Int. J. Electrochem. Sci., 17 **(2022)** Article Number: 220614, doi: 10.20964/2022.06.13

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Blood Coagulation Time Determination by AC Current Phase Shift Measurement

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Received: 18 February 2022 / *Accepted:* 17 March 2022 / *Published:* 7 May 2022

The phase shift of the AC current resulting from the applied AC potential is one of the important parameters determining the shape of the registered curve in the EIS studies. However, its self-sufficient employment is limited while the Nyquist and Bode diagrams are widely applied in the common EIS analysis so far. In this work a new approach was proposed, tested and applied for rapid and precise blood coagulation time determination, a parameter of a great importance for the diagnosis and monitoring of many diseases. The proposed approach is based on AC current phase shift measurement in respect to the applied AC potential with small amplitude as a self-sufficient blood properties characterizing parameter. A specially developed LabVIEW based virtual instrument was used providing results which relative RSD does not exceed 1.9% in the frequency range from 100 Hz up to 10 KHz.

Keywords: Electrochemical impedance spectroscopy (EIS), AC amperometry, AC current phase shift, blood coagulation

1. INTRODUCTION

The blood rheology is an approach already widely applied in medical diagnostic for blood properties characterization [1-3]. However, the rheological results not always allow the distinction of some blood properties alteration. In such cases their combination with electrochemical ones obtained by the application of rather more universal methods such as conductometry and EIS [4-19] enhances the understanding and the interpretation of the rheological results in terms of hematology and blood circulation [1-7].

The AC current phase shift in respect to the applied AC potential is one of the important parameters determining the curve shape registered in the electrochemical impedance spectroscopy (EIS)

but its self-sufficient employment is very limited so far, while the Nyquist and Bode diagrams are widely applied in the common EIS analysis. Unfortunately, the Nyquist curves interpretation is complicated requiring qualified personal. However, the AC current phase shift could bring unique information about samples having complicated heterogeneous structure such as the blood ones which represents a complex mixture of blood cells, organic and inorganic substances dispersed or dissolved in conductive liquid.

The double electrical layer formed on the electrode/electrolyte interface resulting from electrical charges exchange or adsorption or both, possesses electrical capacitance of a few dozen microfarads per centimeter [20]. According to the theoretical models of the double layer structure, the equivalent electrical circuit of an electrode/electrolyte interface is composed by resistors R and capacitors C [20-22]. As known, the electrical circuits containing capacitors or/and inductances represent nonlinear electrical circuits and the application of AC voltage across their terminals results in AC current response, which phase is shifted toward the applied AC voltage [23]. The phase shift amplitude is determined by the electrical circuit structure, its components values and the applied frequency [21].

Being an electrochemical interface forming a double layer, the blood sample/electrode interface represents a nonlinear electrical circuit and that is why it could be expected an AC current phase shift appearing when an AC potential is applied on the electrode. Its amplitude depends on the values of the equivalent electrical circuit components R and C [21] depending on the blood cells concentration and orientation as well as on their adsorption on the electrode surface [24]. So, it could be expected that the AC current phase shift could represent a self-sufficient parameter able to characterize some blood properties.

Based on this assumption the aim of this work is the application of the AC current phase shift measurement in a narrow frequency range or at a single frequency for precise blood coagulation time determination, an important parameter in the diagnosis and monitoring of many diseases

2. EXPERIMENTAL

2.1 Measuring instrument and measuring cell

A virtual measuring instrument based on NI USB 6003 Data Acquisition System (National Instrument, USA) was employed in all the experiments (see Figure 1). Dummycell 2 (Autolab, Switzerland) was employed for measuring instrument precision determination. A software controlled sinusoidal generator was used to produce low amplitude AC voltage (100 mV p-p) with variable frequency in the range from 1 Hz to 10 KHz applied to the electrodes through a booster amplifier. A specially modified measuring cell having 0.8 mL sample capacity developed by the authors earlier [6] to be compatible with the Contraves Low Shear 30 rotational viscometer was employed in all the experiments (see Figure 1). For this purpose, two Pt electrodes were incorporated to the internal wall of the cylindrical viscometer measuring cell (cup) connected to the measuring instrument by the aim of Au sliding contacts. Additionally, thin insulation film was deposited on the metallic viscometer measuring cylinder (bob) immersed in the blood sample to avoid current passage through it during the simultaneous measurements of the phase shift and the viscosity.

