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The intensity reflection coefficient: A complementary method for investigating blood backscattering properties with ultrasound

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Abstract. Parametric imaging of backscatter indices is an active field of research in ultrasound (US) tissue characterization. The US intensity reflection coefficient (IRC) is introduced to characterize porcine blood in a Couette shear flow system. US properties of red blood cells (RBCs) were investigated at shear rates of 5 s^{-1} and 500 s^{-1} for hematocrits ranging from 0 to 54%. The IRC was determined and compared to the integrated backscatter power (IBP) calculated between 8 MHz and 12 MHz. The frequency dependence of both parameters was also explored using their spectral slopes determined in the same frequency bandwidth. The experimental results showed that the IRC is a mirror function of the IBP and their spectral slopes had similar behavior. At a shear rate of 500 s^{-1}, selected to disaggregate RBCs, the IRC exhibited a bi-modal variation with a minimum at 23% hematocrit. The minimum was less pronounced and moved down to 13–17% hematocrits in the case of a shear rate of 5 s^{-1} that promoted aggregation. The IBP presented a sharp peak at 25% hematocrit for the high shear-rate value, while a plateau appeared after 13% hematocrit in the case of the low shear rate. Furthermore, the transition from lower to higher shear rate was accompanied by a diminution in the level of both indices. In the frequency domain, the spectral slope of the backscatter power presented a Rayleigh scattering behavior (value around 4) for all hematocrits at a shear rate of 500 s^{-1}. It dropped to a value of 2 beyond 13% hematocrit when the shear rate was decreased to 5 s^{-1}. At a shear rate of 500 s^{-1}, the spectral slope of the IRC was constant and close to 3.8 below 28% hematocrit and decreased at higher hematocrits. At 5 s^{-1}, it was constant (between 3.7 and 3.9) for hematocrits lower than 17% and then decreased. This study showed the potential of the intensity reflection coefficient to investigate the RBC aggregation phenomenon. An advantage of IRC is its easy computation.

Keywords: Ultrasound, intensity reflection coefficient, integrated backscatter power, spectral slope, red blood cells, aggregation

1. Introduction

Red blood cell (RBC) aggregation is a natural and reversible phenomenon that occurs in the blood circulatory system. It reduces the resistance of blood flowing at the vessel wall by assembling and packing RBCs at the center of the vessel, and placing a cell-poor fluid layer as a lubricant between the

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wall and the aggregated cells. This effect is more encountered in small vessels. It is mainly influenced by a high hematocrit and a high concentration of macromolecular plasma proteins such as fibrinogen [1–3]. Pathologies such as hypertension, diabetes, atherosclerosis, coronary artery disease and certain forms of cancer, are associated with abnormally high levels of RBC aggregation [4–6]. The study and comprehension of the RBC aggregation phenomenon is therefore very important and many methods are used worldwide for this purpose: laser-light scattering [7], microscopic observations [8], viscometry [9] and erythrocyte sedimentation-rate measurements [10]. Because ultrasound (US) is safe, noninvasive, relatively inexpensive and more importantly allows *in vivo* measurements, this modality has been extensively used for the investigation of RBC aggregation. Experimental measurements showed that the US backscatter power increases with the level of RBC aggregation [11]. In the case of aggregating RBCs, such as human or porcine RBCs, the US backscatter power presented a shear-rate dependent behavior [12]; while for nonaggregating RBCs, such as bovine RBCs, the US backscatter power was less affected by the shearing condition [13]. Extensive theoretical and experimental studies were conducted to understand the interaction between ultrasound and the aggregation of RBCs [14–18]. Most of these studies were investigating the US backscatter coefficient, and despite the difficulties inherent to its measurement [19], little interest was dedicated to other acoustical parameters.

In this paper, the intensity reflection coefficient (IRC) is introduced as a new tool for blood tissue investigation. We used the integrated backscatter power (IBP) as a gold standard and did investigate the two parameters versus hematocrit and shear rate. A high shear-rate level that favors the formation of nonaggregated RBCs, and a low shear-rate level that initiates their aggregation were used. We also studied the frequency dependence with the spectral slope of the backscatter power and the intensity reflection coefficient. The spectral slopes were very sensitive for the detection of aggregated RBCs. The comparison between the IRC and backscatter power globally showed equivalence in their behavior as a function of hematocrit and shear rate. The advantage of the new acoustic parameter is its simplicity and sensitivity to probe the formation of organized structures of RBCs.

2. Material and methods

2.1. Blood sample preparation

Porcine whole blood, supplied from a local slaughterhouse overseed by Agriculture Canada, was anticoagulated with EDTA (EthyleneDiamine-Tetraacetic Acid, Sigma Chemical Co., St. Louis, MO, USA) at a concentration of 3 g per blood liter [18]. After centrifugation with a Heamofuge instrument (model 3612, Heraeus Sepatech Instr. GmbH, Germany) to separate the erythrocytes from the plasma and other cells, 50 ml blood samples with hematocrit ranging from 0 to 54% were reconstituted by mixing RBCs with the plasma. The volume fraction of RBCs (hematocrit) was measured manually with a ruler on the microtube containing blood.

