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► **To cite this version:**

Frédéric Ossant, R. Libgot, Pierrick Coupé, P. Lermusiaux, Frédéric Patat. High frequency ultrasound characterization of the coagulation process of whole blood. IEEE Ultrasonics Symposium, Aug 2004, Montréal, Canada. pp.846-849, 10.1109/ULTSYM.2004.1417869 . hal-00645436

HAL Id: hal-00645436

<https://hal.science/hal-00645436>

Submitted on 13 Mar 2023

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High frequency ultrasound characterization of the coagulation process of whole blood

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Abstract –This study was designed to characterize the blood clotting process in vitro by following changes in its acoustic properties. High frequency (from 20 to 45 MHz) ultrasound parameters were measured both in double transmission (sound velocity) and backscattering (backscatter coefficient, integrated backscatter coefficient and effective scatterer size) mode. Whole blood samples were collected from healthy volunteers. The protocol to initiate the coagulation process is describe. During the blood clotting process (two hours' observation), acoustic parameters were measured with 15 seconds time resolution for the transmission parameters and 1 minute for the backscattering parameters. The results clearly showed that changes in several acoustic parameters are able to identify several stages in the *in vitro* blood clotting process, in particular, red blood cell aggregation and blood solidification.

Keywords : high frequency ultrasound , blood clotting, red blood cell aggregation.

I. INTRODUCTION

The clinical interest in studying the blood clotting process is very old and wide : clotting deficiency linked to diseases, hematological or physiological parameters governing thrombus formation, thrombogenicity of material used for implants, etc. Ultrasound techniques have the major advantage of using of non-invasive method to describe the blood clotting process through non-transparent tissue or media. Several studies performed *in-vitro* or *in-vivo* have shown quantitatively the potentials of several ultrasound parameters such as velocity, attenuation coefficient, and backscatter coefficient [1]. Nevertheless, these studies were limited by the ultrasound frequency used (<10 MHz) or the small number of acoustic parameters measured.

Our contribution is to propose *in-vitro* analysis of the interaction of high frequency ultrasound (from 20 to 40 MHz) with whole blood during the clotting process. This analysis was based on the simultaneous measurement of both transmission and backscatter parameters. These measurements were performed with static blood, with reference to previous studies, notably their of Fatkin et al. [2], which confirmed that low or zero flow maximizes formation of red blood cell (RBC) aggregates. Therefore we studied both whole blood coagulation and sedimentation phenomena with several clotting and anti-clotting products.

II. MATERIALS AND METHODS

A. Transducer characteristics

For backscattering measurement, we used a high frequency echographic device named Dermcup2020 [3]. The probe has a single element focused transducer with linear mechanical scanning and provides a real-time image of 5mmx6mm. The transducer has a 20 MHz central frequency with a 55% bandwidth (Fig. 1).

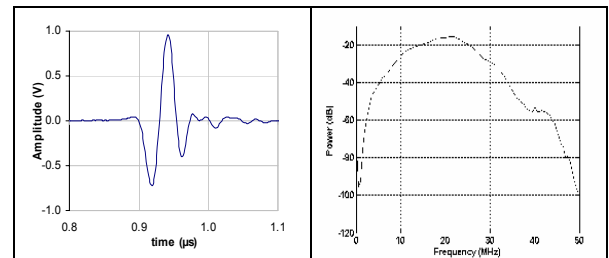


Figure 1: Impulse response (left) and associated power spectrum (right) of transducer used for backscattering measurement.

For transmission measurement, we used a 45 MHz focused transducer with a broadband (200 MHz) pulser/receiver (both designed by Panametrics). The characteristics, i.e. impulse response and power spectrum, are shown in Fig. 2. These characteristics were measured in double transmission and after crossing a division (0.5 mm) dividing blood and water chambers in the measurement cell (see part II.B. Measurement cell).

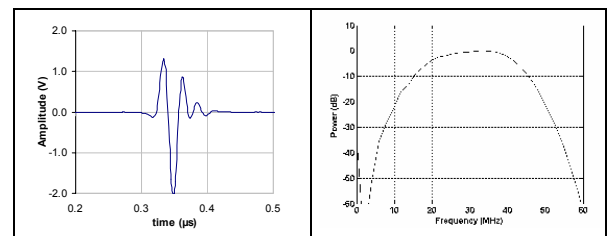


Figure 2: Impulse response (left) and associated power spectrum (right) of transducer used for double transmission measurement (Panametrics)

B. Measurement cell

The measurement cell (Fig. 3) was made in PMMA

(Plexiglas) material. It comprised a 3 ml blood chamber with a propagation path of 5 mm and with water chambers allowing acoustic coupling of the two transducers. The focal depth of the single transducer was located in the middle of the propagation path. Blood and water chambers were divided by a thin layer of plastic (15 μ m) in the case of backscattering measurement and a layer of 0.5 mm (PMMA material) in the case of double transmission measurement. Measurements in both transmission and backscattering were performed at the mid height of the blood compartment.

