Title: Challenges and Opportunities in Blood Flow through Porous Substrate: A Design and Interface Perspective of Dried Blood Spot

Authors: Prasoon Kumar, Prashant Agrawal, Kaushik Chatterjee

PII: S0731-7085(19)30627-2
DOI: https://doi.org/10.1016/j.jpba.2019.07.020
Reference: PBA 12772

To appear in: *Journal of Pharmaceutical and Biomedical Analysis*

Received date: 15 March 2019
Revised date: 9 July 2019
Accepted date: 10 July 2019

Please cite this article as: Kumar P, Agrawal P, Chatterjee K, Challenges and Opportunities in Blood Flow through Porous Substrate: A Design and Interface Perspective of Dried Blood Spot, *Journal of Pharmaceutical and Biomedical Analysis* (2019), https://doi.org/10.1016/j.jpba.2019.07.020

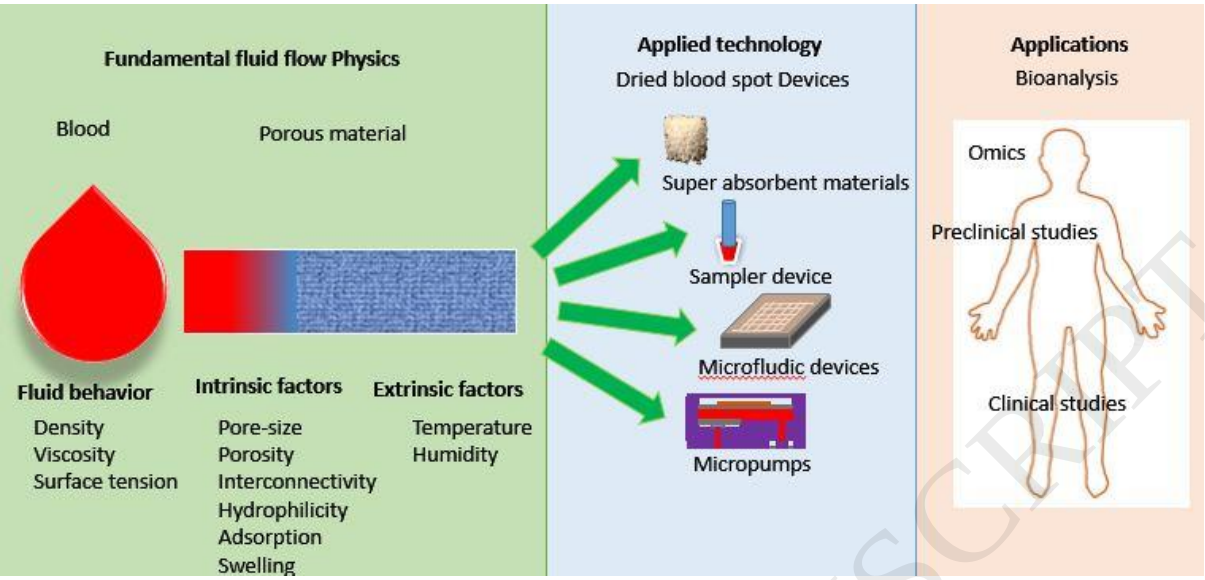
Challenges and Opportunities in Blood Flow through Porous Substrate: A Design and Interface Perspective of Dried Blood Spot

Prasoon Kumar[1, 2*], Prashant Agrawal[3], Kaushik Chatterjee[1#]

[1]Department of Materials Engineering, Indian Institute of Science, Bangalore 560012 India
[2] Department of Medical Devices, National Institute of Pharmaceutical Education and Research, Ahmedabad, Gandhinagar-382355, Gujarat India
[3]Smart Materials and Surfaces Laboratory, Faculty of Engineering and Environment, Northumbria University, Newcastle upon Tyne, NE1 8ST, United Kingdom

[#]corresponding author: kchatterjee@iisc.ac.in; +91-80-22933408

Graphical Abstract



Highlights

- Comparative analysis of current blood microsampling devices is reviewed.
- Intrinsic and extrinsic factors affecting blood flow in porous materials are presented.
- Current advances in the design and development of micro devices and materials for development of blood microsamplers are presented.
- Major clinical and preclinical applications of dried blood spot created by a blood microsampler device are discussed.

Abstract

Blood microsampling is desired in clinical, pharmaceutical and biomedical fields to overcome the challenges of conventional whole blood sampling. One of the popular methods for blood microsampling is the dried blood spot (DBS) kit and the collected sample is subsequently used for bioanalysis. The current practice of DBS is simple to use, cheap and very well standardized from sample collection to analysis. However, DBS suffers from several well

documented challenges related to blood spot formation such as varying hematocrit volume, thin layer chromatography effect and subsequent bio-analysis resulting in a variable and ocassionally high failure rate. A major source of these problems is our limited understanding of blood flow in porous media under different ambient and material conditions. Therefore, it is highly desirable to understand the parameters that affect blood flow in a porous medium to enable a more robust design of DBS and generally blood microsampling kits. In this review, we discuss some existing blood microsampling techniques while focusing on the challenges associated with blood flow dynamics. We also review existing studies on the potential factors that affect the permeation (imbibition or wicking) and spreading of blood in a thin, porous substrate as means to understand and overcome the challenges in designing new DBS kits and blood microsampling devices. Thereafter, we have discussed recent advances in the design of passive flow-based devices to overcome these challenges of current blood microsampling by DBS. Finally, we present a few applications of DBS in clinical and non-clinical studies. This review can benefit researchers working at the interface of complex fluid flow, surface chemistry, and material and device design for biomedical and biological applications.

Keywords: microsampling, dried blood spot, fluid flow, porous substrate, interfaces

1. Introduction

In 1947, Joseph Kleiner invented a glass vacuum tube which he termed as "evacutainer" [1]. Since then, vacutainers have been routinely used by phlebotomists to draw large volume of blood from patients for various diagnostics [2]. These vacutainers are made up of borosilicate glasses that are coated with ethylenediamine triacetate (EDTA) or citrate to prevent clotting of blood and hence, they facilitate temporary storage of blood before any bioanalysis [3]. However, the problem arises when there is a need to draw blood from a geriatric or pediatric patient, where drawing a large volume of blood through a vacutainer would significantly stress the patients and may invite other medical complications [4]. To overcome the above problems, the dried blood spot (DBS) technique was introduced by Robert Guthrie. He used DBS to detect the inborn errors of metabolism such as phenyl ketone urea in pediatric patients[5]. Later, DBS was adopted for the detection of several other analytes in different clinical and non-clinical settings.

DBS is one of the best known alternatives to whole blood sampling for the detection of any biochemical analytes. The major advantage offered by a DBS assay is the requirement of a minimal volume of blood for testing an analyte that is particularly desirable in pediatrics and geriatric patients [6]. Further, the method prevents spillage or transmission of blood-related infection through physical contact [7]. It has been established that viral infections are highly minimized while sampling blood by DBS due to the destruction of a viral capsid on the DBS paper [8]. This method also enables storage of blood and preservation of its analytes for a substantially long period of time; DNA for months, proteins for few days and some chemicals even for years [9]. In addition to the above merits, the DBS technique is quite inexpensive, has a robust supply chain and logistics system and the protocols for the development of DBS have been well documented and practiced across the globe. Owing to the above advantages offered by this technique, it has gained importance in number of applications such as large scale neonatal screening, preclinical drug development for a lead validation, toxicokinetic (TK) and pharmacokinetic (PK) studies, clinical pharmacology, targeted and non-targeted metabolic profiling, therapeutic drug monitoring (TDM), forensic toxicology, doping or environmental contaminant control, and microbiological and epidemiological disease surveillance [5]. Due to its increasing utility in different biomedical applications, the field has been extensively explored in recent years as observed from the volume of literature pertaining to the DBS field. Further, new applications for DBS are being fueled by a rapid and unprecedented increase in the discovery of tools and techniques for the detection of analytes in small samples volume at the nano and picogram level. This increases the ability to translate DBS for biosensors development [10, 11] (Figure 1).

DBS also suffers from various limitations as follows: (a) uneven spreading of the blood drop on a porous substrate due to the differences in the hematocrit volume of blood of different patients [12], (b) formation of clot or blot during the spotting process [13], (c) heterogeneous distribution of analytes of blood on the porous paper due to the thin layer chromatography effect [14], and (d) absence of a non-uniform, effective drying method of the blood spot for the preservation of analytes as humidity leads to contamination and degradation of analytes [15]. These limitations result in a variable and occasionally high failure rate in creating a perfect spot through DBS technique[9]. Such failure in the creation of a perfect spot is attributed to the limited understanding of the blood flow in micro/nano confinements of porous materials.

In the last few decades, efforts to study the fundamentals of blood flow, spreading on a porous sheet and the role of the porous sheet during spotting have gained little momentum [16-19]. These thin porous substrates have been used widely in paper microfluidics and DBS analysis for developing assays for the analysis of blood but there have been very limited studies on understanding the physical mechanisms governing the imbibition of blood in porous substrates [18, 20, 21]. The above systems primarily rely on qualitative blood flow behavior [12, 22-24] or the use of additional external forces for blood fractionation and analysis [25-28]. Many theoretical and numerical models have addressed the effect of the non-Newtonian behavior of blood during capillary imbibition of blood [29-32]. Therefore, these few reported studies have a profound implication in understanding the fundamentals of blood spotting in DBS and would be beneficial for overcoming the challenges associated with DBS.

