Structural and evaporative evolutions in desiccating sessile drops of blood

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We report an experimental investigation of the drying of a deposited drop of whole blood. Flow motion, adhesion, gelation, and fracturation all occur during the evaporation of this complex matter, leading to a final typical pattern. Two distinct regimes of evaporation are highlighted: the first is driven by convection, diffusion, and gelation in a liquid phase, whereas the second, with a much slower rate of evaporation, is characterized by the mass transport of the liquid left over in the gelified biocomponent matter. A diffusion model of the drying process allows a prediction of the transition between these two regimes of evaporation. Moreover, the formation of cracks and other events occurring during the drying are examined and shown to be driven by critical solid mass concentrations.

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I. INTRODUCTION

The evaporation of a colloidal suspension is a very interesting system, allowing the formation of patterns. Early investigations conducted by Deegan et al. [1] revealed the formation of a ringlike pattern of the deposit along the triple line. They pointed out that an outward flow induced by the evaporation of the solvent transports the solute to the self-pinned edge, creating the so-called “coffee stain.” Since this work, several studies [2–5] have investigated fundamental mechanisms influencing deposition phenomena, such as the kinetics of evaporation, internal flow motions, or the triple line dynamic, in order to find a means to control the final pattern. For industrial applications, this is of capital importance in evaporative self-assembly techniques [6,7] or in printing and coating technologies [8,9].

However, when the concentration of solute near the surface reaches a critical value, a skin is formed which is considered a gel phase [10,11]. A sol-gel transition then occurs during the desiccation. This process, also called gelation, is characterized by a change of the rheological behavior with the appearance of a yield stress. The competition between the adhesion of the gel onto the substrate and the increasing internal stresses caused by evaporation leads to the formation of a different final pattern made up of cracks in order to release the excess of stored elastic energy [12,13]. Concentration, ionic strength, the presence of surfactant, the thickness of the system, deformability, and the size of particles are many parameters influencing this nonequilibrium pattern formation. The formation of cracks has mainly been studied in physical systems (polymeric and colloidal suspensions) but this mechanism has also been observed during the drying of biological fluids [14,15]. A regularly radial fracture structure is observed during the drying of bovine serum. A similar pattern has recently been observed for the first time with whole blood [16]. The flow motion induced by the evaporative solvent has been investigated. A capillary flow segregates and transports the cellular matter to the edge leading to a final half-doughnut shape.

In this article, we focus on the dynamics of evaporation and crack formation. Two main types of kinetics of evaporation are reported due to the competition between evaporation and gelation. The transition of the evaporative kinetics is well predicted using a diffusion model. Finally, by observing the concentration of solid mass in the drop during the evaporative process, we demonstrate that crack nucleation is driven by a critical mass concentration of solid.

II. MATERIALS AND METHODS

An experimental setup was designed to provide a characterization of both the mass and geometry of a drop of whole blood deposited onto a microscope glass substrate during its evaporation. Environmental conditions (temperature, pressure, humidity) were recorded using a weather station. Evaporation took place in a parallelepipedic box measuring 100 × 100 × 150 mm³ designed to avoid external flow perturbations. The volume was sufficient to preclude saturation of the atmosphere with the vapor generated. To monitor the drying process, the mass of the drop \( m(t) \) was recorded using a digital high-precision balance with a resolution of 10 μg at 10 Hz (Mettler Toledo XS 205). Moreover, a simultaneous visualization from above was performed using a Canon EOS 7D digital camera fitted with a macro lens in order to follow the morphological evolution of the drop. The blood used in this study was human blood and came from the same donor. The fibrinogen protein was neutralized to avoid coagulation during the process. Blood is a complex fluid made up of a liquid phase (plasma) and of the cellular matter (red blood cells, white blood cells, platelets). The whole of these biocolloids are described in Table I in terms of quantities, sizes, and volumes. These components account for 43.9% of the drop volume based on a hematometry analysis and 23.9% of the mass according to the mass of the deposit on the substrate at the end of the drying process. Physical properties of blood such as viscosity and surface tension had been determined previously [16]. A full cell numeration and biochemistry characterization were performed. Experiments for the set of drops presented here were performed within a period of 48 hours. The solid mass fraction \( \phi_S(t) \) was deduced from the measurement of the mass using a biphasic constituent model. At a given time \( t \), the total sample mass \( m(t) \) is the sum of the solid \( m_S \) and the water \( m_w \) masses: \( m(t) = m_S + m_w(t) \). The solid mass concentration is thus the ratio between the final dried mass \( m_S \) and the...
mass at a given time $m(t)$: $\phi_S(t) = m_S/m(t)$. The volumetric concentration cannot be used in the case of blood since blood cells are shells containing water which will be released during the drying.

