Hysteresis and Negative Resistance Regimes in Membrane Perturbations by Chrysin

Sai Venkatesh Balasubramanian

Sree Sai Vidhya Mandhir, Mallasandra, Bengaluru-560109, Karnataka, India.
saivenkateshbalasubramanian@gmail.com

Abstract

The growing concerns about pro-oxidant nature and toxicity during interaction of flavonoids such as chrysin and associated metal complexes with the cell membrane has led to investigations on membrane perturbations by the same. However, the Cyclic Voltammetry (CV) results show signs indicative of a hysteesis behavior. Taking cue from this, the present work is directed at a nonlinear analysis and characterization of the CV results of membrane perturbations by Chrysin and its copper and iron complexes. Plots of the Voltage-Resistance (V-R) and Voltage-Capacitance (V-C) relations show that while the capacitance is constant independent of voltage, the V-R behavior shows an interesting Hysteresis pattern with negative resistance peaks being seen. Presence of dosage-independent chaos is ascertained using the Lyapunov Exponent. The present work offers an insight into the nonlinear behavior underlying the mechanics of flavonoid-cell membrane interaction and might be the first step in a series of studies focused on the role of nonlinearity and chaos on toxicity.

Keywords: Membrane Perturbation, Chrysin, Cyclic Voltammetry, Bilayer Lipids, Nonlinear Analysis, Hysteresis

1. Introduction

As the flagship of nonlinear science, chaos theory has seen tremendous growth in recent years, thanks to advances in computer simulation and visualizations of ornamental patterns in nature, hitherto unseen [1, 2, 3]. This has led to applications of chaos theory and nonlinear analysis in diverse fields ranging from astronomy to quantum physics [4, 5, 6]. The present work purports to the nonlinear analysis and characterization of membrane perturbations by chrysin and related copper and iron complexes using self-assembled lipid bilayers. After a brief overview of chrysin and published results on membrane perturbation studies, cyclic voltammetry data is analyzed using standard nonlinear analysis techniques such as Lyapunov Exponents and positive values of Resistance variations ascertain the presence of chaotic behavior therein [7]. Furthermore, plots of V-R (Voltage-Resistance) and V-C (Voltage-Capacitance) are plotted and it is seen that for certain values of Voltage, Negative Resistance is seen, akin to that typically seen in Gunn Diode systems. This is seen as a consequence of the hysteresis behavior demonstrated in the cyclic voltammogram results. The observed results establish the behavior of the lipid bilayer as a nonlinear RC filter with a voltage dependent resistance and a fixed capacitance. It is opined that the results described in the present work would be a starting step in series of nonlinear analyses of various membrane perturbation studies and discoveries of novel behavior therein.

2. An overview of Chrysin and Membrane Perturbation Studies

An isoflavone known to possess atioxidant, anti inflammatory, vasodilatory and anticancer properties, and found in flowers such as Passiflora, Chrysin (5,7-dihydroxy flavone) is primarily used by male bodybuilders owing to its prevention of androgen to estrogen conversion by cytochrome P450-dependent aromatase enzyme [8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. Due to the presence of keto and hydroxyl groups, flavonoids exhibit properties of antioxidants, offering protection from deadly ailments such as artherosclerosis, asthma, diabetes and neurogenerative diseases [15, 16, 17, 18, 19]. An issue of concern, though, is the pro-oxidant behavior of flavonoids shown at certain concentrations, shown to the extent of oxidative stress and injury caused by the presence of transition-metal ions [20, 21]. On account of all these observations, it becomes important to investigate pro- or anti- oxidant nature caused by formation of flavonoid-metal complexes.

The interaction of flavonoids with cell membranes, which is the key contributor to the physiological and therapeutic effects of the former, is dependent on the hydrophobicity and localization within the lipid bilayer [22]. Along with concerns regarding the safety of chrysin and induced cell toxicity by low chrysin concentrations, studies have also indicated that the blocking of chrysin by the lipid bilayer may be the reason for the pro-oxidant behavior [22, 23, 24]. This necessitates investigation into the molecular level interactions of chrysin with the membrane both in the presence and absence of transition-metal ions.