In this review article, we focus on understanding the role of device design, blood properties, physiochemical nature of porous materials and ambient condition on the formation of a DBS. We first provide an overview of different blood microsampling techniques currently practiced for blood spotting/ collection, storage and transportation. We then briefly discuss the technical challenges in obtaining a perfect robust blood spot, highlighting the importance of blood rheology and the substrate material and construction properties. We also present the reader with considerations and challenges related to blood flow dynamics in a DBS device that can hinder or enhance the formation of a perfect spot. Furthermore, recent advances in design, materials and methods to enhance the lateral imbibition of blood are also presented. Finally, we briefly discuss a few major applications of DBS in pharmaceutical and biomedical applications. An understanding of the effect of different factors on the imbibition of blood in a porous substrate may assist in a rational design of a DBS device. A detailed discussion on application of DBS is beyond the scope of the present review. However, readers can refer to several recent papers exploring the promise of DBS in the field of analytical chemistry [6-8, 33].

2. Blood micro-sampling methods

Several innovations in microsampling devices have been reported in recent years[34]. Some of the popular current blood microsampling techniques widely used in industry are compiled in Figure 2.

2.1 Vacutainer

Traditionally, blood collection from patients has been done through vials and vacutainers after venipuncture through trained medical practitioners or certified paramedical staff. An experienced phlebotomist inserts a needle in a lumen of a vein in the fore-arm while the other end of the needle is connected to a tube which is inserted in a vacutainer tube. These vacutainer tubes are color-coded to enable blood collection for different bioanalysis. Thereafter, due to a vacuum in the tube, blood is drawn in to the tube by suction pressure (Figure 2 (a)). In majority of the cases, the defined volume of blood collected in a vacutainer needs to be processed to extract plasma or serum prior to performing any diagnostics. This procedure of blood collection is only possible provided the patients are available in-person at the site of the diagnostic lab or requires the collection and transportation of blood through a mobile blood collector and subsequent cold chain transportation. The major advantages of this method are that it facilitates the analysis of blood for a wide array of bio-analytes to assess the health of the patient, availability of large

number of trained medical practitioners or certified paramedic staff and health workers, availability of a wide array of instruments that can process blood tubes in an automated and semi-automated manner, and the existence of a well-established work flow for blood sampling and usage. Hence, this method of blood sampling is still one of the principal methods to perform diagnosis. However, its major limitation lies in the fact that this method has huge health implication on pediatric and geriatric patients where repeated or large volume of blood cannot be drawn for bioanalysis. In some cases, this method of blood sampling cannot be performed on chronic diabetic or hemophilia patients. The challenges of locating the vein and inserting a 22G needle in a lumen of vein significantly decreases patient compliance [35]. Furthermore, this method is also expensive, needs trained manpower, has the risk of contamination (compromised biosafety) and finally, poses logistics challenges associated with transporting frozen blood from remote geographical locations that can significantly limit access. The key players in the supply of these vacutainers are Terumo Corp.; Sekisui; Becton, Dickinson and Company; Medtronic; NIPRO Medical Corp.; FL medical; SARSTEDT AG & Co. KG, among others.

2.2 Capillary tube

Capillary tube based micro-sampling is a method to draw few microliters of blood [36]. A trained medical practitioner or paramedical staff pricks the heel or finger with a needle and places a glass capillary tube over the oozed blood. The capillary pressure guides the blood to rise in a capillary tube till the weight of the blood in tube balances the capillary pressure[37] (Figure 2(b)). Sometimes, it is essential to squeeze and massage the pricked heel/ finger to draw the blood of dehydrated patients with a high hematocrit volume. However, excess massage or squeezing the finger before microsampling may result in hemolysis, cross-contamination of the blood by nearby interstitial or intracellular fluid, and may lead to obstruction of blood flow[38]. Thereafter, post processing of the capillary tube containing blood is carried out by centrifugation process to separate plasma and blood cells. A modification in capillary tube have been proposed by C L Bowen et al. to separate blood cells and plasma before performing any routine blood diagnostics[39]. The benefits include an easy and simple procedure to conduct and extract a good volume of blood[40]. However, post processing before the diagnostic assay is a quite laborious task, which leads to blood wastage and generally, only few diagnostic assays can be performed. Furthermore, the volume of blood extracted is limited by the capillary number of the tube, material of the capillary tube, skill of the paramedic staff, and hematocrit volume of the blood in patient. There is also an associated risk of contamination and problem with storage and transportation in microsampling of blood through capillary tubes. The key players supplying capillary blood collection devices are Danaher Corporation (U.S.A), Sarstedt AG & Co (Germany)., Becton, Dickinson and Company (U.S.A) , Medtronic (U.S.A), Thermo Fisher Scientific Inc (U.S.A)., Kabe Labortechnik GmbH (Germany), Terumo Medical Corporation (Japan), Greiner Bio One International GmbH (U.S.A), and Improve Medical Technology Co (Guangzhou). F. Hoffmann-La Roche Ltd (Switzerland), Abbott Diagnostics Inc. (U.S.A), Beckman Coulter, Inc. (U.S.A), Bio-Rad Laboratories, Inc. (U.S.A) and others[41].

2.3 Automated microsampling technique

This microsampling technique involves a computer controlled robotic system that is capable of drawing blood, plasma and body fluid from an animal model for *in vivo* drug development studies. This method allows integrative pharmacology design by utilizing micro volumes of biofluids from fewer animals[42]. It involves a catheter that is inserted in a blood vessel of an animal and then connected to a collector via multiple tubes. The integrated micropump draws samples from the catheter via collecting tubes to be deposited in dialysate collectors. The catheter contains a microseparation or ultraseparation membrane, which allows the diffusion of analytes from the convective blood to the dialysate solution. These solutions are analyzed in real time and displayed through a computer (Figure 2 (c)). Such drawing of samples from the animal can be programmed through a time scheduler, which periodically draws samples through robotic arm and then transfers it to a refrigerated sample collector until ready for processing and analysis. All these activities are performed through a computer controlled robotic machine [43]. This method offers minimal sample collection and processing with low wastage within a limited time and minimal effort. However, this method is presently utilized only in pharmacological studies and limited to laboratory animals. Hence, in its current format, it cannot be used for blood microsampling from human patients. It is expensive, bulky and has limited usage.

2.4 Volumetric absorptive microsampling

The quest to extract a defined volume of blood on a DBS paper led to modifications such as microfluidic channels in DBS collection device[44-46]. Denniff and Spooner introduced a technique called volumetric absorptive microsampling (VAMS) technique where they advocated the use of defined, uniform porous, hydrophilic material to extract definitive volume of blood during microsampling[47]. This method utilizes the absorption of a 10 µl volume of blood obtained after pricking a heel or a finger in a proprietary material present at proximal end of a VAMS microsampler (Figure 2 (d)). The lower distal part of VAMS microsampler supports connection with a pipette for better handling during blood microsampling and post-processing procedure. Thereafter, these are stored at a room temperature for drying within 2 to 3 hours before shipping in a specialized box called clamshell. The blood from microsampler can be analyzed through automatic and semi-automatic mode. This is a simple, minimally invasive technique that can sample defined volume of blood as per the requirement. Given hydrophilic nature of the material, the adsorption of proteins in blood to the substrate is minimal and hence, it facilitates easy recovery of proteins/ lipids for routine analysis [48]. The limitation of this technique is the inability to separate red blood cells from plasma, which may be a necessity for certain analysis procedures[49]. The stability of different analytes in VAMS needs to be considered and evaluated before including VAMS for analyte microsampling. The commercial player for VAMS is Phenomenex Inc, USA.

2.5 Membrane based microsampling

This method employs a hollow fiber membrane with microseparation or ultraseparation capabilities. It is introduced in the tissue space or blood vessel from where microsampling is performed and then connected by a perfusion system such as a flow controlled syringe pump [43]. Thus, due to diffusion, the analytes from the tissue/ blood diffuses in the hollow fiber membrane, which are then carried away by a perfusing liquid to be collected in a collector

(Figure 2 (e)). This technique is particularly useful to sample a variety of body fluids and collect analytes of different sizes. It has a huge implication in pharmacological application for drug screening and discovery on small laboratory animals. They can be integrated with automatic/ semi-automatic analyzers for PK and PKD studies. However, the method is invasive, requires surgery, high expertise to perform and is expensive; hence, it cannot be used for blood microsampling of patients in its current form.

2.6 Dried blood spot

DBS was introduced for the first time for screening of babies for inborn metabolic errors. Later, it was adopted for biochemical analysis in laboratories due to the advancement in bioanalytical systems. It is based on the principle of wicking of blood in a microporous filter paper by capillary action to form a spot (Figure 2 (f)). The DBS has been successful in sampling and collecting blood for diagnostic screening from remote areas due to their easy supply chain and logistics. The DBS technique is inexpensive, easy to use, and minimally invasive and has been nearly perfected through practice and established protocols over several decades. The blood sampled through DBS allows the estimation of a large variety of analytes such as DNA, RNA, proteins, metabolites, drugs and other chemical species. The major limitations of DBS as a microsampling technique have been described above.

