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Pattern formation in drying drops of blood

D. BRUTIN[1]†, B. SOBAC[1]
B. LOQUET[2] AND J. SAMPOL[3]

[1]Université de Provence, IUSTI UMR 6595 CNRS, 13453 Marseille, France
[2]Laboratoire d'Analyses Médicales Bio 13, 13013 Marseille, France
[3]UMR U608 INSERM, Faculté de Pharmacie, 13385 Marseille, France

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The drying of a drop of human blood exhibits coupled physical mechanisms, such as Marangoni flow, evaporation and wettability. The final stage of a whole blood drop evaporation reveals regular patterns with a good reproducibility for a healthy person. Other experiments on anaemic and hyperlipidaemic people were performed, and different patterns were revealed. The flow motion inside the blood drop is observed and analysed with the use of a digital camera: the influence of the red blood cells motion is revealed at the drop periphery as well as its consequences on the final stage of drying. The mechanisms which lead to the final pattern of the dried blood drops are presented and explained on the basis of fluid mechanics in conjunction with the principles of haematology. The blood drop evaporation process is evidenced to be driven only by Marangoni flow. The same axisymmetric pattern formation is observed, and can be forecast for different blood drop diameters. The evaporation mass flux can be predicted with a good agreement, assuming only the knowledge of the colloids mass concentration.

Key words: bioconvection, blood flow, colloids

1. Introduction

Several patterns can be observed in dried drops of a different nature. Coffee drops have been widely studied in the past by demonstrating the influence of colloids in the final pattern observed (Deegan *et al.* 1997). Regarding human blood serum (the clear portion of any liquid separated from its more solid elements), the dried drop of blood serum presents different patterns depending on the individual's health condition. The specific regular pattern characteristics of a healthy individual do not appear in a dried drop of the blood serum from a person with blood disease (Martusevich, Zimin & Bochkareva 2007; Shabalin & Shatokhina 2007).

Experimental observations of dried drops of biological fluids (except whole human blood) have been performed and published in medical journals. The drying process is not analysed in the literature; they only focus on the final pattern in order to differentiate healthy people from patients with diseases. The drying technique has been used to store samples in locations, where appropriate storage conditions are not available: field studies, third world countries, deserts, etc. (Zhuang *et al.* 1982). The method was used to detect hepatitis B infection in serum dried on filter paper. Also,

† Email address for correspondence: david.brutin@polytech.univ-mrs.fr

experimental observations of dried drops of serum have been performed (Yakhno 2008). The author identified the origin of the patterns observed in dried drops of serum from the phase transition (gellification) of proteins. A strong attachment of the dried serum due to a biological reaction of the protein with the glass plate of the microscope and salt crystallization were found in the dried drop, these phenomena have been explained.

Whole blood is a complex colloidal suspension which behaves like a non-Newtonian fluid. Since the colloids of blood have complicated shapes, the individual colloid behaviour is hardly predictable. Pozrikidis (2006) demonstrate the existence of a molecular adhesion component to explain the flipping behaviour of a platelet over a plane wall. Besides the flow around these colloids, the biochemistry is also present, the red blood cells (RBCs) evidence repulsive behaviour near a wall as they do in the human body to avoid the vessel obstruction. When drops of blood evaporate, all the colloids are carried by the flow motion inside the drop and interact. These interactions are governed by fluid mechanics, biology and chemistry. Any modification in the drop evaporation process can reveal a given disease, either an RBC-related disease for whole blood drop evaporation or another disease for serum drop evaporation.

Similar to those phenomena which we observe with whole blood are presented in the case of the desiccation of a colloidal suspension (Pauchard, Parisse & Alain 1999). Crack patterns formed by desiccation of a colloidal suspension in a sessile drop placed on a glass surface have been observed. Pauchard *et al.* (1999) demonstrate that the crack formation is related to the salinity of the suspension and interpreted the pattern obtained for large drops as buckling instability. The experimental observations with mica colloids in deionized water (Deegan 2000) were theoretical confirmed by Popov (2005).

The biological suspension used in this study is real human whole blood from which the coagulation protein (fibrinogen) has been removed. RBCs are observed thanks to the relative transparency of a thin layer of blood (less than 500 μm). In this paper, we will mainly present the dynamic of the process of evaporation using a top view visualization and the drop mass evolution during the drying process.