2.2. Couette shear flow system

The acoustic parameters were measured using a Couette shear flow system (Fig. 1). It consisted of a stationary inner cylinder surrounded by a concentric rotating outer cylinder. Both cylinders were made of Plexiglas. The 2 mm space between the walls of the two coaxial cylinders was filled with blood experiencing controlled shear rates depending on the angular speed of the rotating cylinder. A 10 MHz center frequency focused transducer (model V312-SM, Panametrics, Waltham, MA, USA) with a diameter of

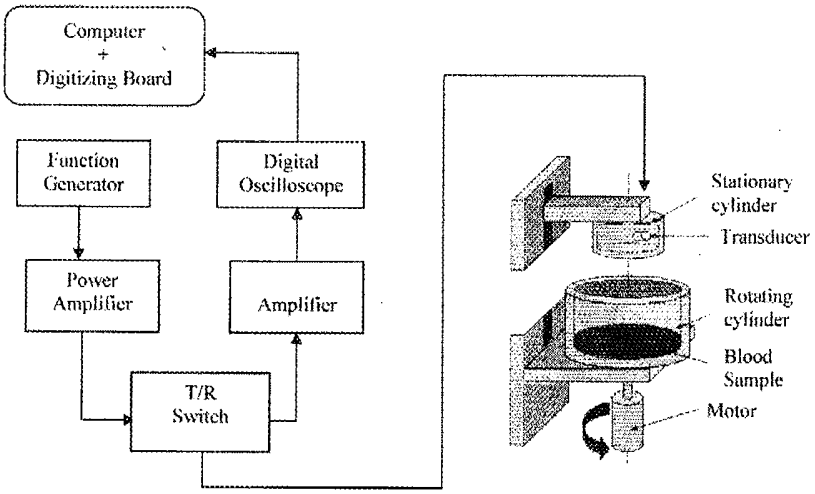

Fig. 1. Block diagram of the pulse echo measurement system and the Couette shear flow apparatus. The stationary inner cylinder was slid down within the outer rotating cylinder for measurements. (Color online.)

6 mm and a focal length of 12 mm was mounted in the side wall of the stationary inner cylinder. This was done in such a way to direct the US beam perpendicular to the wall of the outer rotating cylinder. The cavity between the transducer and blood contained agar gel (3%) to which glycerol (8%) was added to control the acoustic mismatch impedance between the transducer and blood. To measure the IRC, the ultrasound beam was focused at the interface between agar gel and blood in order to maximize the power of the reflected pulse. In the case of the backscatter power measurements, the ultrasound beam was focused at the center of the interstice between the two cylinder walls where blood was located. The agar gel surface between the transducer and blood matched the curvature of the inner cylinder for not disturbing the flow.

2.3. *Experimental set-up and data acquisition*

The experimental arrangement for the pulse echo system to measure the backscatter power and IRC is shown in Fig. 1. A waveform generator (model 33250A, Agilent Technologies, Santa Clara, CA, USA) was used. Pulses of ultrasound were sent out in a sinusoidal tone burst mode of 10 MHz frequency. They had a pulse repetition frequency (PRF) of 1 kHz, and contained 5 cycles per pulse. They were amplified with a broadband radio-frequency (RF) power amplifier (model 75A250, Amplifier Research, Souderton, PA, USA), and supplied to the 10 MHz transducer via a T/R (Transmit/Receive) switch (model RDX-6HF, Ritec, Warwick, RI, USA). The backscatter RF signal from blood samples was received and amplified (model 5900PR, Panametrics, Waltham, MA, USA) prior to its digitization and storage in a computer for an off-line processing. The signal was acquired using a high speed digitizing board (model CS 8500, Gage Applied Sciences Inc., Montreal, Qc, Canada) at a sampling frequency of 500 MHz.

2.4. *Experimental protocol*

The first part of the experimental protocol was aimed to acquire RF backscatter signals from blood. The transducer was focused at the center of the interstice between the two cylinders. Then the blood

sample with the proper hematocrit was poured into the Couette instrument (outer cylinder). Commands were sent to the rotating cylinder via a LabView program (version 7, National Instruments, Austin, TX, USA) to obtain the appropriate shear rates. For each blood sample, US backscatter signals corresponding to the shear rate of 500 s^{-1} were first acquired. According to [20], this shear rate was sufficient to completely disaggregate RBCs of porcine blood. The first recordings were followed by the acquisition of US backscatter signals at a shear rate of 5 s^{-1}. At that shear rate, a time period of 5 minutes was allowed for the RBCs to form aggregates before collecting RF signals. Note that a lower shear rate was not used because blood sedimentation could affect the interpretation of our results. The second part of the protocol was to collect reflected US signals from the interface between agar gel and blood. The position of the transducer was then shifted back in such a way to focus on that interface. The same procedure as for the backscatter signals was applied to acquire the reflected signals. All measurements were done at room temperature.

