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Regular Article

Effect of protein adsorption on the radial wicking of blood droplets in paper

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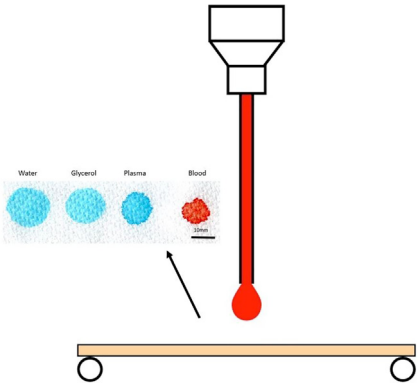
GRAPHICAL ABSTRACT



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ABSTRACT

Hypotheses: (1) The equilibrium size and characteristics of a radially wicked fluid on porous material such as paper is expected to be dependent on the fluid properties and therefore could serve as a diagnostic tool. (2) The change in wicked stain size between biological fluids is dependent on a change in solid-liquid surface interfacial energy due to protein adsorption.
Experiments: Sessile droplets of increasing volume of blood, its components, and model fluids were deposited onto paper and the equilibrium stain size after coming to a halt was recorded. The contact angle of fluid droplets on model cellulose surfaces was measured to quantify the effect that blood protein adsorption at the solid-liquid interface has on radially wicked equilibrium size. Finally the significance of droplet evaporation for the time scale of interest was analysed.
Findings: The final stain area of all fluids tested on paper scales remarkably linearly with droplet volume. Different fluids were compared and the gradient of this linear relation was measured. Model fluids varying in surface tension and viscosity all behave similarly and exhibit a constant gradient. Blood and its components produce smaller stains, demonstrated by lower gradients. The gradient is a function of protein concentration, thus the mechanism of this phenomenon was identified as protein adsorption at the cellulose-liquid interface. The slope of the area/volume relationship for droplets is an important quantitative mechanistic variable.

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1. Introduction

The wicking of fluids through porous media is of fundamental importance in many processes and applications such as printing, textiles, agriculture and more recently the medical field for its applications in paper based blood diagnostic tools [1,2].

Blood is a suspension of cells that are weakly electrostatically stabilised in plasma, the liquid component of blood. Plasma consists mostly of water (90 wt%), proteins (approximately 6 wt%) and a small amount of lipids, carbohydrates and electrolytes [3]. There are different types of proteins in blood of which the most abundant are albumin (38.7 g/L) [4], globulin (25 g/L) and fibrinogen (3 g/L) [3]. The composition and protein concentrations in blood vary greatly as a function of health as well as natural variation among populations [5,3,6]. Many blood proteins are surface active and readily adsorb onto surfaces [7]. There are three main types of cells in blood: erythrocytes (red cells), lymphocytes (white cells) and platelets. The majority of cells in blood are red blood cells. Healthy red blood cells are biconcave disks approximately 8 μm in diameter and 3 μm in thickness [8]. The shape, abundance and mechanical properties (deformation) of these cells are responsible for the unique viscoelastic and shear thinning behavior of blood [9]. By centrifugation the majority of cells can be removed from blood allowing the analysis of solely the plasma component. Although plasma exhibits weak viscoelastic properties [10], most analyses assume Newtonian behavior to no significant loss in accuracy. By testing plasma instead of whole blood the effect of protein adsorption can be isolated from the non-Newtonian properties of blood cells as a suspension (shear thinning, viscoelasticity and granularity).

The radial wicking of a drop has been studied previously [11–15]. However all previous studies have considered highly idealised systems ignoring evaporation and the complex properties of multicomponent systems. This study aims to identify the parameters and phenomena controlling the final stain area achieved by a single droplet of blood radially wicking onto paper. This is of direct interest in the development of blood diagnostics for several reasons. The surprising reproducibility and sensitivity of results suggest that drop stain analysis can serve as a convenient and inexpensive method to identify fluid properties. Whether these property changes are caused by protein content, specific antibody-antigen reaction or hematocrit levels will determine the nature of the sensor. The effect of protein concentration is examined here; however, this type of analysis is applicable to many sensing applications. Further a robust fundamental knowledge of the blood stain/volume relationship on paper/porous media will allow more sensitive, faster and more accurate diagnostic methods to be developed and guide the optimisation of existing devices. This study is also relevant to any system involving the contact or deposition of a wetting liquid over a porous material.