The phase shift between the applied AC potential and the resulted AC current was calculated after the data acquisition termination and their transfer to the computer. The Discrete Wavelet Transform (DWT) applying the Denoise function of Lab View (LV) was employed for the curve smoothing [25, 26]. The main advantages of the DWT are keeping the shape, the slope and the amplitude of the "noisy" source curve, as well as prevention of the precision degradation of the phase shift determination caused by curve displacement over the X axis during the curve processing, a common disadvantage of the other curve smoothing methods.

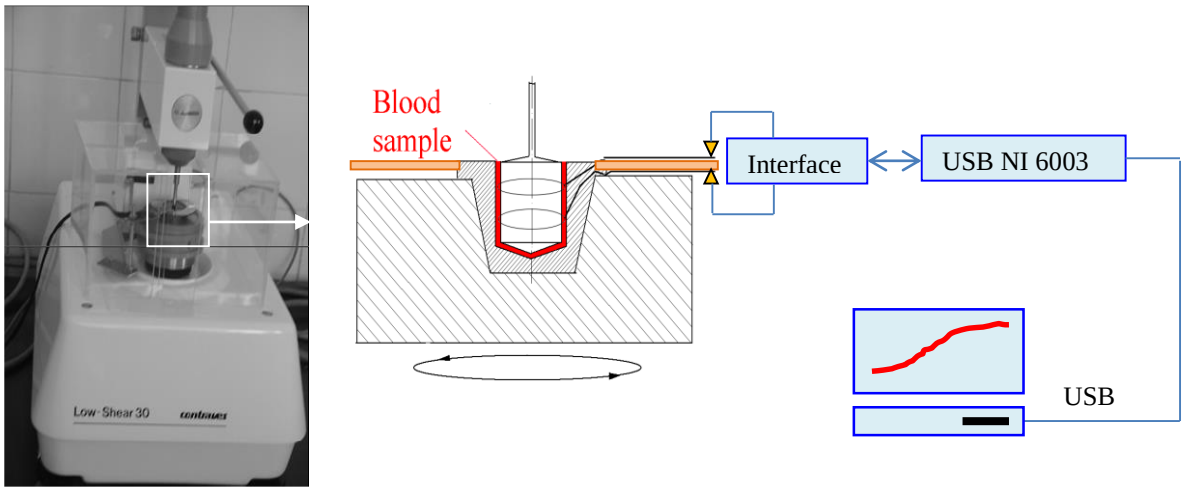

Figure 1. Measuring cell holder (Contraves Low Shear 30 rotational viscometer) photo (left); measuring cell and virtual instrument block diagram (right)

2.2 Reagents

The blood samples were taken in in a closed system using factory prepared 10 mL sterile tubes with Li heparin deposited on the tube walls to avoid the natural blood coagulation before the experiments. 2% solution of $CaCl_2$ in DI H_2O was used as coagulation provoking agent.

2.3 Method

The measurement of AC current phase shift was done in parallel with the hemorheological investigation applying a very simple procedure consisting of the following steps: 1. Addition of the blood sample of about 0.8 mL into the measuring cell (cup). The sample took the gap between the internal cell wall and the viscometer measuring cylinder (see Figure 1); 2. Turning on the measuring cell rotation; 3. Application of the AC potential with sweeping frequency; 4. Simultaneous data acquisition of the imposed AC potential and resulting AC current during the frequency scan; 5. Treatment of the saved data (curve smoothing) and phase shift angle evaluation. The hemorheological experiments were performed within 3 hours after the venepuncture applying a rotational viscometer (Couette viscometer

Contraves Low Shear 30) under conditions of steady blood flow at shear rates (γ) within the range of 0.0237 s^{-1} and 128.5 s^{-1}.

