Real time analysis of erythrocyte sedimentation by photothermal methods

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Abstract: Whole blood is an inhomogeneous, thermally fragile liquid suspension. It was shown that photothermal radiometry yields meaningful results, but required irradiation level might disturb the sedimentation process. Despite the necessity for direct contact, photopyroelectric method is more sensitive and also easier and cheaper to implement for routine measurements. All the experimental results are in a good agreement with developed theoretical models.

1. INTRODUCTION

The *in vitro* time evolution analysis of blood physical structure (such as for example erythrocyte sedimentation process), is still nowadays a difficult task. In the past, blood sedimentation has been [1-4] the object of several investigations by photothermal (PT) and photoacoustic methods (PA). However, the major problem one encounters with this type of biological samples is their instability to thermal influences, leading to convective perturbation of the sedimentation and eventually to irreversible degradation.

2. PHOTOTHERMAL MODEL FOR THE SEDIMENTATION PROCESS

Blood is a suspension of solid particles (red cells, leucocytes, platelets, etc.) in a liquid (plasma). In a first step, we assume that the sample of blood is equivalent to a two-layer system, the thickness of the first layer of plasma increasing in time. The layer beneath (which contains an increasing concentration of red cells) has a thickness that decreases in time. The sedimentation rate is a complex process which depends on many parameters. A suspension of latex spheres in water can be study in order to simulate the physical properties of blood. The sedimentation rate is then proportional to the square of their diameter and to the density difference between polymer and liquid. In the case of red cells, one has to take into account their aggregation during the process which may markedly affect [5] the sedimentation rate.

When the sample is exposed to a sine modulated excitation, the laser beam with Gaussian profile is essentially absorbed at the plasma/blood interface. The superficial plasma layer is semi-transparent for the (visible) excitation wavelength but opaque for the IR re-emitted radiation. The resulting oscillating temperature field is detected either remotely, from above (front-side, Photothermal Radiometry (PTR)), or at rear, via direct thermal contact (photopyroelectric (PPE) method). The front-side radiometric signal has been calculated [6] by Potier for semi-transparent multilayer systems and depends, among many other parameters, on the thickness of layers. For similar conditions and following the same approach as previous one-dimensional models [7-8], we obtained the two-dimensional temperature field with axial symmetry at the back-side of the sample. The complex expression of the PPE signal is then [9]:

$$\tilde{V}_{PPE}(\omega) = -\frac{p}{\epsilon} q \left[\frac{\tilde{X}_{i0}}{\tilde{\sigma}_{i0}} - \frac{\tilde{Y}_{i0}}{\beta_i} \right] + 2 \sum_{n=1}^{\infty} \frac{J_1(\lambda_n r_c)}{\lambda_n r_c} \left[\frac{\tilde{X}_{in}}{\tilde{\sigma}_{in}} - \frac{\tilde{Y}_{in}}{\beta_i} \right] A(\omega) e^{j\omega t} \quad (1)$$

$$\text{with: } \tilde{X}_{in} = \tilde{A}_{in} (e^{-\tilde{\sigma}_{in,1}} - e^{-\tilde{\sigma}_{in,1-1}}) + \tilde{B}_{in} (e^{\tilde{\sigma}_{in,1-1}} - e^{\tilde{\sigma}_{in,1}}), \quad (2)$$

$$\tilde{Y}_{in} = \tilde{E}_{in} (e^{-\beta_{i,1}} - e^{-\beta_{i,1-1}}) + \tilde{G}_{in} (e^{\beta_{i,1-1}} - e^{\beta_{i,1}}), \quad (3)$$

where p , ϵ , q are the pyroelectric coefficient, the dielectric constant and the sample/sensor surface ratio,

respectively. $A(\omega)$ is the transfer function of the equivalent electrical network [10] and β_i is sample's optical absorption coefficient. The temperature expression is represented in terms of infinite series of Bessel functions J_0 . The constants $\tilde{E}_{in}$ and $\tilde{G}_{in}$ are obtained from the heat source terms. The boundary conditions imposed on z and r allow for determination of $\tilde{A}_{in}$ and $\tilde{B}_{in}$ constants. With α_n being the n -th zero of $J_1(r)$, one has: $\lambda_n = \alpha_n / r_c$ and $\sigma_{in}^2 = \lambda_{in}^2 + j\omega/a_i$, where r_c is the sample radius, a_i is thermal diffusivity and ω is the pulsation frequency.