2. Materials and methods

2.1. *Experimental set-up*

An experimental set-up was designed to provide a complete geometric characterization of a posed drop of blood during its evaporation on a substrate. The drops were deposited on Bioblock glass plates. Environmental conditions (temperature, pressure, humidity) were recorded using a meteorological station. Evaporation took place in a parallelepipedic box measuring $100 \times 100 \times 150\,\mathrm{mm}^3$ which remains covered during the whole duration of the experiment to avoid external flow perturbations. The box volume is not sufficient enough to saturate due to the generation of drop vapour phase. The image analysis was made by means of a Canon EOS 7d digital camera coupled with a $\times 1$ to $\times 5$ macro lens. This camera enabled us to obtain 5184×3456 pixels on an area of $22.3 \times 14.9\,\mathrm{mm}^2$. With an enlargement of $\times 1$, the resolution was 4.30 μm, and with the greatest enlargement of $\times 5$ the resolution was 0.86 μm. For films with 30 images s^{-1}, spatial resolution had to be reduced to 1920 by 1080 pixels (full HD) with a pixel size of 2.30 μm. This optical diagnosis allowed a visualization of the drop from above in enough detail for further analysis of specific areas. Drop evaporation was performed using a cold cathode back light at 5000 ± 270 K without any surrounding light to avoid reflection on the blood drop interface. The effect of the

density of light on the drying process was studied to avoid any biological or chemical disturbance of the phenomenon. To follow the drying process, the experiment is performed on a digital accurate weighting balance with a resolution of 10 μg up to 81 g (Mettler Toledo XS 205). The drop is deposited on a microscope glass substrate (5 g) and then the total mass of the set-up is recorded on a computer at 1 Hz.

2.2. *Blood: biological and physical properties*

Blood samples were taken in a nearby medical laboratory and stored in their original 10 μl sterile tubes (BD Vacutainer 9NC 0.109M) in a refrigerator at +4°C, so that several experiments could be carried out at different times. Experiments were performed at any time from 1 h to 6 days after the sample was drawn, results for the same person remained almost the same showing that these storage conditions do not influence the main drying process. The blood used in this paper was taken from volunteers of whom some were in good health and others had a disease: anaemia (reduction below normal of the number of RBCs, quantity of haemoglobin or the volume of packed RBCs in the blood) or hyperlipidaemia (elevated blood lipids, particularly triglycerides, after carbohydrate ingestion). The biological fluid properties have been determined in order to correctly analyse the flow behaviour observed and the pattern formation. The whole blood and the serum have been analysed in terms of fluid viscosity using a Physica MCR 501 'Anton Paar' and the surface tension has been determined using the pendant drop method. At the same time, a full haematology and biochemistry analysis of the blood has been performed to obtain the blood characteristics in terms of comosition (haematocrit, lipidaemic balance). The blood density varies slightly for different people in between 1020 and 1060 $\mathrm{kg\,m^{-3}}$ depending on the blood composition. Considering this small variation in density (less than 4 %), one assumes a constant whole blood density of 1040 $\mathrm{kg\,m^{-3}}$ in this study. The blood rheology is obtained in the range of shear stresses from 0.1 to 100 $\mathrm{s^{-1}}$ and is given in (2.1) as

$$\tau = k\dot{\gamma}^n, \tag{2.1}$$

with n equal to 0.82 ± 0.01 and k is a constant. This is in agreement with typical results obtained, indicating that whole blood viscosity is driven by the RBCs concentration (the haematocrit) (Thieret 2008). For the blood serum, we obtain $n = 1.01 \pm 0.01$, which is more consistent with a Newtonian fluid. The blood serum does not contain micrometre-sized colloids, such as RBCs or WBCs, but only ions and proteins. Of the whole blood drop, 43.9 % volume is RBCs based on haematological analysis. Mass-wise, 23.9 % of whole blood is composed of colloids which will be remain on the glass substrate at the end of the drying process. The main blood colloid distribution is as follows.

(i) Erythrocytes (red blood cells) with 4.9×10^6 units $\mathrm{mm^{-3}}$ and a typical size of 8 μm represent 97 % of the volume of colloids,

(ii) Leukocytes (white blood cells) with 4.3×10^3 units $\mathrm{mm^{-3}}$ and a typical size of 15 μm represent 2 % of the volume of colloids,

(iii) Platelets with 2.4×10^5 units $\mathrm{mm^{-3}}$ and a typical size of 3 μm represent 1 % of the volume of colloids.

For our analysis in § 3, we will assume that the fluid is mainly composed of 8 μm diameter colloids at high concentration (about 45 %). Whole blood also contains ions and proteins which represent 11.6 % by mass of the final dried deposit (statistical values obtained for all experiments were performed using the same blood sample).