3. RESULTS AND DISCUSSIOIN

3.1 Typical AC current phase shift response

The phase shift approach subject of the present work was applied for blood coagulation time determination comparatively with the shear stress (apparent dynamic viscosity) registration at different rotational rates in the range from 300 to 50 rpm, starting from the highest to the lowest value. The blood, being electro-conductive but heterogeneous system possesses different properties at different rotational rates corresponding to different shear rates, because of red blood cell (RBC) blood cells aggregates formation and RBC deformability. This requires the highest rate to be applied first to destroy the already formed aggregates homogenizing this way the blood sample.

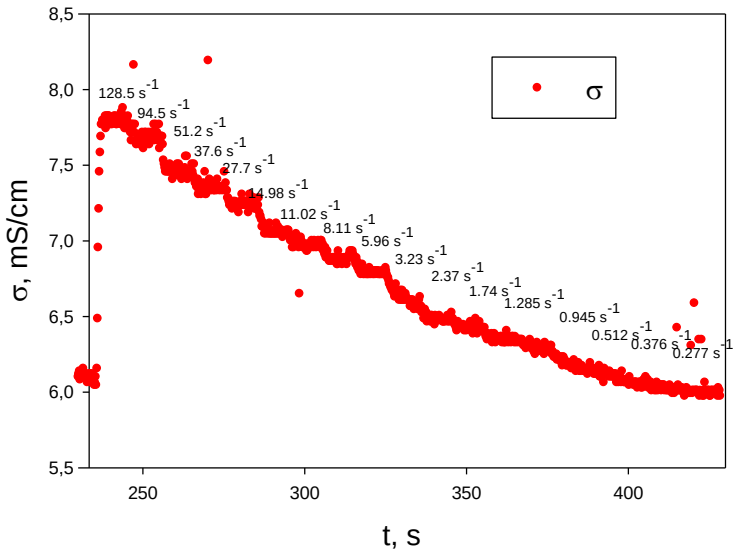

Figure 2. Whole blood sample conductivity vs. the time at rotation rate in the interval from 128.5 s^{-1} to 0, 0237 s^{-1} decreasing gradually with a step of 10 rpm s^{-1} every 10 s. Ht (hematocrit)=45,4 %, T=37 ºC.

The conductometry was applied to determine the appropriate frequency scan range yielding maximal phase shift response. For this purpose the especially modified measuring cell (cup) suitable for conductometric measurements were applied rotated by the Contraves Low Shear 30 rotational viscometer (see Figure 1). Theoretically, the phase shift get ahead of the imposed AC voltage and its phase shift Φ = -90° in case of strictly capacitive loads. However, in case of mixed resistive-capacitive loads the phase shift angle lies in the interval between 0° and -90° and its value is determined by the

capacitors C and the active resistors R values. The capacitor C value determines the capacitive resistance X_C of the electrode/liquid phase interface impedance according to the equation $X_C = 1/\omega C$ (Equation 1) [27, 28]. In case of capacitor and resistor connected in series (which is the case of the electrode/liquid sample interface) the AC current phase shift Φ can be calculated from the vector impedance triangle and expressed by the following equation: $\Phi = \tan^{-1}(\boldsymbol{X_C/R})$ or $\Phi = \tan^{-1}(\boldsymbol{1}/\ \omega C\boldsymbol{R})$ (Equation 2) [27]. The active resistor value R depends on the measuring cell rotational rate because it affects the formed blood cells aggregates and hence the thickness of the adsorbed blood cells on the electrode surface which according to the Ohm law determines the active resistance R value. The blood sample conductivity (1/R value) was registered along the time decreasing the rotational shear rate gradually from 128.5 s^{-1} to 0, 0237 s^{-1} with a step of 10 rpm s^{-1} every 10 seconds and the curve is presented in Figure 2. Contraves Low Shear 30 rotational viscometer was employed for the measuring cell rotation with precisely controlled rate.

