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Morphology of drying blood pools

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ABSTRACT

Often blood pools are found on crime scenes which may provide information concerning the events that took place on the scene. However, there is a lack of knowledge concerning the drying dynamics of blood pools. This study focuses on the drying process of blood pools to determine what relevant information can be obtained for the forensic application. We recorded the drying process of blood pools with a camera while measuring the mass. We found that the drying process can be separated into five different stages: coagulation, gelation, rim desiccation, centre desiccation, and final desiccation. Moreover, by normalizing the mass and drying time we show that the mass of the blood pools diminish similarly and in a reproducible way for blood pools created under various conditions. In addition, we verify that the size of the blood pools is directly related to its volume and the wettability of the surface. Our study clearly shows that blood pools dry in a reproducible fashion. This preliminary work highlights the difficult task that represents blood pool analysis in forensic investigations, and how internal and external parameters influence its dynamics. We conclude that understanding the drying process dynamics would be advancement in time line reconstitution of events.

1. Introduction

Bloodstain pattern analysis is a forensic tool used by investigators to determine, among others, what, where and how a crime took place [1]. One of the most common types of bloodstains found on a crime scene following a deadly blood shedding event, is the blood pool (Fig. 1). Ante- and post-mortem it is often the case that a victim bleeds out, thus accumulating blood in one or multiple areas. Currently, when a blood pool is found, it is classified as such and an investigator can conclude that the blood donor was bleeding at that location for any reasonable period of time for the pool to be created, be it seconds, minutes or even hours. Previous studies have investigated if it was possible to determine what the volume of a blood pool was, to determine if such a loss of blood volume could constitute loss of life [2], or for other crime scene reconstruction purposes [3–6]. However, almost no studies have been performed concerning the drying of an entire pool of blood. Such studies can be very useful for determining, e.g., the time that the blood shedding event occurred, any actions that may have occurred during the blood shedding event or the physiological state the subject was in. For example, Fig. 1) shows two crime scene pictures of the same pool, 22 h apart. In the first (top) picture, the edges and the bottom of the pool have started drying. In the second picture the pool has completely dried. Information obtained from how fast the blood dried could be crucial to determine when the pool was created.

There have been several studies concerning the drying of singular blood droplets [7–11]. To our knowledge only Ramsthaler et al. investigated the drying of blood *pools* [12]. In their study they focused on the drying and morphology of diluted blood droplets and pools to be able to distinguish between diluted and whole blood. In this paper we report on the morphology of drying blood pools. Pools of blood, obtained from healthy volunteers were deposited on linoleum surfaces. Based on our results we are able to distinguish five different stages of drying. In addition, we report the universal properties of drying blood pools, but also distinguish anomalies, which can differ between pools.

2. Background theory

Once bleeding occurs, blood being ex vivo, it will coagulate and dry. During the coagulation (clotting) process, fibrin strands are formed creating a solid structure of the blood, the clot. During drying water evaporates from the blood pool until only the solid matter, mainly red blood cells (RBCs), remains. Depending on the size of the pool and environmental conditions, the time the pool

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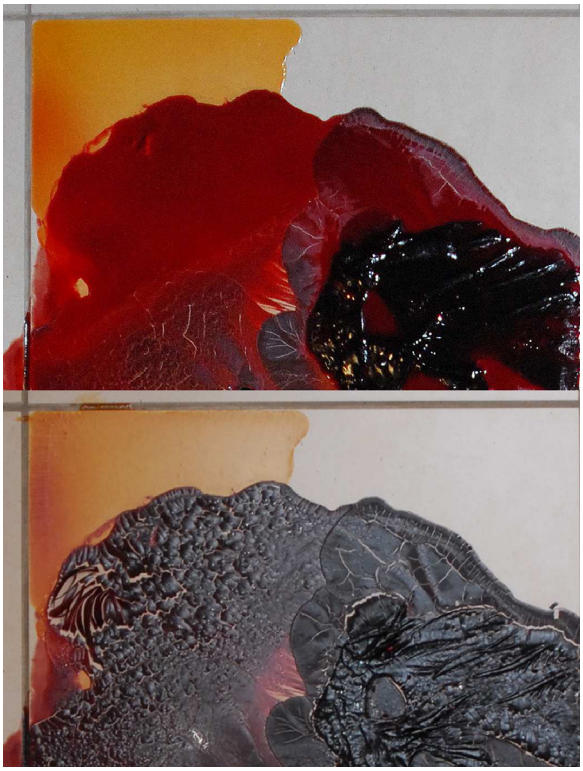

