Photoacoustic monitoring of real time blood and hemolymph sedimentation

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The dynamics of blood and hemolymph sedimentation is studied in real time using the photoacoustic technique. A modified configuration of a conventional photoacoustic cell is used, where the advantage of this methodology is that the sample is not illuminated directly and that the process can be monitored through the measurement of the thermal contact between a reference material and the blood. It is demonstrated that during the process the thermal effusivity decreases at the region of contact between the sample and the reference materials. The usefulness of these results in real time monitoring using photothermal techniques is discussed. © 2003 American Institute of Physics. [DOI: 10.1063/1.1512982]

I. INTRODUCTION

Blood sedimentation rate is a usual indicator in clinical diagnosis; this quantity does not give a nonambiguous result about the presence of a specific disease, but it is helpful as an auxiliary method to indicate that an underlying disease may be present. Conventional methods for blood sedimentation analysis are based on the measurement of the settling of red blood cells in a thin tube during a given time.¹

Blood consists of solid particles, erythrocytes, platelets, leucocytes, etc. suspended in liquid plasma.¹ In invertebrates, hemolymph plays a role similar to blood. It presents a transparent cloudy aspect. It is composed essentially of a kind of plasma and cells mainly phagocytes and hemocytes (which carry the respiratory pigment hemocyanine).²

The process of sedimentation in blood and hemolymph generates a layered system. In the case of blood, three layers can be easily differentiated: the upper layer formed by plasma, an intermediate layer formed by leucocytes, and a third one on the bottom formed mainly by erythrocytes.¹

Photothermal methods have been used for the study of sedimentation blood rates. It has been shown that photothermal techniques are very well suited for the study in real time of these phenomena. In this case the sedimentation rate has been followed using photoacoustics.³ Photopyroelectric and photothermal radiometry methods have also been used for real time monitoring of blood sedimentation, directly illuminating the blood.⁴ In these cases the results have been interpreted as the result of changes in the optical properties of the system, taking into account the formation of a two layer system. However, heat and light transfer in the layered system, which evolves during the sedimentation process, is very complex. The formation of a layer of leucocytes between the plasma and the bottom layer (formed mainly by erythrocytes) makes difficult the interpretation of the experimental data. The changes of density and composition of the layers also involve a dynamic behavior of the thermal properties of the layers. It is of primary interest, in photothermal experiments, to evaluate the contribution of the change of the thermal properties during sedimentation. On the other hand it is necessary to explore the possibility of monitoring the sedimentation process, considering only the evolution of the thermal process.

In this article, a method is proposed that permits us to study the sedimentation process by analyzing the thermal evolution of the system without involving the changes in the optical absorption coefficient. The method consists of positioning the blood in contact with a reference material which is illuminated with modulated light, and detecting the photothermal signal in the reference material using a modified conventional photoacoustic (PA) cell.⁵ In order to show the capabilities of our method, the sedimentation process in transparent samples has also been monitored and the important case of oyster hemolymph is presented. For this system the monitoring of the sedimentation process by optical methods would be extremely difficult.

II. MATERIALS AND METHODS

Samples of blood were taken from the dorsal spine of fish of the species Tilapia nilotica, with syringes with heparin in order to prevent coagulation. Hemolymph samples were obtained, using a syringe, from the muscle of oysters of the species Crassostrea virginica. The samples obtained in this form were immediately deposited on the PA cell to be measured. The PA cell used is shown in Fig. 1. It has been previously used in the determination of the thermal properties of liquids and in the study of curing processes.⁶⁷ It consists of a conventional photoacoustics cell, closed on one side, by a transparent window and on the other by a reference material. In order to guarantee a fast heat transfer to the sample, a high diffusivity material must be chosen as a reference material. In our case a thin 50 μm aluminum foil has

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been chosen as the reference material, given that it fulfills the above conditions and permits us to avoid the thermoelastic contribution to the process. The sample (blood or hemolymph) is deposited on top of the external surface of the reference material and inside of an acrylic ring.

The experimental setup consists of a 20 mW He–Ne laser at 632.8 nm (Uniphase 1135P), whose beam was modulated by a chopper (Stanford Research Systems 540), at a constant frequency of 11 Hz, and sent onto the opaque reference material, through the transparent window on the other side of the cell. A lock-in amplifier (Stanford Research Systems, model SR830) interfaced with a personal computer was used to record the amplitude and phase of the photoacoustic signal as a function of time. The modulation frequency of 11 Hz was chosen in order to avoid the harmonics of the line frequency, and corresponds to the lower modulus of the line frequency range of our experimental system.

It has been shown that the amplitude of the PA signal for the configuration shown in Fig. 1 is given by

$$\delta p(t) = \frac{A}{\sqrt{1 + \varepsilon_i(t)} P + \varepsilon_f(t) P^2/2}. \quad (1)$$

With $A$ a quantity that does not depend on the thermal properties of the sample, $P = 1/(\varepsilon_i a/\alpha_R)$, with $\varepsilon_i = \sqrt{k_i \rho_i C_i}$, the thermal effusivity of the medium $i$, $k_i$ is the thermal conductivity, $\rho_i$ is the density, and $C_i$ is the heat capacity of medium $i$, $i = s, R$. $s$ corresponds to the sample and $R$ corresponds to the aluminum reference sample. $a = \sqrt{\pi f/\alpha_R}$, where $l_R$ and $\alpha_R$ are the thickness and thermal diffusivity of the aluminum reference sample, respectively, and $f$ is the modulation frequency of the incident light.