It was found from the experimental results that the blood sample conductivity decrease from 7.7 to 6.0 mS cm in the range of shear rates from 128.5 s^{-1} to 0, 0237 s^{-1}. These values of the conductivity correspond to active resistance interval from 129.8 to 166.6 Ohms. Taking into account that the double layer capacitance is about 40 10^{-6} F/cm as found by many authors [29 - 31] and that the surface area of the Pt electrodes integrated to the measuring cell is 0.52 cm^2 one may found that the capacitance of the Pt electrode/blood sample interface is 20.8 10^{-6} F. Replacing these values in Equation 2 one can obtain: $\Phi = \tan^{-1}(\boldsymbol{1}/\ \omega\ 20.8\ 10^{-6}\ 115\ 10^3) = \tan^{-1}(1/\ 2.39\ \omega)$. This equation shows that it could be expected greater phase shift response Φ if higher frequency is applied. However, the AC phase shift altering will be more significant at higher frequencies and will result in lower precision due to the blood sample aggregates existence. That is why a moderate frequency interval was chosen to be applied in phase shift measuring experiments: from 100 to 1000 Hz avoiding this way the mentioned problem.

First, a coarse preliminary frequency scan was performed in the range from 100 to 1000 Hz simultaneously registering the resulting AC current phase shift and the blood shear stress (apparent dynamic viscosity) at different rotational rates. Families of Bode diagrams in the frequency range from 100 to 1000 Hz registered along the time after the addition of the coagulation agent $CaCl_2$ to the blood sample are presented in Figure 3. The registered curves were obtained by the repetitively application of frequency scans in the mentioned frequency interval every 3 minutes.

As expected the initial phase shift values were negative due to the still predominant capacitive component of the interface impedance (the sample still was keeping its liquid state). This result correlates very well with the theoretical models and the equivalent electrical circuit configuration of an electrode/electrolyte interface [20-22]. The structural changes of the blood sample later caused additional phase shift in negative direction due to augmenting of the interface capacitive component value domination probably resulted from adsorption occurring on the electrode surface.

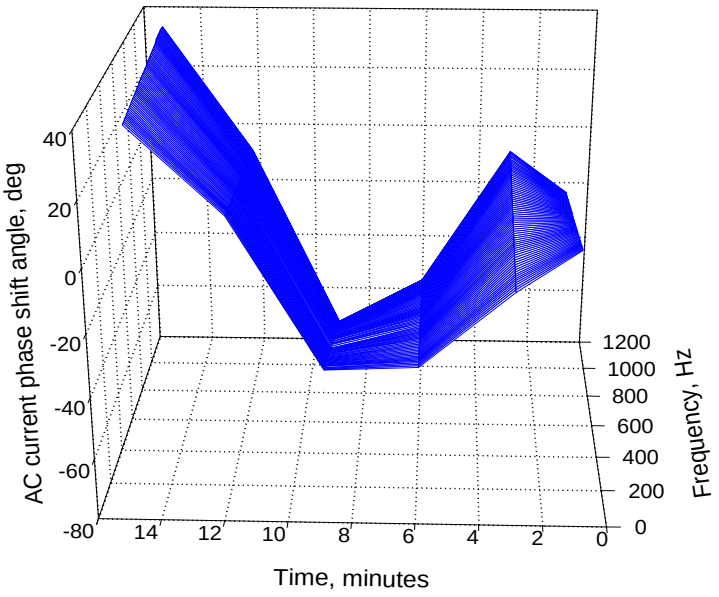

Figure 3. AC current phase shift in the frequency range from 100 to 1000 Hz vs. the time after addition of $CaCl_2$ as a coagulation agent to the blood sample

The beginning of the coagulation process however causes progressive blood sample solidification and corresponding increase of the active resistor value at the expense of the capacitor one forming the interface equivalent electrical circuit provoking this way a rapid increase of the AC phase shift in positive direction. This increase continues up to the termination of the coagulation process which makes the resistive component of the interface impedance totally predominant.

The 3D plot presented in Figure 3 showed that the phase shift curves vs. the time keep the same shape in the entire frequency scan range between 100 and 1000 Hz but providing different AC phase shift response amplitudes. This fact allows the employment of a single frequency value yielding the maximal response which can make shorter the determination time. The analysis of the results showed that the AC current phase shift amplitude registered at 1000 Hz was the maximal: rising from –61.5 up to 40.3 degrees allowing precise determination of the coagulation time (see Figure 4). This frequency value was chosen to be applied in all the further experiments. The coagulation time was found to be 7 minutes and 15 seconds using the data presented in Figure 4. This result differs from the natural coagulation time due to the presence of the anticoagulant agent (sodium heparin) added to the sample to preserve its coagulation before the experiment.