Fig. 1. Picture of a real pool of blood found on a crime scene, (top) before the body was removed and (bottom) 22 h later. The yellow liquid is serum which was separated during clotting and the black mass in the top picture is a large formed clot.

completely evaporates may take hours to days. On the crime scene, pools can be found in the order of millilitres to litres. We, however, focus on pools in the order of millilitres, simply because pools with a volume of several litres, without any additives like antic-oagulants, would require a very large donation of a volunteer which is not a viable option. Prior to our investigation into drying blood pools we require some general knowledge about fluid dynamics of evaporating liquids.

When a droplet is deposited upon a surface it will spread. The area (A) the droplet spreads over depends on the physical properties of both the surface and liquid, where the surface tension and contact angle are the most important parameters (see supplementary materials). The surface tension (γ) is defined as the amount of energy required to increase the surface area by one square meter. In other words, increasing the area of a droplet requires energy and the higher the surface tension, the more energy this takes. As the droplet or pool lays upon a surface, the surface tension acts upon the triple-line (the line around droplet or pool where liquid, surface and air meet, see Fig. 2a and b). How a droplet or pool spread upon a surface can be deduced from Young's equation [13]:

$$S = \gamma(\cos\theta - 1) \tag{1}$$

Here, S is the so-called spreading parameter, γ is the surface tension between liquid and gas interface and θ the contact angle between liquid and surface (see Fig. 2c). When the contact angle is much smaller than 90° (S is positive) the surface wetting, i.e., the liquid can easily spread over the surface and the surface is presumed wetting. When the contact angle is much larger than 90° (S is negative) the liquid cannot spread over the surface easily and the surface is presumed non-wetting. With a small contact angle, the pool will cover a much larger area and have a larger perimeter,

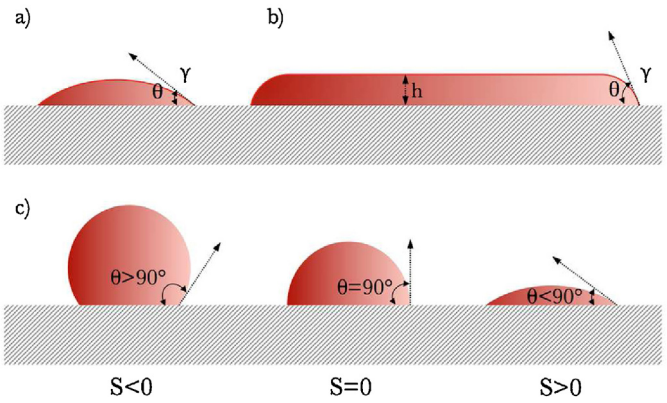

Fig. 2. A schematic representation of the cross-section of (a) a single droplet, (b) a pool and (c) three droplets on surfaces varying in wettability.

which should significantly increase the rate of evaporation. Therefore, the contact angle is a very important parameter concerning the drying of blood pools.

Deegan et al. [14] demonstrated very accurately the principles of the coffee ring effect that is observed during the drying of a droplet of a colloidal suspension. This study showed how the flow arising from the evaporating liquid induced the characteristic ring formation. A study by Brutin et al. [7] focused on the drying of sessile whole blood droplets which showed that it is very similar to the drying of a droplet of a colloidal suspension, blood being a colloidal fluid. During the drying of a blood drop the formation of cracks is observed. Moreover, the study showed that a drop of blood dries following two different regimes and goes through five different stages. The first regime (first three stages) is being driven by convection, diffusion and then gelation. At the moment the drop is deposited, RBC's are evenly distributed inside the droplet, but then the solvent starts evaporating inducing an evaporation flux at the interface and an internal flow transporting particles inside the drop. This leads to the formation of a gel once the concentration of particles is high enough. Additionally, this flow induces the formation of the so-called biological deposit on the periphery of the droplet; indeed RBCs are driven from the inner part of the droplet to its rim. Then the transition phase takes place and leads to the gelation of the entire drop. A sharp decrease in the drying rate is observed, whereas gelation is rapid.

