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Short communication

Ultrasonic monitoring of droplets' evaporation: Application to human whole blood

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ABSTRACT

During a colloidal droplet evaporation, a sol–gel transition can be observed and is described by the desiccation time τ_D and the gelation time τ_G. These characteristic times, which can be linked to viscoelastic properties of the droplet and to its composition, are classically rated by analysis of mass droplet evolution during evaporation. Even if monitoring mass evolution versus time seems straightforward, this approach is very sensitive to environmental conditions (vibrations, air flow...) as mass has to be evaluated very accurately using ultra-sensitive weighing scales. In this study we investigated the potentialities of ultrasonic shear reflectometry to assess τ_D and τ_G in a simple and reliable manner. In order to validate this approach, our study has focused on blood droplets evaporation on which a great deal of work has recently been published. Desiccation and gelation times measured with shear ultrasonic reflectometry have been perfectly correlated to values obtained from mass versus time analysis. This ultrasonic method which is not very sensitive to environmental perturbations is therefore very interesting to monitor the drying of blood droplets in a simple manner and is more generally suitable for complex fluid droplets evaporation investigation.

1. Introduction

In literature concerning ultrasound and droplets evaporation monitoring, QCM (Quartz Crystal Micro-balance) has already been largely applied. Since the first works of Sauerbrey [1], and of Kanazawa [2], the literature available on this subject is extensive but one can refer for instance to the works of Zhuang [3,4] or Yakhno [5]. In particular, Yakhno and co-workers [6,7] focused their study on various liquids using a specific uncoated QCM device and the acoustic-mechanical impedance of the droplet. More recently, in order to reduce the volume analyzed during the evaporation process, micromechanical QCM systems have been proposed leading to results on femtoliter droplets [8,9]. To our knowledge, the ultrasonic shear reflectometry method, which uses ultrasonic echoes directly and not the resonance curve of a piezoelectric crystal is used less or not at all for evaporation monitoring but its interest for phase transition study has been underlined by several authors [10–12]. We therefore propose to test this method and to analyze its potentialities to monitor the drying of droplets. We have focused our attention on the modulus of the shear reflection coefficient at the interface (substrate/droplet). Based on its evolution versus time, we have assessed desiccation and gelation times. The range of evaporation times investigated is very wide (∼1000–10,000 s) and humidity effect has also been investigated. As shear reflection coefficient evolution is an indicator of rheological properties of the fluid under study, it is related to fluid viscosity evolution during evaporation. For the specific case of blood, viscosity is related to the etiology of cardiovascular diseases. So, on a medical point of view it is of interest for all pathologies related to blood clothing and thrombosis. Furthermore, it can be thought that evaporation process is also influenced by blood cell morphology and by interactions between blood cells. Hence this simple and straightforward ultrasonic method could eventually constitute a complementary tool for blood microstructure investigation.

2. Material and methods

2.1. Experimental setup [13]

When an ultrasonic shear wave is reflected off a (solid/viscoelastic material) interface, its amplitude decreases and the wave undergoes a phase shift which is generally extremely small.

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Both amplitude modification and phase shift are linked to viscoelastic moduli of the material [14]. From an experimental point of view, as indicated in [15] the reflection configuration is the most interesting. The use of shear waves is suitable because the sensitivity to sol–gel transitions is emphasized. We used two shear commercial wave transducers with incorporated delay lines (Olympus-V220-BA-RM and V222-BB-RM). The V220-BA-RM had a central frequency of 5 MHz with a bandwidth of ±1 MHz and the V222-BB-RM model presented a central frequency of 20 MHz with a large bandwidth reaching ±4 MHz. Electrical excitation was provided by an Olympus 5073 PR pulse generator. An additional delay line (1 mm glass plate for investigation at 5 MHz and 400 μm for 20 MHz study) coupled with honey to the silica delay line was added on the sensor. Such an approach offers several advantages: firstly, the high quality ultrasonic shear transducer with no longitudinal parasite modes ensures plane waves emission in far field at the end of the incorporated silica delay line. Secondly, having an additional disposable delay line is very useful when studying aggressive materials, or to evaluate the influence of delay line nature on evaporation... More details concerning this specific configuration can be found in [16]. In order to measure mass and ultrasonic parameters simultaneously, the ultrasonic sensor and its additional delay line were placed on a precision weighing scale Ohaus Explorer E10640 with an accuracy of 0.1 mg. At last, hygrometry was measured with a T3319 Comet Web sensor hygrometer. A schematic representation and photography are given in Fig. 1.

