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Influence of haematocrit level on the kinetics of blood spreading on thin porous medium during dried blood spot sampling

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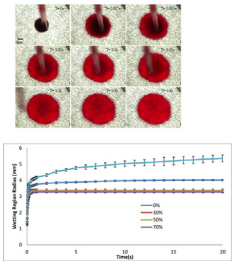
HIGHLIGHTS

- Blood spreading kinetics on porous medium is studied.
- Pig's blood was used to mimic the behaviour of human blood.
- Blood droplet height, base radius and contact angle are universal functions of dimensionless time.
- Prediction and control of spreading behaviour of blood on thin porous substrates.
- Haematocrit effects on the spreading dynamics of blood are determined.

GRAPHICAL ABSTRACT



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ABSTRACT

Dried blood spotting (DBS) is a convenient blood collecting and sampling method which is widely applied in newborn screening and blood analysis. At the moment, the practice is to try to keep the blood within a marked circle in a thin porous filter paper. However, it is not always possible to predict exactly how the blood spot spreads inside the filter papers and it depends on many factors including the properties of the filter papers, blood properties and how the blood is deposited on the filter paper. In this paper, we aim to identify the relationships between the physical properties and the spreading behaviour of blood on a typical DBS filter paper (Whatman 903). Pig's blood was used to mimic the behaviour of human blood and investigate the spreading/imbibition processes of blood drops on the filter paper. Both top and side views were used to analyse the spreading/imbibition behaviour. The experimental data present the haematocrit effect on the spreading dynamics of blood for dried blood spot sampling. The results obtained prove that the spreading/imbibition time dependences of droplet height, droplet base radius and contact angle are universal function of dimensionless time.

1. Introduction

Dried blood spotting (DBS) is a convenient blood collecting and sampling method, which is widely applied in newborn screening and blood analysis. The ease of its use and a number of other benefits derived from advanced analytic technology have led to a rapid growth in the application of DBS in traditional screening methods (e.g., large scale neonatal screening) and others, e.g., preclinical test and, pharmacokinetic (PK), toxicokinetic (TK) and therapeutic drug monitoring (TDM) [1–8]. DBS provides many benefits compared to conventional whole blood collection or plasma sampling, such as low cost, ease of transport and storage, etc. [9]. These benefits are derived from the ability of DBS to collect, handle and store blood samples of micro-volumes from which qualitative and quantitative data can be obtained at a later date [1,2,10]. However, this method suffers from two main problems. First, the dispersion

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of blood analytes over filter paper is often found to be unequal which may cause inaccuracies in the clinical analysis of the collected blood [11]. Second, the current DBS methods may not be applied to analyse certain analytes due to the small sampling volume and low recovery. The latter problem has been benefitted from advanced analytical methods recently, such as liquid chromatography tandem mass spectrometry (LC–MSMS) and high performance liquid chromatography-ultraviolet (HPLC-UV) [1,2,6,10]. However, the issues mentioned above have always been the bottleneck of practical application of DBS.

According to a number of review papers [3,6,7,10,12,13], most researches of DBS are focussed on the metabolic disorder and clinical disease analyses, and studies on their fluid dynamical behaviour (e.g., spreading kinetics of blood above and within the filter paper) are much less visible [5–8]. At the moment, the practice is to try to keep the blood samples within a marked circle in a thin porous filter paper. Recently, a significant amount of work has been spent trying to find out how much the blood spot will spread (spreading behaviour) without trying to quantify the kinetics of the wetting properties of the filter paper [2,9,11,12,14,15]. Also, a large amount of the work seems to have been spent on trying to develop techniques for measuring concentrations of solute/molecules from the collected blood sample on filter papers [2,7,10,12]. However, it is well known that the spreading behaviour of blood droplets are not always possible to predict exactly on the filter papers and it depends on many factors including the properties of the filter papers, blood properties and the way how blood is deposited on the filter paper. Therefore, there is no well-defined relationship between the theoretical and experimental parameters in the published DBS literature and only few researches have considered the influence of spreading dynamics on the DBS without quantifying these behaviour in detail [3,9,16]. In order to understand the spreading processes accurately new model based on both theoretical and experimental methods should be developed since the study of spreading behaviour of the blood drop over DBS filter paper had not been developed before. In addressing these points, the spreading and imbibition of blood droplets on thin porous media, namely, DBS filter paper is studied in this paper.