Substrate	θ_{blood}	θ_{serum}	θ_{water}
Glass without coating	14.8° ± 16.2 %	9.5° ± 21.1 %	–
Glass with PTFE coating	140.0° ± 2.6 %	128.5° ± 2.9 %	143.6° ± 3.4 %
Glass with epoxy coating	84.9° ± 2.4 %	74.0° ± 2.7 %	88.1° ± 1.7 %

TABLE 1. Drops of blood and serum contact angles (water contact angle on glass substrate are not measurable with sufficient accuracy).

The blood surface tension was measured using the pendant drop method with a capillary tube of 1 mm diameter. The exact capillary tube diameter was measured to an accuracy of 0.02 mm and blood drop mass was determined to be 10 µg using an electronic balance. This method gives a statistical value of 69.8 mN m^{-1} ± 4.6 % over 72 drops for the full blood and 58.8 mN m^{-1} ± 4.9 % over 24 drops for the serum. These values agree with the theoretical values found in the literature, and they indicate that full blood surface tension is very close to the value obtained for water, while serum surface tension is of a much lower value. Contact angles were determined using the sessile drop method where whole blood, serum and water were dropped onto three substrates: a microscope glass plate without any coating or with PTFE or epoxy coating, to confirm the surface tension measurements. The values obtained were close to the value obtained with pure ultra-filtered water as presented in table 1. Based on physical properties, the full blood used in this study is very close to water except for the fluid viscosity, which demonstrates non-Newtonian behaviour (thixotropic fluid).

3. Experiments

3.1. *The pattern formation for healthy people*

The different stages of blood drop evaporation are shown in figure 1. The initial drop diameter is 5.9 mm and the enlargement used is ×4.8 to allow visualization of RBCs. The images for the film did not undergo any processing except for cropping as the original size of each picture was 5184 pixel × 3456 pixel. However, due to the experimental protocol, the digital camera started taking pictures up to 3 min after the drop was placed on the plaque. Nevertheless, this delayed starting time was compensated and is $t = 0$ min. We present a film of the whole evaporation process online with a playing time which is seven times the real duration, the external drop diameter is 6.5 mm (supplementary movies available at journals.cambridge.org/flm).

The evaporation/desiccation process can be divided into five stages which are detailed below. During the evaporation process, the drop is always pinned on the substrate. The total evaporation time for the case presented is 36 min and the durations of the stages are presented as a percentage of the total time of evaporation.

(i) Stage 1 (from 0–20 %): the RBCs move out from the centre of the drop to the desiccation line which recedes. A red deposit is observed at the edge of the drop. The measured receding speed of the desiccation line is 0.7 µm s^{-1}.

(ii) Stage 2 (from 20 to 50 %): crystallization appears at the edge of the drop and propagates inwards. At the same time, the desiccation process continues from the edge towards the inside of the drop. A dark red torus is observed which demonstrates a different fluid composition. This torus contains a high concentration of RBCs. These cells are now clearly observed moving from the centre to the edge of the drop.

