Ultrasonic Assessment of Platelet-Rich Plasma by Digital Signal Processing Techniques

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Abstract. This paper presents the implementation of an ultrasonic non-invasive and non-destructive system for the acoustic characterization of bovine Platelet-Rich Plasma based on advanced digital signal processing techniques. The system comprises the development of computational procedures that allow spectral estimation of parameters such as the angular coefficients with linear frequency dependence and the measurement of the speed of sound of regions of concern in sample studies. The results show that the relationship of acoustic parameters obtained from backscattered ultrasonic signals contributes to the hematological prediction of platelet concentration based on linear regression model.

Keywords: Attenuation · Backscattering · Blood

1 Introduction

Platelets rich plasma (PRP) is an important bioactive substance in processes that stimulate tissue regeneration and healing, acting as an autologous source of growth factors and in turn stimulating the secretion of proteins involved in tissue regeneration [1]. Currently, the characterization of plasma for therapeutic applications in animals [2] as well as in humans causes great interest, since the quality of the platelet concentration present in the PRP should be sufficient to guarantee the regenerative potential [3]. Considering the different methods of platelet quantification, based on the direct observational analysis performed under a conventional microscope (susceptible to errors by the observer's appreciation), to automated methods with hematology equipment able to identify different cell types from a single whole blood sample [4], it is well known that the methods mentioned above present variability in sensitivity and specificity, as well as disadvantages in relation to the costs of operation, maintenance and negative impacts generated to the environment, by the use of chemical reagents [5], which suggests the need of looking for alternative methodologies or techniques.

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On the other hand, ultrasound-based technologies are shown to be advantageous in the non-invasive characterization of tissues, fluids and materials [6], in addition to having an optimal cost-benefit ratio. Characterization of biological fluids has been proposed recently, particularly at the hematological level, in which blood coagulation [7], erythrocyte aggregation [8, 9] and estimation of glucose concentration parameters [10] have been proposed, among others performed in humans and animal models [11], reflecting the scope of ultrasonic characterization techniques.

Taking into account the potential of high frequency mechanical wave characterization techniques and their validation for the estimation of concentrations of substances, the present study presents the implementation of a non-invasive non-destructive system of quantitative characterization of animal blood plasma, based on spectral energy loss estimation algorithms and time of flight measurements, which provide information associated with platelet concentration indices.

2 Materials and Method

2.1 Hematological Samples

Twelve samples of bovine blood each one with 5 ml were obtained from the jugular vein in the faculty of veterinary medicine of the Antonio Nariño University (UAN). All experimental tests were carried out with the consent of the research group on welfare, health and animal production (UAN). The protocol for obtaining samples of platelet-rich plasma consisted first of the provision of hematological samples in EDTA tubes at rate of centrifugation of 2400 rpm during 10 min. The samples were processed for histological analysis, mounted on 75 mm × 25 mm slides, and then treated with Masson's trichrome staining. They were analyzed under an optical microscope at 100x, followed by the corresponding quantification of platelets in Neubauer's chamber (see Fig. 1).

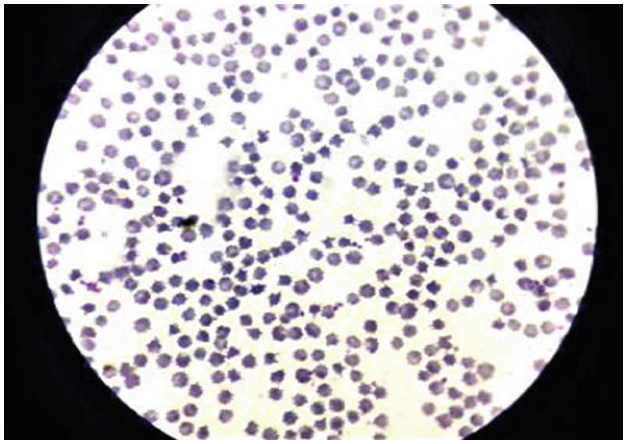

Fig. 1. Morphological analysis and platelet count. 100× (Masson's trichrome staining).

