On study of nonlinear viscoelastic behavior of red blood cell membrane.

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Abstract

The linear viscoelastic behavior of the red blood cell membrane of mammal and human was studied in previous works proposing different experimental methods to determine their viscoelastic parameters. In the present work the nonlinear component of dynamic viscosity of the red blood cell membrane by nonlinear time series analysis is used. For such aim, it obtained time series of test in vitro of samples of humans and rats red blood cells using the Erythrodeformeter in oscillating regime. The signal filtrate suppresses any linear behavior as well as represented by a system of linear ordinary differential equations. The test shown as much in humans as in rats resonance frequencies associated to an attractor of unknown nature independently of excitation in the physiological range. The preliminary studies shown that attractor could be correspond to a complex form bull. These results allow to extend the present knowledge on dynamic of the cellular membrane to similar stimulus which happens in the blood circulation and it will allows to make better models of the same one.

Keywords: Nonlinear viscoelasticity, recurrence plots, Erythrocyte membrane, dynamic viscoelasticity, erythrodeformeter.

PACS numbers: 05.45.-a
I. INTRODUCTION

In previous works we have demonstrated that the diffractometric method is adapted to make the complex viscoelastic analysis of human red blood cell (RBC) and turns out to be a very useful tool to investigate the properties of these cells. The complex viscoelastic parameters of human RBC determined by laser diffractometric have been and continue being very useful for the detection of alterations produced in different levels from the RBC membrane or by diseases such as the diabetes, some anemia or the arterial hypertension, thus also like the induced ones in vitro by synthetic lectins, glicosilation or polications.

The measurements made with the Erythrodeformeter in oscillating regime have allowed to verify that for frequencies and shear rate within the physiological rank the RBC deformation varies sinusoidal since it makes the tension of cut with a shift phase $\phi$ that is function of the oscillation frequency, it is to say that the RBC for that rank of shear stress can actually be like a linear viscoelastic materials. Nevertheless the results show the existence of underlying nonlinear viscoelastic component.

The method of the recurrent plots (RP) allows to visualize recurrent patterns in a time series of data. This technique was in first time propose by Eckman et al. in 1987 and to find complex and hidden correlations between the data. At the moment it is applied in the diverse fields as much of physics, chemistry, the economy etc.

A. Theoretical Foundations

An RP is an injective application of a single reconstructed trajectory to Boolean matrix where each value pairs $y_i$ and $y_j$ coming from a time series is related with a numerical pair $(i, j)$ called recurrence point. Let us consider $N$ join values from a time series given by:

$$Y = \{y_0, y_1, \ldots, y_{N-1}\} \quad (1)$$

with $N$ large enough in order to evaluate the embedding dimension $d > 2$ by using the false nearest neighbor algorithm and the time delay $\tau > 1$ by looking at the relative minimum in the mutual information. Following Takens’ embedding theorem the dynamics can be appropriately mapped by the phase space trajectory reconstructed by using the time delay vectors:

$$A_k = \{y_k, y_{k+\tau}, y_{k+2\tau}, \ldots, y_{k+(d-1)\tau}\} \quad (2)$$
with *false-neighbours* algorithm, it is possible to obtain dimension embedding \( d \).

Its symmetric recurrence matrix is:

\[
R_{(i,j)} = \Theta(\delta_h - ||A_i - A_j||_{\infty}) - \Theta(\delta_l - ||A_i - A_j||_{\infty})
\]  

(3)

where \( \Theta \) is the *Heaviside* function. This means a RP is built by comparing all delay vectors with each other. When \( \delta_l < ||A_i - A_j||_{\infty} < \delta_h \) the \( R_{(i,j)} \) component is set to “1” value, otherwise it is set to “0” value. The interval \([\delta_l, \delta_h]\) is known as *threshold corridor*. The choice of this interval is critical, if too large produce a saturation of RP including irrelevant points, and if too narrow loses information. A good criterion is the suggested by Zbilut\(^5\) in which the scale fractal zone is used to choose the possible values of this passage. This criterion is used in this work to find the advisable values of \( \delta_l \) y \( \delta_h \). Webber *et al.*\(^{10}\) in order to characterize and analyze RP introduced a set of quantifiers which are collectively called *recurrence quantified analysis* (RQA).

**a)** The first one is the *percentage of recurrence* (%REC), it is defined as:

\[
\%REC = 100 \frac{N_r}{N_t}
\]  

(4)

where \( N_t = \text{dim}(R) \) (every possible points) and \( N_r \) is number of recursive points given by

\[
N_r = 2 \# \{(i, j) / R_{(i,j)} \> 0 \text{ and } i < j \}.
\]  

(5)

where \# is set cardinal function. The slope of linear region in the S-shaped %REC vs corridor width is the correlation dimension.

