



## Short communication

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

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## ARTICLE INFO

## Article history:

Received 16 June 2015

Received in revised form 29 February 2016

Accepted 2 March 2016

Available online 2 March 2016

## Keywords:

Ultrasound

Shear waves

Evaporation

Droplets

Human whole blood

## ABSTRACT

During a colloidal droplet evaporation, a sol–gel transition can be observed and is described by the desiccation time  $\tau_D$  and the gelation time  $\tau_G$ . 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  $\tau_D$  and  $\tau_G$  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.

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## 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 ( $\sim 1000$ – $10,000$  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 clotting 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  $\pm 1$  MHz and the V222-BB-RM model presented a central frequency of 20 MHz with a large bandwidth reaching  $\pm 4$  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  $\mu\text{m}$  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  $n^\circ i$ , 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  $\tau_D$  and  $\tau_G$  were assessed.  $\tau_D$  represents the beginning of the sol–gel transition and  $\tau_G$  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  $\mu\text{L}$  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%.

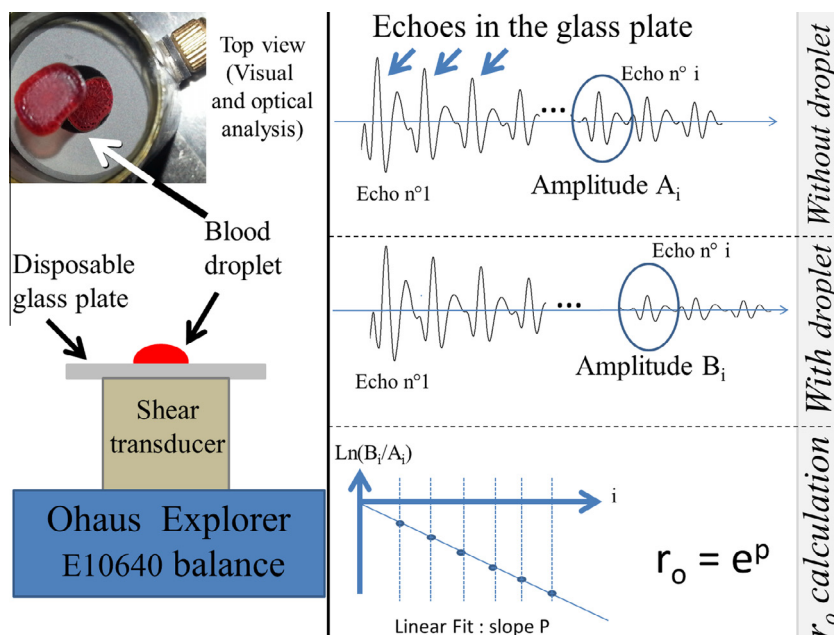


Fig. 1. Schematic representation of the experimental device and ultrasonic echoes.

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