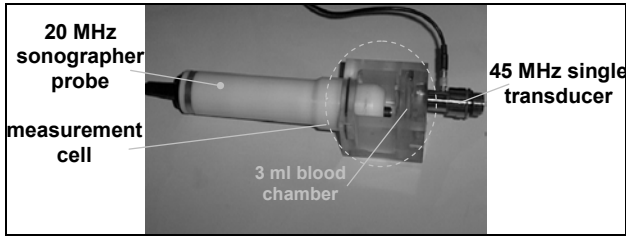


Figure 3: Photograph of the measurement cell, the 45 MHz transducer and the 20 MHz probe.

To avoid liquid evaporation and to maintain a constant temperature in the measurement cell, a lid of molded silicon was placed in the cell during measurement.

C. Ultrasound transmission parameters

For double transmission measurement, acoustic signals were digitized at 500 Mhz with 8-bit resolution (LC534A, Lecroy). The transmission parameters were measured with 15 seconds time resolution. In order to reduce noise of signals and thus increase measurement precision, acoustic signals were time averaged (averaging was performed with 16 signals).

- *Acoustic Velocity*

The technique used to measure acoustic velocity (m/s) was a classical differential pulse echo method. The ultrasound pulse was transmitted into the blood sample through the water/blood division and reflected by the bottom of the blood chamber. We then measured the time interval between ultrasound pulses reflected from the sides of the water/blood division in contact with blood (t_1) and the bottom of the blood chamber (t_2). As the time interval from the water/blood division was constant (for a constant temperature), variations in the differences in the two time intervals was due to changes in velocity in the blood sample along the propagation path (L_B). The blood velocity, V_b , can thus be estimate by the equation (1).

$$V_b = 2L_B / (t_2 - t_1) \quad (1)$$

We used a cross-correlation method to estimate variations in time interval t_2 . Cross-correlation points were interpolated

to increase resolution. Precision in velocity measurement was estimated better than 1 cm/s.

D. Ultrasound Backscattering parameters

For backscattering measurement, RF signals were digitized at 100 MHz with 8-bit resolution. Parameters were estimated in a region of interest (ROI) positioned 100 μ m below the 15 μ m layer with 224 x 252 pixels (i.e. 8.6 mm²). The focal depth of the probe was positioned in the middle of the ROI. The backscatter parameters were measured with 1 minute time resolution

- *Backscatter coefficient*

The backscatter coefficient, σ_b , represents the quantity of acoustic energy backscattered by a volume of tissue. σ_b was calculated following the expression established by Insana et al. [4]. The ROI was subdivided into 13 windows, each window representing 32x252 pixels in which the signal was thought to be stationary, the windows overlapping by 50 %. In part III Results, we will present σ_b value averaged on the 13 values estimated in the 13 windows. This parameter was attenuation and diffraction-corrected. The two parameters below were assessed from the backscatter coefficient.

- *Integrated backscatter coefficient (IBC)*

The integrated backscatter coefficient, $IBC(\text{sr}^{-1} \cdot \text{cm}^{-1})$, was calculated according to equation (2) :

$$IBCo = \int_{f_{\min}}^{f_{\max}} \frac{\sigma_b(f, z)}{f_{\max} - f_{\min}} df \quad (2)$$

- *Effective scatterer size (ESS)*

Effective scatterer size (μ m) was determined from the frequency dependence of the backscatter coefficient following the method described by Insana [2]. As for σ_b , 13 ESS values were calculated for each ROI and averaged values are presented in part III.

Note that attenuation coefficient, IBC and EES were estimated in the 10-30 MHz frequency range.

E. Sampling of whole blood and preparation protocol

- *Coagulation protocol of the whole blood:*

Whole blood samples were collected from healthy volunteers into 3.5 ml vacuum sodium citrate tubes (3.2 %). Each tube was initially heated at $37 \pm 0.1^\circ\text{C}$ in an incubator and replaced in the incubator with the blood sample for 30 min. After this time, the coagulation process was initiated by adding calcium chloride (100 μ l at 0.5M concentration), the prepared blood sample was then transferred to the blood chamber of the cell measurement and acoustic measurement began immediately.

All measurements were performed in the incubator at $37 \pm 0.1^\circ\text{C}$. Hematocrit and fibrin concentration were determined for each blood sample.