The second regime is much slower since it is diffusive. The final two stages correspond to the drying and the formation of cracks that are nucleating and propagating. This extensive work on drying of droplets gives precious information about the process and shows accurately that desiccation starts at the periphery of the drop, and then dries towards the centre of the droplet. The work presented in this study no longer focuses on droplets but on pools. To understand the dynamics occurring during the drying of a pool, the size of the blood pool must be considered. As long as the volume is low, in the case of droplets, the surface tension forces are dominant resulting in a curved surface. In contrast, if the volume is large enough, the gravitational forces will dominate over the surface tension forces producing a flat surface on top of the pool. Similar to a droplet, a pool will have a contact angle with the substrate on the edges, but in contrast is flat otherwise (Fig. 2b). The area the pool spreads over is directly dependent on the contact angle (see Appendix A). In order to understand the phenomena and the dynamics driving the drying of blood pools, we performed experiments with small blood pools (about 4 mL), which were recorded by taking pictures every 2 min. Foremost, the purpose of our experiment is to identify the different drying stages of blood pools.

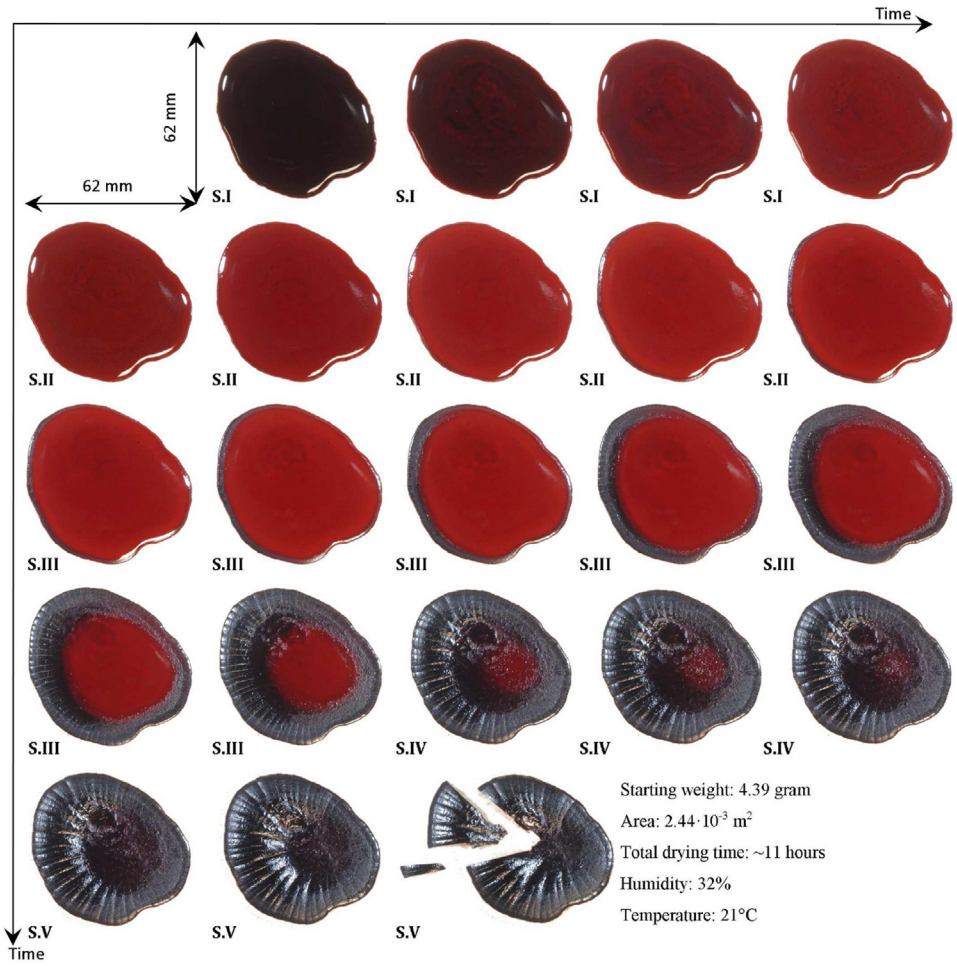

Fig. 3. Time-lapse of a drying pool of blood from a healthy person, at 21 °C with a relative humidity of 32%. For a movie of the drying blood pool, see Supplementary Material. Note that the elapsed time between pictures is different for each stage. The time of drying for each picture is shown in Fig. 5.

3. Methods and materials

To follow the drying of a blood pool we required the environment to be monitored and as constant as possible. Therefore each blood pool was created in a glovebox (Jacomex T-Box, $V = 700$ L). The humidity and temperature were recorded during drying by means of a hydrometer (Teslo AG, 175-H2, Datalogger, Germany). The temperature was constant at 22 ± 0.5 °C during all of the experiments. Within the glovebox, a camera (Nikon D200, resolution: 2592 × 3872 pixels or Nikon D300s resolution: 2848 × 4288 pixels both with a 60 mm 1:2.8 lens) was suspended directly above the blood pool. The camera's enabled us to take a single picture, every 2 min, with a resolution of roughly 26–30 pixels per millimetre. For each