2.5. Data processing

In the case of the backscatter and reflected signals, one hundred RF signal lines were recorded for every blood sample at both shear rates. The characterization of the backscatter signal was based on a calculation of the relative integrated backscatter power (IBP) using the FFT transformation:

$$IBP = \frac{1}{f_2 - f_1} \frac{\int_{f_1}^{f_2} S_b^2(f)\,\mathrm{d}f}{\int_{f_1}^{f_2} S_0^2(f)\,\mathrm{d}f}, \tag{1}$$

where $S_b^2(f)$ is the power spectrum of the backscatter signals from blood over a frequency bandwidth ranging from f_1 (8 MHz) to f_2 (12 MHz). A normalization of the power spectrum $S_b^2(f)$ was done with respect to the power spectrum $S_0^2(f)$ of the backscatter signal from a flat reflector made of stainless steel. The FFT transformation was performed within a time window of 2 μs, which was centered on the backscatter signal from blood. To improve the signal to noise ratio, average power spectra were calculated over the 100 RF lines.

The computation of the relative IRC was derived from the measurement of the reflected pulse amplitude $s_r(t)$ at the interface agar gel–blood, which was normalized with respect to the reflected pulse amplitude $s_0(t)$ at the interface agar gel–plasma :

$$IRC = \frac{\int_{t_1}^{t_2} s_r^2(t)\,\mathrm{d}t}{\int_{t_1}^{t_2} s_0^2(t)\,\mathrm{d}t}. \tag{2}$$

The relative IRC was calculated within a time window (t_1, t_2) centered on the reflected signal and having a duration of 2 μs.

The calculation of the backscatter power spectral slope was derived from the average power spectrum of the backscatter signal from blood, which was normalized with respect to a reference power spectrum (using a flat reflector made of stainless steel). A log–log representation of the normalized backscatter power versus frequency between 8 MHz and 12 MHz allowed the determination of the spectral slope using a linear regression. The same procedure was applied to study the frequency dependence of the intensity reflection coefficient. The power spectrum of the reflected signal at the interface agar gel–blood was calculated, averaged and then normalized with respect to the power spectrum of the reflected

signal from the flat reflector. A log–log representation over the same frequency range as it was done for the backscatter power with a linear regression was used to compute the spectral slope of the intensity reflection coefficient.

2.6. Statistical analyses

One-way analyses of variance (ANOVA, SigmaStat, version 3.11, Systat software, San Jose, CA, USA) with the Bonferroni test for multiple comparisons were performed to support results of Figs 2–5 on IBP and IRC, and their spectral slopes. A significance level of $p < 0.05$ was considered to be statistically significant.

3. Results

The key feature of the present work was the possibility to determine the relative integrated backscatter power and the relative intensity reflection coefficient versus the hematocrit of porcine blood at two shear rates; and simultaneously study the frequency dependence of both parameters. The IBP and IRC were not compensated for attenuation and were thus relative to a 0 dB reference. Error bars in all figures correspond to the standard deviation of five measurements with different blood samples.

3.1. Backscatter power

3.1.1. Relative integrated backscatter power

Figure 2 shows the relative IBP in decibels (dB) versus hematocrit at shear rates of 5 s^{-1} and 500 s^{-1}. For the lower shear-rate value, the relative integrated backscatter power increased with increasing hematocrit and reached a plateau at 13% hematocrit ($p < 0.05$ only for multiple comparisons between

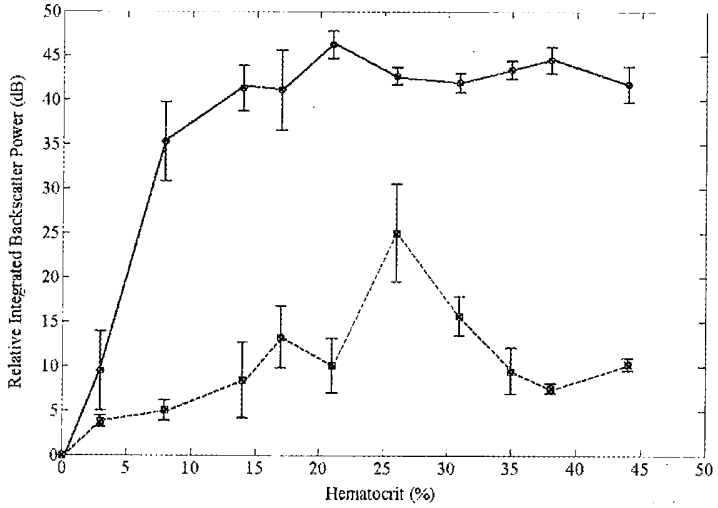

Fig. 2. The relative integrated backscatter power in dB versus hematocrit of porcine RBCs at shear rates of 5 s^{-1} (●) and 500 s^{-1} (■). The integration was done over a frequency bandwidth ranging from 8 MHz to 12 MHz and the 0 dB reference value was attributed to the integrated backscatter power from plasma. Results are mean ± one standard deviation from five experiments.