**b)** The second quantifier is called *percentage of determinism* (%DET), it is defined as:

\[
\%DET = 100 \frac{N_l}{N_r}
\]  

(6)

where \( N_l \) is called the number of periodic dots given by

\[
N_l = 2 \# \{(i, j) / (i, j) \in d_c(k, b), i < j, \forall c, k, b > 0.\}
\]  

(7)

and a periodic line with length \( b \), origin \( k \) and zone \( c > 0 \) is defined as:

\[
d_c(k, b) = \{(i, i + c) / \prod_{i=k}^{k+b} R_{(i,i+c)} > 0.\}
\]  

(8)

The %DET is related with the organization of RP.
c) The third quantifier is called entropy (S), it is defined as the first rate cumulants of periodic lines:

\[ S = - \sum_{b=1}^{H} P_b \log_2(P_b) \]  

(9)

The label entropy is just that, a label, not to be confused with Shannon’s entropy. \( H \) is the length of maximum periodic line and \( P_b > 0 \) is the relative frequency of periodic lines with \( b > 0 \). Webber assume that \( S \) is related with Shannon’s entropy if and only if the system is chaotic and the embedding dimension large enough.

d) The fourth and last quantifier is \( H \). Eckman et al.\(^4\) claim that line lengths on RP are directly related to inverse of the largest positive Lyapunov exponent. Short lines values are therefore indicative of chaotic or stochastic behavior.

Recently Castellini and Romanelli\(^1\) have developed an effective algorithm to evaluate RQA applied to the study of transitions in chaotic chemical reactions. The objective of the present work is to extend that methodology to the study of the viscoelasticity nonlinear of the RBC membrane as much for human RBC beings like of RBC rats. The election of this animal must to that the linear answer of the RBC is similar one of RBC human and it is used habitually for studies of diseases induced in alive. In this work it is tried to determine if there are significant differences between both species when nonlinear behavior by means of the RQA as by power spectra studies as much within the physiological rank.

II. METHOD AND MATERIAL

A. Human RBC

The human RBC was obtained from whole of healthy givers and anticoagulated blood with EDTA. The samples were maintained to 4\(^\circ\)C until their use which was made according to the recommendations of the Society International of Clinical Hemorheology and within the 48 later hours to its extraction.

B. Rat RBC

The originating blood of 10 male rats of line ”m” was extracted post morten of the anticoagulated heart and with heparin. Previously 6 of the rats were injected by intraperitoneal
route with aloxane which induces diabetes and the other four were considered like controls. Glicemia of the rats dealt with aloxane went of 2.90±0.80 to the 48 hours of the injection and it stayed within that rank during the two weeks of the work.

C. Preparation of the suspension

The whole blood sample was suspended in isotonic viscous means constituted by a solution to 5% w/v of polivinil-pirrolidone (360 PVP Sigma, p.m. 360 kDa) in buffer saline phosphate (PBS: 0.150 M NaCl, and 0.005 M; pH 7.4±0.05; 295±8 mOsmol/kg). The viscosity of means was fit to 22.0±0.5 cp to 25°C.

D. System of measurement

A thin layer of RBC suspension is placed between both concentric horizontal discs and of the Erythrodeformeter. The superior disc is fixed and the inferior one is movable. An adjustable power supply provides to the motor of a stationary voltage or an oscillating sinusoidal voltage. In stationary regime the inferior disc spins at constant speed. In dynamic conditions the inferior disc spins at speeds that can vary sinusoidal way with six pre-established frequencies: 0.5, 1.0, 1.5, 2.0, 2.5, and 3 Hertz. A laser beam perpendicularly crosses the sample to RBC suspension producing a diffraction pattern that is to circulate for RBC without shear stress and elliptical under shear stress.

E. Time series analysis

The obtained temporary series of data was filtered to eliminate all well-known sinusoidal frequency response. For it a frequency groove was used\textsuperscript{12}. The resulting remainder \( r(t) \) again it was filtered with a \textit{Savitzky-Golay}\textsuperscript{13} filter of sixth order which allows to eliminate white noise phenomena in the data. It is possible to emphasize that this it is a F.I.R. filter (Finite Impulse Response) and not a I.I.R. filter (Infinite Impulse Response) reason that attractor behavior is not affected by this type filtering\textsuperscript{14}. Then the data to null average \( \mu = 0 \) and variance unity \( \sigma = 1 \) were standardized. The filtered temporary series contains the behavior nonlinear looked for with an optimal relation signal noise.
Table I

| Excitation frequency (Hz) | $f_1$ (Hz) | $f_2$ (Hz) | $f_3$ (Hz) | $f_4$ (Hz) |
|---------------------------|------------|------------|------------|------------|
| 0.5                       | 0.921      | 1.043      | 1.087      | 1.14       |
| 1.0                       | 1.234      | 1.322      | 1.82       | 2.087      |
| 1.5                       | 1.108      | 1.171      | 1.234      | 2.861      |