blood pool created, blood of a healthy volunteer was drawn by a certified nurse in a 4.5 ml evacuated blood collection tube (VenoSafe, Terumo, France). Immediately after blood collection the tube was emptied above the target substrate (linoleum) creating a pool of blood of roughly 4 mL each time. Moreover, blood pools were created by slowly dripping blood directly from the tube/needle connected to the arm to imitate the most realistic blood shedding event possible. Accordingly, blood pools created had a starting weight $3.5 \text{ g} < m_i < 7$ g. No differences were observed in the drying process of the pools between the two methods of blood deposition. Of several blood pools the substrate was on top of a balance (Mettler Toledo, ML802, Switzerland) to determine exactly the mass (m) of the blood pool during the entire drying process (one measurement every minute). By means of a reference length next to the pool it was possible to determine the area of the pool, by using a program written in Matlab.

4. Results and discussion

In Fig. 3 we show a time-lapse of a drying blood pool deposited on a linoleum surface (see supplementary movie for the complete time-lapse). First of all the simple observation of the pictures obtained in the experimental conditions described previously allowed the identification of five distinct phases (Fig. 3): (I) coagulation stage, (II) gelation stage, (III) rim desiccation stage, (IV) centre desiccation stage, (V) final desiccation stage (see supplementary materials for a point-wise summary).

(I) When blood is deposited upon a surface, the RBC's are evenly distributed throughout the pool, which then will sediment. At this point the blood has a dark red colour and starts to coagulate [15]. It is possible that due to wetting and capillary action, the area of the blood pool increases as the blood spreads slowly over the surface during the initial 15–30 min. During this first stage there is a change in colour from dark red to lighter red, mainly due to coagulation. (II) As fluid evaporates from the blood pool red blood cells, that are not constricted in the fibrin web, are transported to the rim of the stain and deposited, due to flow caused by evaporation [14]. The transition from the fluidal to a gel state is referred to as the gelation front. The second stage starts when the gelation rim is created around the pool. The gelation front propagates inwards, towards the centre of the stain, as the pool