The planar lipid bilayer is typically chosen as a model system to investigate the influence of chrysin and its copper and iron complexes on the membrane integrity [25, 26]. This has led to studies on the interactions of chrysin and its copper and iron complex elucidating the extent of its protective effect on membrane integrity by measuring the change in electrical properties of the bilayer.

After preparing the chrysin-copper complex and forming the bilayer lipid membrane using a modified Singleton and Gray method, electrochemical measurements consisting of cyclic voltammetry (CV), chronoamperometry (CA) and admittance spectra have been performed [7]. The CV pattern of the lipid bilayer is shown in Fig. (1) for addition of 0-100 μm of chrysin displaying the characteristic pattern of a parallel plate capacitor, whose capacitive nature is not altered by the presence of chrysin. A similar behavior is observed for addition for chrysin-copper complex as well, as shown in Fig. (2). The corresponding Nyquist plots have been shown to display a progressive reduction of resistance and capacitance with increasing chrysin concentrations. Based on these and related results, it is concluded that the copper complex of chrysin displays a greater membrane fluidizing effect dependent on dosage, where it could stabilize membranes at concentrations below 20 μM, whereas higher concentrations could render it cytotoxic [7].

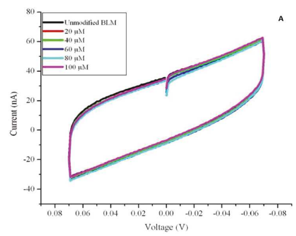

Figure 1: Cyclic Voltammetry results for addition of different concentrations of Chrysin

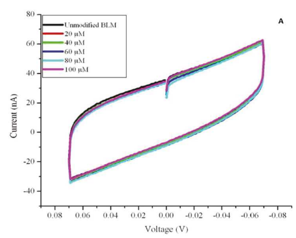

Figure 2: Cyclic Voltammetry results for addition of different concentrations of Chrysin

3. Nonlinear Analysis of the Cyclic Voltammetry Results

From a preliminary inspection of Fig. (1) and Fig. (2), it is seen that the Current responses for the rising and falling Voltage trends are different, and this behavior is typically an indicator to the presence of hysteresis in the system. In order to further investigate the presence of hysteresis and other nonlinear aspects, two plots, namely the Voltage-Resistance (V-R) plot, where R is obtained as $R = V/I$, and the Voltage-Capacitance (V-C) Plot where C is obtained from $C = Q/V$, are plotted for 20 μM and 100 μM concentrations of Chrysin, Chrysin-Copper and Chrysin-Iron in Fig. (3) - (8).

From these plots, the following can be inferred:

1. All the V-C plots show an 'open-ended' behavior, with purely mathematical singularities seen at V=0 values.
2. Apart from the singularities at V=0, the capacitances are fairly constant and independent of changes in V.
3. The V-R curves show a looping behavior, confirming the fact that the hysteresis seen in the CV plots comes from the Resistive and not the capacitive component, as well as establishing the fact that the resistance varies with voltage in a nonlinear fashion, as seen from the sudden spikes.
4. The presence of negative spikes are seen in the V-R plots, confirming negative resistance regions of operation for particular values of V. In microwave systems such as the Gunn Diode, such negative resistance regions are seen to generate signals in the microwave regime [27, 28, 29]. Taking cue from this, it might be assumed that the negative resistance region indicates a transfer of charge from the cell membrane to the probe, rather than in the reverse.

