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

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Introduction

- Dried blood spotting (DBS) is a convenient blood collecting and sampling method which is widely applied in newborn screening and blood analysis.
- The spreading behaviour of blood droplets depends on many factors including the properties of the filter papers, blood properties and the way how blood is deposited on the filter paper.
- The relationships between the physical properties and the spreading behaviour of blood on a typical DBS filter paper (Whatman 903) was analysed by image analysis with different haematocrit levels.
- 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 results obtained prove that the spreading/imbibition time dependences of droplet height, droplet base radius and contact angle are universal function of dimensionless time.

Method

Preparation of different haematocrit levels of blood

- Blood of different haematocrit levels was prepared according to the procedures presented by Baskurt et al. [1].
- The blood samples were centrifuged to separate the red blood cells (RBC) and plasma.
- After the separation, the plasma was kept for re-suspension later and the cells were washed by the sterile saline solution three times to remove buffy coat layer..
- The washed cells were then re- suspended in plasma at the required haematocrit levels, namely, 0%, 30%, 50% and 70%. 2.3. Spreading experiment

Spreading experiment

The experimental set up of spreading experiment is shown in Fig. 1. The experiments were processed in the following order for obtaining both side and top views of blood spreading:

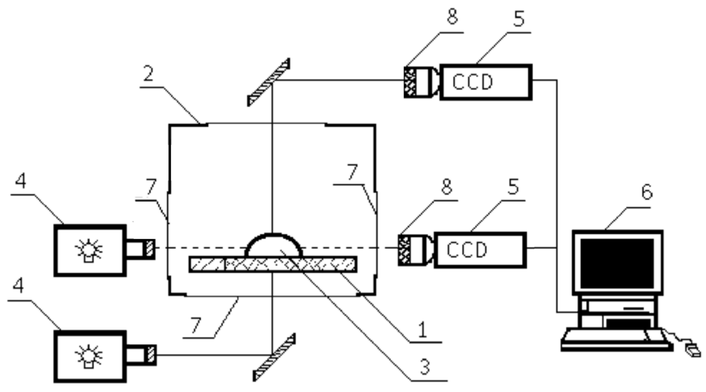

Fig. 1. Sketch of the experimental set-up: 1, porous substrate; 2, hermetically closed, thermostated chamber, 3, liquid drop; 4, light sources; 5, CCD cameras; 6, PC; 7, optical windows; 8, optical objectives.

a) The porous substrate under investigation (filter paper) was placed in the chamber.

b) Optical equipment was adjusted to focus on the sample depositing spot and the spreading process area, where the equipment include: CCD camera lenses, light source based on working distance.

c) A droplet of investigated sample (blood) was placed on the substrate by syringe and the whole process was recorded by CCD cameras.

d) Calibration of known distance in each image was done with identified micro-scale.

e) The image was analysed to obtain experimental data via image processing software (ImageJ and Vision builder (National Instrument, UK)) , where the volume of the spherical cap and the contact angle formed by the fluid with substrate was calculated as follows [2]:

$$r_c = \left(L(t)^2 + {h_c}^2\right)/2h_c$$

$$\theta = 90 - \frac{180}{\pi}\sin^{-1}\left(\frac{r_c - h_c}{r_c}\right)$$

f) These calculated data are able to record specific parameters such as droplet radius, height and wetting region and quantify the obtained data as shown in Fig. 2.

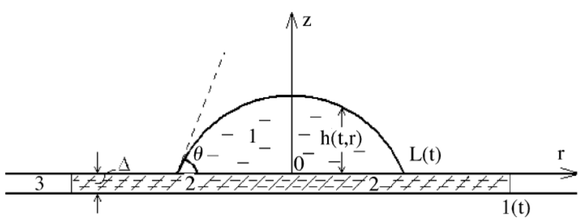

Fig. 2. Cross-section of the axis-symmetric spreading drop over initially dry filter paper with thickness Δ. 1 -liquid drop; 2 - wetted region inside the porous substrate; 3 - dry region inside the porous substrate. L (t) - radius of the drop base; l (t) - radius of the wetted area inside the porous substrate; Δ-thickness of porous substrate, r, z co-ordinate system; h (t, r) -profile of the spreading drop; ***θ***-contact angle.

Results and Discussions

- Fig.4 indicates that the presence of RBCs change considerably the spreading behaviour of blood inside the filter paper
- The result of spreading radius shown in Fig. 6. suggests that the spreading kinetic decreases as the haematocrit level increases or the same as the viscosity increases.
- Figs. 7–9 demonstrate that the time evolution of spreading radius of blood on time can be divided into two stages in the case spreading/imbibition process of blood droplet over filter paper as in the case of Newtonian liquids.

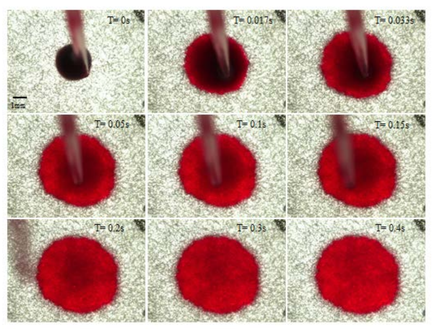

Fig.3. Top views of 30% haematocrit blood spreading on filter paper. Each image was captured at 60 fps from the time the first image was taken.