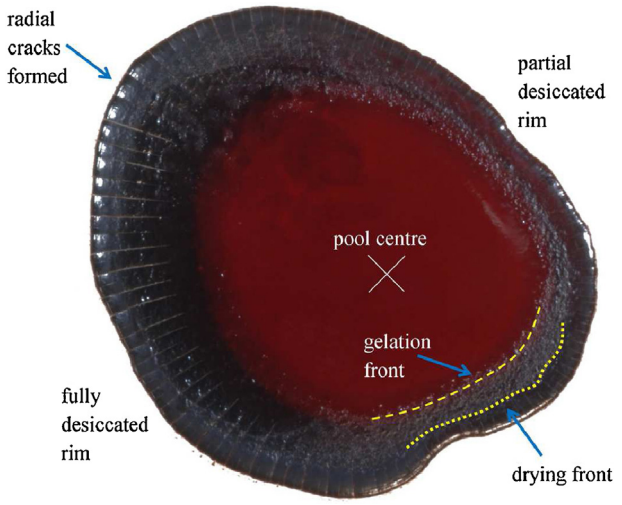

Fig. 4. Close-up view of a drying pool, with several defined properties of the pool. The yellow dashed and dotted lines represent the gelation and drying front, respectively. (For interpretation of reference to color in this figure legend, the reader is referred to the web version of this article.)

continues to dry. (III) The third stage starts as soon as the rim turns black and starts to crack, indicating that the rim is desiccating. The transition from the red to black colour is referred to as the drying front. During this stage both the gelation and drying front propagate towards the centre of the stain. (IV) Once the gelation front reaches the centre of the stain, the entire stain has gelified and evaporation of fluid is mainly driven by the porous media drying dynamics [7]. The drying front and cracks propagate towards the middle of the stain. (V) Finally, the drying front reaches the centre of the pool. The pool has almost completely desiccated. During this last stage, the entire pool is black in colour. As the last liquid evaporates, the remains contract and the cracks reach the middle of the stain. Accordingly, flakes are separated and partially or completely detach from the surface. We have observed the five stages described above for every pool we created. However, it should be clear that a pool does not dry in a uniform manner. Instead, one part of the blood pool may be fully desiccating (left side Fig. 4) while another part is still in a gel-like state (right side Fig. 4). Consequently, the centre of the pool, i.e., the location where all cracks come together, does not necessarily has to be the geometrical centre of the pool. Furthermore, the duration of any one stage and complete desiccation can differ between blood pools, depending on the humidity, temperature, shape and size of the pool and the kind of surface.

During the drying of the blood pool, the mass (m) of the pool was measured with a balance, every minute (Fig. 5). In the first and second stage the pool loses roughly 40% of its mass. Liquid is evaporating and the height of the pool diminishes over time. A linear function was fitted to the data points for the first six hours of drying, with fitting parameters A the initial droplet mass equal to 4.39 g and B the mass loss per hour equal to 403 $\pm$ 3 mg/h. It is clear that the mass loss during the first six hours scales linear with time. Only once the drying front is significantly formed, does the decrease in mass stop scaling linear with time. This effect can be explained as follows. The pool is pinned to the surface, i.e., the contact line cannot move. As liquid from the pool evaporates, the volume diminishes, but because the contact line is pinned, the pool can only decrease in height. Moreover, to compensate for the volume loss at the contact line, there is a flow from the middle of the pool towards the rim. This flow causes particle (RBCs) transport throughout the pool which are deposited at the contact line creating a characteristic rim around the pool. As the pool dries, the contact angle should change which in turn should decrease the evaporation rate [16]. We, however, have not observed any change in evaporation rate due to this effect. As long as the area of the pool does not change, the evaporation rate does not change. Only once a critical amount of liquid has been depleted from the pool, in this case more than 55%, does the evaporation rate change. At this point the liquid and gel areas of the pool are diminishing, accordingly the evaporation rate diminishes as well, explaining the deviation from the linear trend after six hours in Fig. 5. The largest visual change happens during stage III where the entire stain transforms from a liquid/gel to a solid. Finally, 21% of the initial mass remains in this case.