### III. RESULTS AND DISCUSSION

The morphological evolution of a drying drop of blood is provided in Fig. 1. The drop was deposited onto the substrate and the edge was pinned throughout the drying process as shown by the constant wetting diameter. Indeed, in the case of biological fluids, proteins are adsorbed on the substrate leading to an anchoring of the triple line [18]. The evolutions of the drop mass and drying rate are presented in Fig. 2. An analysis of the kinetics of the drying rate reveals three different regimes, which are linked to physical processes. During the first regime, an outward flow induced by solvent evaporation is observed, which displaces biocolloids toward the edge of the drop [16]. This leads to a typical cross section of the deposit in the shape of a half-doughnut. Simultaneously, a gel-like skin phase forms at the edge and a gelation front moves inwards (see Fig. 1 at time $t_C$). This mechanism is clearly shown both in Fig. 2, with the nonlinear decrease of the mass indicating that another process (gelation) disturbs the pure evaporation of the liquid phase (see the dashed line, which refers to the drying time of a pinned drop of pure liquid $t_D$), and in Fig. 1 at time $t_C$ with the color contrast between the solid ring-like area (light red) and the fluid area (dark red). Considering the time for a red blood cell to radially move to the edge $t_f$ and the time to sediment across the thickness $t_s$, the sedimentation process is negligible in this problem since $t_f/t_s \approx 0.2$ [16]. At the end of this first regime, the central area of the drop is still liquid while the drop edge is gellified. Then a transition regime is observed which is characterized by a sharp decrease in the drying rate and a rapid gelation of the center as shown by the shrinking of the fluid part between the $t_{C2}$ and $t_{C1}$ images of Fig. 1. Similar transition behavior is usually observed during phase change transitions such as solidification. The last regime of drying is characterized by a very slow drying rate, which is explained by the evaporation of the remaining liquid in the gellified cellular matter.

The typical drying rates extracted from Fig. 2 are, respectively, 5.2 $\mu g/s$ (12.3 mg in 2300 sec) for the first regime of evaporation and 0.092 $\mu g/s$ (0.25 mg in 2700 sec) for the last regime of drying. The kinetics of evaporation of the last regime is about 56 times slower than the first, indicating a process that is only related to the evaporation of the liquid phase diffusing through the gellified porous media. Indeed, during the drying process, these biocolloids accumulate and are compacted, which traps liquid inside the solid matrix. Similar kinetics of evaporation has been revealed in suspension films [13]. Our process clearly reveals a competition between evaporation and gelation. Considering the characteristic times of the problem, one can notice that the desiccation time $t_D$ is the same order of magnitude as the gelation time $t_G$ since the ratio $t_G/t_D \approx 1.25$. This is in agreement with the fact that a gelled foot builds up near the drop edge while the central part stays fluid at first and then recedes [2]. In the work of Pauchard et al. [2], with the same ratio of $t_G/t_D$, the authors evidence a buckling instability, as in their case the buckling time is shorter than the gelation time. For our situation, gelation continues in the central area of the drop, avoiding the development of buckling instability. The gelation time $t_G$ has been taken as the time at the end of the solid-gel transition. The desiccation time $t_D$ can be evaluated using the mean water flux evaporated per surface unit $w = -\frac{1}{2} \frac{dV}{dt}$, where $S$ and $V$, respectively, are the free surface and the volume of the drop at a time $t$. In the situation where the wetting radius remains constant during the drying and considering a
spherical cap shape, the desiccation time is expressed: 

