrement occurs in the 3rd week and then rises slightly in the 4th week; however, its values are still low compared to control. One can see that in the LDR has infiltrated into the biosystem smoothly and raised the total enthalpy of the Hb molecule resulting in an increase in the enthalpy change during the experiment. On the other hand, the HDR exposure caused a mess in the 1st week rising the enthalpy of the Hb molecule which induced the bio-system to react using all the aid it obtained from the moringa leaves extract's contents to stand against the harm occurring resulting in a total control of the situation that decreased the enthalpy change to levels below the control values from the 2nd week till the end of the experiment.

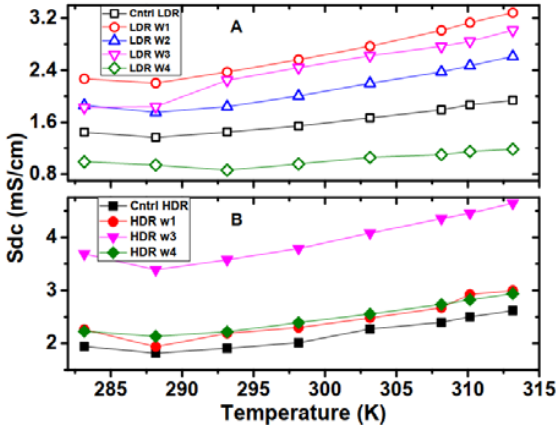

Fig. 3 Direct current conductivity, Sdc, as a function of temperature and time for LDR group (A) and HDR group (B)

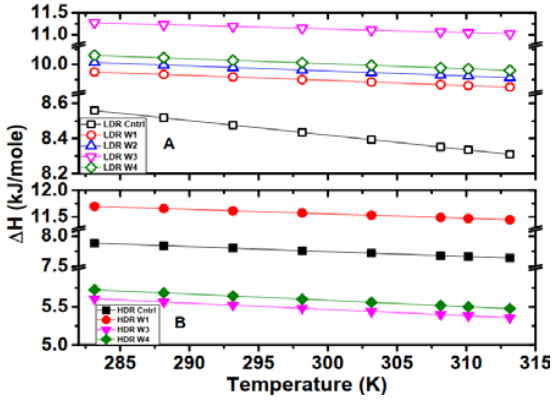

Fig. 4 Enthalpy change, ΔH, as a function of temperature and time for LDR (A), and HDR (B)

Activation energy, as shown in Fig. 5, shares elevation in the 1st week with respect to control, at "0" time. for LDR group, a slight difference between activation energies obtained from tau and dc conductivity where Ea rises steadily till the 4th week except for the 4th week Ea resulting from DC conductivity which decreased to 1st week's level. HDR group had higher values for activation energy than the LDR group where the Ea resulting from tau records the highest value in spite of it has the lowest value for control, then the Ea in the HDR group performed a steep decrement in the third week and a slight rise in the 4th week. Thus, the effect of MOLE was to reverse the response of the biosystem to the enthalpy

change except for the 1st week in the HDR group and 3rd one in the LDR group. It raised the Ea for LDR group in the 1st week to approach HDR group. So it reversed the effect and /or reversed the biosystem's response to the LDR group in the 1st week. Then, it affected the Ea of both groups, after the 1st week, rising that of LDR and decreasing Ea of HDR along the experiment time. As for the relaxation time, the major effect of moringa leaves extract was on the HDR group. The extract decreased the relaxation time along the experiment period. Unlike the effect of higher dose rate irradiation, in the absence of the extract, which caused elevation of relaxation time, data to be published soon. In other words, the biosystem was able to reverse the effect of higher dose rate irradiation upon the administration of moringa leaves extract. Accordingly, the effect on the direct current conductivity was as expected from the relaxation time results where moringa affected the HDR group, and most data for both groups were increased. All previous remarks indicate strong influence for the moringa leaves extract especially on the exposure to high dose rate.

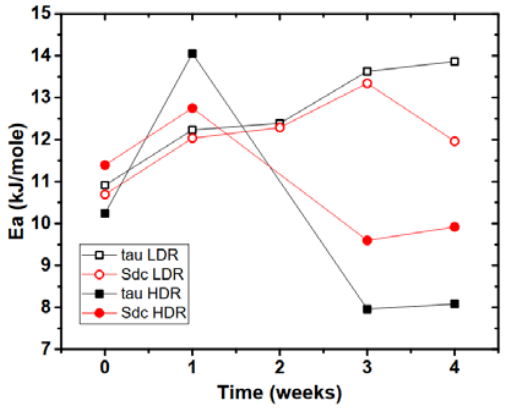

Fig. 5 Activation energy, Ea, as a function of time for both LDR and HDR groups

Finally, referring to the mortality rate, moringa leaves extract decreased the mortality from 45% to 20% for the HDR group [14] while it made no changes in mortality rate, 6.7%, for the LDR group. Also, the presence of diarrhea in HDR group was observed more than it is in LDR.

IV. Conclusion

Moringa leaves extract administration caused significant changes for measured physical parameters, relaxation time, Sdc, ΔH and Ea of the Hb molecule especially for the HDR group. This is mostly due to providing the living organism with proteins, trace elements, antioxidants thus enabling the organism to repair damages and manufacture antioxidants and enzymes that deactivate free radicals and detoxifies hydrogen peroxide. From our previous research (submitted to PBMB) and this work, we advise the use of moringa leaves extract along with radiation therapy and after accidental exposure, especially to high dose rates where its impact is greater and the biosystem is in desire need for assistance to overcome the catastrophic situation.

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