2.2. Experimental protocol for mass and ultrasonic signals acquisition and treatment

First of all, ultrasonic echoes in the small glass plate were acquired with no droplet. Then, after obtaining a droplet blood sample directly from a finger using a diabetic monitoring kit, we used a micropipette (Eppendorf Stream) to deposit a precise volume of blood on the small glass plate (previously cleaned with ethanol) and the measurement began. During evaporation, echoes and mass droplet $m(t)$ were acquired at regular time intervals on a personal computer via an USB/GPIB interface using homemade Labview software. Echoes amplitudes were then measured and $r_o(t)$ calculated as follows. As the attenuation is small in glass, multiple echoes are generated in the glass plate. Hence, if A_i is the amplitude of the echo n° i, in the case of the interface (glass plate/air) and if B_i is the amplitude for the interface (glass plate/droplet), then the modulus of the shear reflection coefficient can be calculated with the following relationship:

$$r_o = \left(\frac{B_i}{A_i}\right)^{\frac{1}{i}} \quad (1)$$

Therefore, if $\ln(B_i/A_i) = f(i)$ is plotted, a linear adjustment passing through the origin can be performed. If "P" represents the slope of this adjustment, then r_o is given by e^P (Fig. 1).

Concerning mass versus time analysis, we followed the method described by B. Sobac and co-worker in [17]. From this analysis, for each evaporation experiment, the two specific times τ_D and τ_G were assessed. τ_D represents the beginning of the sol–gel transition and τ_G the end of the process.

2.3. Experimental protocol for hygrometry analysis

Recently, Bou Zied et al. [18] published data concerning the influence of humidity on blood droplets evaporation. All the results were obtained thanks to the evolution of mass versus time. In order to validate the ultrasonic method and to give an example of investigation of a specific relevant parameter (humidity in this case), a second set of experiments was performed with controlled relative humidity. The ultrasonic device was placed in a KB 53 incubator (BINDER GmbH) in order to work with a perfectly constant temperature equal to 25 °C. The volume of the droplet was 20 μL for each experiment. For these experiments it was not possible to assess the mass because vibrations created by temperature control system of the incubator disturbed the mass measurement (the weighing scale being too sensitive). Before the experiments, desiccators or a tank of water were placed in the incubator in order to modify the relative humidity. We performed experiments with relative humidity levels from 16% to 93%.