As it can be seen in figure-1 for different physiological frequencies of excitation they appear a few of resonance sharp. In table I is the numerical value of the frequency for these sharp in human RBC. We only write the values of more dominant frequencies. The values emphasized in bold correspond to greater amplitude sharp. With different values from frequency were analogs results in red RBC of healthy and aloxane rats occur. This suggests the presence of a attractor that is sensible to small changes of its environment and the species to which investigates. The RQA allows to quantify the attractor topologic behavior.

Before applying algorithm RQA it was determined as the minimum of the mutual information is the average delay $\tau$ investigating. The value obtained in sampling passage turned out to be $\tau = 10$. The concrete value in seconds depends on the sampling frequency and it is of little relevance in this type of analysis. Previous to calculate embedding dimension by false-neighbors method the Theiler’s window in sampling passage was considered whose value was $\tau_t = 15$. The embedding dimension calculated was $d = 5$. Based on these data the time delay vector series was generated to apply RQA.

III. RESULTS AND DISCUSSION

The correlation dimension $C_2$ by means of the Grassberger and Procaccia’s algorithm was calculated. The tests made with human RBC gave to a value of $C_2 = 3.48 \pm 0.68$. As the region in which can be appreciated in figure-2 the estimation of $C_2$ becomes independent of $d$ and $\epsilon$ is reduced giving a not convincing result. However when RQA was used, figure-3 to evaluate the dimension of correlation and the wide of passage the S-shaped zone is independent not only from the frequency of excitation also from the species in individual in the case of RBC of healthy individuals. This suggests that topology of the attractor is unique with a calculated dimension of $C_2 = 3.5 \pm 0.3$ that agrees in probability with the value before obtained. An interesting detail to consider is that in the case of aloxanized rats
this dimension is increased to a value $C_2 = 4.0 \pm 0.3$. Based on this result the wide one of the calculated passage was $\delta_h - \delta_l = 0.64$. Then RQA was used to calculate the maximum length $H$ and entropy $S$ as it is shown in table II.

| Sample                          | $H$ (Dots) | $S$ (bits/ut) |
|---------------------------------|------------|---------------|
| Human to 0.5 Hz                 | 285        | 1.82          |
| Human to 1.0 Hz                 | 241        | 1.66          |
| Human to 1.5 Hz                 | 165        | 1.66          |
| Healthy Rat to 0.5 Hz           | 343        | 2.21          |
| Healthy Rat to 1.0 Hz           | 845        | 2.51          |
| Healthy Rat to 1.5 Hz           | 843        | 2.13          |
| Rat Dealt with to 0.5 Hz        | 304        | 1.77          |
| Rat Dealt with to 1.0 Hz        | 234        | 1.30          |
| Rat dealt with to 1.5 Hz        | 319        | 1.62          |

IV. CONCLUSIONS

Results obtained previously in samples of rats dealt with aloxane, they did not show to alterations in the linear hemorheologic parameters like the deformability and the aggregation due to the diabetes induced by this treatment\(^\text{17}\). Nevertheless, in this work we found that the dimension of correlation $C_2$ measured with RQA allowed to observe differences in relation to the controls. On the other hand as it is appraised in Table II the entropy shows differences between healthy human RBC and healthy rats RBC indicating that these last ones display a non-linearity as ratifies parameter $H$. However it is interesting to emphasize the sensitivity of the method when the rats are affected by diabetes induced by the aloxane because the entropy is much smaller for these RBC of altered rats that the controls. Equal result is observed in $H$. The reasons for this behavior until have not been now deciphered considering these restlessness for future studies. In this study one concludes that the parameters determined by means of the use of the RQA are great sensitivity to detect alterations in RBC membrane no detectable by other techniques. Consequently these results would be
of great applicability in the biomedical investigation.

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FIG. 1: Power spectra of Human RBC in different excitation frequencies. It is possible to appreciate the different sharp that correspond to their frequencies of resonances. Where their values depend on the excitation frequency.
FIG. 2: The correlation dimension $C_2$ by means of the Grassberger and Procaccia’s algorithm. It is possible to appreciate the region in which $C_2$ estimation becomes independent of $d$ and $\epsilon$ is reduced.
FIG. 3: The correlation dimension $C_2$ by means of RQA analysis. The slope of this curve in S-shaped is $C_2$. It is possible appreciate that curves corresponding to healthy individuals are almost parallel in S-shaped zone.