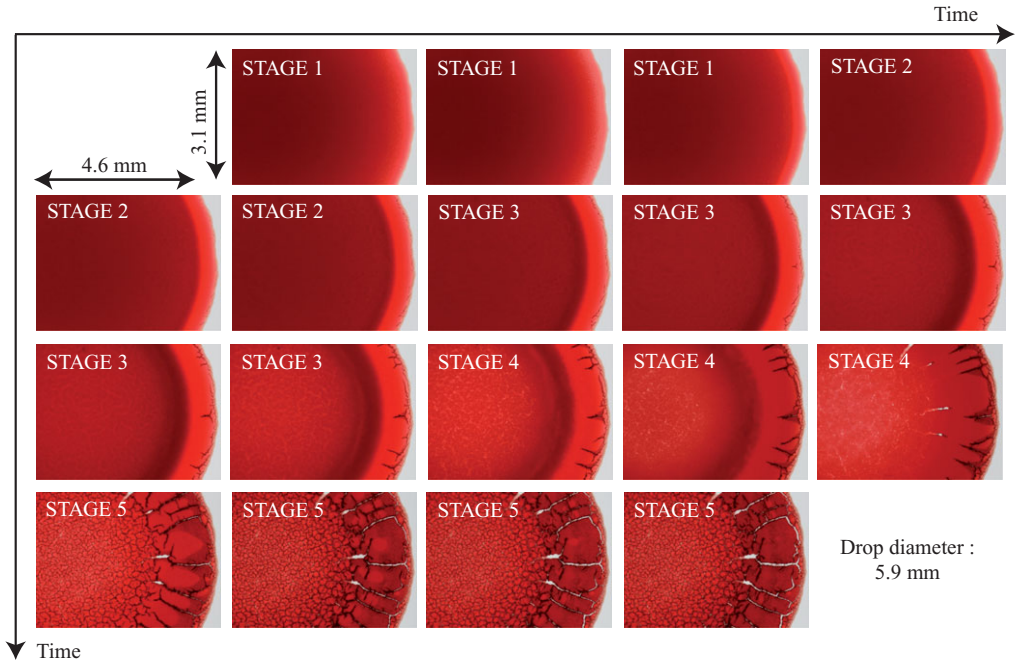

FIGURE 1. Mechanisms involved in a drying drop of blood from a healthy person (drop diameter, 5.9 mm; room temperature, 22°C; microscope glass substrate; 100 s between frames). A movie is provided online with a playing speed increased by 60. See figure 4 for a schematic cut view of the drop.

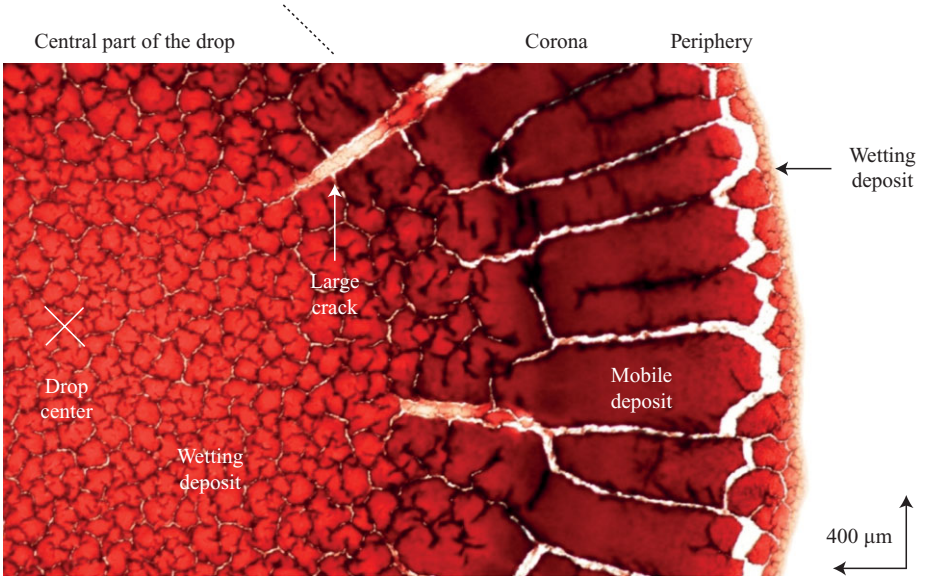

FIGURE 2. Closer view of patterns: different characteristic pattern sizes exist (drop diameter 5.9 mm; room temperature, 22°C; microscope glass substrate; field of view, 4.6 mm × 3.1 mm).

(iii) Stage 3 (from 50 to 70 %): the dark red torus desiccates rapidly and simultaneously the colour of the central part of the drop changes to an increasingly lighter red. The drop is almost totally desiccated and the first crack appears around the drop between the future corona and central part of the drop.