2.2 Characterization System

The acoustic non-invasive and non-destructive characterization system comprises: (a) an ultrasonic field emission system (*Olympus 5072PR*), (b) a positioning machine and (c) a data acquisition and processing system. The ultrasonic field emission system is configured in pulse-echo mode to provide an excitation energy of 26 μJ for an ultrasound transducer (*Panametrics A019s*) with a central frequency of 5 MHz at bandwidth of 6 MHz at −6 dB. It is stimulated with a pulse repetition frequency of 100 Hz. The positioning machine uses a ROHS stepper motor (28BYJ-48) driven by an *Arduino* microcontroller, which allows a control step size of 1 mm by coupling of an endless screw. This system also includes a mechanical support to hold the ultrasound transducer and an acoustic glass tank on which a liquid is attached aiming the coupling between the acoustic wave transfer and the hematological sample. At last, the data acquisition system includes a *Gw Instek GDS-1102 A-U* oscilloscope, which stores the ultrasonic backscatter signals in the interest region (RoI) with a sampling rate of 50 m/s. The stored signal files are subsequently processed by computational procedures implemented using *Matlab* (*R2014b*). Figure 2 describes the experimental set up components.

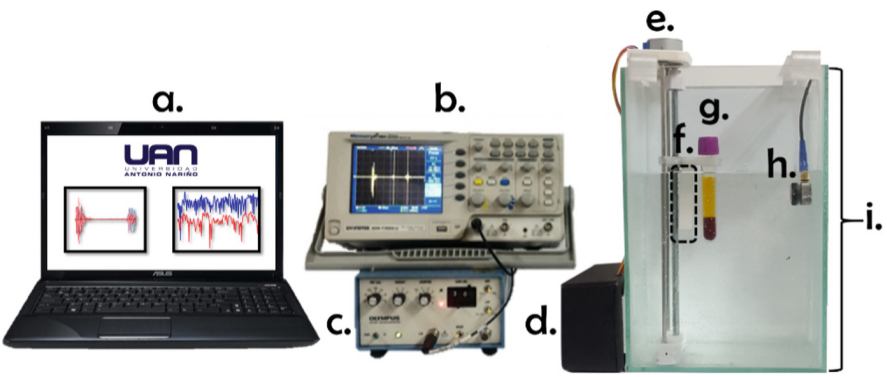

Fig. 2. Ultrasonic characterization system design and implemented system. a. Processing system. b. Oscilloscope. c. Pulse generator. d. Microcontroller. e. Stepper motor and endless screw. f. Backing. g. Hematological tube. h. Transducer. i. Acoustic tank.

2.3 Ultrasonic Inspection

The bovine platelet-rich plasma samples contained in the clinical hematology tubes were subjected to a high frequency and low power acoustic field at a focal length of 6 cm, where a more uniform wave front with minimization of the diffraction effects is guaranteed. For each sample, twenty signals were obtained during the vertical sweep

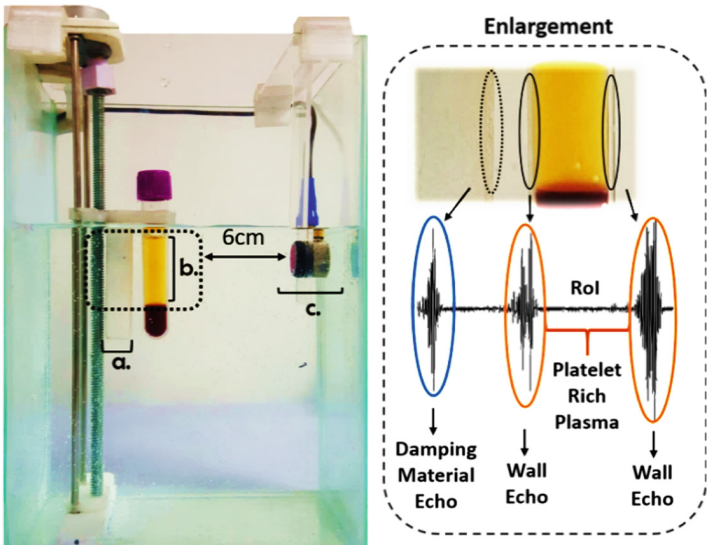

Fig. 3. Backscattering signal in RoI of platelet-rich plasma.

from the basal – line of blood plasma aiming the digital signal processing. An example of the ultrasonic backscattering signal collected in the RoI is shown in Fig. 3.