The advantages and disadvantages of these different microsampling techniques have been qualitatively listed in the Table 1. A number of blood microsampling techniques and devices have been introduced recently, which have significantly increased commercial interest in the blood microsampling arena. Some of the new organizations such as Phenomenex, Spot-on-Science, Boston microfluidics, Trajan and HemaPEN are rapidly changing the landscape of blood microsampling. However, the basic principle underlying the majority of these techniques is the capillary wicking of blood in a hydrophilic, porous substrate such as capillary glass tube, hydrophilic porous polymer, microporous cellulose filter paper and porous hollow fibers. A fundamental understanding of the role of various factors that affect the wicking of blood and formation of dried blood can drive the development of new generation microsampling devices.

3. Technical challenges in current dried blood spot

As mentioned above, DBS relies on a porous substrate, predominantly a cellulose based filter paper to imbibe blood over a marked region (Figure 2 (f)). The blood spot is obtained by pricking the finger, heel or toe and spotting the blood on a pre-printed circular mark on the DBS card. The blood imbibes and spreads on the card radially due to capillary pressure in the pores of the filter paper. The spotting is done on 4 to 5 different preprinted circular spots. The card is then left for air drying at ambient temperature, sometimes on a moisture absorbent surface, for approximately 1 to 3 hours. The DBS cards are then stored in a plastic bag, sometimes with a

desiccant (to ensure a complete dry-out of the blood spot). The DBS cards are then stored from anywhere between -80 °C to room temperature depending on the type of analytes to be tested.

The above-mentioned procedure is conducted to ensure that a large uniform, circular, uncontaminated and a dried spot is obtained. The uniformity and shape of the blood spot is determined by the dynamics of blood flow in the porous substrate [51]. The spreading of blood on a porous substrate is broadly dependent on three critical factors: (1) rheological properties of blood, (2) substrate properties and (3) environmental conditions. We first discuss the rheological properties of blood.

3.1 Properties of blood

Blood is a non-Newtonian flow fluid [52] and its rheology depends on cellular morphology, mechanical properties, cellular interactions and plasma composition[53]. It is a heterogeneous mixture of multiple components and phases and comprises of two main components: the fluid medium (or plasma) and colloidal cellular components. The plasma consists mostly of water whereas the rest comprises of solutes such as sodium electrolytes, organic and inorganic compounds and proteins (albumin, fibrinogen and globulin) [54, 55]. The cellular components comprise of red blood cells, white blood cells, and platelets. Variations in each of these components directly affects the non-Newtonian nature and therefore, the flow behavior of blood in any medium.

As blood is comprised of both liquid and solid components, its rheological properties depend on the mechanical properties of the solid components and their interaction in and with the liquid phase. For instance, blood is a viscoelastic liquid due to the mechanical deformability of the red blood cells and the presence of proteins in blood plasma[56, 57]. Viscoelasticity imparts it a time-dependent shear rate directly affecting its flow and spreading properties in various configurations [56, 58]. It has been observed that blood viscosity increases exponentially at low shear rate (below 50 sec^{-1}) due to the formation of rouleaux, a rod-shaped stacks of red blood cells (RBCs). Further, it has been observed that apparent viscosity of blood changes dramatically even with slight hemolysis of blood. This is because red blood cell aggregation is facilitated by hemoglobin released by hemolysis during finger or heal massage while blood spotting [38, 59]. This results in an increased viscosity of blood that further affects its permeation in porous media. Hence, it has been recommended that hemolysis should be minimized during blood spotting [60]. Moreover, hemoglobin release from hemolysis may interfere with quantitative analysis of blood proteins[6]. The presence of high molecular weight plasma proteins changes as globulin and fibrinogen overcomes the zeta potential of erythrocytes and assist in aggregate formation called rouleaux[61]. However, at a higher shear rate above (230 sec^{-1}) blood cells tend to get aligned with flow direction whereas at a critical shear rate, blood cells tend to rotate[62]. As a result, blood behaves as a shear thinning liquid, that is, its viscosity decreases with increasing viscous friction (or increasing flow velocity) or shear rate. Therefore, it is imperative to design a device for microsampling that offers enough viscous shear stress to enable smooth flow of blood without blood cell lysis or aggregation of blood cells. Properties such as plasma constitution and more importantly, blood hematocrit affect its shear rate and consequently, its viscosity in different flow conditions [63, 64]. It has been reported that hematocrit varies with sex, age and

ethnicity. The variability in blood hematocrit volume is also reflected in healthy individuals and blood diseases in patients[65]. As mentioned above, the role of hematocrit in DBS is significant, which may be ascribed to its effect on the viscosity [12, 66]. Thus, blood microsampling process across the globe must be uniform and should not get affected by variability in blood hematocrit volume and its rheological properties.

3.2 Blood flow in a porous medium and effect of ambient conditions

Imbibition in a porous medium is a competition between the viscous resistance to the flow (proportional to liquid viscosity) and the driving capillary pressure [67, 68]. As the spreading distance increases, the viscous resistance increases and the pressure gradient decreases leading to a slow moving wicking front [69]. Assuming the pores mimic flow in a capillary tube, the capillary pressure, driving the imbibition can be simplistically represented as $p_c = 2\gamma \cos\theta \,/r$, where, γ is the surface tension of the blood in air, r is the radius of the pores in the porous medium, θ is the dynamic contact angle [70] of blood with the material of the porous medium. The above driving and resisting forces are dependent on blood rheology, substrate properties and environmental conditions. For instance, the non-Newtonian flow property results in a dynamic inter-dependence of the viscosity and flow velocity. As mentioned above, the imbibition speed decreases as the wetting front advances. In case of a shear thinning flow, this implies an increase in the viscosity, which would increase the viscous resistance and provide a negative feedback to reduce the imbibition speed even further.

Substrate properties also alter the capillary pressure and the resistance to flow. For instance, parameters such as porosity, pore geometry [71], tortuosity [72] and pore distribution [73], affect the resistance to flow in the porous medium. These parameters are collectively often characterized by the term permeability [74], which signifies the ease of flow in a porous medium. Additionally, substrate properties such as material wettability (measured by the contact angle θ), material absorptivity, fiber distribution in the substrate, pore size distribution (affects r) and porosity (ϕ) directly affect the capillary pressure driving the imbibition, and indirectly affect the viscosity depending on the imbibition speed. For instance, the effect of pore size is visible in the imbibition of blood in a woven fabric, which behaves like a porous substrate, as shown in Figure 3 (a) and (b). Woven fabrics are generally orthotropic, i.e., they have yarns (or threads) with different pore spacing (or diameter) in perpendicular directions, referred to as the warp and weft directions. The warp direction of the fabric has smaller pore sizes than in the weft direction. Hence the imbibition speed in the warp direction is higher than that in the weft direction. This speed difference can be observed in the case of linear imbibition, where the blood spreads in only one direction along the fabric, as shown in Figure 3 (a) [75]. However, in DBS, blood spreads from the source in all directions on the DBS card, i.e., radial imbibition. In this case of radial imbibition, the orientation of fibers in the substrate not only affects the spread of the blood but the shape of the spot, as seen in Figure 3 (b) and (c) [75-77]. An orthotropic/ anisotropic substrate can lead to an elliptical blood spot as opposed to a circular spot in an isotropic substrate [75, 78]. A filter paper, such as that used in DBS, behaves similar to an isotropic substrate; therefore, the above concepts on blood imbibition dependence on pore size of a substrate are also valid.

As discussed above, the material wettability determines the contact angle θ between the porous material and the imbibing blood. For a drop spreading on a porous sheet such as that depicted in Figure 4 (a), the material wettability affects the size of the drop above the porous substrate as it imbibes into the substrate as well as the imbibition speed inside the pores[17]. After deposition on the substrate, the blood drop quickly spreads to a maximum radius (Stage 1 in Figure 4 (b)) at the advancing contact angle. After this quick spreading, the spreading of the drop can follow two scenarios depending on the material wettability, as depicted in Figure 4 (a) and (b) [17]. On substrates with high wettability (hydrophilic), spreading of the drop follows a complete wetting scenario, where it retains its maximum spread radius (contact angle with the substrate decreases) while imbibing in the substrate [20]. On substrates with low wettability (hydrophobic), the drop's radius shrinks during imbibition (contact angle remains constant at the receding contact angle), which is characterized as the partial wetting scenario [17]. For blood flow inside the pores, the contact angle of blood with the material governs its imbibition speed. The contact angle on a hydrophobic material would be higher than that on a hydrophilic material, resulting in relatively reduced capillary pressure [79, 80] and thus, a smaller spot size. For instance, the hydrophobicity of cellulose based paper, pore size distribution and porosity determines the degree of spread of fluid over the substrate and its penetration inside the pores [81]. Chemical treatments to fibers and the presence of surfactants also alter the material wettability and consequently, affect the spread of blood in a porous medium [75-77]. These surface treatments can either increase or decrease the wettability of the fibers and therefore, increase or decrease the spread of blood, respectively.