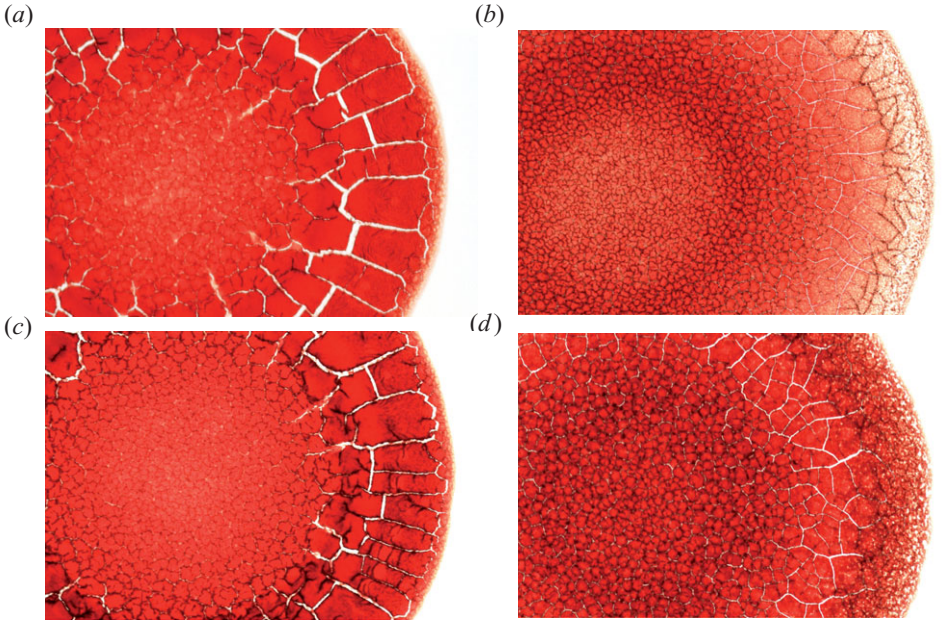

FIGURE 3. Four drops of blood: (*a*) sample from a 27-year-old woman in good health, (*b*) person with anaemia, (*c*) sample from a 31-year-old man in good health and (*d*) person with hyperlipidaemia.

(iv) Stage 4 (from 70 to 85 %): the central part of the drop desiccates producing much smaller plaques. Desiccation of the corona finishes and circular drying spots are observed around the corona.

(v) Stage 5 (from 85 to 100 %): the large plaques of the corona move slightly as soon as they are totally desiccated. This concludes the desiccation of the drop and no further changes are observed.

All patterns observed with dried drops of blood present similar characteristics: the central part of the drop, the wide mobile plaques of the corona with wide white cracks, and the fine periphery (figure 2). Whereas the central part of the drop and the fine periphery stick onto the glass plate due to the absence of RBCs, the corona is where mobile deposits form and these do not adhere to the substrate. The explanation of this wetting phenomenon lies in the proteins which exist on the RBCs (glycoproteins). The function of these proteins is to avoid wettability with a wall and, in particular, the internal wall of organs, veins.

At the beginning of drop evaporation, the fluid is homogeneous in colloids, this is why a thin periphery can dry on the glass plate. However, since Marangoni convection is occurring, the RBCs (and other heavy colloids) accumulate at the drop triple line, which is receding. The RBCs accumulate by Marangoni convection to form a solid deposit which will be called hereafter 'the corona'. The remaining fluid, which consists mainly of serum without heavy colloids, leads to typical small-sized patterns adhering to the glass substrate after its evaporation.

3.2. *Repeatability and reliability about whole blood experiments*

The pattern formation observed in drying drops of blood is totally different for individuals in good health and for those who have a disease. However, for healthy people, the patterns observed are quite similar and are presented in figure 3. They are as follows.

(i) Cases (*a*) and (*c*): case (*a*) is a 27-year-old woman and case (*c*) is a 31-year-old man, neither smokes or consumes alcohol on a regular basis. The results of drying drops of blood for these two cases reveal the same patterns which will be detailed in § 4.

(ii) Case (*b*): the person is anaemic. The external part of the drop corona is very light-coloured compared to the central part; furthermore, the formation of dark lines is observed in this area and then the appearance of white lines in a central torus which corresponds to a non-wetting situation of the blood on the glass substrate. There are small-sized plaques on the central part of the drop.

(iii) Case (*d*): the person is hyperlipidaemic. The external part of the drop corona is thick and 'greasy'. This external zone is followed by the formation of intermediate plaques with white lines which also demonstrate a non-wetting situation on the glass substrate. There are small-sized plaques on the central part of the drop.

4. Discussion

4.1. *Physical mechanisms involved*

Based on the biological and physical properties of blood obtained in the previous section, we can estimate the dominating forces. RBC speed can be measured using flow visualization and is determined to be $\sim 8\ \mu m\, s^{-1}$. With the blood viscosity and density, we obtain a Reynolds number for the internal drop flow of $Re = \varrho U D/\mu \approx 3 \times 10^{-3}$, where ϱ is the whole blood density, U is the average speed estimated using a RBC displacement, D is the drop radius and μ is the blood viscosity. The capillary number which compares the viscous and the capillary forces is: $Ca = U\mu/\sigma \approx 1.1 \times 10^{-6}$, where σ is the blood surface tension. The Rouse number which compares the sedimentation fall velocity and the shear velocity is: $Ro = U_s/(k * \sqrt{\tau/\varrho}) \approx 8.2 \times 10^{-5}$, where U_s is the sedimentation fall velocity ($1\ mm\, h^{-1}$ during the first hour) and k is the Von Kármán constant. Consequently, the flow inside the blood drop is driven only by Marangoni convection.