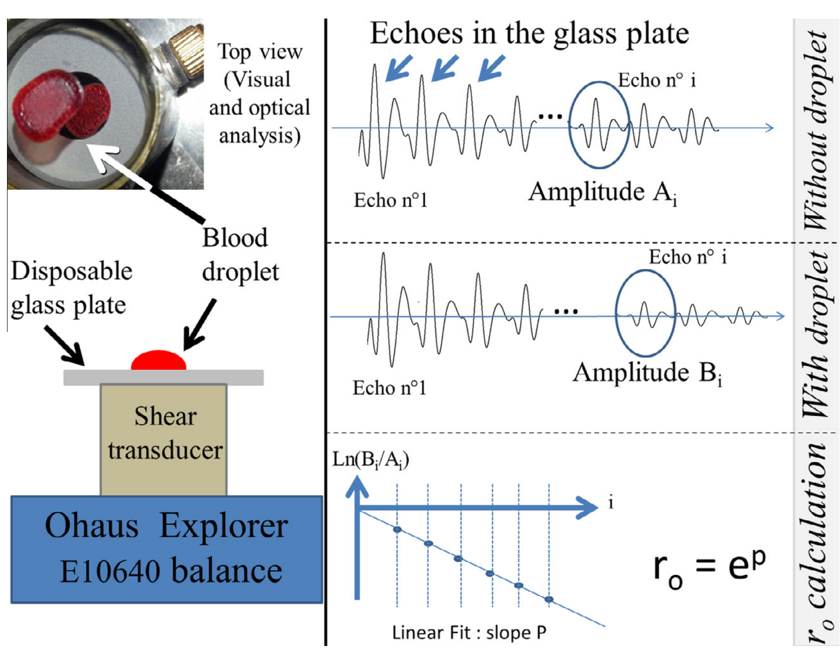

Fig. 1. Schematic representation of the experimental device and ultrasonic echoes.

3. Results

3.1. Correlations between τ_D and τ_G measured with ultrasound and with m(t) analysis

Depending on weather conditions, temperature varied from 19 to 28 °C. Hygrometry ranged between 26% and 46%. Furthermore we also carried out measurements with various droplet volumes (8–50 μl). This resulted to evaporation times between ~1000 and 10,000 s. In order to comment ultrasonic results, one has to bear in mind that between $t = 0$ and $t = \tau_D$, mass evolves considerably with a water loss of more than 80%. After τ_D, the mass evolves very slightly. One can refer to Ref. [17] for more details.

Regarding ultrasonic data collected, for all the experiments carried out, the evolution of $r_o(t)$ was very characteristic (Fig. 2) and obtained with a high level of accuracy. On this figure a sliding average on 20 points has been added to reduce measurement noise. At the beginning of the experiment $r_o(t)$ evolves very slightly. This very small evolution can be attributed to a small densification of the fluid due to water evaporation, and consequently to a very small increase of its acoustical impedance modulus which influences $r_o(t)$. Then, after τ_D, $r_o(t)$ decreases considerably. This evolution of $r_o(t)$, which is caused by an important evolution of fluid acoustical impedance with no significant variation of density is a manifestation of a high variation of ultrasonic shear velocity in blood droplet due to the sol–gel transition which occurs in the fluid. For the ultrasonic signal this is seen as an hardening of the fluid and the waves can pass from the glass plate into the droplet studied leading to a decrease of $r_o(t)$. Hence, the time for which $r_o(t)$ begins to decrease significantly has been defined as $\tau_{D(US)}$ (Fig. 2). The subscript US stands for UltraSounds. When gelation time is reached, if relative humidity is less than 60–65%, then large cracks or delamination clearly appear under or in the droplet [18]. The ultrasonic signals cannot therefore propagate properly in the desiccated droplet and hence, $r_o(t)$ increases considerably again. This time has been defined as $\tau_{G(US)}$ (Fig. 2). If relative humidity is above 60–65%, the number of cracks decreases in the desiccated droplet and with relative humidity above 80% they totally disappear [19]. Therefore, $r_o(t)$ increases very little after τ_G or does not increases at all and attains a plateau. In this case, the time $\tau_{G(US)}$ has been defined as the beginning of this plateau (Fig. 2). Experiments were conducted on 27 droplets. Graphs of $\tau_{D(US)}$ versus τ_D, $\tau_{G(US)}$ versus τ_G and a last, τ_G versus τ_D (measured with mass or ultrasounds) are presented in Figs. 3–5. The accuracy on $\tau_{D(US)}$ and $\tau_{G(US)}$ is ±3 min and corresponds to the size of the dots. Eight evaporation experiments were performed with the 5 MHz transducer and the other (19 droplets) with the 20 MHz sensor. As no significant difference concerning the ultrasonic estimation of specific times as a function of frequency was observed, the distinction is not made on the graphs. Such an element is important because every commercial sensor in the range of a few MHz can be used. Regarding the slopes and R^2 values in Figs. 3 and 4, it is clear that $\tau_D = \tau_{D(US)}$ and $\tau_G = \tau_{G(US)}$. This lead us to the conclusion that the criteria taken to assess desiccation and gelation times with $r_o(t)$ are correct and reliable. Furthermore (see Fig. 5) we obtained $1.19 < \tau_{G(US)}/\tau_{D(US)} < 1.25$ and $1.24 < \tau_G/\tau_D < 1.32$. So there is no significant difference between these two experimental methods and our results are in line with the value 1.25 given by Sobac and co-workers [17].