The above discussion highlights the complexity in the flow of blood just because of its flow properties as a single-phase continuum fluid. The presence of particulate matter such as cells further adds complexity to the non-Newtonian flow behavior. The possibility of channel clogging by the cells makes the speed of imbibition a crucial factor [21]. A slow-moving imbibition front will lead to higher chances of clogging of the pores by the blood cells near the blood source. This would add resistance to blood flow thereby affecting its viscosity and reducing the net spread. A fast moving imbibition front can drag the cellular material and the analytes more effectively and increase their spread around the blood source. Consequently, the hematocrit of the blood is critical in determining the blood spread and poses a significant challenge in DBS analysis. An increase in the hematocrit content increases the viscosity and visco-elasticity of blood [83-85]. Combining this with the possibility of channel clogging and increased resistance, it has been shown that the spreading time of blood drop radius increases with an increase in the hematocrit level of the blood (Figure 4 (c)) [17, 18, 21]. Channel clogging also leads to plasma separation [17], which, if uncontrolled, is undesirable for DBS application. Asymmetric clogging of the pores can also lead to an asymmetric spot size as shown in Figure 3 (c) [77, 78, 86]. This not only results in a bad spot shape for analysis but also alters the distribution of analytes in the spot sample.

Blood also absorbs in the fiber of substrates made of natural fibers such as cotton whereas it remains in the channels/ pores in substrates made from synthetic fibers such as polystyrene [76]. This alters the local pressure differences driving the blood spread and consequently, size and

shape of the macro spot [87]. Environmental conditions, primarily humidity, also play an important role in blood spread (Figure 4 (d)) [82]. In a humid environment blood spreading increases due to the presence of water molecules either as a pre-wetted layer on synthetic fibers or imbibed in natural fibers [88]. The ambient temperature affects the speed of drying and therefore, the maximum spread of blood on the porous substrate is achieved a low temperature. However, the increase in temperature of blood, for instance due to hyperthermia condition in patients, from 36.5°C to 39.5°C results in a decrease in the viscosity of blood by 10.38%, which results in an increased flow velocity through porous substrate. It is for this reason, a finger or a foot heal is treated with lukewarm water during blood sampling. Further, at low temperature such as 22°C, the viscosity of blood increases by 26.13% [89]. Such an increase in viscosity significantly lowers the flow of blood through the porous substrate. Hence, the temperature of blood is significantly important in determining its flow and spreading behaviors in a porous substrate. It should be noted that drying of blood on a porous paper negatively affects cellular hematological testing due to altered blood cells (erythrocytes, leukocytes, platelets, etc.)[6].

4. Advances in device design and material for DBS

4.1. *Design modifications in DBS technique*

Considering the challenges of permeation and uniform spread of blood in a microporous substrate to achieve a perfect DBS, efforts can be directed to modify the design of a device having DBS paper to draw and spread blood. However, an increase in the hematocrit will always increase the viscosity and density of blood making it more hostile for flow in a microporous substrate[90]. Modifications in the DBS paper through an introduction of nanoporous membrane over the DBS paper have been proposed by Sturm et al. to separate blood cells and plasma during a spot formation. Thus, plasma component of blood with even high hematocrit value could permeate and spread in a DBS paper resulting in the formation of spot called dried plasma spot (DPS) that is suitable for the detection and analysis of many different analytes [91, 92]. This may be a good strategy where bioanalysis has to be performed only from plasma. It should be noted that the plasma derived from these separation and / or lateral flow type devices is likely to be different to that derived by classical centrifugal methods. Hence, this might affect the measurement of the analytes of interest. However, there are certain bioanalysis procedures where blood cells are required. Hence, it is imperative to tailor the design of the device to facilitate the permeation of whole blood before dry-out in ambient conditions. Considering the challenges associated with tailoring the porosity, pore size distribution, tortuosity of a DBS paper, researchers suggested the use of pre-punched DBS paper and place it in a device where the pre-punched DBS paper does not make contact with the floor of the device [93, 94]. Although the cause of hematocrit invariance has not been addressed directly, but the underlying substrate on which the pre-cut cards are kept might play a role in the uniformity and imbibition speed. Furthermore, given approximately 10 μl sample of blood, a small pre-cut disk ensures that the blood drop has a finite area to spread on completely compared to a seemingly infinite incomplete spread area on a DBS card. In this regard, the pre-cut area might ensure a more uniform spread. Hence, these devices enabled the formation of spots independent of blood hematocrit. Another probable reason for the hematocrit invariance on analyte detection in these methods might be because they analyze the entire spotted blood sample volume as opposed to traditional DBS

where only a 3 mm diameter area is punched from the spot for analysis as suggested by Neto et al [95]. Li et al also employed the radially perforated DBS paper to overcome the limitations due to varying hematocrit [35]. These technologies require an accurate of volume of blood dispensing on the substrate as opposed to DBS where the blood volume is not controlled. Therefore, these methods benefit from the analysis of a defined and known volume of blood in the dried spot over traditional DBS where the unknown volume of blood on the 3 mm punch may vary with factors such as hematocrit and even the method of deposition. Furthermore, a different shape of the porous paper (fan shaped filter paper) has been explored to provide better accuracy and precision than traditional DBS [96, 97]. These modified substrate designs provide better accuracy by forcing the blood to imbibe in a confined, pre-defined area and consequently achieve a consistent spot shape and size compared to the traditional DBS.

However, owing to the complex nature of blood, the current DBS technique also suffers from thin layer chromatography effect. When the analytes/components of blood having selective affinities to the substrate and the mobile phase (blood) while moving through a substrate and they get distributed non-uniformly along the length of the sample substrate. This phenomenon is termed as "chromatographic effect. Further, due to the variations in the density, viscosity and component's concentration of blood in different individuals, the movement of the mobile phase (blood) and its components is further affected in a porous substrate. This results in an uneven spread of analytes over the area of blood spot forming a region of blood cells and plasma separately. This effect is particularly profound when the hematocrit volume is high. Moreover, the skill and experience of the trained personnel become critical while punching from the DBS to ensure a homogeneous area in a blood spot is processed [12]. Thus, it is desirable that blood first spreads on the DBS paper and subsequently permeates in the pores by diffusion, similar to the complete wetting case in Figure 4 (a). Such even spreading of blood can be induced by strategies such as distributing blood through a network of microchannels connected to a porous substrate where blood flow in the microchannels is greater than its permeation velocity in porous substrate [44, 98]. This will enhance the distribution of blood irrigated onto a porous substrate. This method can be adopted to drive the blood over a porous substrate in an efficient manner by adequately choosing the channel dimensions and then allowing the blood to permeate through the DBS paper. However, increase in viscosity and density of blood due to dehydration or high hematocrit poses a significant challenge. The technologies discussed here rely on an accurate volume of blood being added to the substrate, which a classic DBS approach does not employ.

4.2. *Design modification to facilitate capillary pumping of high density and viscosity fluids*

Recently, few researchers have proposed modifications in the porous paper based devices through its integration with other systems to accelerate the flow of highly dense and viscous fluids. Such enhanced passive pumping of fluids has potential applications in the diagnostic industry. Shou and Fan proposed that the nanofibrous mesh of W-shape can be integrated with a conventional microporous substrate or nanofibrous membrane to form a W-shaped cross-section capillary channels [99]. These capillaries demonstrated an almost 6 to 8 times higher wicking speed as compared to the standalone microporous/ nanoporous paper. Such behavior was ascribed to the increased capillary pressure generated by the higher curvature of the capillary wedge in the W-shaped channel. Although they reported the flow of water as a fluid, they claim

that the system can be used for driving any fluid. Similarly, Camplisson *et al.* suggested the use of a wax printed porous paper to create a microchannel by bonding two printed papers side-by-side through few layers of a toner, i.e., a layer of toner is sandwiched between wax printed parts of the paper. This arrangement led to increased fluid movement in the microchannel created between the porous papers as compared to a standalone porous paper [100]. However manufacturing such devices on a large scale may pose a challenge [101]. These devices have not been tested for other types of complex liquids except aqueous dye solutions. In complex liquids there is an additional challenge to drive the liquid by capillary action with a constant velocity. Guo *et al* suggested the introduction of nanocapillary in front of microcapillaries to drive the fluid with constant velocity irrespective of its viscosity. They demonstrated the normalized filling time of fluid (different water-glycerol combination) with increasing viscosity in a porous media is nearly constant in the modified design as compared to normal porous media (Figure 5 (a) and (b)). Such deviation from Lucas-Washburn capillary filling is due to the occurrence of gas inertia dominated flow. They have also proposed a theoretical model to design such systems as per the applications involving different liquids [102]. They also suggested modifications in the design when a device comprises of a channel connected to an absorbent pad through an intermediate liquid separator. When the pumping liquid is drawn by capillary forces in the absorbent pad, it creates a pressure drop in the sample channel, thereby drawing the sample fluid from the reservoir into the sample channel. They further demonstrated that this micropump can pump fluid of varying viscosity and density with a constant speed (Figure 5 (d)) [103, 104]. In an another study, researchers have suggested the use of a converging tube to increase the capillary flow velocity [105]. Such systems can be investigated for the blood flow studies where the hematocrit significantly affects the viscosity of blood and thereby the capillary flow velocity. Further modifications in the DBS kit can be proposed based on the findings discussed above to overcome the hematocrit issue in the current DBS technique.