To confirm the different mechanisms involved in the drying process, we compared the evaporation mass flux of three drops: blood, serum and pure water on a glass substrate. The stages of the drops evaporation are all reported in figure 4, where it appears that the drying process is mainly driven by the Marangoni flow, since the blood and serum curves are in agreement with the pure water curve. The colloidal suspension curves (blood and serum) are corrected by dividing the evaporation mass flux by the real fluid mass concentration (which is 76.1 % for blood and 88.1 % for serum, water fluid mass concentration is 100 %, since we use pure distilled ultra-filtered water). Since the evaporation process is related to the fluid phase transition to vapour phase, the solid phase of the suspension is not of concern. One notices that when the blood drop is deposited on the glass substrate, the evaporation mass flux is slightly more important compared with the pure water case. Then this enhancement tends to disappear with time while the colloid concentration changes.

At the beginning of the experiment, the blood drop is deposited and its shape is hemispherical. The RBCs are homogeneously dispersed inside the drop which is about 0.5 mm in thickness and 6 mm in diameter. Marangoni flow is acting (see the supplementary movie), the RBCs movement from the inner of the drop to the periphery is clearly observed. The blood drop desiccates since the water contained in it is evaporating. At the same time due to Marangoni flow, the RBCs are accumulating at the drop periphery creating a layer of biological deposit (cellular components). From 50 % of evaporation time, the drop shape is no more classical with a central

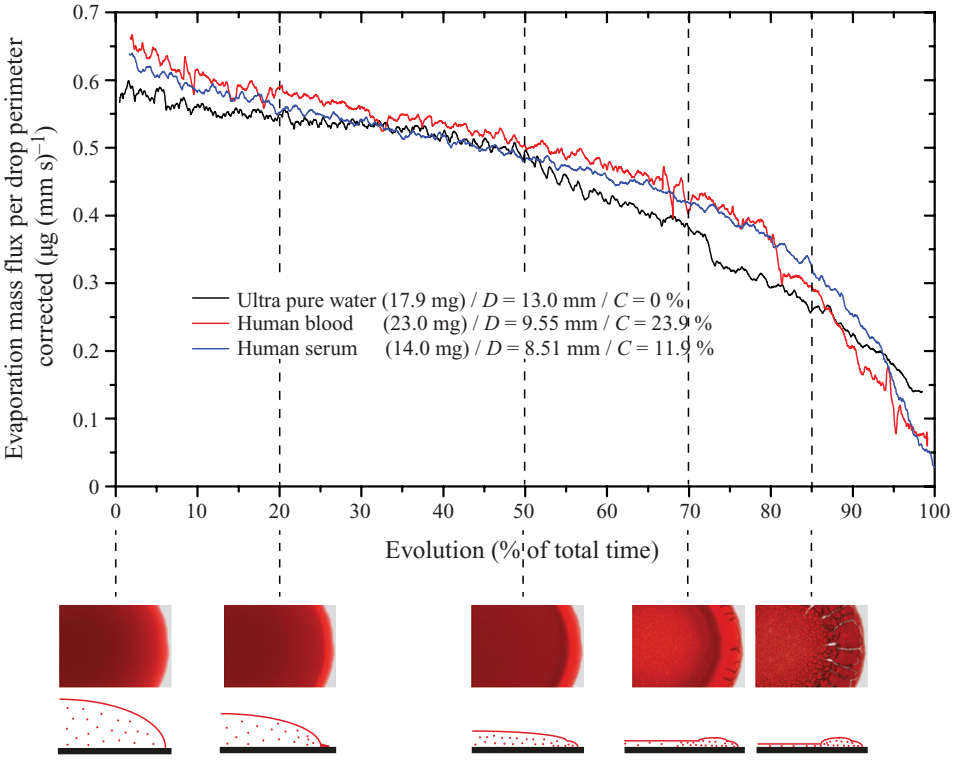

FIGURE 4. Evolution of the evaporation mass flux per drop perimeter: a correction for the solid mass fraction (C) is applied for blood and serum taking into account the real fluid mass under evaporation (room temperature, 29°C; microscope glass substrate).

flat area. Marangoni flow is no longer possible and the remaining RBCs are trapped into the central area of the drop, while most of the cellular components have been deposited in the drop corona area. At 70 % of the total evaporation time, the central area of the drop starts to completely desiccate and evidences small-sized pattern formation. This last stage of the process is characterized by a sharp decrease of the evaporation mass flux, since the remaining water is that which is inside the RBCs. Finally, after 85 % of the total evaporation time, the RBCs which are just shells containing the haemoglobin, crack and free the remaining liquid which can evaporate. This leads to a typical cross-section deposit which can also be called a 'donut' shape.