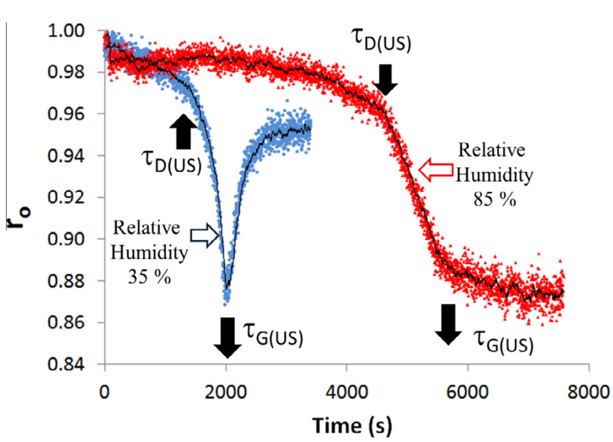

Fig. 2. Typical $r_o(t)$ analysis for $\tau_{D(US)}$ and $\tau_{G(US)}$ evaluation for two values of relative humidity (35% and 85%).

3.2. Humidity effect

Compared to relative humidity study by Bou Zeid at al. [18] the range of investigation has been considerably extended (from 16% to 93%). Values of $\tau_{D(US)}$ and $\tau_{G(US)}$ are presented in Fig. 6. Here again the general trend observed by ultrasonic shear reflectometry is in agreement with data deduced from $m(t)$ analysis presented in [18]: if relative humidity is inferior to 60%, desiccation and gelation times are not considerably affected by hygrometry. On the contrary, as soon as relative humidity is greater than 60% its influence is considerable. More in details, evaporative mass flux is inversely proportional to the relative humidity due to two phenomena: first the spreading diameter is more important and thus the contact angle is lower, second the more humidity there is in the surround air the more difficult it is to evaporate. These two combined phenomena induce a nonlinear variation of the evaporative mass flux as a function of humidity. It has been evidenced in [19]. If the evaporative mass flux decreases a lot, thus the time to evaporate a constant mass is greatly increased. Furthermore, according to this reference, the evaporative flux is a complex function of relative humidity, involving polynomial and trigonometric functions. In order to obtain a simple and straightforward formulation, we propose to adjust the evolution of $\tau_{D(US)}$ and $\tau_{G(US)}$ by a 3 order polynomial expression up to a relative humidity equal to 60%. Such a polynomial expression presents a good trend and correctly describes the plateau observed regarding our data. The regressions are given by relationships (2) and (3) where RH represents the relative humidity and is ranging between 16% and 60%:

$$\tau_D = 23\,667\mathrm{RH}^3 - 27\,197\mathrm{RH}^2 + 10\,582\mathrm{RH} \tag{2}$$

$$\tau_G = 32\,091\mathrm{RH}^3 - 36\,770\mathrm{RH}^2 + 15\,306\mathrm{RH} \tag{3}$$

If RH > 60% we propose to use the relationships (4) and (5) which tend to infinity if RH tends to 1. These regressions have been reported in Fig. 6.

$$\tau_D = \frac{1309.53}{(1 - \mathrm{RH}^3)} \tag{4}$$

$$\tau_G = \frac{2256.40}{(1 - \mathrm{RH}^3)} \tag{5}$$

Regarding these evolutions of characteristic times versus relative humidity, as previously explained in introduction, if evaporations are carried out in order to compare various blood droplets to detect pathologies related to blood viscosity or to blood cells morphology, experiments should be conducted with a relative humidity below 60%.