4.3. Role of materials in blood microsampling

The heterogeneity in analytes spread can be eliminated provided the flow of blood through a porous material becomes independent of the material properties. Several studies have reported an improvement in porous materials for blood microsampling applications. For example, five different DBS cards have been explored for the analysis of six immunosuppressants [106]. It suggested that choice of materials for DBS must be in line with type of analytes to be tested. Materials such as glass fiber membranes have been proposed due to their high hydrophilicity and non-absorbent nature as compared to cellulose paper that absorbs biofluids. These glass fiber membranes do not swell and maintain the native pore size distribution during blood permeation [81]. The changes in surface chemistry of glass due to aging and other environmental factors affect the capillary flow of blood [107]. Materials such as woven polyester have the potential to overcome the hematocrit effect during permeation and spreading of blood due to increase in hydrophilicity either through adsorption of plasma proteins or by use of surface modification agents. Hence, this leads to creation of a more homogenous blood spot with increased size [45, 90]. The pattern of weaving of fibers and type of yarn in a woven fabric affects the pore size and its interconnectivity. This results in the formation of a different pattern of the blood spot [108, 109]. There have been reports on the use of nitrocellulose membranes for creation of dried plasma spot[17]. The super-absorbent, hydrophilic polymers such as polyurethane sheets, synthetic paper and cellulose hydrogels can be an alternative materials for blood absorption and hence, can be used materials for blood spot creation[110-112]. The summary of the

modifications in DBS that potentially overcome its limitations such as the TLC effect and hematocrit volume of blood are listed in Table 2.

5. Application of dried blood spots

Owing to the several advantages offered by DBS over other techniques, a few important applications of DBS are discussed below. For detailed discussion on the applications of DBS, the readers are directed to other reviews in this field [5, 6, 113-115].

5.1 Omic applications

The study in the omics field (genomics, proteomics and metabolomics) has been accelerated through whole blood sampling. In such cases, microsampling can play a vital role while overcoming issues with whole blood sampling such as analyte stability, contamination, problems of collection and storage and limited volume of sample [50]. However, microsampling of plasma has been generally practiced for omics studies due to problems associated with microsampling of whole blood. Nevertheless, Chambers *et al* demonstrated the identification of 243 proteins in DBS through LC-MS/MS as compared to 181 proteins in plasma based investigation [116]. This result guided them to develop immunoassays for thyroglobulin and prostate-specific antigens [117]. Other groups have been successful in applying DBS for the discovery of biomarkers through untargeted metabolomics studies for breast cancer and breastfeeding [118, 119]. DBS techniques have also been successful in identification of proteins of diagnostic interest such as hemoglobin in Sickle-cell disease and other hemoglobinopathies, apolipoproteins and ceruloplasmin to diagnose Wilson's disease [120-122]. Other peptides and protein biomarkers discovered using DBS include hepcidin [123], peginesatide [124] and insulin-like growth factor-1 [125].

5.2 Pre-clinical applications

The preclinical studies like pharmacokinetic (PK), pharmacodynamic (PD), toxicokinetic (TK) and toxicodynamic (TD) are performed on the sampled blood of laboratory animals to quantitatively evaluate the properties of drugs before initiating human trials. However, the cost of these studies is huge considering the large number of animals used during conventional sampling and analysis. Moreover, inter-animal variability in the control groups and the sample groups are also unavoidable in the results obtained from such studies. This results in a sacrifice of large number of animals and huge compromise on the well-being of the animals. For instance, in a typical PK study, significant volume of blood has to be sampled at multiple time points from a small sized animal either by sacrificing the animal or by collecting the blood from the same animal repeatedly. These methods significantly increase the number of animals sacrificed or live a compromised life. [126]. Similarly, the toxicological studies, requiring lot of blood samples, are mostly conducted on two groups of laboratory animals; the test group of animals are used to evaluate the risk of different drug-dosing intakes (TD study) while the satellite groups are used to determine the relationship between drug concentrations and adverse drug effects (TK study) [127, 128]. Thus, there are chances of the results of the TD study getting affected if the same animals are not used for both the studies. In such cases, DBS method is quite handy in the

collection of small blood volume in a less invasive manner, improving the quality of results of bioanalysis, reducing the failure rate of such drugs in human clinical trials and eventually and overcoming the challenges of large volume sampling.

5.3 Clinical applications

5.3.1 Newborn screening: The newborn babies need screening for genetic/ blood-borne diseases after their birth for appropriate therapeutic interventions. However, drawing of large volume of blood may bring fear, stress and pain in infants. Therefore, the European Medical Agency recommends the use of minimally invasive ways to draw blood or drawing 1% of blood from newborn with not greater than 3% per week [33]. In such cases, DBS based microsampling has been quite easy, economical and non-traumatizing means (for the parents and the infant) of extracting minimal volume of blood through heel prick. This method of blood drawing avoids wastage of blood for post-processing and quite amenable for detection of analytes such as DNA, proteins, metabolites, lipids and others through manual and automated methods.

5.3.2 Therapeutic drug monitoring: The therapeutic drug monitoring (TDM) is a step closer to personalized medicine where the focus is primarily to optimize five rights as follows; right patients, right doses, right drugs, and right route at the right time. The therapeutic exposure of drugs given to a patient should be in the right therapeutic window. It is evident that there will be inter-individual pharmacokinetic variations. However, the aim is not to minimize these PK variations, but rather to understand these variations between individuals and hence, administer the correct dose. During any clinical trial of new drug, inter-individual pharmacokinetic variation need to be minimized. For this, drug concentration has to be monitored by periodic sampling and analysis of drug from plasma or serum. Every time blood needs to be sampled, the patient has to go through stressful conditions such as multiple needle pricks, loss of certain volume of blood, discomfort and others [129]. DBS can provide blood sampling through a minimally invasive means leading to high patient compliance for the patients on a long-term medication. Furthermore, blood collected through DBS can be analyzed through conventional biochemical assays. Pattet *et al* demonstrated the application of LC-MS/MS techniques for the detection of 15 antipsychotics and 7 of their metabolites in TDM studies [130].

5.3.3. Forensic toxicology: This field amalgamates the disciplines of toxicology, clinical and analytical chemistry to investigate the role of small molecules in drug abuse, death and poisoning. In these studies, biofluids such as blood, urine or saliva is collected and analyzed. The on-site blood collection by competent authorities through conventional venipuncture is quite challenging due to the need for high expertise and resource rich setting. Further, the whole blood may pose other issues such as drug instability, contamination and biosafety. Therefore, DBS may provide an inexpensive, fast and easy route to sample the blood and test it for analysis. It has been known that the stability of drugs such as zopiclone, benzodiazepines and cocaine are compromised in whole blood due to the hydrolysis of their amide or ester bond. In such cases, DBS offers increased stability of metabolites and drugs over a longer period of time [131]. For the population-based screening to estimate the toxicity/ abuse of drugs/ chemical agents, DBS is simple, inexpensive and better logistically (especially in resource and infrastructure constrained places) than sampling through whole blood. Furthermore, DBS offers an excellent platform to

automate the screening of humans/ wildlife for the toxicity of heavy metals through inductively coupled plasma mass spectroscopy [132].

6. Conclusion

The field of microsampling encompasses different application areas such analytical chemistry, clinical chemistry, biomedical engineering, pharmaceutical sciences and others. Several microsampling techniques have emerged in recent years. DBS continues to remain popular due to its inexpensive, easy, established bioanalysis protocols and supported logistics. However, due to the less explored area of fluid physics in DBS techniques, it suffers from problems such as thin layer chromatography effect, hematocrit effect and others. Blood being a colloidal solution with particles of varying sizes exhibits a complex flow behavior in a microporous substrate. Furthermore, environmental factors and fluidic properties pose challenges in maintaining the desired flow and spread of blood in porous media. Few modifications in the design of DBS kit device, modification based on microfluidic principles and choice of materials for the porous substrate have indeed helped to overcome the problems of DBS. Owing to the huge applications and simplicity of the DBS technique, it is desirable to overcome these fluidic challenges in a scalable manner to harness the full potential of DBS in blood microsampling.

Competing Interests Statement

The authors have no competing interests to declare.

Novelty Statement

*Manuscript "**Challenges and Opportunities in Blood Flow through Porous Substrate: A Design and Interface Perspective of Dried Blood Spot**"*

The dried blood spot (DBS) technique for blood collection is well established and has received renewed interest among researchers in multidisciplinary fields. Recent reviews have primarily addressed the challenges, opportunities and application of DBS techniques for analyte detection or on the developments in other microsampling techniques.

However, the challenges behind the fundamental principles of DBS and strategies to overcome these challenges have not been reviewed systematically, to the best of our knowledge. We take this opportunity to review the principles of the formation of a DBS focusing on the design, materials and interface perspectives.

Acknowledgements

PK acknowledges Department of Science and Technology (DST, SERB), India for the National Postdoctoral Fellowship. The funding from the Department of Biotechnology (DBT), India for the Bioengineering and Biodesign Initiative − Phase 2 is gratefully acknowledged.

References

[1] J.-M. Boeynaems, D. Leener Anne, B. Dessars, R. Villa-Lobos Hector, J.-C. Aubry, F. Cotton, P. Thiry, Evaluation of a new generation of plastic evacuated blood-collection tubes in clinical chemistry, therapeutic drug monitoring, hormone and trace metal analysis, Clinical Chemistry and Laboratory Medicine (CCLM), 42(1) (2004) 67-71.

[2] A. M. Lilienfeld, Irwin D. J. Bross, Philip E. Sartwell, Observations on an Outbreak of Infectious Hepatitis in Baltimore During 1951, Am J Public Health Nations Health 43(9) (1953) 1085–1096.