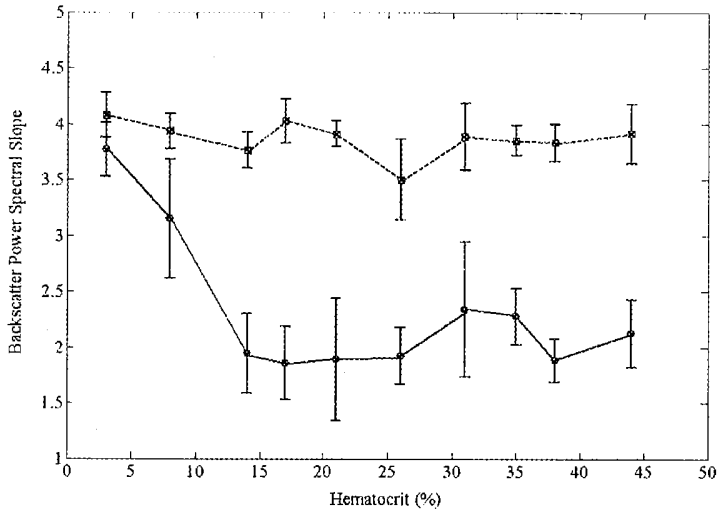

Fig. 3. The spectral slope of the backscatter power versus hematocrit for porcine RBCs at shear rates of 5 s^{-1} (●) and 500 s^{-1} (■). Results are mean ± one standard deviation from five experiments.

hematocrits of 3%, 7% and 13%). In the case of the higher shear-rate level, it peaked sharply for 25% hematocrit ($p < 0.001$) and then decreased when hematocrit was further increased. The relative IBP was shear-rate dependent, decreasing when the shear rate was increased at all hematocrits. At the particular value of 43% hematocrit, the decrease level presented an average of 32 dB.

3.1.2. Backscatter power spectral slope

Figure 3 shows the behavior of the backscatter power spectral slope versus hematocrit and shear rates. At a shear rate of 500 s^{-1} where no significant RBC aggregates are expected, the backscatter power spectral slope presented a constant value close to 4 over the whole explored hematocrit range ($p > 0.05$ expect for slopes at hematocrits of 3% versus 25% and 17% versus 25%). In the case of the lower shear-rate value (5 s^{-1}), the spectral slope was hematocrit dependent ($p < 0.001$). It decreased from a value close to 4 at low hematocrits to a value around 2 for hematocrits higher than 13%, where a plateau was reached ($p > 0.05$ for all multiple comparisons between hematocrits of 13% to 44%).

3.2. Intensity reflection coefficient

3.2.1. Relative intensity reflection coefficient

Figure 4 shows that at the shear rate of 5 s^{-1}, the relative IRC was hematocrit dependent ($p < 0.001$). It decreased smoothly until 13–17% hematocrits and then started to increase with increasing hematocrits. In the case of the shear rate of 500 s^{-1}, the minimum value of the relative IRC was shifted to 23% hematocrit ($p < 0.001$). The relative IRC was shear-rate dependent and decreased with increasing shear rate. The relative IRC dropped by a magnitude of 6 dB between shear rates of 5 s^{-1} and 500 s^{-1} at 40% hematocrit.

3.2.2. Intensity reflection coefficient spectral slope

Figure 5 shows the behavior of the relative IRC spectral slope. At a shear rate of 5 s^{-1}, the spectral slope of the relative IRC varied almost linearly as a function of the hematocrit. All values differed

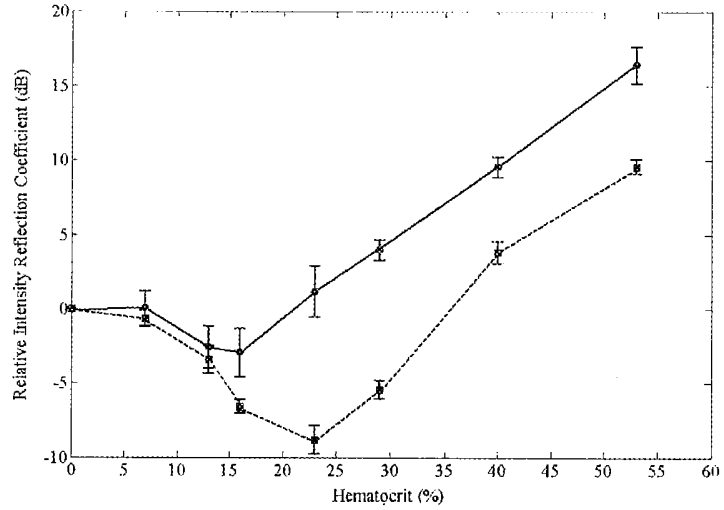

Fig. 4. The relative IRC in dB versus hematocrit at shear rates of 5 s^{-1} (●) and 500 s^{-1} (■). The 0 dB reference value corresponds to the intensity reflection coefficient at the interface agar gel–plasma. Results are mean ± one standard deviation from five experiments.