- *Sedimentation protocol for whole blood:*

Whole blood samples were collected from healthy volunteers into 3.5 ml citrated and 6 ml heparin tubes under the same temperature conditions as above. Blood samples were then transferred from heparinized or citrated tubes to the measurement chamber and acoustic measurement began immediately.

III. RESULTS

The curves shown below are typical of 20 experiments for coagulation and 10 experiments for sedimentation of whole blood.

A. Coagulation of whole blood

- *Ultrasound transmission parameters*

Temporal variations in the acoustic velocity parameters during the coagulation process are shown in Fig. 5 for two volunteers. Several characteristics differentiate of these two coagulation dynamics : the time from which the velocity increased strongly (about 40 and 20 min for volunteers 1 and 2, respectively) and the final velocity value reached at 120 min (1625 and 1610 m/s, respectively).

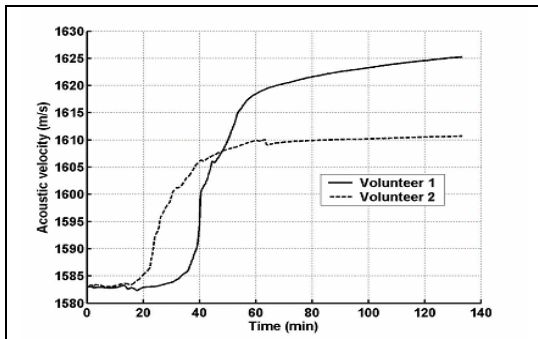


Figure 5 : Acoustic velocity as a function of time measured for volunteers 1 (Ht=40,8%) and 2 (Ht=36%) during the coagulation process.

- *Ultrasound backscatter parameters :*

Integrated backscatter coefficient (broken line) and effective scatterer size (continuous line) as a function of time during the coagulation process are shown in Fig. 6. Typically, IBC values increased rapidly over the first minutes and then decreased. Initial ESS values were zero (corresponding to Rayleigh scattering) for a few minutes and then increased strongly during the next few minutes to reach a plateau with values typically in the order of $35 \mu\text{m}$.

B. Sedimentation of whole blood

In order to validate and understand variations in the

parameter values of the clotting process better, we also measure acoustic parameters during the sedimentation of the whole blood (see *II.E*).

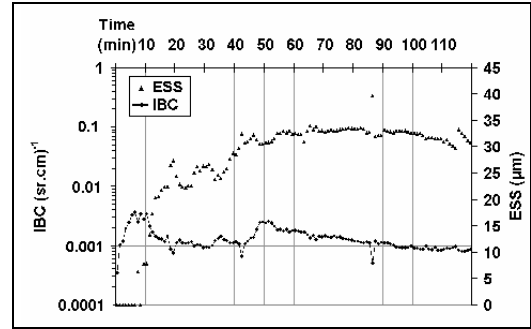


Figure 6 : Integrated backscatter coefficient (broken line) and effective scatterer size (continuous line) as a function of time for one volunteer during the whole blood clotting process.

Acoustic velocities as a function of time are shown in Fig. 7, for one volunteer. We first observed that the amplitude of the variations with sedimentation were very low (a few m/s in 140 min). The kinetics of these curves were different, with a quasi linear variation for citrated blood (dashed line) in contrast to heparinized blood (continuous line).

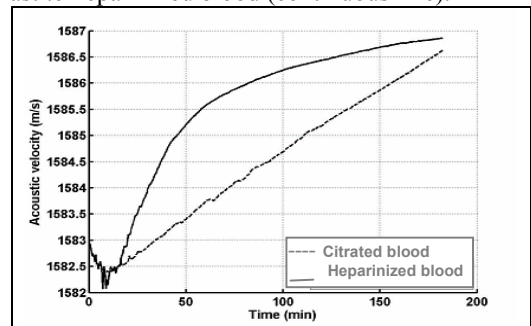


Figure 7 : Acoustic velocity as a function of time measured for one volunteer during sedimentation of two blood samples : citrated blood (dashed line) and heparinized blood (continuous line).

IBC as a function of time is shown during blood sedimentation for two volunteers in Fig. 8. Variations in values measured in citrated blood (broken line) were noised and decreased slowly. Values measured in heparinized blood (continuous line) were noised only during the first 40 minutes and then the curve was smooth with a slow decrease.

Finally, we compared ESS values as a function of time for citrated blood (broken line) and heparinized blood (continuous line) for one volunteer (Fig. 9). For citrated blood, ESS values remained at zero (corresponding to Rayleigh scattering) for ten to twenty minutes and then increased to values between 10 and $15 \mu\text{m}$ with a noised curve. For heparinized blood, values rapidly increased from zero to about $25\text{-}30$ and then slowly decreased to $20 \mu\text{m}$ with a smooth curve.