Multiple blood pools were created and recorded with varying haematocrit values and under different humidities. The mass of those pools are shown in Fig. 6b(inset). Not surprisingly, it is clear that the larger the mass of a pool, the longer it takes for the pool to dry. The mass decreases linearly with time until roughly 50–70% of the mass has been depleted, at which point the slope diminishes and the mass becomes constant as the last liquid evaporates from

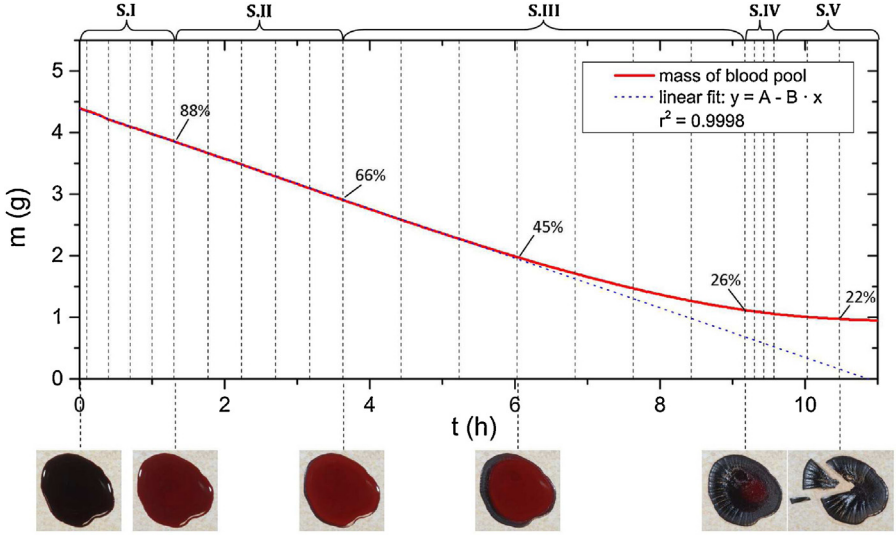

Fig. 5. The mass of a blood pool as a function of time with some pictures of the pool at several moments in time. The vertical dashed lines represent the time at which the pictures in Fig. 3 where taken. The red line is the mass of the pool, the dashed line a linear fit to the data, from t = 0 till t = 8.24 h, the time the pool transitioned into stage III. The percentages are the amount of mass left at that specific point in time.

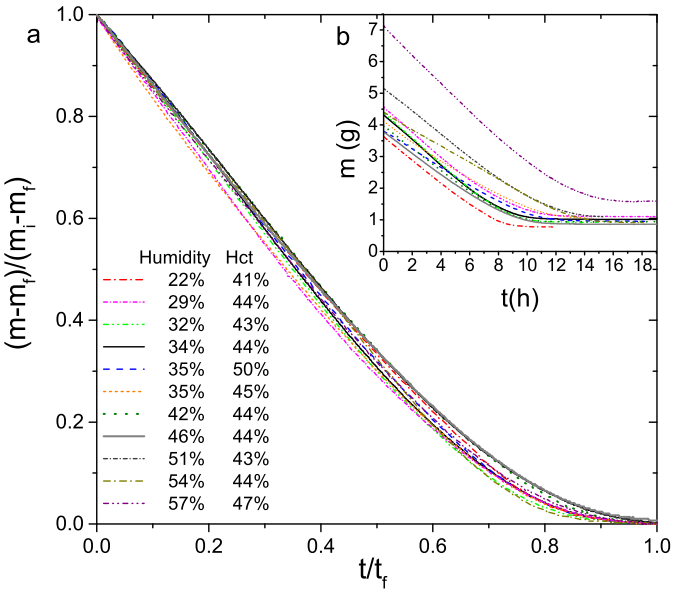

Fig. 6. (a) The normalized mass as a function of normalized time of the blood pools and (b) the mass of the pools as a function of time (inset), for different humidities and haematocrit values.