The kinetics of the spreading of other liquid drops over porous medium has been investigated in previous studies [17–19]. According to the previous studies, the drop spreading over dry porous layers is considered as two competitive processes: (i) the spreading of the drop over an saturated porous surface and (ii) the imbibition of the liquid from the drop into the porous substrate [20]. In this paper an axisymmetric experimental model of liquid drop spreading over a thin porous layer is adopted as discussed in the next section. In the experiments, the dynamic contact angles, droplet base radius and profile have been measured to characterize the spreading process.

Although some of the above studies reported the spreading/imbibition of droplets of Newtonian liquids on thin porous media, there is little or no study that has reported the spreading behaviour of non-Newtonian fluids in general and, more specifically, blood in the context of DBS. Therefore, the experimental investigation on blood spreading behaviour is essential. As mentioned earlier, there are a number of parameters which affect the spreading behaviours of blood on filter papers. Firstly, the physical properties of a filter paper, such as an average pore size and, thickness on the layer affect the capacity and spreadability of blood on the filter paper. Considering the consistency of the properties of DBS filter papers, the performance of filter paper was monitored by the Newborn Screening Quality Assurance Program (NSQAP) at the Centers for Disease Control and Prevention (CDC) (Atlanta, USA) to ensure that new filter paper are consistent with established guideline [21]. Secondly, the properties of blood, including, blood rheology, haematocrit level (i.e., the volume fraction of red blood cells in blood) and drop volume affect the performance of blood spreading/imbibition.

It is well known that the blood rheology is affected by the haematocrit level [22,23]. Further, the significance of haematocrit level to dried blood sampling has been discussed earlier [9,11,24–26]. For example, it was reported that the levels of most amino acids and free carnitine were higher in the blood drop periphery than in the central spot with lower haematocrit level [11]. Denniff and Spooner [9] reported that a bias was observed in the concentration of two analytes at different haematocrit levels and the area of DBS samples decreased linearly with increasing haematocrit levels. [9]. O'Mara et al. [24] reported that a significant bias (>15%) existed due to the haematocrit effects and unequal distribution across the spot. This shows that the influence of haematocrit levels on the concentration of analytes would be case-dependent, i.e., analyte concentration could vary in different cases of the DBS samples, which could be caused by unequal distribution of analytes in plasma, red blood cells or both [25]. In order to utilize DBS accurately in clinical analyses, the haematocrit effects should be investigated as a method development and validation for individual analyte. Generally speaking, it is expected that the influence of the spreading kinetics of blood droplet at different haematocrit level to the DBS sampling is much more consistent although the spreading kinetics is determined by the rheology of blood, which again depends on haematocrit level. Nevertheless, the spreading kinetics of blood at different haematocrit levels should be investigated further to provide a better consideration of the influence of haematocrit level differences to the whole DBS sampling and analysis process.

In addressing the above issues, a series of experiments is presented in this paper to investigate the spreading/imbibition behaviour of blood droplets with different haematocrit levels on DBS filter papers. The experiments are aimed at recording blood drop spreading/imbibition behaviour over the filter paper using a high speed camera and analysing the spreading droplet radius, volume, wetted area inside the filter paper and contact angle by an image analytic software [17,19,27]. The whole process requires special conditions in which they are carried out as the spreading/imbibition experiment data may be influenced by environmental factors such as, gas flow, vibration and horizontal level. Therefore, in our experiment, a special hermetically isolated chamber has been designed and installed on a vibration-protected table to eliminate the environmental effects during the drop spreading experiments. In order to quantify the blood spreading process, we observe the wetted region on the surface of filter paper (top view) as well as the droplet spreading and absorption behaviour (side view). The wetting region on the surface of the filter paper is known as the dried blood spots sample area. According to Starov et al. [19], the spreading behaviour of liquid droplet over porous layer (filter paper in our case) could be considered as overlapping of two different processes: one is the spreading of blood over the filter paper; another is the capillary motion inside the matrix of the filter paper. In consistent with this study, the time evolution of the radius of the wetting region, the drop base, the drop volume and the contact angle were monitored in our experiments.

We use pig's blood with different haematocrit levels to simulate the behaviour of human blood, and observe the spreading process of the liquid drops on DBS filter paper to analyse its spreading behaviour. The selection of the animal blood as a simulant for human blood in our experiments is based on the similarity of its rheological properties to those of human blood, namely, the blood viscosity, plasma viscosity, erythrocyte aggregation and others [28]. The easy availability of pig's blood from nearby abattoir and the ethical policy on minimum or no use of human blood for laboratory experiments are other considerations in our design of experiments. Accordingly, pig's blood is considered as the most