[3] R. A. R. Bowen, A.T. Remaley, Interferences from blood collection tube components on clinical chemistry assays, Biochemia Medica 24(1) (2014) 31–44.

[4] SR. Howie, Blood sample volumes in child health research: review of safe limits, Bull World Health Organ 89(1) (2011) 46-53.

[5] P.A. Demirev, Dried Blood Spots: Analysis and Applications, Analytical Chemistry 85(2) (2013) 779-789.

[6] S. Lehmann, C. Delaby, J. Vialaret, J. Ducos, C. Hirtz, Current and future use of "dried blood spot" analyses in clinical chemistry, Clinical Chemistry and Laboratory Medicine, 51(10) (2013), 1897-909.

[7] R.J. Meesters, G.P. Hooff, State-of-the-art dried blood spot analysis: an overview of recent advances and future trends, Bioanalysis 5(17) (2013) 2187-2208.

[8] S. Kr. Ivanova, Svetla G. Angelova, Elitsa N. Golkocheva-Markova, Petia D. Genova-Kalou , Adelina P. Pavlova, Irina L. Georgieva, A Review on Dried Blood Spots (DBS) as Alternative, Archival Material for Detection of Viral Agents (Measles, Mumps, Rubella, Hepatitis B Virus), American Scientific Research Journal for Engineering, Technology, and Sciences 30(1) (2017) 161-172.

[9] J. Mei, Dried Blood Spot Sample Collection, Storage, And Transportation, Editor: Wenkui Li Mike S. Lee (Ed.), Dried Blood Spots: Applications and Techniques, John Wiley & Sons, Inc.2014, pp. 21-31.

[10] S.C.C. Shih, H. Yang, M.J. Jebrail, R. Fobel, N. McIntosh, O.Y. Al-Dirbashi, P. Chakraborty, A.R. Wheeler, Dried Blood Spot Analysis by Digital Microfluidics Coupled to Nanoelectrospray Ionization Mass Spectrometry, Analytical Chemistry 84(8) (2012) 3731-3738.

[11] J. Lu, D. Spasic, F. Delport, T. Van Stappen, I. Detrez, D. Daems, S. Vermeire, A. Gils, J. Lammertyn, Immunoassay for Detection of Infliximab in Whole Blood Using a Fiber-Optic Surface Plasmon Resonance Biosensor, Analytical Chemistry 89(6) (2017) 3664-3671.

[12] P. Denniff, N. Spooner, The effect of hematocrit on assay bias when using DBS samples for the quantitative bioanalysis of drugs, Bioanalysis 2(8) (2010) 1385-1395.

[13] T.C. Dainty, E.S. Richmond, I. Davies, M.P. Blackwell, Dried Blood Spot Bioanalysis: An Evaluation of Techniques and Opportunities for Reduction and Refinement in Mouse and Juvenile Rat Toxicokinetic Studies, International Journal of Toxicology 31(1) (2012) 4-13.

[14] A. F. Kadjo, Stamos BN C. Phillip Shelor, Jordan M. Berg, Benjamin C. Blount, and Purnendu K. Dasgupta, Evaluation of Amount of Blood in Dry Blood Spots: Ring-Disk Electrode Conductometry, Analytical Chemistry 88(12) (2016) 6531−6537.

[15] E.H. BW Adam, M Sternberg, TH Lim, SR Flores, S O'Brien, D Simms, LX Li, VR De Jesus, and WH Hannon, The Stability Of Markers In Dried-Blood Spots For

Recommended Newborn Screening Disorders In The United States, Clinical Biochemistry 44(0) (2011) 1445-1450.
[16] T.C. Chao, O. Arjmandi-Tash, D.B. Das, V.M. Starov, Spreading of blood drops over dry porous substrate: Complete wetting case, Journal of Colloid and Interface Science 446 (2015) 218-225.
[17] T.C. Chao, O. Arjmandi-Tash, D.B. Das, V.M. Starov, Simultaneous spreading and imbibition of blood droplets over porous substrates in the case of partial wetting, Colloids and Surfaces A: Physicochemical and Engineering Aspects 505) (2016) 9-17.
[18] A. Trybala, V. Starov, Kinetics of spreading wetting of blood over porous substrates, Current Opinion in Colloid & Interface Science 36 (2018) 84-89.
[19] J.B. Lee, Droplet dynamics on non-porous and porous media: impact, spreading and absorption, Physics, ETH Zurich, Swiss Federal University of Technology, 2015, p. 218.
[20] T.C. Chao, O. Arjmandi-Tash, D.B. Das, V.M. Starov, Spreading of blood drops over dry porous substrate: complete wetting case, J Colloid Interface Sci 446 (2015) 218-25.
[21] T.C. Chao, A. Trybala, V. 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: Physicochemical and Engineering Aspects 451 (2014) 38-47.
[22] W. Li, F.L. Tse, Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules, Biomed Chromatogr 24(1) (2010) 49-65.
[23] B.G. Keevil, The analysis of dried blood spot samples using liquid chromatography tandem mass spectrometry, Clin Biochem 44(1) (2011) 110-8.
[24] M. Holub, K. Tuschl, R. Ratschmann, K.A. Strnadova, A. Muhl, G. Heinze, W. Sperl, O.A. Bodamer, Influence of hematocrit and localisation of punch in dried blood spots on levels of amino acids and acylcarnitines measured by tandem mass spectrometry, Clin Chim Acta 373(1-2) (2006) 27-31.
[25] M.S. Maria, P.E. Rakesh, T.S. Chandra, A.K. Sen, Capillary flow-driven microfluidic device with wettability gradient and sedimentation effects for blood plasma separation, Sci Rep 7 (2017) 43457.
[26] H. Li, D. Han, G.M. Pauletti, A.J. Steckl, Blood coagulation screening using a paper-based microfluidic lateral flow device, Lab Chip 14(20) (2014) 4035-41.
[27] X. Yang, O. Forouzan, T.P. Brown, S.S. Shevkoplyas, Integrated separation of blood plasma from whole blood for microfluidic paper-based analytical devices, Lab Chip 12(2) (2012) 274-80.
[28] J.H. Son, S.H. Lee, S. Hong, S.M. Park, J. Lee, A.M. Dickey, L.P. Lee, Hemolysis-free blood plasma separation, Lab Chip 14(13) (2014) 2287-92.
[29] S. Chakraborty, Dynamics of capillary flow of blood into a microfluidic channel, Lab Chip 5(4) (2005) 421-30.
[30] J. Siddique, D.M. Anderson, Capillary Rise Of A Non-Newtonian Liquid Into A Deformable Porous Material, 14(12) (2011) 1087-1102.
[31] S. Cito, Y.-C. Ahn, J. Pallares, R.M. Duarte, Z. Chen, M. Madou, I. Katakis, Visualization and measurement of capillary-driven blood flow using spectral domain optical coherence tomography, Microfluidics and nanofluidics 13(2) (2012) 227-237.
[32] R.M. Digilov, Capillary Rise of a Non-Newtonian Power Law Liquid: Impact of the Fluid Rheology and Dynamic Contact Angle, Langmuir 24(23) (2008) 13663-13667.

[33] Y. Enderle, K. Foerster, J. Burhenne, Clinical feasibility of dried blood spots: Analytics, validation, and applications, Journal of Pharmaceutical and Biomedical Analysis 130 (2016) 231-243.
[34] M. Barfield, The Application of Dried Blood Spots in Toxicokinetic and Pharmacokinetic Studies, School of Chemistry, University of Lincoln, Lincoln, England, 2017, p. 188.
[35] F. Li, J. Zulkoski, D. Fast, S. Michael, Perforated dried blood spots: a novel format for accurate microsampling, Bioanalysis 3(20) (2011) 2321-2333.
[36] O. Jonsson, R.P. Villar, L.B. Nilsson, C. Norsten-Höög, J. Brogren, M. Eriksson, K. Königsson, A. Samuelsson, Capillary microsampling of 25 µl blood for the determination of toxicokinetic parameters in regulatory studies in animals, Bioanalysis 4(6) (2012) 661-674.
[37] D. Coleman, G. Smith, R. Lawrence, D. McManus, S. Diaram, J. Edwards, Capillary microsampling in nonclinical safety assessment: practical sampling and bioanalysis from a CRO perspective, Bioanalysis 9(10) (2017) 787-798.
[38] A.D. Jasna Lenicek Krleza, Ana Grzunov, and Miljenka Maradin, Capillary blood sampling: national recommendations on behalf of the Croatian Society of Medical Biochemistry and Laboratory Medicine, Biochemia Medica 25(3) (2015) 335–358.
[39] C.L. Bowen, H. Licea-Perez, M.Z. Karlinsey, K. Jurusik, E. Pierre, J. Siple, J. Kenney, A. Stokes, N. Spooner, C.A. Evans, A novel approach to capillary plasma microsampling for quantitative bioanalysis, Bioanalysis 5(9) (2013) 1131-1135.
[40] O. Jonsson, R.P. Villar, L.B. Nilsson, M. Eriksson, K. Königsson, Validation of a bioanalytical method using capillary microsampling of 8 µl plasma samples: application to a toxicokinetic study in mice, Bioanalysis 4(16) (2012) 1989-1998.
[41] Capillary Blood Collection Devices Market Research Report- Global Forecast Till 2023, Market Research Future, 2018, p. 85.
[42] Q. Zhang, D. Tomazela, L.A. Vasicek, D.S. Spellman, M. Beaumont, B. Shyong, J. Kenny, S. Fauty, K. Fillgrove, J. Harrelson, K.P. Bateman, Automated DBS microsampling, microscale automation and microflow LC–MS for therapeutic protein PK, Bioanalysis 8(7) (2016) 649-659.
[43] L. David Hopper, Automated Microsampling Technologies and Enhancements in the 3Rs, ILAR Journal 57(2) (2016) 166-177.
[44] G. Lenk, S. Sandkvist, A. Pohanka, G. Stemme, O. Beck, N. Roxhed, A disposable sampling device to collect volume-measured DBS directly from a fingerprick onto DBS paper, Bioanalysis 7(16) (2015) 2085-2094.
[45] Y. Mengerink, J. Mommers, J. Qiu, J. Mengerink, O. Steijger, M. Honing, A new DBS card with spot sizes independent of the hematocrit value of blood, Bioanalysis 7(16) (2015) 2095-2104.
[46] L.A. Leuthold, O. Heudi, J. Déglon, M. Raccuglia, M. Augsburger, F. Picard, O. Kretz, A. Thomas, New Microfluidic-Based Sampling Procedure for Overcoming the Hematocrit Problem Associated with Dried Blood Spot Analysis, Analytical Chemistry 87(4) (2015) 2068-2071.
[47] P. Denniff, N. Spooner, Volumetric Absorptive Microsampling: A Dried Sample Collection Technique for Quantitative Bioanalysis, Analytical Chemistry 86(16) (2014) 8489–8495.
[48] N. Spooner, A. Olatunji, K. Webbley, Investigation of the effect of blood hematocrit and lipid content on the blood volume deposited by a disposable dried blood spot collection device, Journal of Pharmaceutical and Biomedical Analysis 149 (2018) 419-424.