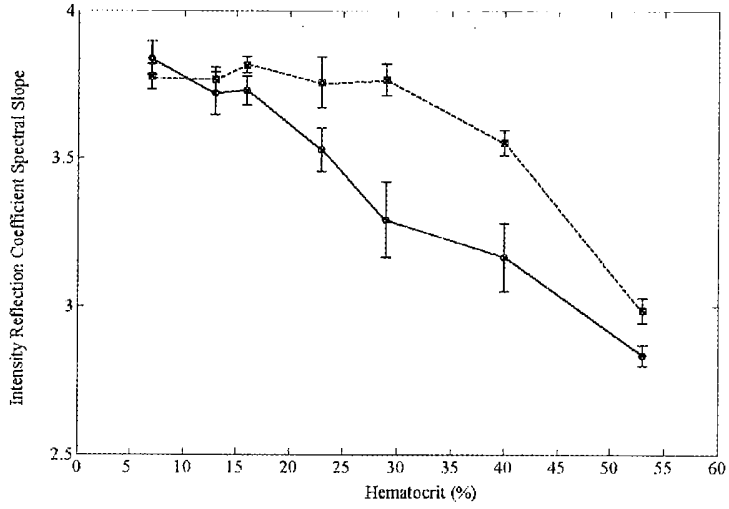

Fig. 5. The intensity reflection coefficient spectral slope versus hematocrit of porcine RBCs at shear rates of 5 s^{-1} (●) and 500 s^{-1} (■). Results are mean ± one standard deviation from five experiments.

($p < 0.02$), except for hematocrits of 7% versus 13% and 17%, 13% versus 17% and 28% versus 40%). It was close to 3.8 at low hematocrits and dropped to 2.8 at a hematocrit of 54%. For the higher shear-rate value of 500 s^{-1}, the spectral slope was constant until 28% hematocrit ($p > 0.05$ for all multiple comparisons between hematocrits of 7–28%), and then decreased but less than in the previous case.

4. Discussion

This study investigated porcine whole blood using the integrated backscatter power and the intensity reflection coefficient. The hematocrit dependence of the two parameters was equivalent and strongly dependent on the shear rate. We also explored the frequency dependence of the two indices that also varied with the hematocrit and shear rate.

4.1. The integrated backscatter power and intensity reflection coefficient

The backscatter coefficient from blood has been reported to vary linearly for low hematocrits. As the hematocrit is increased, the relationship becomes non linear with the appearance of a peak backscatter position, which depends on the nature of the flow [21]. Figures 2 and 4 showed a nonlinear behavior for both IBP and IRC. Furthermore, the two parameters were symmetrical to the horizontal axis (mirror relationships), especially at a shear rate of 500 s^{-1}. Table 1 summarizes the symmetry between the integrated backscatter power and the intensity reflection coefficient, and gives the hematocrit values where they had extrema.

Previous studies in tube flow showed that the ultrasound power backscattered by nonaggregated porcine RBC suspensions peaked near 13% hematocrit under laminar condition [13]. Under Couette flow conditions, we observed that the integrated backscatter power peak was shifted towards a higher hematocrit (25%) in the case of a high shear rate where RBC disaggregation is promoted. This difference was probably related to the appearance of flow disturbance at elevated shear rates, as suggested by Shung et al. [22]. In the presence of flow instabilities, they showed that the backscatter peak moved to 20% hematocrit. The backscatter power of porcine aggregating RBCs under laminar flow has also been reported to be affected by the flow velocity (i.e. the shear rate) [23]. At low flow velocity, a plateau was observed beyond 13% hematocrit, which is consistent with our results of Fig. 2 at a shear rate of 5 s^{-1}. As it was documented in several studies [21], the level of IBP was found to drop when the shear rate was elevated. The decrease level at 43% hematocrit was 32 dB in the present study, whereas Shung et al. [23] noticed a diminution of 6 dB in the magnitude of the backscatter power following the transition from low to high flow velocities. The difference between the two results maybe related to the experimental conditions involved in each case. The authors [23] could not precisely assess the mean shear rate in tube flow where US recordings were performed, which probably differed from the values used here (i.e., 5 and 500 s^{-1}). This decrease level seems to be very sensitive to the experimental conditions and "time history" of the applied constant shear rate. Cloutier et al. [11] found a different decrease value (12.4 dB) when they varied the flow rate in a tube containing porcine RBCs so that the mean shear rates across the tube were dropping from 74 s^{-1} to 5.7 s^{-1}.

Table 1 also shows that the IBP and the IRC had extrema for similar hematocrits. The equivalence between them can be explained by considering the energy conservation at the interface agar gel–blood. This interface is characterized by R, the intensity reflection coefficient. Let us assume that E_i is the US

Table 1

Comparison between the hematocrit values where peaked the relative integrated backscatter power and the relative intensity reflection coefficient

	Shear rate of 5 s^{-1}	Shear rate of 500 s^{-1}
Relative IBP	Plateau after H = 13%	Maximum for H = 25%
Relative IRC	Minimum for H = 13–17%	Minimum for H = 23%

incident energy, then the transmitted energy at the considered interface is $E_i(1 - R)$. It is split between the backscatter energy E_{back} and the energy losses E_{loss}:

$$E_i(1 - R) = E_{back} + E_{loss}. \tag{3}$$

The term E_{loss} groups all forms of energy that are lost and not captured by the transducer as backscatter energy. It includes the energy lost in the medium by thermal dissipation as well as the US energies scattered in space directions other than the backward orientation. If we divide the two components of equation (3) by E_i, then it becomes:

$$1 - R = \frac{E_{back}}{E_i} + \frac{E_{loss}}{E_i}. \tag{4}$$

By definition, the backscatter coefficient is the average power backscattered per steradian by a unit volume, it is proportional to the energy fraction term E_{back}/E_i [24]. Equation (4) shows that the backscatter power, which is not corrected for attenuation (equivalent to E_{loss}/E_i), is equivalent to $1 - R$. This explains why IRC (or R) had a mirror relationship with respect to IBP.