$$\tau_D = \frac{V_0}{\frac{1}{2} \rho \omega} = \frac{1}{6} \frac{(1 - \cos \theta_0)(2 + \cos \theta_0) R_0^2}{\omega E}$$

where the subscript 0 specifies that parameters are considered at the initial time. With the approximation of weak contact angles ($\theta_0 \approx 14.8^\circ$ [16]), the expression of the desiccation time can be simplified and finally written

$$\tau_D \approx \frac{\theta_0}{4} R_0 \rho S_0$$

with $\rho$ the density of the fluid. The obtained numerical value $\tau_D = 2.5 \times 10^3$ s fits with the experimental value extracted from Fig. 2 where $\tau_D = 2.3 \times 10^3$ s. As for the total time of evaporation $\tau_F$, it is defined by a constant signal of the mass along with a null mean signal of the drying rate.

The influence of the drop mass on the evaporation process was investigated. Figure 3 provides dimensionless evolutions of the liquid mass evaporated $[m_0 - m(t)]/[m_0 - m_S]$ as a function of the dimensionless time $t/\tau_F$ for different initial drop masses. The proportion of the second regime of drying becomes bigger at the expense of the first regime with the increase of the drop mass. This can be explained by the increase of the gel deposit thickness, the wetting diameter evolving with the drop mass to the power of 1/3 [see Eq. (2)]. Consequently, more water is trapped in the gellified cellular matter and the diffusion time through this porous media becomes longer.

In our experimental situations using a low-volatility fluid drying under natural conditions without heating, evaporation is limited by diffusion of water into air. Thus, a diffusion model was used to predict the time of drying transition, which also corresponds to the gelation time $\tau_G$. For blood as a working fluid and for the range of drop diameters, the contact angle is assumed to be constant at $\theta = 14.8^\circ$ [16]. Using the spherical cap shape assumption, it is thus possible theoretically to relate the drop diameter to the drop mass by

$$d_0 = K(\theta)m_0^{1/3}$$

with $K(\theta) = (\frac{24}{\rho \pi} \frac{\sin \theta}{(1 - \cos \theta)^2(2 + \cos \theta)})^{1/3} = 0.331 \text{ m}^2 \text{s}^{-1} \text{kg}^{-1/3}$ (the fit of experimental data gives $0.323 \text{ m}^2 \text{s}^{-1} \text{kg}^{-1/3} \pm 0.75\%$). From the simplified version of Stefan’s law, the total time of evaporation transition $t_{\tau_F}$ is given by

$$t_{\tau_F} = \frac{V_0}{\frac{1}{2} \rho \omega} = \frac{1}{6} \frac{(1 - \cos \theta_0)(2 + \cos \theta_0) R_0^2}{\omega E}$$

FIG. 2. (Color online) Evolution of mass $m$, drying rate $|dm/dt|$, and solid mass concentration $\phi_S$ (inset) for an drying drop of blood (same conditions as Fig. 1). Regime I: drying regime driven by convection, diffusion, and gelation. Regime II: drying regime only driven by diffusion.

FIG. 3. (Color online) Evolution of the dimensionless liquid mass evaporated $[m_0 - m(t)]/[m_0 - m_S]$ for a range of drop masses: an indication of the proportion of each of the two evaporation regimes in the drying time.
FIG. 4. (Color online) Variation of the gelation time $\tau_G$ and the wetting diameter of the drop of blood $d_0$ as a function of the initial blood drop mass $m_0$: experimental results (points) and theoretical models (dashed lines) (room temperature $= 23.1 \pm 0.1 ^\circ C$, humidity varying between 7.0% and 26.5%).