The drying process behaviour based on the evaporation mass flow rate is not modified by the drop diameter as presented in figure 5. After this, the evaporation mass flux sharply decreases. This second dynamic is clearly related to the absence of convection, since the drop is now mainly a solid with a small amount of liquid remaining inside the red blood cells. The last stage of the drop evaporation consists of the red blood cells shell cracking, which releases the haemoglobin. This last stage of evaporation takes more time since the fluid has to migrate through the solid deposit, which does not wet the glass microscope plate.

As evidenced in figures 4 and 5, the evaporation dynamics can be scaled assuming that the blood is a colloidal suspension composed of pure water and 23.9 % by mass RBCs 8 μm in diameter and the serum as a colloidal suspension of pure water

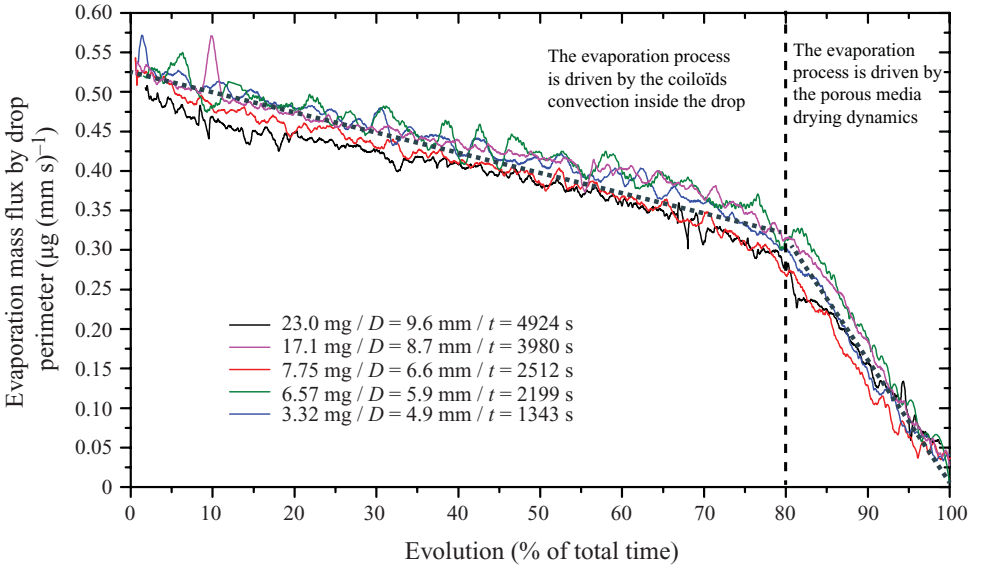

FIGURE 5. Evolution of the evaporation mass flux per drop perimeter for five blood drops. The blood comes from the same tube, thus the initial colloid concentration for each drop is identical (room temperature, 29°C; microscope glass substrate; colloid mass concentration, $C = 23.9\,\%$).

and 11.9 % by mass proteins 1 μm in diameter. For several drop diameters of blood investigated, the same evaporation dynamics is observed like for pure water. Thus, the total evaporation time can be predicted based on the blood haematologic composition by comparison with pure water.

4.2. *Axisymmetric pattern formation*

Depending on the diameter, cracks can be observed as shown in figure 6, where we present drops with different diameters formed simultaneously from the same blood with the same experimental conditions. The drop with a diameter of 3.8 mm shows no wide axisymmetric cracks, whereas the drop which is 5.4 mm in diameter clearly shows periodic cracks around the corona. This cracking phenomenon is only observed above a critical diameter which for this case is 4.2 mm. The mechanical stresses inside the solid deposit desiccating can be advanced as the origin of this cracking. The formation of wide cracks is similar to the desiccation of drops of nanoparticles on glass plates studied by Pauchard, Abou & Sekimoto (2009) to which the RBCs in the present case may easily be compared. Pauchard *et al.* (2009) observed cracking which depended on the initial concentration and deformability of nanoparticles. In our study, the concentration and shape of RBCs can lead to the same consequences on the final patterns observed. The corona of the drop is mainly composed of RBCs which have been deposited by internal drop convection. Thus, the central part of the remaining blood is less and less rich in RBCs. After stage 4, the liquid remaining in the central area of the drop contains mainly proteins, white blood cells, blood platelets and a few amount of RBCs. All these elements present hydrophilic behaviour with glass and wet the substrate.