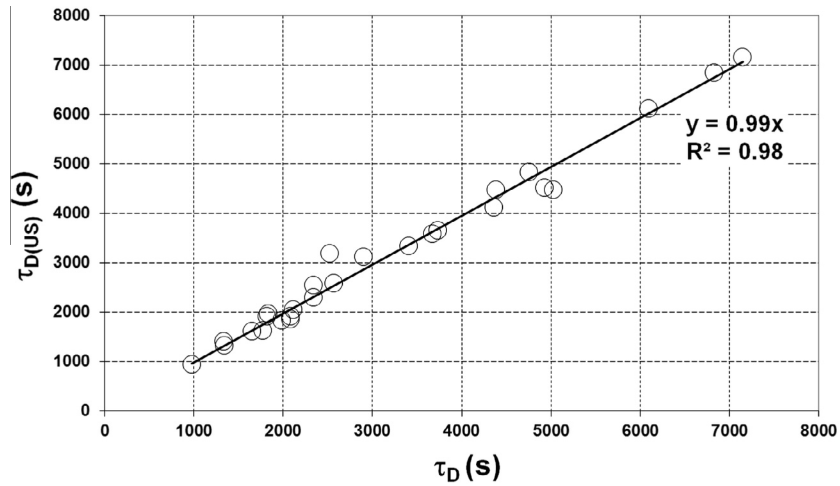

Fig. 3. Evolution of $\tau_{D(US)}$ as a function of τ_D.

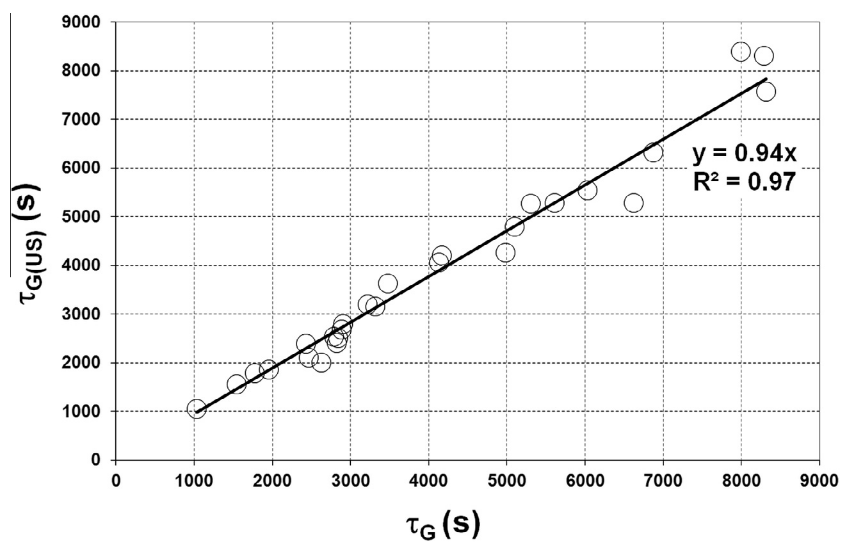

Fig. 4. Evolution of $\tau_{G(US)}$ as a function of τ_G.