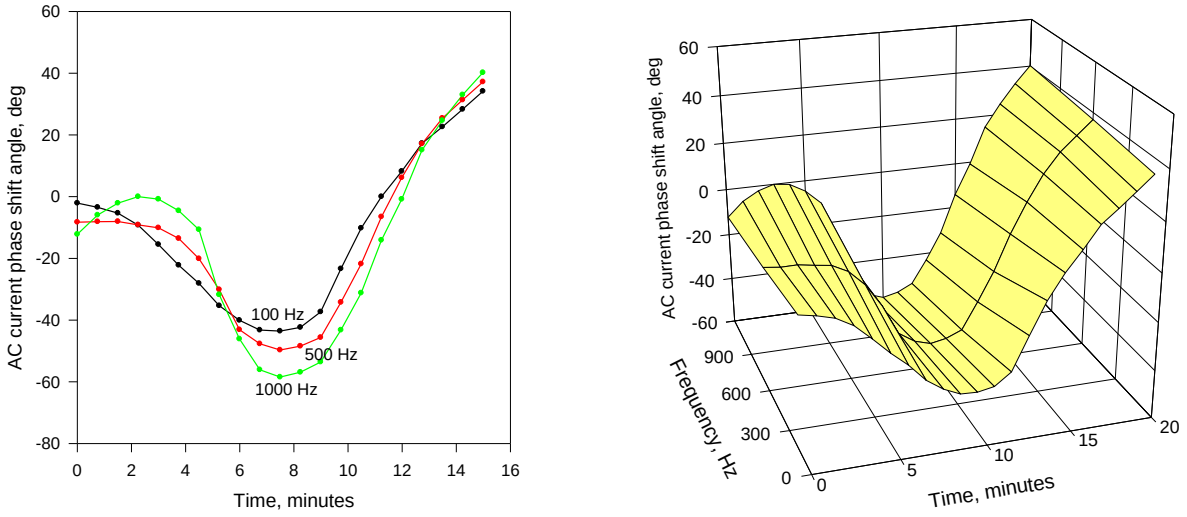

Figure 4. 2D and 3D graphics of the Phase shift vs. the Time at 100, 500 and 1000 Hz for whole blood samples at gradually decreasing rotational rates from 128,5 s^{-1} to 0,277 s^{-1}, after addition of 100 µL 2% solution $CaCl_2$ to 0.8 mL blood sample (1 minute measuring intervals)

Preliminarily the dynamic viscosity of a normal whole blood sample containing sodium heparin only was registered in the gradually decreasing rotational rates interval from 128,5 s^{-1} to 0,277 s^{-1}; see Figure 5 right. There the blood sample dynamic viscosity is high at the beginning of the experiment but decreases with the rotational rate decreasing due to the blood sample homogenization caused by the cell aggregates decomposition at the initial high rotational rate.

The dependence of the whole blood apparent dynamic viscosity vs. the time at the mentioned above shear rates interval was registered after the coagulation agent addition and the curve is presented in Figure 5 left. As seen there the blood coagulation is not able to yield a sharp jump of the viscosity response because the blood sample viscosity was already suppressed by the initial aggregates decomposition by the high rotational rate applied first. As a result, a wave shaped response curve (blood viscosity vs. the time) was registered which slope does not allow the precise determination of the beginning of the blood coagulation. In contrast a peak shaped curve was registered employing the phase shift method, subject of the present work. The X coordinate (time) of the peak clearly marks the moment of the blood coagulation beginning. The comparison of the results obtained by the rheological and phase shift methods showed that the phase shift registration can be a very useful supplement of the already widely applied rheological methods in case of unsatisfactory results obtained by the rheological methods application.