4.2. *The spectral slopes*

Some equivalence between the integrated backscatter power and the intensity reflection coefficient also seemed to exist in their spectral behavior. So far, only spectral slopes of the ultrasound backscatter coefficient were investigated theoretically and experimentally [13,25–27]. A comparison between spectral slopes of IRC and of backscatter power is presented below for the case of shear rates of 5 and 500 s^{-1}.

4.2.1. *Shear rate of 500 s^{-1}*

According to the Rayleigh scattering theory, when scatterers are much smaller than the ultrasound wavelength, RBCs' cross section presents a f^4 frequency variation. At a shear rate of 500 s^{-1}, red blood cells are expected not to aggregate and can thus be considered as Rayleigh scatterers at a mean frequency of 10 MHz. As expected, our results for the spectral slope of the backscatter power are in good agreement with the Rayleigh theory. For instance, a value close to 4 was calculated at that shear rate over the explored hematocrits. Wang and Shung [28] found similar results when studying porcine whole blood, which was constantly stirred with a magnetic stirrer to avoid aggregation, in the frequency bandwidth between 5 MHz and 30 MHz. For a hematocrit lower than 30%, their spectral slopes varied between 3.9 and 4.7.

The situation slightly differed for the case of the spectral slopes of IRC. At 500 s^{-1}, it was constant and close to 3.8 for hematocrits $\leqslant$28% and then slightly decreased to reach a value of 3 for the higher explored hematocrit of 54%. Although the reason for this is unclear at the moment, it is reasonable to think that the increase in the number of RBCs may create more possibilities for the formation of organized patterns of blood cells, even in the absence of aggregation. The spectral slope of the IRC is probably sensitive to detect these small packed structures.

4.2.2. *Shear rate of 5 s^{-1}*

As shown in Figs 3 and 5, the spectral slopes of the backscatter power and IRC presented a deviation from the Rayleigh scattering theory at a shear rate of 5 s^{-1}. A value of 2 was found in the case

of the backscatter power spectral slope beyond a certain hematocrit (13%). Yuan and Shung [27] already observed that the frequency dependence for porcine whole blood, in the frequency range between 3.5 MHz and 12.5 MHz, was significantly smaller than a fourth power dependence for a mean shear rate of 2 s^{-1}, and hematocrits of 4.5%, 25% and 45%. They were promoting RBC aggregation by changing the fibrinogen concentration in a tube flow. The tendency for the spectral slope to diminish in the case of aggregated structures is a well established fact; it is even more striking when studying the aggregation phenomenon at higher frequencies. For instance, Van der Heiden et al. [29] measured a spectral slope as low as 1.3 for human blood in a Couette flow for frequencies between 22 MHz and 37 MHz. Performing experiments at a higher frequency band (30–70 MHz) with human blood, still under Couette flow, Foster et al. [30] observed a very low spectral slope of 0.4. Most of the investigations on the aggregation of RBCs have been focusing on the spectral dependence at a normal hematocrit (around 40%). According to Figs 3 and 5, the promotion of RBC aggregation by reducing the shear rate to 5 s^{-1} seems to be enhanced at hematocrit above 13–17%, approximately. This can be explained by the higher rate of collisions between red blood cells as the hematocrit is increased.

4.3. Potential advantages of the intensity reflection coefficient

The intensity reflection coefficient could be a valuable complementary tool to the physical parameters involving backscatter properties (backscatter coefficient, integrated backscatter power, Doppler backscattered power, etc.) for the investigation of biological tissues. The main advantage of this parameter lies in the simplicity of its measurement. The intensity reflection coefficient is defined as the ratio between the reflected and transmitted energies at an interface. The reflected energy is equivalent to an average quantification of the whole US backscatter process taking part beyond the considered interface; therefore the IRC could be considered only as an average estimator of the backscatter behavior. The intensity reflection coefficient depends on the mass density and sound velocity of the explored medium. The mass density is related to the number of RBCs while the sound velocity is influenced by their organization structure. This provides an explanation why the IRC is sensitive to hematocrit and shear-rate variations.

The process involved in the interaction between RBCs and ultrasound is still much complex to allow the existing mathematical models to give a satisfying comprehension of the phenomenon. These models have been focusing on the backscatter aspect of the interaction between ultrasound and RBCs. It would be interesting to adopt more mathematical approaches involving the intensity reflection coefficient as an equivalent tool to study the scattering properties of blood cells. A pioneering work towards this direction has been made to model the relation between the backscattering properties and hematocrit [31]. In this model, an ultrasonic wave was propagated in plasma normal to randomly placed slabs of RBCs, and the corresponding intensity reflection coefficient was calculated.