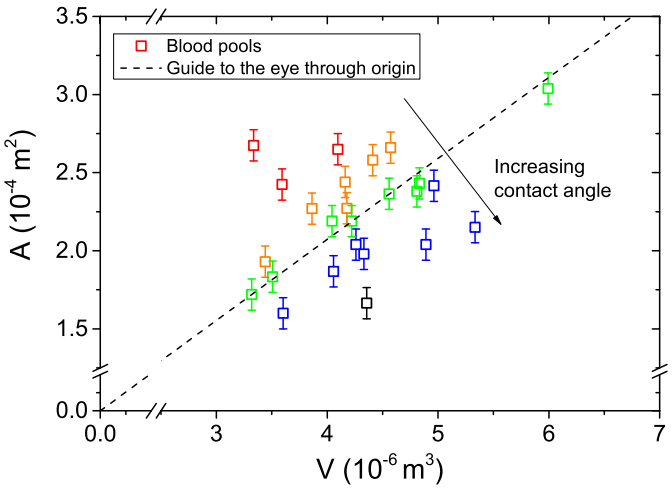

Fig. 7. The area A and as a function of the volume V of the blood pools. The colours represent the contact angle of the blood with the surface, red $30^\circ < \theta < 37^\circ$, orange $40^\circ < \theta < 45^\circ$, green $45^\circ < \theta < 50^\circ$, blue $50^\circ < \theta < 60^\circ$, black $\theta = 65^\circ$. Errors in volume are smaller than the size of the symbols. (For interpretation of reference to color in this figure legend, the reader is referred to the web version of this article.)

the pool. Moreover, the left-over mass is dependent on both the initial volume of the pool and the haematocrit value of the blood, which is in accordance with the findings of [5,6]. As the pools were deposited under various environmental humidities; these findings indicate that the drying speed, i.e., the slope of the linear part of the drying curves, becomes steeper with decreasing humidity. In other words, the higher the humidity, the longer the blood pool takes to dry. However, a more in-depth investigation is required to quantify how the drying speed depends on the humidity and other factors, as temperature, contact angle and size of the pool might influence the drying speed a lot. To have a better insight into the drying of the pools, we normalized the mass according to:

$$m_{norm} = \frac{m - m_f}{m_i - m_f} \tag{2}$$

Here m_i is the initial mass, m_f is the final mass and t_f is the final drying time defined as the point in time where the change in mass is less than 0.01 g/h. In Fig. 6a, the normalized mass was plotted as a function of the normalized time to show that independent of humidity, mass, haematocrit value, or total drying time the mass diminishes similarly for every pool. We show that this dimensionless rescaling is valid for a range of masses (3.5 g $< m_i <$ 7 g) and a range of humidities (22% $< H <$ 57%). This rescaling is the first step towards a unified theory concerning the drying of blood pools and maybe even pools in general.

Although the literature concerning the drying and evaporation of blood pools is poor, there has been extensive studies concerning the drying of gels [17–20], which show considerable similarities. The drying process of gels has three distinct drying stages. During the first stage the evaporation rate is constant and the volume decrease of the gel is equal to the volume of liquid lost by evaporation [18,19]. Once a critical point is reached, the volume of the gel stops to decrease and cracking may occur. According to a study of Dwivedi [20], the evaporation rate of water from alumina gels during this first stage is comparable to the evaporation rate of pure water. Subsequent to the first stage, gels undergo two so-called 'falling rate' stages where the evaporation rate decreases towards zero. During the first falling rate stage the liquid flows through partially empty pores, followed by a second falling rate stage, where the liquid diffuses its vapour to the surface. Comparably, drying blood exhibits a similar behaviour to water and a drying gel. After blood pool creation, blood dries with a constant rate of evaporation (Fig. 5). Subsequently, the drying rate decreases and cracking occurs, similar to a gel. Consequently, drying blood has analogous characteristics to that of both water and a gel.

The size of the pool is dependent on two important parameters. The first one is the volume of the blood, namely, the larger the volume of the blood, the larger the area will be the blood spreads over (Fig. 7). The second parameter is the contact angle θ, i.e., the wettability of the surface the blood lies on, which was calculated using the approach described in the supplementary materials (Eq. (S6)). If a surface is wetting ($\theta < 90^\circ$) then the blood spreads over a much larger area then if the surface was non-wetting ($\theta > 90^\circ$). Accordingly, the larger the contact angle between blood and surface, the smaller the area covered by the blood. This is reflected well by our results (Fig. 7) which show that blood pools with a larger contact angle deviate from the average (dashed line), i.e., they have a smaller area. These factors are very important for the drying dynamics. Namely, a higher volume blood pool takes a longer time to dry, whereas an increase in area and a lower contact angle can both speed up the drying process.