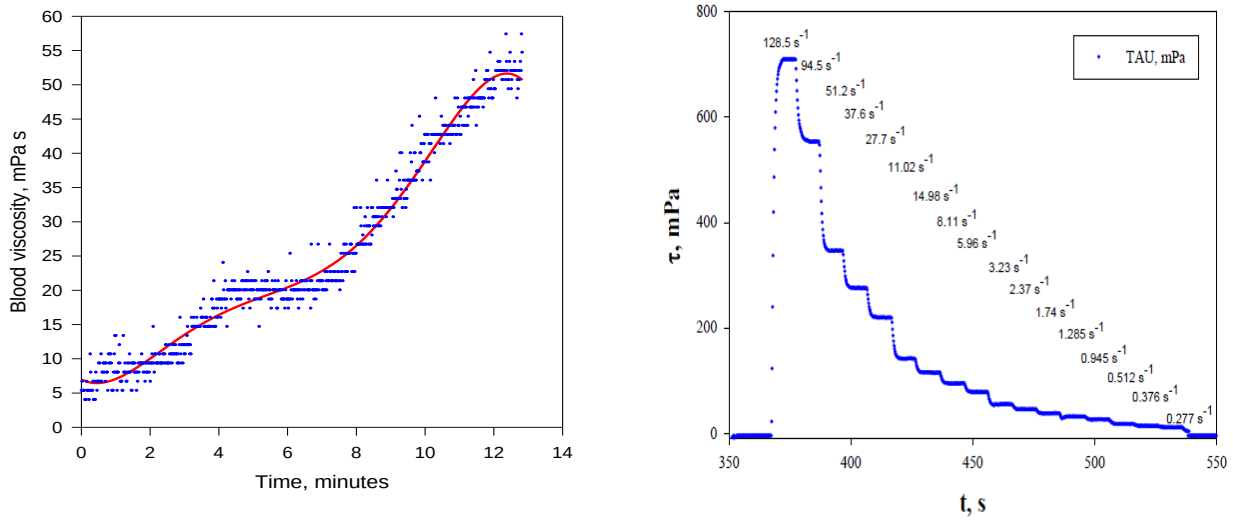

Figure 5. Left: Whole blood dynamic viscosity vs. the time after addition of 100 µL 2% solution $CaCl_2$ to 0.8 mL blood sample, Ht =34,4 %, T=37 ºC. Right : blood sample shear stresses vs. time at gradually decreasing rotational rates from 128,5 s^{-1} to 0,277 s^{-1}, Ht =45,4 %, T=37 ºC.

3.2 Precision of the determination

The precision of the determination which is a crucial parameter for each measuring method including the AC current phase shift measurement was determined over the entire frequency range between 1 Hz and 10 KHz and the results are presented below. For this purpose, a standard "dummy cell" widely used for such purposes in the electrochemistry was employed. A "dummy cell" was chosen because of the impossibility to prepare real standards samples with known properties. AC voltages with constant amplitude of 100 mV (p-p) and frequency 1, 10, 100, 1000 and 10 000 Hz were applied on its terminals. The obtained relative standard deviations of the determined phase shift at these conditions are presented in Table 1.

Table 1. Precision of the phase shift measurement determined by "dummy cell" application

Frequency, Hz	1	10	100	1000	10 000
RSD, %	1.9	1.85	1.8	1.7	1.8

The best precision was obtained at 1 KHz but the results obtained at the other frequency values are very similar, and an average value of 1.81 % was calculated for the entire frequency range.

4. CONCLUSION

A new approach was proposed and tested: AC current phase shift measurement as blood self-sufficient characterizing parameter. A virtual instrument for its application was developed and

characterized in the frequency range between 1 Hz and 10 KHz. The precision of the results determined as Relative Standard Deviations does not exceed 1.9% in the entire frequency range.

The proposed approach was applied for blood coagulation time determination comparatively with the shear stress (dynamic viscosity) method. Better results in terms of precision were obtained applying the proposed method. The frequency of 1000 Hz was found to provide maximal response and that is why only it was applied in the experiment instead of frequency scan decreasing thus the measuring time.

ACKNOWLEDGEMENTS
This study has been supported by the Basic Research Project 2018 (КП-06-Н27/13): "Development of an experimental microfluidic system and methodology for the assessment of the microrheological properties of blood. Analysis of the peripheral vasomotor reactivity and vascular endothelial functions in patients with type 2 diabetes mellitus", funded by the Bulgarian National Science Fund.

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