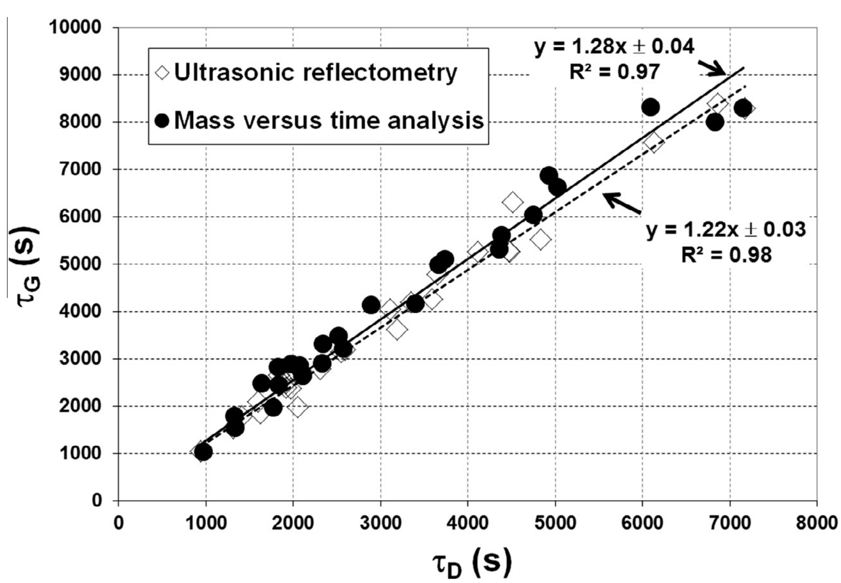

Fig. 5. Plot of τ_G versus τ_D for $m(t)$ and $r_o(t)$ analysis.

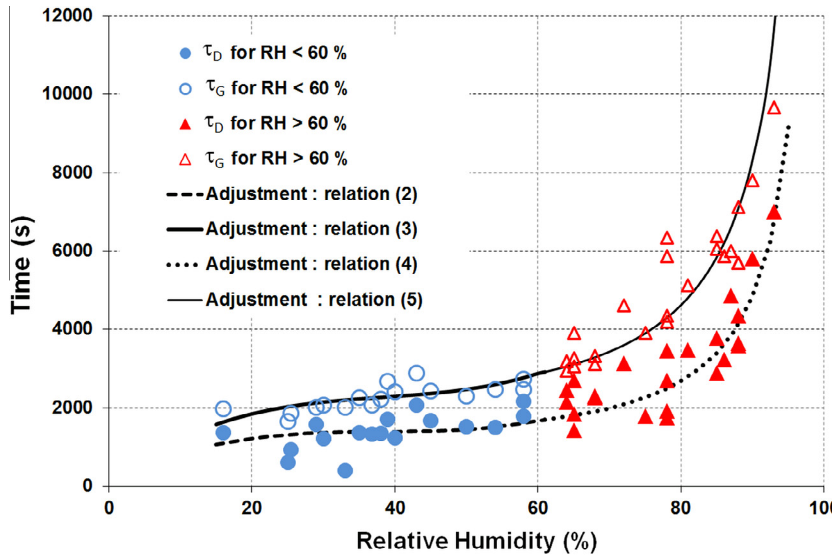

Fig. 6. Evolution of $\tau_{D(US)}$ and $\tau_{G(US)}$ versus relative humidity in the range 16–93%.

4. Conclusion

In the case of a whole blood droplet evaporation we have shown that ultrasonic shear reflectometry is an interesting tool for estimation of classical relevant times τ_D and τ_G. As it is a non-destructive approach which can be easily implemented on other experiments (mass analysis, infrared metrology, optical studies...) we recommend it to obtain relevant data linked to rheology of blood or more generally to investigate complex fluid droplets evaporation.

5. List of symbols and variable definitions

– τ_D: desiccation time rated by droplet mass analysis: time for which the major part of water has been evaporated. At this time it can be considered that sol–gel transition begins.
– τ_G: gelation time rated by droplet mass analysis: time for which the sol–gel transition is finished. On a rheological point of view the sol–gel transition can also be defined as $G'' = G'$, if G' and G'' represent the real and imaginary parts of the complex modulus G^*.
– $\tau_{D(US)}$ and $\tau_{G(US)}$: desiccation and gelation times assessed using ultrasonic method.
– r_o: modulus of the shear ultrasonic reflection coefficient. It represents the ratio between the amplitude of the reflected echo and the amplitude of the incident ultrasonic pulse.

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