Evaporation (in seconds) based on the first regime driven by a convection, diffusion, and gelation mode is expressed by

$$\tau \approx \frac{4RT}{\pi \Delta P d_0} \approx \frac{4RT}{\pi \Delta P K(\theta)} \approx \left(\frac{2.036}{D}\right) m_0^{2/3} \quad (3)$$

with $D$ the coefficient of diffusion, $T$ the air temperature, $R$ the gas constant, and $\Delta P$ the difference between the saturated pressure and the air pressure. The solute phase diffuses into the air during the first regime of evaporation with a diffusion coefficient that is extracted from our experimental results with the previous relation: $D_{C1} = 5.5 \times 10^{-7} \text{ m}^2/\text{s}$. This is in agreement with the two extreme cases: pure water diffusion into air ($D_{wa} = 2.19 \times 10^{-5} \text{ m}^2/\text{s}$) and diffusion through a colloidal gel made of stacked spheres ($D_{cg} = 5.0 \times 10^{-10} \text{ m}^2/\text{s}$ [19]). Figure 4 summarizes both these experimental results and models. A good agreement is found with the experimental observations made for whole blood, which was shown to behave like a colloidal suspension. The last drying regime previously described can be clearly observed for heavy drops. However, for small drops this stage of evaporation is not easily noticeable although it does exit. Water diffuses through the desiccated cellular components at an evaporation rate that is 56 times slower during the second regime than during the first regime. Consequently, the diffusion coefficient for the second regime is $D_{C2} = D_{C1}/56 = 9.8 \times 10^{-9} \text{ m}^2/\text{s}$. This second regime diffusion coefficient is in good agreement with the values obtained in the situation of colloidal gel diffusion into air [19] where the value is between $10^{-9}$ and $10^{-10} \text{ m}^2/\text{s}$.

The structural evolution of a drying drop of blood is shown in Fig. 1 with a set of pictures. Several stages are observed and are described here for the case presented. As previously described, a gelled part builds up at the edge and extends inwards while the central fluid part shrinks progressively. Because of the competition between the adhesion of the deposit onto the substrate, which limits the reorganization of the solid phase, and the evaporation of solvent, the internal stresses increase inside the matter. At a certain moment, this internal stress is too great and regularly spaced radial fractures appear so as to release mechanical energy. The first cracks form at the gel periphery after 25% of the total time of evaporation $\tau_{C1}$. Then, secondary radial cracks appears at the center at 44% of the evaporation time $\tau_{C2}$. During the drying, cracks propagate toward the center and become wider. At the time $\tau_{G}$, the drop is almost dried. This pattern is explained by the presence of orthoradial stress, which is a consequence of the adhering ring of gelled blood around the fluid central area [2].

Considering the solid mass fraction during desiccation, experiments reveal that crack formation, as other steps, appears at a critical mass concentration of solid whatever the initial drop mass. The results are summarized in Table II. The first mechanical crack of the dried cellular matter at the periphery is observed at 29.6%, while the other central radial cracks nucleate at 65.4%. These cracks propagate inward as stretch by the motion of the gelation front. The transition to the last regime of evaporation occurs at a critical concentration of 88.3%. At this time, almost all the liquid has been evaporated. The remaining 11.7% is trapped inside the cellular components.

<table>
<thead>
<tr>
<th>Event</th>
<th>$t$</th>
<th>$\phi^*_s$</th>
<th>$\Delta \phi^*_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance of 1st radial cracks</td>
<td>$\tau_{C1}$</td>
<td>29.6%</td>
<td>3.4%</td>
</tr>
<tr>
<td>Appearance of central radial cracks</td>
<td>$\tau_{C2}$</td>
<td>65.4%</td>
<td>5.0%</td>
</tr>
<tr>
<td>Drying regime transition</td>
<td>$\tau_{G}$</td>
<td>88.3%</td>
<td>4.8%</td>
</tr>
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</table>
gellified onto the substrate and dries slowly until the end of the evaporation.

IV. CONCLUSION

Two regimes of evaporation are clearly evidenced during the drying of drops of blood. After a first stage mainly driven by convection, diffusion, and gelation, a transition phase occurs with the complete gelation of the system. Then, a second regime appears, which is only diffusive. The transition is correctly predicted by means of a model of diffusion through the liquid evaporating into the air. Finally, during the drying, cracks nucleate and propagate, leading to the formation of a typical regularly spaced radial pattern. By observing the concentration of solid mass in the drop during drying, we observe that fractures’ formation occurs at a critical mass concentration of solid.

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