It is well known that RBC aggregation is enhanced by the low shear rates that may occur at arterial bends and bifurcations, in the recirculation zone beyond arterial stenoses but also in the vein vessels, which may be relevant to venous thrombogenesis; in such conditions shear rates can be as low as 1 s^{-1} [32,33]. Our group has also demonstrated that there is a significant difference in the level of RBC aggregation between veins and arteries when studying normolipidemic and hyperlipidemic individuals [34]. Patho-physiologically, a vicious cycle promoting the acceleration of RBC aggregation is induced in the venous region [2]. The venous system seems to be an ideal place to study RBC aggregation. Since its vessel wall is less subject to diseases as it is the case for most arteries, it would be interesting when

performing *in vivo* backscatter measurements to simultaneously measure the IRC. At that point, the intensity reflection coefficient would only reflect the backscatter behavior of blood instead of any abnormal or unhealthy state of the vessel.

5. Conclusion

The integrated backscatter power and the intensity reflection coefficient of whole porcine blood under Couette flow conditions were varied with the hematocrit and the shear rate. The two acoustical parameters had a nonlinear behavior as a function of the hematocrit. They were symmetrical and had extrema for similar hematocrit values. They both increased when the shear rate was decreased. In the frequency domain, the spectral slope for the two parameters also presented similar behavior over the explored range of hematocrits. The backscatter power spectral slope seemed to be a good probe for the detection of RBC aggregation beyond 13% hematocrit. The spectral slope of the IRC was not only depending on the shear rate but also on the hematocrit.

This study showed the potential of the new parameter, the US intensity reflection coefficient, to investigate the RBC aggregation phenomenon. Under some circumstances, this parameter may be more sensitive than the backscatter power to probe RBC aggregation. It had the advantage of being less complicated to measure. This new method could be used as a complementary tool to the backscatter coefficient. The *in vivo* investigation of RBC aggregation in venous blood vessels using simultaneously the backscatter coefficient and the intensity reflection coefficient may bring new insights for the comprehension of the phenomenon.

Acknowledgments

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References

[1] H. Kitamura, B. Sigel, J. Machi, E.J. Feleppa, J. Sokil-Melgar, A. Kalisz and J. Justin, Roles of hematocrit and fibrinogen in red cell aggregation determined by ultrasonic scattering properties, *Ultrasound Med. Biol.* **21** (1995), 827–832.
[2] N. Maeda, Erythrocyte rheology in microcirculation, *Jpn. J. Physiol.* **46** (1996), 1–14.
[3] A.R. Pries, T.W. Secomb and P. Gaehtgens, Biophysical aspects of blood flow in the microvasculature, *Cardiovasc. Res.* **32** (1996), 654–667.
[4] C. Le Devehat, M. Vimeux, G. Bondoux and T. Khodabandehlou, Red blood cell aggregation in diabetes mellitus, *Int. Angiol.* **9** (1990), 11–15.
[5] W. Koenig and E. Ernst, The possible role of hemorheology in atherothrombogenesis, *Atherosclerosis* **94** (1992), 93–107.
[6] H.C. Kwaan and A. Bongu, The hyperviscosity syndromes, *Semin. Thromb. Hemost.* **25** (1999), 199–208.
[7] H. Zhao, X. Wang and J.F. Stoltz, Comparison of three optical methods to study erythrocyte aggregation, *Clin. Hemorh. Microcirc.* **21** (1999), 297–302.
[8] L. Dintenfass, H. Jedrzejczyk and A. Willard, Application of stereological methods to the evaluation of aggregation of red cells in 12.5 micrometer slit: a photographic and statistical study, *Biorheology* **18** (1981), 387–404.
[9] S. Chien, Blood viscosity: Influence of erythrocyte aggregation, *Science* **157** (1967), 829–831.