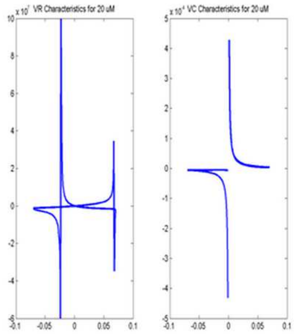

Figure 3: V-R and V-C plots for 20 μM Chrysin

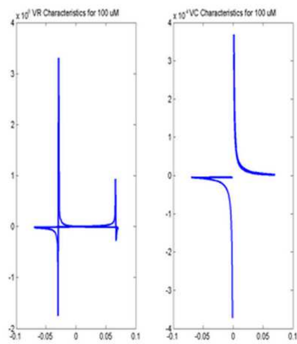

Figure 4: V-R and V-C plots for 100 μM Chrysin

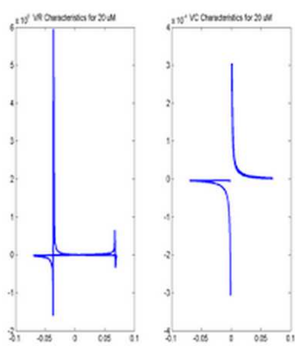

Figure 5: V-R and V-C plots for 20 μM Chrysin-Copper

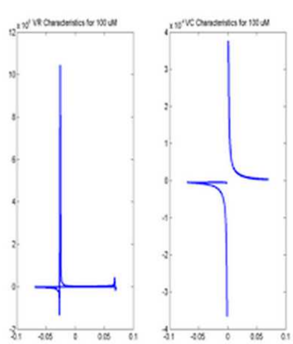

Figure 6: V-R and V-C plots for 100 μM Chrysin-Copper

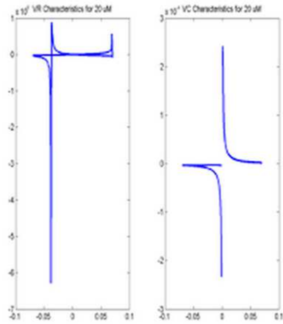

Figure 7: V-R and V-C plots for 20 μM Chrysin-Iron

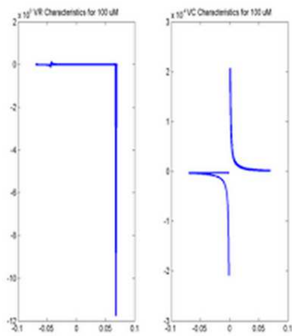

Figure 8: V-R and V-C plots for 100 μM Chrysin-Iron

The presence of negative resistance regions as well as a hysteretic voltage dependent resistance are key indicators of nonlinear behavior in the membrane-flavonoid interactions. The best measure to confirm the presence of chaos is the largest Lyapunov Exponent(LLE), a measure of a system's sensitive dependence on initial conditions [30]. Rosenstein's algorithm is used to compute the Lyapunov Exponents λ_i from the voltage waveform, where the sensitive dependence is characterized by the divergence samples $d_j(i)$ between nearest trajectories represented by j given as follows, C_j being a normalization constant [31]:

$$d_j(i) = C_j e^{\lambda_i(i\delta t)} \tag{1}$$

The values of LLE obtained for various concentrations of Chrysin, Chrysin-Copper and Chrysin-Iron are tabulated in Table 1.

Table 1: Concentration dependent LLE Values for Chrysin, Chrysin-Copper and Chrysin-Iron

Concentration (μM)	Chrysin	Chrysin-Copper	Chrysin-Iron
0	4.15	4.24	5.82
20	4.12	4.32	5.63
40	4.02	4.37	5.72
60	4.12	4.28	5.64
80	4.07	4.31	5.85
100	4.09	4.25	5.92

From the table, while the positive values of LLE confirm the presence of chaos in all cases of membrane-flavonoid interactions, it is seen that the variation in concentrations of Chrysin and its complexes does not significantly affect the nature of chaos. However, the values of Chrysin-Iron complex are significantly higher than the other two cases, and this might be related to the V-R behavior observed in Fig. (8), in which, the R is almost fully negative.

4. Conclusion

The behavior and significance of chrysin is explored briefly and an overview of established results on the membrane perturbation studies of chrysin and its copper complex is presented. Following this, the Cyclic Voltammetry (CV) results are studied using nonlinear analysis, by plotting Resistance and Capacitance curves as a function of voltage (V-R and V-C). From the results it is clear that C remains constant independent of V except for mathematical singularities, whereas R varies with V in a trademark hysteretic fashion, with the most interesting observation being the presence of negative resistance peaks for certain values of V. Following this, chaotic characterization of the Resistance variations is carried out using Lyapunov Exponents, and the positive values observed indeed ascertain the presence of chaos although the nature of chaos is generally unaltered with change in concentration levels. The detection and characterization of nonlinear behavior in membrane-flavonoid interactions throw new light on the underlying mechanism, and it is opined that a detailed investigation into the nature of such interactions might reveal the cause and course of the pro-oxidant behavior of chrysin in the presence of transition metal ions.

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