[49] M. Protti, R. Mandrioli, L. Mercolini, Tutorial: Volumetric absorptive microsampling (VAMS), Analytica Chimica Acta 1046 (2019) 32-47.
[50] G. Nys, M.G.M. Kok, A.-C. Servais, M. Fillet, Beyond dried blood spot: Current microsampling techniques in the context of biomedical applications, TrAC Trends in Analytical Chemistry 97 (2017) 326-332.
[51] W. Li, M.S. Lee, Dried Blood Spots: Applications and Techniques First Edition, John Wiley & Sons, Hoboken, New Jersey, 2014.
[52] M. Brust, C. Schaefer, R. Doerr, L. Pan, M. Garcia, P.E. Arratia, C. Wagner, Rheology of human blood plasma: viscoelastic versus Newtonian behavior, Phys Rev Lett 110(7) (2013) 078305.
[53] J. Enderle, J. Bronzino, S. Blanchard, Introduction to Biomedical Engineering, 2nd Edition ed., Academic Press, San Diego, 2005.
[54] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, Molecular Biology of the Cell, 4th edition, Garland Science, New York, 2002.
[55] F.R. Smith, D. Brutin, Wetting and spreading of human blood: Recent advances and applications, Current Opinion in Colloid & Interface Science 36 (2018) 78-83.
[56] M. Brust, C. Schaefer, R. Doerr, L. Pan, M. Garcia, P.E. Arratia, C. Wagner, Rheology of Human Blood Plasma: Viscoelastic Versus Newtonian Behavior, Physical Review Letters 110(7) (2013) 078305.
[57] S. Chien, R.G. King, R. Skalak, S. Usami, A.L. Copley, Viscoelastic properties of human blood and red cell suspensions, Biorheology 12(6) (1975) 341-346.
[58] A. Kolbasov, P.M. Comiskey, R.P. Sahu, S. Sinha-Ray, A.L. Yarin, B.S. Sikarwar, S. Kim, T.Z. Jubery, D. Attinger, Blood rheology in shear and uniaxial elongation, Rheologica Acta 55(11-12) (2016) 901-908.
[59] A. Seiyama, Y. Suzuki, N. Maeda, Increased viscosity of erythrocyte suspension upon hemolysis, Colloid and Polymer Science 271(1) (1993) 63-69.
[60] Overview of the History and Applications of Dried Blood Samples, Dried Blood Spots, pp. 1-15.
[61] J. Stuart, M.W. Kenny, Blood rheology, Journal of clinical pathology 33(5) (1980) 417-429.
[62] A.M. Robertson, Rheological models for blood, in: L. Formaggia, Quarteroni, Alfio, Veneziani, Alessandro (Ed.), Cardiovascular Mathematics, Springer-Verlag Mailand2009, pp. 211-241.
[63] S. De Gruttola, K. Boomsma, D. Poulikakos, Computational Simulation of a Non-Newtonian Model of the Blood Separation Process, Artificial Organs 29(12) (2005) 949-959.
[64] S. Charm, G. Kurland, Viscometry of Human Blood for Shear Rates of 0-100,000 sec−1, Nature 206 (1965) 617.
[65] E. Beutler, C. West, Hematologic differences between African-Americans and whites: the roles of iron deficiency and alpha-thalassemia on hemoglobin levels and mean corpuscular volume, Blood 106(2) (2005) 740-745.
[66] E.M. Hall, S.R. Flores, V.R. De Jesus, Influence of Hematocrit and Total-Spot Volume on Performance Characteristics of Dried Blood Spots for Newborn Screening, Int J Neonatal Screen 1(2) (2015) 69-78.
[67] S. Whitaker, Flow in porous media I: A theoretical derivation of Darcy's law, Transport in Porous Media 1(1) (1986) 3-25.

[68] H. Huinink, Fluids in Porous Media, Transport and phase changes, Morgan & Claypool Publishers, 2016.
[69] E.W. Washburn, The Dynamics of Capillary Flow, Physical Review 17(3) (1921) 273-283.
[70]D. G, Pierre-Gilles, B.-W Francoise, Q. David, Capillarity and Wetting Phenomena: Drops, Bubbles, Pearls, Waves, Springer-Verlag, New York, 2004.
[71] C. Grzelakowski, D. Ben Jazia, B. Lebeau, L. Vonna, D. Dupuis, H. Haidara, On the influence of pore structure on the free-imbibition of sessile drops into nanoporous substrates, Langmuir 25(10) (2009) 5855-60.
[72] J. Cai, B. Yu, A Discussion of the Effect of Tortuosity on the Capillary Imbibition in Porous Media, Transport in Porous Media 89(2) (2011) 251-263.
[73] M. Alava, M. Dubé, M. Rost, Imbibition in disordered media, Advances in Physics 53(2) (2004) 83-175.
[74] J. Bear, Dynamics of Fluids in Porous Media, Courier Dover Publications, New York, 1972.
[75] P. Agrawal, L. Barnet, D. Attinger, Bloodstains on woven fabric: Simulations and experiments for quantifying the uncertainty on the impact and directional angles, Forensic Sci Int 278 (2017) 240-252.
[76] T.C. de Castro, D.J. Carr, M.C. Taylor, J.A. Kieser, W. Duncan, Drip bloodstain appearance on inclined apparel fabrics: Effect of prior-laundering, fibre content and fabric structure, Forensic Sci Int 266 (2016) 488-501.
[77] T.C. de Castro, M.C. Taylor, J.A. Kieser, D.J. Carr, W. Duncan, Systematic investigation of drip stains on apparel fabrics: The effects of prior-laundering, fibre content and fabric structure on final stain appearance, Forensic Sci Int 250 (2015) 98-109.
[78] K.L. Adams, W.B. Russel, L. Rebenfeld, Radial penetration of a viscous liquid into a planar anisotropic porous medium, International Journal of Multiphase Flow 14(2) (1988) 203-215.
[79] M.S. Maria, P.E. Rakesh, T.S. Chandra, A.K. Sen, Capillary flow of blood in a microchannel with differential wetting for blood plasma separation and on-chip glucose detection, Biomicrofluidics 10(5) (2016) 054108.
[80] M.S. Maria, P.E. Rakesh, T.S. Chandra, A.K. Sen, Capillary flow-driven microfluidic device with wettability gradient and sedimentation effects for blood plasma separation, Scientific Reports 7 (2017) 43457.
[81] A. Metelkin, D. Kuznetsov, E. Kolesnikov, K. Chuprunov, S. Kondakov, A. Osipov, J. Samsonova, Membrane materials for storing biological samples intended for comparative nanotoxicological testing, IOP Conference Series: Materials Science and Engineering 98(1) (2015) 012019.
[82] W. Bou-Zeid, D. Brutin, Effect of relative humidity on the spreading dynamics of sessile drops of blood, Colloids and Surfaces A: Physicochemical and Engineering Aspects 456 (2014) 273-285.
[83] K. Perktold, G. Karner, A. Leuprecht, M. Hofer, Influence of Non-newtonian Flow Behavior on Local Hemodynamics, ZAMM - Journal of Applied Mathematics and Mechanics / Zeitschrift für Angewandte Mathematik und Mechanik 79(S1) (2011) 187-190.
[84] K.M.D. Hell, A. Balzereit, U. Diebold, H.D. Bruhn, Importance of Blood Viscoelasticity in Arteriosclerosis, Angiology 40(6) (1989) 539-546.