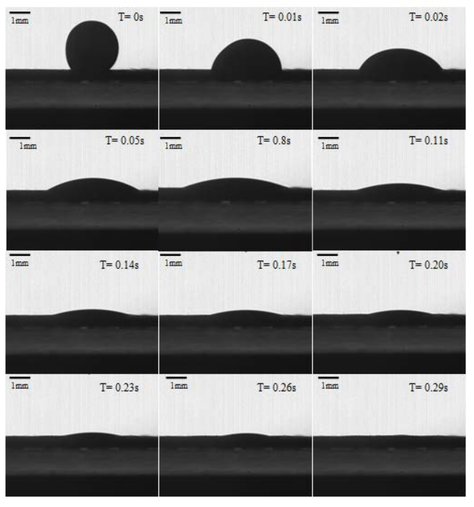

Fig. 5. Side views of 30% blood spreading over Whatman 903 filter paper. Each image is captured at 600 fps from the time the first image is taken. The time scale for first three pictures is 0.01 s and for following sequence is 0.03 s.

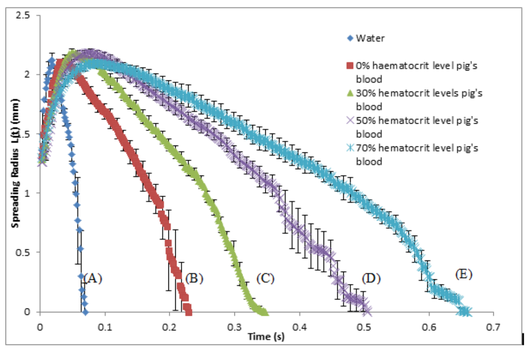

Fig.6. Radii of the drop bases as function time: (A) 10 µl water, (B) 10 µl 0% blood (C) 10 µl 30% blood (D) 10 µl 50% blood (E) 10 µl 70% blood on Whatman 903 filter paper

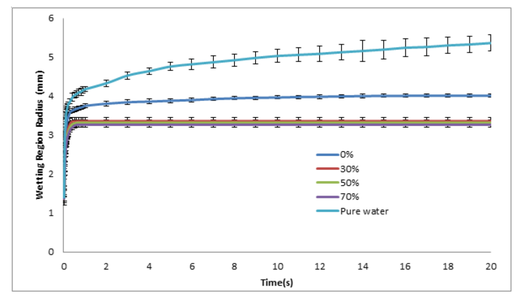

Fig.4. Dimensionless droplet heights of water, blood 0%, 30%, 50% and 70% haematocrit blood as function of dimensionless time

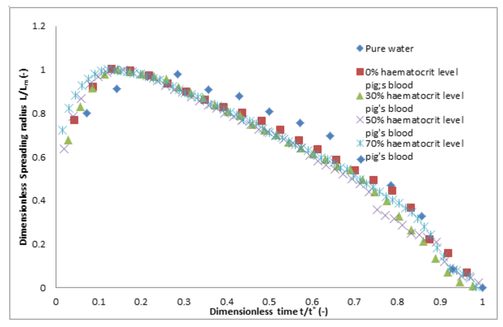

Fig. 7. Dimensionless radii of the drop base as function of dimensionless time for water and blood samples at 0%, 30%, 50% and 70% haematocrit levels

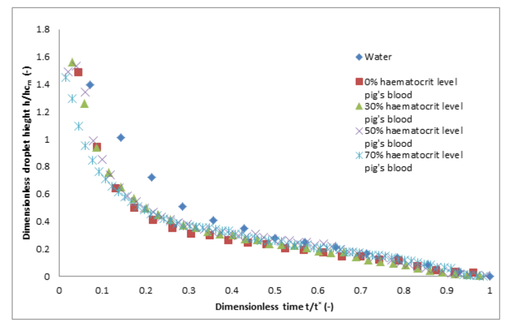

Fig. 8. Dimensionless droplet heights of water, blood 0%, 30%, 50% and 70% haematocrit blood as function of dimensionless time

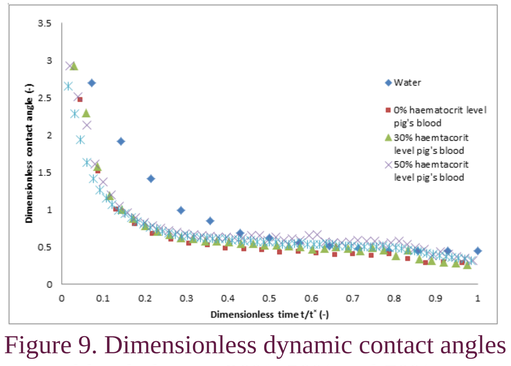

Figure 9. Dimensionless dynamic contact angles of water, blood plasma, 30%, 50% and 70% haematocrit blood as function of dimensionless time

Conclusion

- The experimental data present the haematocrit effect on the spreading/imbibition dynamics of DBS sampling.
- The results suggest that all the spreading/imbibition dependences such as droplet height, droplet base radius and contact angle can be presented as universal functions of dimension less time.
- This behaviour of blood spreading allows us to control and calculate several spreading parameters of DBS sampling, such as, wetting region, retention volume, imbibition volume, liquid retention time above filter paper, etc.
- The development of a theoretical model based on the experimental data of spreading behaviour for determining the distribution of specific analytes is being considered and will be presented in our future work.

References

[1] O.K. Baskurt, in: O.K. Baskurt, M.R. Hardeman, M.W. Rampling, H.J. Meisel- man (Eds.), Handbook of Hemorheology and Hemodynamics, vol. 69, IOS Press, Amsterdam, Netherlands, 2007, pp. 1–455.

[2] V.M. Starov, S.R. Kostvintsev, V.D. Sobolev, M.G. Velarde, S.A. Zhdanov, Spreading of liquid drops over dry porous layers: complete wetting case, *J. Colloid Interface Sci*, 252, 2002, pp. 397–408.

[3] T.C. Chao, A. Trybala, V.M. Starov, D.B. Das, Influence of haematocrit level on the kinetics of blood spreading on thin porous medium during dried blood spot sampling, Colloids and Surfaces A. 451, 2014, pp. 38-47.

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