For small drops below a critical value, drop evaporation which is related to the volume to be dried, is much faster. For large drops with a diameter of more than 4.2 mm, evaporation takes place over 30 min and Marangoni convection observed

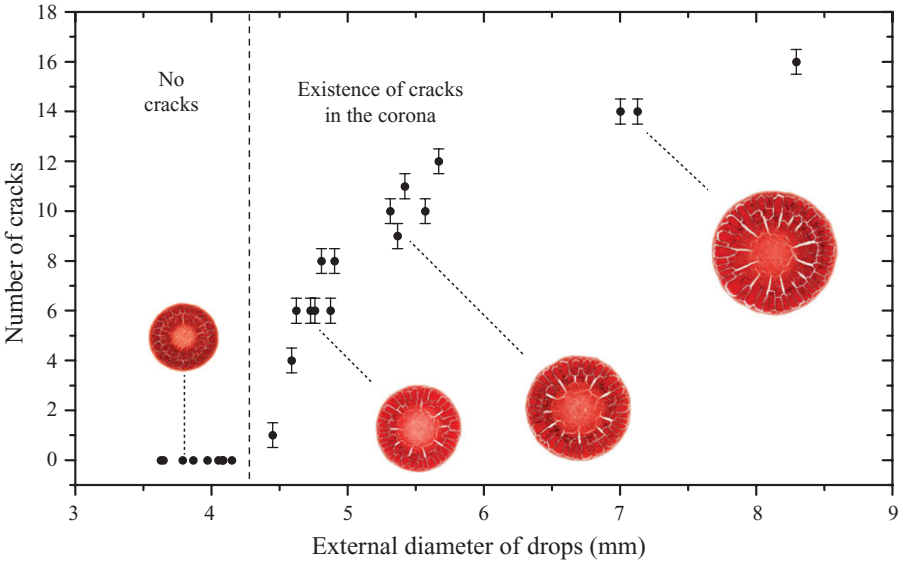

FIGURE 6. Dependency of the number of cracks in the corona on the external diameter of the drop (blood coming from the same tube taken from a healthy subject; microscope glass substrate; room temperature, 22°C; humidity, 47 %).

through RBC motion is noticeable. RBCs accumulate at the edge of the drop. When the desiccation phenomenon occurs on the glass substrate, a decrease in volume is observed due to the large amount of water required. During the desiccation process, stress inside the solid deposit of desiccated blood built up and induced surface cracks periodically releasing the stress. Furthermore, the RBCs present at their surface proteins induce a hydrophobic behaviour with all materials. This is observed with glass. The dried RBCs at this position in the drop do not wet the substrate and reveal an hydrophobic behaviour. This enables a cracking phenomenon linked with the diameter of the drop, and consequently with the perimeter of the corona. For small drop perimeters, the stress inside the RBCs solid deposit is small compared with large drop diameters.

5. Concluding remarks

Observations of the Marangoni flow motion inside a drop of blood using a digital camera at a good resolution (about 2.3 μm) enable us to show that the motion of RBCs at the edge of the drop is the main mechanism of blood drop evaporation and deposition. The mechanisms involved in the blood drop evaporation are confirmed through the evaporation mass flux which is measured to be in agreement with a pure water drop evaporation. Other biological elements (white blood cells, proteins) are transported to the edge of the drop with the RBCs and contribute to the pattern formation through the deposit wettability with the substrate and mechanical properties. The blood evaporation dynamic can be predicted by a comparison with the pure fluid evaporation case corrected by the mass concentration in colloids. Totally different patterns are formed according to whether the person is healthy or suffers from anaemia or hyperlipidaemia. This is leading our research to correlate the pattern formation with blood diseases. An analysis of the corona of the drop reveals the presence of large solid plaques measuring several hundred micrometres which are

clearly visible for a normal individual, but are absent for someone with a disease. This simple, reliable and inexpensive indicator could be used for the diagnosis of blood diseases. The fluid mechanics involved in this real colloid suspension include convection of the main colloids (cellular components), but also evidences hydrophobic behaviour between the RBCs and the substrate, which modify the pattern observed after the drop has desiccated. An explanation for nonlinear evolution of the number of cracks with increasing diameter is currently being prepared, in conjunction with elements of solid mechanics.

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Supplementary movies are available at journals.cambridge.org/flm.

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