3. EXPERIMENTAL

With the PTR method, all experiments were performed on well stirred whole human blood samples, in free atmosphere and at ambient temperature. The first study was performed with random excitation [4]. Figure 1 shows a typical measurement with 100 mW excitation from an Argon ion laser (Spectra Physics, 488 nm). The agreement with the theoretical two-layer model is good. The noise level is very large, especially at the end of sedimentation process. With lock-in detection and sine modulation at low frequencies (0.1 Hz), the minimum excitation level still providing acceptable signals was 10 mW (Figs. 2). A theoretical investigation of the maximum irradiation level of the sample that does not disturb the sedimentation process was made. According to Rayleigh theory, the temperature difference between two sides of a 5 mm thick blood sample must be less than 1 K in order to avoid convection. Generally speaking, the Rayleigh number should be as low as possible. Based on our theoretical model, maximum allowable excitation power was estimated to about 1 to 2 mW. Using an AGEMA model 880 LW camera, we observed the surface of the blood sample submitted to such excitation level by IR thermography. The temperature values are in a good agreement with predicted ones. It was possible to visualize the sedimentation process for some minutes, but with appreciable noise.

Schematic layout of the PPE cell and the set-up are shown in Figs. 3. The bottom of the cell is a 25 μm thick metallized PVDF pyroelectric foil, which is in a direct thermal contact with the liquid sample. The signal is fed to a current preamplifier optimized for very low frequency operation (0.01 -10 Hz). The radiation source is a 1.5 mW green He-Ne laser (Melles Griot 05 LGR 193, 543.5 nm), sine modulated by an ISOMET acousto-optic modulator (model 1205C-2). The digital lock-in amplifier is Stanford SR850. To validate the theory, the two-layer sample system was simulated by immersing a thin, blackened metallic foil in water, into position parallel to the PVDF sensor and at a well controlled distance above it. In such a way, sample can be regarded as being a two-layer system of variable geometry (the upper layer being transparent and the lower layer opaque), both having the thermal properties of water. Fig. 4 demonstrates the possibility to acquire depth information from up to three thermal diffusion lengths. This implies 0.5 Hz modulation frequency for a blood layer of about 1 mm. Other test series were made with suspensions of calibrated (3.5 μm in diameter, 1 g / 20 ml) red latex spheres. The origin of the phases was taken to coincide with the value obtained at the beginning of sedimentation. Figure 5 illustrates that the PPE method is able to monitor the sedimentation from its beginning to its end.

The results from standard human blood are displayed in Figs. 6. The theoretical model accounts for increase of optical absorption coefficient of the second layer as its thickness decreases in time: $\beta = \beta_0 \cdot x$ (sample thickness/sediment thickness), with $\beta_0 = 235 \text{ cm}^{-1}$. From Fig. 6.(c), one obtains 0.35 for the ratio between the final thickness of the blood sediment (0.65 mm) and total initial sample thickness (1 mm). This is in a good agreement with the hematocyte figure. From this curve, we derive time dependence of the plasma thickness (Fig. 6.(c)). Using a sigmoid type function for the fit in Fig. 6.(c), time dependence of the sedimentation velocity (Fig. 6.(d)) was obtained. Generally, it is possible to assume that the erythrocytes settling follows the Stokes law during the first minutes of the process. However, the role of the aggregation becomes rapidly dominant in our case as seen in Fig. 6.(d). Because of high concentration, velocity increases until repulsion between the red cells saturates the process. The velocity values for the first two minutes of sedimentation process (between $1.10 \cdot 10^{-7} \text{ ms}^{-1}$ and $4.5 \cdot 10^{-7} \text{ ms}^{-1}$) agree very well with those estimated by the photoacoustic methods [2]. The results are characterized by good reproducibility and very good signal/noise ratio, especially few minutes after initialization of sedimentation process.

4. CONCLUSION

Photothermal radiometry has the advantage of remote detection, but it requires important excitation levels of radiation. The PPE method, which is a contact microcalorimetric technique, is not only more sensitive but cheaper and more reliable. It provides the fundamental parameter of blood sedimentation, i.e. the first layer thickness, via an adequate two-dimensional model with axial symmetry. Subsequently, the time evolution of the blood sedimentation velocity was determined during the entire process.

Fig. 1. Frequency dependence of PTR signal 27 minutes from the onset of sedimentation

(a)

(b)

Figs. 2. The amplitude (a) and phase (b) of PTR signal at 0.1 Hz during the sedimentation process. ("o", 2D theoretical simulation)

Fig. 3. (a) Schematic layout of the PPE cell

Fig. 3. (b) PPE experimental set-up

Fig. 4. PPE signal (1 Hz) vs. the thickness of an ideal absorbing layer ("—" theoretical curve; "o" exp. data)

Fig. 5. The amplitude (A) and phase (Φ) of the PPE signal (0.5 Hz) determined experimentally during sedimentation of latex spheres

Fig. 6. (a) Calculated amplitude and phase of PPE signal vs. the plasma thickness, during the sedimentation of blood.

Fig. 6. (b) Experimental PPE signal amplitude and phase during the sedimentation of blood

Fig. 6. (c) Time dependence of plasma thickness during the sedimentation of blood, based on data from Figs. 6. (a) and 6. (b).

Fig. 6. (d) Time dependence of velocity of the red cells during their sedimentation (time derivative of curve in Fig. 6. (c))

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