[10] M. Zlonis, The mystique of the erythrocyte sedimentation rate. A reappraisal of one of the oldest laboratory tests still in use, *Clin. Lab. Med.* **13** (1993), 787–800.
[11] G. Cloutier, Z. Qin, L.G. Durand and B.G. Teh, Power Doppler ultrasound evaluation of the shear rate and shear stress dependences of red blood cell aggregation, *IEEE Trans. Biomed. Eng.* **43** (1996), 441–450.
[12] R.E.N. Shehada, R.S.C. Cobbold and L.Y.L. Mo, Aggregation effects in whole blood: Influence of time and shear rate measured using ultrasound, *Biorheology* **31** (1994), 115–135.
[13] Y.W. Yuan and K.K. Shung, Ultrasonic backscatter from flowing whole blood. I: Dependence on shear rate and hematocrit, *J. Acoust. Soc. Am.* **84** (1988), 52–58.
[14] M. Boynard, J.C. Lelievre and R. Guillet, Aggregation of red blood cells studied by ultrasound backscattering, *Biorheology* **24** (1987), 451–461.
[15] G. Cloutier and Z. Qin, Shear rate dependence of ultrasound backscattering from blood samples characterized by different levels of erythrocyte aggregation, *Ann. Biomed. Eng.* **28** (2000), 399–407.
[16] I. Fontaine, D. Savéry and G. Cloutier, Simulation of ultrasound backscattering by red cell aggregates: Effect of shear rate and anisotropy, *Biophys. J.* **82** (2002), 1696–1710.
[17] D. Savéry and G. Cloutier, A point process approach to assess the frequency dependence of ultrasound backscattering by aggregating red blood cells, *J. Acoust. Soc. Am.* **110** (2001), 3252–3262.
[18] K.K. Shung, R.A. Sigelmann and J.M. Reid, Scattering of ultrasound by blood, *IEEE Trans. Biomed. Eng.* **23** (1976), 460–467.
[19] E.L. Madsen, F. Dong, G.R. Frank, B.S. Garra, K.A. Wear, T. Wilson, J.A. Zagzebski, H.L. Miller, K.K. Shung, S.H. Wang, E.J. Feleppa, T. Liu, W.D. O'Brien, K.A. Topp, N.T. Sanghvi, A.V. Zaisev, T.J. Hall, J.B. Fowlkes, O.D. Kripfgans and J.G. Miller, Interlaboratory comparison of ultrasonic backscatter, attenuation, and speed measurements, *J. Ultrasound Med.* **18** (1999), 615–631.
[20] X. Weng, G. Cloutier, P. Pibarot and L.G. Durand, Comparison and simulation of different levels of erythrocyte aggregation with pig, horse, sheep, calf, and normal human blood, *Biorheology* **33** (1996), 365–377.
[21] G. Cloutier and Z. Qin, Ultrasound backscattering from non-aggregating and aggregating erythrocytes: A review, *Biorheology* **34** (1997), 443–470.
[22] K.K. Shung, Y.W. Yuan, D.Y. Fei and J.M. Tarbell, Effect of flow disturbance on ultrasonic backscatter from blood, *J. Acoust. Soc. Am.* **75** (1984), 1265–1272.
[23] K.K. Shung, G. Cloutier and C.C. Lim, The effects of hematocrit, shear rate, and turbulence on ultrasonic Doppler spectrum from blood, *IEEE Trans. Biomed. Eng.* **39** (1992), 462–469.
[24] M.F. Insana and D.G. Brown, Acoustic scattering theory applied to soft biological tissues, *Ultrasonic Scattering in Biological Tissues*, K.K. Shung and G.A. Thieme, eds, CRC Press, Boca Raton, 1992.
[25] I. Fontaine and G. Cloutier, Modeling the frequency dependence (5–120 MHz) of ultrasound backscattering by red cell aggregates in shear flow at a normal hematocrit, *J. Acoust. Soc. Am.* **113** (2003), 2893–2900.
[26] D. Savéry and G. Cloutier, Effect of red cell clustering and anisotropy on ultrasound blood backscatter: A Monte Carlo study, *IEEE Trans. Ultrason. Ferr. Freq. Control* **52** (2005), 94–103.
[27] Y.W. Yuan and K.K. Shung, Ultrasonic backscatter from flowing whole blood. II: Dependence on frequency and fibrinogen concentration, *J. Acoust. Soc. Am.* **84** (1988), 1195–1200.
[28] S.H. Wang and K.K. Shung, An approach for measuring ultrasonic backscattering from biological tissues with focussed transducers, *IEEE Trans. Biomed. Eng.* **44** (1997), 549–554.
[29] M.S. van der Heiden, M.G. de Kroon, N. Bom and C. Borst, Ultrasound backscatter at 30 MHz from human blood: influence of rouleau size affected by blood modification and shear rate, *Ultrasound Med. Biol.* **21** (1995), 817–826.
[30] F.S. Foster, H. Obara, H.T. Bloomfield, L.K. Ryan and G.R. Lockwood, Ultrasound backscatter from blood in the 30 to 70 MHz frequency range, *IEEE Ultrason. Symp. Proc.* (1994), 1599–1602.
[31] E.Y. Lui, J. Tavakkoli and R.S. Cobbold, Influence of boundary conditions on a one-dimensional ultrasound backscattering model of blood, *Ultrasound Med. Biol.* **27** (2001), 571–578.
[32] G.D.O. Lowe, Agents lowering blood viscosity, including defibrinogenating agents, in: *Cardiovascular Thrombosis*, M. Verstraete, V. Fuster and E.J. Topol, eds, Lippincott-Raven Publishers, Philadelphia, New York, 1998.
[33] J. Machi, B. Sigel, J.C. Beitler, J.C.U. Coelho and J.R. Justin, Relation of in vivo blood flow to ultrasound echogenicity, *J. Clin. Ultrasound* **11** (1983), 3–10.
[34] G. Cloutier, X. Weng, G.O. Roederer, L. Allard, F. Tardif and R. Beaulieu, Differences in the erythrocyte aggregation level between veins and arteries of normolipidemic and hyperlipidemic individuals, *Ultrasound Med. Biol.* **23** (1997), 1383–1393.