III. RESULTS

In Fig. 2, the photoacoustic signal amplitude as a function of time for a typical sample of fish blood is shown. As can be seen from this figure, the signal grows and after 1000 s reaches a maximum and remains constant.

In Fig. 3, the photoacoustic signal amplitude as a function of time for a typical sample of mollusk hemolymph is shown. It presents a behavior similar to the blood sample, however the time required for reaching the constant value is higher, about 1500 s. This result can be expected given that hemolymph presents a lower number of dissolved solids as compared with blood.

The increase of the signal as a function of time for both cases (see Figs. 2 and 3) can be interpreted as a consequence of the growth of the thermal resistance between the reference material and the sample, due to the sedimentation process. The evolution of the thermal effusivity of the sample can be supposed to follow a logistic-like behavior in time, beginning at an initial high thermal effusivity (low thermal resistance) to a lower thermal effusivity (high thermal resistance) at the final stages of the process. Based on this, it can be assumed that the evolution of the thermal effusivity of the samples, follows a decreasing logistic-like behavior in time,

$$\varepsilon(t) = (\varepsilon_0 - \varepsilon_f)/(1 + (t/t_0)^r) + \varepsilon_f, \quad (2)$$

where, $\varepsilon(t)$ is the thermal effusivity of the system at any time, $\varepsilon_f$ is the thermal effusivity when the process has settled down, $\varepsilon_0$ is the thermal effusivity at the beginning of the process, the parameter $t_0$ will provide the stabilization time, and the factor $r$ is proportional to the velocity of the growth of the thermal effusivity, specially at the beginning of the process. This functional behavior for the sample effusivity was included in Eq. (1) to fit the experimental data. Given that the study of the proportion of the change of thermal
effusivity was the objective of this work, the initial thermal effusivity values of the samples ($\varepsilon_0$) were supposed to be nearly equal to the thermal effusivity value of water.

In Fig. 3, the continuous line represents the fitting of the experimental data using Eqs. (2) and (3) for the sample a typical sample of fish blood. The parameters obtained from the fit are: $\varepsilon_f = 0.10 \, \text{W} \, \text{s}^{1/2} / \text{cm}$, $t_0 = 260 \, \text{s}$, and $p = 0.73$. This value of $p$ indicates that thermal effusivity presents a continuous decrease and stabilizes for times longer than $t_s = 520 \, \text{s}$. On the other hand for oyster hemolymph, the same procedure can be followed and the results obtained are: $\varepsilon_f = 0.117 \, \text{W} \, \text{s}^{1/2} / \text{cm}$, $t_0 = 380 \, \text{s}$, and $p = 0.84$. As in the case of the blood, this value of $p$ indicates that thermal effusivity presents a continuous decrease and stabilizes for times longer than $t_s = 760 \, \text{s}$.

Thermal effusivity as a function of time were calculated with Eq. (2), using the fitted parameters, for the case of blood and hemolymph. The results are shown in Fig. 4. As can be expected hemolymph thermal effusivity decreases more slowly than blood and its change during the whole sedimentation is smaller.

It is important to make the following remarks in order to understand these results. If it is considered that thermal diffusivity for blood is around $\alpha = 0.0015 \, \text{cm}^2 / \text{s}$, at the modulation frequency of 11 Hz, the thermal diffusion length for blood would be about $\mu = 70 \, \mu\text{m}$. As a consequence of this, using our approach, it is only possible to analyze the process occurring at this length. Using the fact that our results show a settling down of about 500 s for blood, it is straightforward to obtain the velocity of sedimentation, corresponding to $1.4 \times 10^{-7} \, \text{m/s}$. This result is in agreement with the velocity values for the first minutes of sedimentation reported in the literature.\footnote{P. Helander and I. Lundstrom, J. Photoacoust. 1, 203 (1982).}

In the case of the oyster hemolymph the velocity is about $0.92 \times 10^{-7} \, \text{m/s}$, as expected, given that the hemolymph density is lower.

It has been shown that the thermal properties are changing during the sedimentation process of blood and hemolymph. They are useful to study the sedimentation process without involving the analysis of the absorption coefficient evolution of the sample. In order to observe additional details, related to the complex process of sedimentation including the role of aggregation, it is necessary to make use of methodologies being able to provide larger thermal diffusion lengths. It would also be desirable to take into account our results in the existing methodologies of sedimentation monitoring by direct illumination of blood.

**IV. CONCLUSIONS**

Using photoacoustic spectroscopy, the process of sedimentation of blood and hemolymph can be monitored using an approach that involves analysis of the evolution of thermal effusivity. Our results indicate that this quantity decays after a time interval to a constant value in a thermal diffusion length.

Given that our methodology does not involve direct illumination of the sample, it presents two advantages. On one hand it permits the noninvasive analysis of the system; this aspect is crucial in the case of blood because it is light sensitive and direct illumination could induce degradation. On the other hand, given that our approach is also adequate for the study of semitransparent systems, it is possible, in the case of hemolymph, to explore whether the rate of sedimentation is related to specific diseases as happens in the case of blood.

This methodology could be adapted to be used in clinical and practical applications because it does not involve complex measurement systems. However our methodology only permits the analysis of the process in a limited region near the bottom of the sample. In order to perform the analysis of all the layers of the system, it becomes necessary to extend the method to the range of lower modulation frequencies.

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