2.4 Computational Procedures

The characterization of the study samples was performed using digital signal processing algorithms, which estimate three acoustic parameters: speed of sound, acoustic attenuation and the backscatter integrated coefficient, being the last two based on spectral analysis techniques. The calculation of the speed of sound is important taking as it contributes to the understanding about the relationship between the acoustic impedance and the density of the study medium, as well as provides information that might be associated with the compressibility characteristics and particles concentration of biological fluids studied, which allows its characterization. Experimentally, the speed of sound (c) was determined by measuring the time of flight (ToF) of a wave travelling along the propagation distance (x) in the RoI, detecting echoes of high amplitude produced by the high reflection coefficient of the polypropylene walls of the hematological tube, associated with acoustic impedance change between the tube wall and the blood plasma. According to the *ToF*, the speed of sound was estimated based on the expression:

$$c = \frac{2x}{ToF} \tag{1}$$

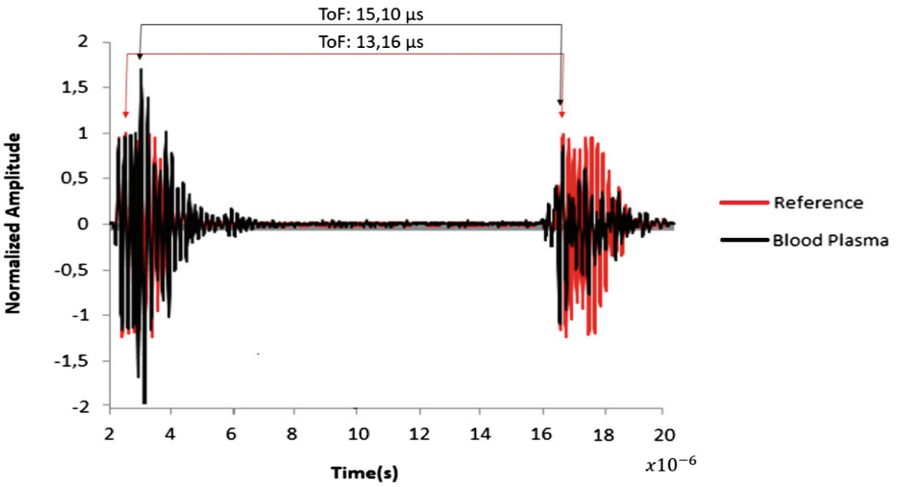

Fig. 4. Ultrasonic backscattering signals from platelet-rich plasma and reference medium.

Figure 4 shows an example of the echoes selected on the walls of the blood test tube aiming the calculation of the speed of sound. The distance of propagation in the colloidal system is twice the diameter of the hematological tube (considering that the acoustic inspection was carried out in pulse echo mode). The speed of sound algorithm was adjusted from experimental measurements in water which was used as reference liquid.

The calculation of the acoustic attenuation coefficient is a parameter that describes the loss of acoustic energy caused by the propagation medium. Its value depends on the medium in question. In soft tissue characterization procedures its calculation is given by the following equation:

$$\alpha = 10log\left(\frac{Vx}{Vi}\right)/2 \tag{2}$$

Considering the power law $\alpha = \beta f^n$, the frequency-dependent attenuation coefficient (β) can be estimated assuming that experimentally, the frequency dependence index n for soft tissues can be approximated linearly.

Computationally, the acoustic attenuation is measured based on the estimation of the angular coefficient given by the linear adjustment between the normalized power spectral subtraction obtained from ultrasound echoes in platelet-rich plasma and water. The spectral subtraction guarantees the elimination of energy loss effects caused by the hematological tube walls and alignment errors in the incident ultrasonic field (Fig. 5).