2. Experimental section

2.1. Materials

Tests were performed on Kimberly Clark Scott hand towel (4419) and Whatman 41 filter paper. Wet, dry and oven dried (O.D) weights of 100 cm^2 sections of paper are given in Table 1. The wet and dry thicknesses were also measured. Analytical grade glycerol, and ethanol were purchased from Merck, and Thermo Fisher Scientific, respectively. BSA (bovine serum albumin) solutions were diluted from 30% (wt/v) solutions from BioCSL, PBS (phosphate buffered saline) was made to 0.9 wt% with tablets from Sigma. Human blood with EDTA anti-coagulant was provided by the Australian Red Cross following established best ethics practice. Deionized water for tests and dilutions was purified from tap water with a Direct-Q water purification system to a minimum resistivity of 18.2 MΩ cm.

2.2. Methods

2.2.1. Paper characterisation

Paper oven dry (O.D) weight was measured using TAPPI standard 412 by placing samples in a 105 °C oven until weight stabilised. The weight at this point was recorded. The given values are the mean of 3 recordings for each type of paper. Dry Weight was measured using TAPPI standard 402, where the weight of the samples after equilibration in 23 °C 50% relative humidity (RH) conditions for at least 24 h was measured. The values reported are the mean of 6 recordings for each type of paper. Wet weight was determined using a variation of testing standard TAPPI 441. This involved submersing the paper in water for 2 h, then pressing each between two pieces of blotting paper with 2 passes of a 10 kg roller to remove excess water. The paper was then weighed. This process was repeated until weight stabilised. The values for wet weight presented in Table 1 are the mean of 3 recordings for each type of paper. Wet and dry thicknesses were measured using a L & W micrometer. Thickness values are the mean of 10 measurements.

2.2.2. Equilibrium stain size

Reproducible droplets of volume ranging from 2 to 40 μL were created with an adjustable needle and a syringe pump (Fig. 1a). The syringe pump was used to provide a constant flow rate to the needle which created repeatable small drops. Drop size could be controlled by needle geometry. These droplets were directed onto a paper surface that was suspended in 23°C 50% relative humidity (RH) air. Setup took sufficient time to assume fluids were at room temperature during tests. The paper was then imaged using an Epson perfection V370 office scanner and analysed to give the area of each stain. Droplets were recorded in groups of at least 7 onto one piece of paper that was moved between each drop. The volume of the created droplets was measured 4 times before and after being directed onto paper to ensure the drops' volume remained a constant. Prior to tests with biological fluids, needles were soaked for at least 2 h in the fluid to be tested. This was to ensure protein adsorption onto the needle's surface was at equilibrium which improved the uniformity of drops. The difference in area between stains and the variation in measured drop volume is given as error bars in Fig. 2a and b. All property values were found in literature [16–20], except the viscosity and surface tension of blood plasma and BSA solutions, these were measured with an Ostwald viscometer and a pendant drop surface tensiometer [21]. The

Table 1
Paper properties.

Paper	O.D weight (g)	Std Dev (g)	Dry weight (g)	Std Dev (g)	Wet weight (g)	Std Dev (g)	Dry thickness (μm)	Std Dev (μm)	Wet thickness (μm)	Std Dev (μm)
Hand towel	0.2558	0.0030	0.2671	0.0031	0.5537	0.0180	98	2	76	2
Filter paper	0.8537	0.0065	0.8794	0.0174	1.8086	0.0208	226	5	218	5