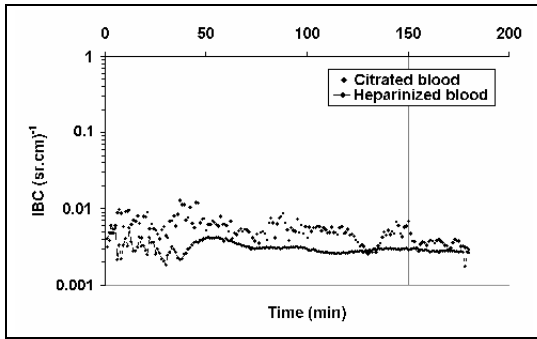


Figure 8 : IBC as a function of time measured for two volunteers during sedimentation : citrated blood (Ht=40.6%, broken line) and heparinized blood (Ht=44.3%, continuous line).

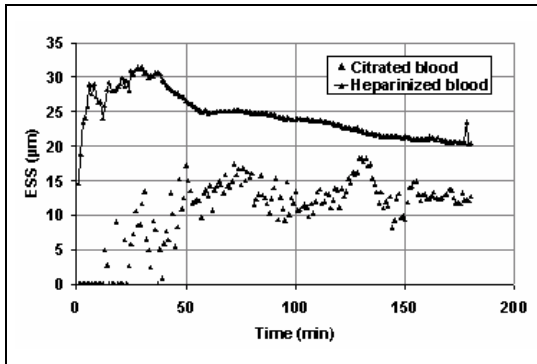


Figure 9 : ESS as a function of time measured for two volunteers during sedimentation : citrated blood (Ht=40.6%, broken line) and heparinized blood (Ht=44.3%, continuous line).

C. Discussion

The findings linked to whole blood sedimentation (Fig. 7, 8 & 9) confirmed several phenomena :

- ESS values for heparinized blood immediately increased to reach about 30 μm . This was due to RBC aggregate formation (rouleaux) in heparinized blood, an established hematological phenomenon. The slow decrease in ESS values from about 40 min might be linked to the sedimentation of these RBC aggregates;
- As a result of the lack of RBC aggregates with citrated blood (established phenomenon), ESS values increased only after 25 minutes with small size (about 12 μm) and might only have been the effect of sedimentation.
- Time velocity variations were low compared to those of the blood clotting process (Fig. 5), because no major mechanical property of blood is affected during sedimentation. Note also that the difference between the kinetics of the two curves in Fig.7, mentioned above and underlying more rapid sedimentation of heparinized blood, was probably due to the presence of RBC aggregates in this blood sample.

The findings linked to whole blood clotting emphasized specific phenomena (Fig. 5, 6 & 10):

- Increase in ESS values starting after a few minutes reflected RBC aggregates with an effective size 10 times larger than a RBC;
- Sudden increase in velocity specifically emphasized the blood clotting process (blood solidification). The fact that this clotting did not affect ESS is an important result and probably means that clot structures are governed by RBC aggregates preceding clot formation.
- Increase in IBC values during the first minutes could be linked to the formation of small RBC aggregates (not measurable at 20 MHz).

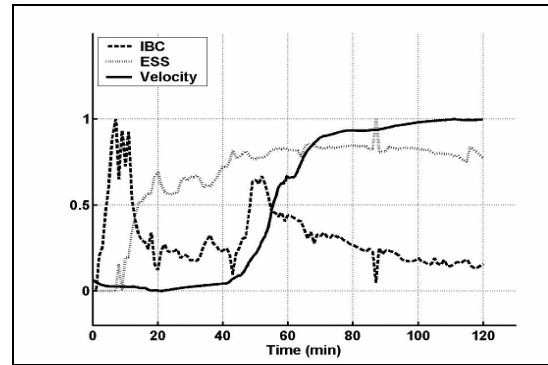


Figure 10 : Velocity, IBC and ESS parameters (normalized linear scale) as a function of time measured for one volunteer (same as in figure 6) during blood clotting process.

IV. CONCLUSION

Measurement of transmission and backscattering parameters allowed us to describe the blood clotting process both in terms of mechanical properties (blood solidification) and spatial distribution of structures (RBC aggregates). Future investigations with standardized blood samples will provide better understanding of stages in the overall blood clotting process.

ACKNOWLEDGMENT

We thank Professor Y. Gruel (Hematology Dept., Univ. Hospital, Tours) for his help in the implementation of the whole blood clotting protocol.

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