It is possible that during stage II, due to clotting, serum is forced out of the main pool, also known as serum separation [1]. In this study, we did not encounter any serum separation from our blood pools. This is not a surprise considering the findings of Ramsthaler et al. [12] who reported no serum separation unless the volume of the pool was larger than 10 mL, while in our study all pools were smaller than 7 mL.

We have to stress that the five stages of blood pool drying reported in this study are not the same stages as reported for single sessile droplets [7]. The drying stages observed in our experiments are very similar to those of drying blood droplets, however there are several distinct differences. First of all, the droplets reported in [7,10,11] contain anti-coagulant and therefore do not clot, while clotting is a very important parameter, as it may inhibit particle transport due to evaporative flow. On crime scenes blood almost always clots once it has left the body and the drying dynamics could change a lot if the blood did not clot, e.g., serum separation would not occur at all. In addition, during clotting (stage I) the colour of blood turns from a dark red to a bright red, something which was not observed for non-coagulating blood droplets.

Secondly, pools are much larger than droplets which change the evaporation dynamics and the final appearance of the remains.

Drying blood droplets show mobile fragmented cracking patterns at the corona and fine cracking patterns at the middle and periphery of the droplet [7]. The cracking patterns of blood droplets have been extensive investigated and resemble the cracking patterns of colloidal gels [10,11,21], i.e., the peripheral cracks of a blood droplet divide the remains in polygonal shaped cells. However, the cracking patterns of an entire (coagulating) blood pool are quite dissimilar from a single blood droplet. With blood pools we observe long elongated cracking patterns which propagate towards the middle of the stain, that turn black when completely desiccating. Once more, the size and thickness of the pool and coagulation of the blood can be the main reasons why blood pools show very different cracking patterns from those of single blood droplets. The cracks of a blood pool are similar to those observed by Pauchard et al. for a colloidal suspension (with a low ionic strength $I = 0.4$ mol/l) [22] and for a drying suspension of latex particles (0.1 μm) resulting from directional growth of fractures [23]. Cracks closely follow the drying front and the width between cracks is directly related to the thickness of the sample, with a prefactor depending on gel thickness, physicochemical properties, adhesion onto substrate and desiccation conditions [23,24].

Finally, a single blood droplet only shows a gelation front which partly propagates from the rim towards the middle. In contrast, a drying blood pool shows both a gelation front and a drying front that is created at the rim and completely propagates towards the middle of the stain. It is specifically the evolution of the drying fronts that define our different stages.

This study showed clearly that the drying dynamics of a pool of blood could be identified. However many more parameters would need to be investigated in further studies. Such parameters would be the influence of humidity and temperature, but as well the influence of the substrate which would mainly change the contact angle, and thus drying rate since it would change the spreading of the pool. Moreover it would be interesting to consider more the influence of the shape of the pool. Finally, size of the pool is very important. In these experiments, each pool was in the order of 4 mL, while in practice they might be much larger or even much smaller. For the latter, it is necessary to distinguish when a volume of liquid is considered a pool or a droplet. We suggest that a pool should be defined as having a flat surface. Accordingly, in our experiments given a contact angle of roughly 45°, the minimum volume of a pool should be much more than 170 μl (see Supplementary Materials, Eq. (S11)), which is the case in our experiments.

5. Conclusion

In this study, for the first time the drying dynamics of pools of whole blood were investigated, for a range of 3.5–4.5 mL. We were able to distinguish five different drying stages, each with their own characteristics. The mass of a blood pool diminishes in a very reproducible manner, first linearly in time and then approaches a constant value. Additionally, we were able to collapse all mass curves onto a single curve by normalizing the mass and time of drying. The general knowledge concerning blood pools within the field of bloodstain pattern analysis is very limited at most. This work is a step forward in the classification and characterization of blood pools within this field. Prospectively, the results of this study may be used for crime scene reconstruction or for future investigations into determining the time the blood shedding event occurred. Finally, we verified that the size of the blood pools is directly related to its volume and the wettability of the surface. This result could be used to estimate the original volume of a dried blood pool, to answer the question if the amount of blood could constitute loss of life. We anticipate this study to be of considerable importance for forensics and bloodstain pattern analysis as a whole.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.forsciint.2016.08.005.

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