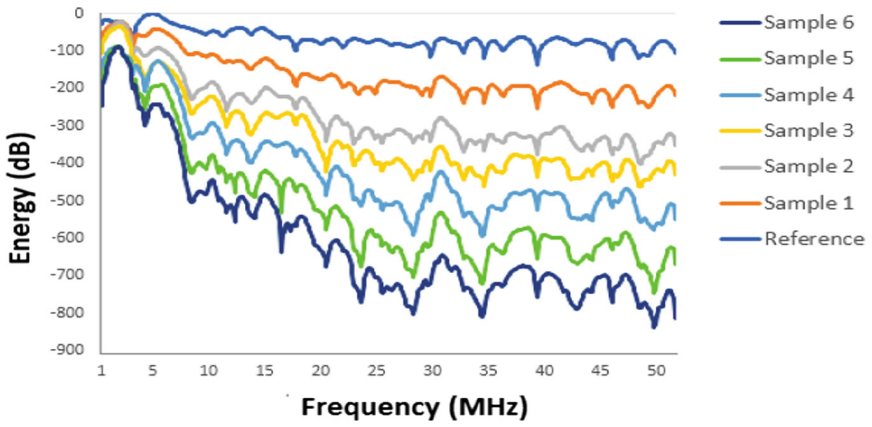

Fig. 5. Spectral differences for different platelet-rich plasma samples and its respective comparison with reference spectrum in water.

3 Results

Acoustic parameters values were estimated for blood plasma samples. These values are displayed in Table 1.

A linear relationship between platelet concentration and experimental estimation of speed of sound and loss of spectral energy (attenuation) was found in Fig. 6. The speed of sound can be adjusted linearly showing the increase of plasma platelet concentration. For the speed of sound, the linear regression is described by the mathematical model seen in Fig. 6, $y = 0.9061x + 1218$, indicating a growth rate of 1 m/s for each 1.103×10^3 platelet per mm^3. The results obtained were compared with existing data reported in the literature [12], for measurements of speed of sound for blood cell concentration [13, 14], which also indicates a linear proportionality. On the other hand, attenuation estimation presented a linear relation according to the increase of platelets, evidencing an angular coefficient of 2×10^{-6} that describes the resolution for predicting concentration values.

It is clearly observed that these acoustic descriptors present a proportional relation to the cell concentration and allow a better understanding of the interaction process wave - colloidal system, from which it is possible to predetermine and quantify platelet concentration.

Table 1. Acoustic parameters estimated by signal processing techniques

Sample	Speed of sound average (m/s)	Attenuation average (dB/cm-MHz)	Platelets (10^3/mm^3)
1	1.398	0.000308	287
2	1.590	0.000281	283
3	1.433	0.000163	280
4	1.438	0.000134	225
5	1.431	0.000141	225
6	1.385	0.00011	209

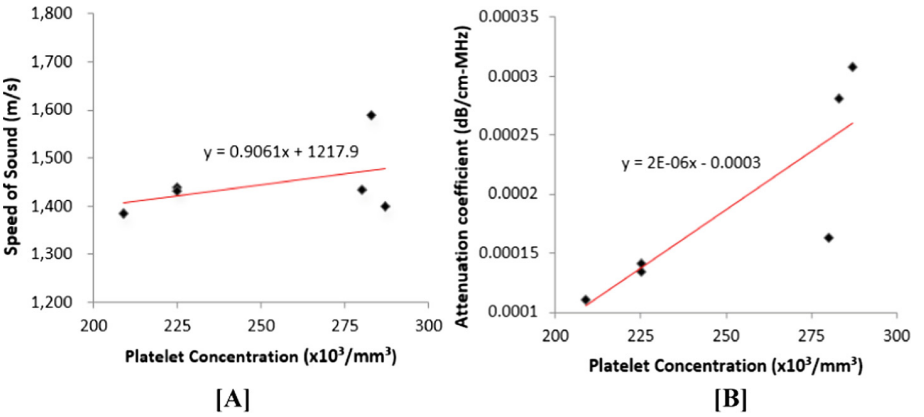

Fig. 6. A. Average speed of sound profile for different platelet concentration. B. Profile of the acoustic attenuation average for different platelet concentration.

4 Conclusions

The computational procedures for the ultrasonic characterization of Platelet-Rich Plasma from bovine blood samples showed the potential of acoustic parameters such as speed of sound and attenuation for the estimation of platelet concentration. The calculations made possible the estimation of a linear regression model that allowed to infer platelet concentration values, evidencing that for speed of sound variations of approximately 53 m/s there is an approximate variation of 55.18×10^3 platelets per mm^3. Finally, the experimental results showed a linear proportionality between the concentration cells and the estimated parameters demonstrating the relevance of the ultrasonic quantitative characterization technique to infer in measurement of substance concentrations in other applications.

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