[85] C.L. Morris, D.L. Rucknagel, R. Shukla, R.A. Gruppo, C.M. Smith, P. Blackshear, Evaluation of the yield stress of normal blood as a function of fibrinogen concentration and hematocrit, Microvascular Research 37(3) (1989) 323-338.
[86] E.M. Williams, M. Dodds, M.C. Taylor, J. Li, S. Michielsen, Impact dynamics of porcine drip bloodstains on fabrics, Forensic Sci Int 262 (2016) 66-72.
[87] T.J. Verdon, R.J. Mitchell, R.A. van Oorschot, The influence of substrate on DNA transfer and extraction efficiency, Forensic Sci Int Genet 7(1) (2013) 167-75.
[88] N. Weisbrod, M.R. Niemet, J.S. Selker, Imbibition of saline solutions into dry and prewetted porous media, Advances in Water Resources 25(7) (2002) 841-855.
[89] Y. Çinar, A.M. Şenyol, K. Duman, Blood viscosity and blood pressure: role of temperature and hyperglycemia, American Journal of Hypertension 14(5) (2001) 433-438.
[90] M.J. Hertaeg, R.F. Tabor, G. Garnier, Effect of protein adsorption on the radial wicking of blood droplets in paper, Journal of Colloid and Interface Science 528 (2018) 116-123.
[91] R. Sturm, J. Henion, R. Abbott, P. Wang, Novel membrane devices and their potential utility in blood sample collection prior to analysis of dried plasma spots, Bioanalysis 7(16) (2015) 1987-2002.
[92] H.J. Li Y, Abbott R, Wang P., The use of a membrane filtration device to form dried plasma spots for the quantitative determination of guanfacine in whole blood, Rapid Communications in Mass Spectrometry 26(10) (2012) 1208-1212.
[93] N. Youhnovski, A. Bergeron, M. Furtado, F. Garofolo, Pre-cut dried blood spot (PCDBS): an alternative to dried blood spot (DBS) technique to overcome hematocrit impact, Rapid Communications in Mass Spectrometry 25(19) (2011) 2951-2958.
[94] R.J. Meesters, J. Zhang, N.A.v. Huizen, G.P. Hooff, R.A. Gruters, T.M. Luider, Dried matrix on paper disks: the next generation DBS microsampling technique for managing the hematocrit effect in DBS analysis, Bioanalysis 4(16) (2012) 2027-2035.
[95] R. Neto, A. Gooley, M.C. Breadmore, E.F. Hilder, F. Lapierre, Precise, accurate and user-independent blood collection system for dried blood spot sample preparation, Analytical and Bioanalytical Chemistry 410(14) (2018) 3315-3323.
[96] J.H. James Hill, Biological sample collection and preservation, in: U.S. Patent (Ed.) Spot on Sciences, United States of America, 2017.
[97] S.D. Polley, D. Bell, J. Oliver, F. Tully, M.D. Perkins, P.L. Chiodini, I.J. González, The design and evaluation of a shaped filter collection device to sample and store defined volume dried blood spots from finger pricks, Malaria Journal 14(1) (2015) 45.
[98] P.S.G. Prasoon Kumar, Mainak Majumder, Enhanced capillary pumping through evaporation assisted leaf-mimicking micropumps, arXiv preprint arXiv:1807.11464 (2018).
[99] D. Shou, J. Fan, Design of Nanofibrous and Microfibrous Channels for Fast Capillary Flow, Langmuir 34(4) (2018) 1235-1241.
[100] C.K. Camplisson, K.M. Schilling, W.L. Pedrotti, H.A. Stone, A.W. Martinez, Two-ply channels for faster wicking in paper-based microfluidic devices, Lab on a Chip 15(23) (2015) 4461-4466.
[101] C.-H. Choi, S. Krishnan, W. TeGrotenhuis, C.-H. Chang, Capillary Rise of Nanostructured Microwicks, Micromachines 9(4) (2018).
[102] W. Guo, J. Hansson, W. van der Wijngaart, Capillary Pumping Independent of Liquid Sample Viscosity, Langmuir 32(48) (2016) 12650-12655.

[103] W. Guo, J. Hansson, W. van der Wijngaart, Capillary pumping independent of the liquid surface energy and viscosity, Microsystems & Nanoengineering 4(1) (2018) 2.
[104] W. Guo, J. Hansson, W. van der Wijngaart, Viscosity Independent Paper Microfluidic Imbibition, The 20th International Conference on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS 2016, 9-13 October 2016, Dublin, Ireland, MicroTAS, 2016, pp. 13-14.
[105] W. van der Wijngaart, Capillary pumps with constant flow rate, Microfluidics and Nanofluidics 16(5) (2014) 829-837.
[106] R.A. Koster, R. Botma, B. Greijdanus, D.R. Uges, J.G. Kosterink, D.J. Touw, J.-W.C. Alffenaar, The performance of five different dried blood spot cards for the analysis of six immunosuppressants, Bioanalysis 7(10) (2015) 1225-1235.
[107] F. Lapierre, A. Gooley, M. Breadmore, Principles around Accurate Blood Volume Collection Using Capillary Action, Langmuir 33(50) (2017) 14220-14225.
[108] P. Agrawal, L. Barnet, D. Attinger, Bloodstains on woven fabric: Simulations and experiments for quantifying the uncertainty on the impact and directional angles, Forensic Science International 278 (2017) 240-252.
[109] X. Li, J. Li, S. Michielsen, Effect of yarn structure on wicking and its impact on bloodstain pattern analysis (BPA) on woven cotton fabrics, Forensic Science International 276 (2017) 41-50.
[110] T. Shimamoto, Polyurethane sheet: a potential substitute of surgical cotton gauze, Journal of cardiothoracic surgery 6 (2011) 26-26.
[111] J. Ma, X. Li, Y. Bao, Advances in cellulose-based superabsorbent hydrogels, RSC Advances 5(73) (2015) 59745-59757.
[112] J. Hansson, H. Yasuga, T. Haraldsson, W. van der Wijngaart, Synthetic microfluidic paper: high surface area and high porosity polymer micropillar arrays, Lab on a Chip 16(2) (2016) 298-304.
[113] A.J. Wilhelm, J.C.G. den Burger, E.L. Swart, Therapeutic drug monitoring by dried blood spot: progress to date and future directions, Clinical pharmacokinetics 53(11) (2014) 961-973.
[114] J.D. Freeman, L.M. Rosman, J.D. Ratcliff, P.T. Strickland, D.R. Graham, E.K. Silbergeld, State of the Science in Dried Blood Spots, Clinical Chemistry 64(4) (2018) 656.
[115] K. Gupta, R. Mahajan, Applications and Diagnostic Potential of Dried Blood Spots, International journal of applied & basic medical research 8(1) (2018) 1-2.
[116] A.G. Chambers, A.J. Percy, D.B. Hardie, C.H. Borchers, Comparison of Proteins in Whole Blood and Dried Blood Spot Samples by LC/MS/MS, Journal of The American Society for Mass Spectrometry 24(9) (2013) 1338-1345.
[117] S. Velghe, Sara Capiau, and Christophe Stove, Opening the Toolbox of Alternative Sampling Strategies in Clinical Routine : a Key-role for (LC-)MS/MS., TrAC Trends in Analytical Chemistry 84 (2016) 61–73.
[118] Q. Wang, T. Sun, Y. Cao, P. Gao, J. Dong, Y. Fang, Z. Fang, X. Sun, Z. Zhu, A dried blood spot mass spectrometry metabolomic approach for rapid breast cancer detection, OncoTargets and therapy 9 (2016) 1389-1398.
[119] P. Prentice, A. Koulman, L. Matthews, C.L. Acerini, K.K. Ong, D.B. Dunger, Lipidomic Analyses, Breast- and Formula-Feeding, and Growth in Infants, The Journal of Pediatrics 166(2) (2015) 276-281.

[120] F. Boemer, O. Ketelslegers, J.-M. Minon, V. Bours, R. Schoos, Newborn Screening for Sickle Cell Disease Using Tandem Mass Spectrometry, Clinical Chemistry 54(12) (2008) 2036.
[121] C.M. Henderson, J.G. Bollinger, J.O. Becker, J.M. Wallace, T.J. Laha, M.J. MacCoss, A.N. Hoofnagle, Quantification by Nano Liquid Chromatography-Parallel Reaction Monitoring-Mass Spectrometry of Human Apolipoprotein A-I, Apolipoprotein B, and Hemoglobin A1c in Dried Blood Spots, Proteomics. Clinical applications 11(7-8) (2017) 10.1002/prca.201600103.
[122] S. Lehmann, A. Picas, L. Tiers, J. Vialaret, C. Hirtz, Clinical perspectives of dried blood spot protein quantification using mass spectrometry methods, Critical Reviews in Clinical Laboratory Sciences 54(3) (2017) 173-184.
[123] V. Houbart, G. Cobraiville, A.-C. Servais, A. Napp, M.-P. Merville, M. Fillet, Hepcidin determination in dried blood by microfluidic LC–MS/MS: comparison of DBS and volumetric absorptive microsampling for matrix effect and recovery, Bioanalysis 7(21) (2015) 2789-2799.
[124] I. Möller, A. Thomas, H. Geyer, W. Schänzer, M. Thevis, Development and validation of a mass spectrometric detection method of peginesatide in dried blood spots for sports drug testing, Analytical and Bioanalytical Chemistry 403(9) (2012) 2715-2724.
[125] H.D. Cox, J. Rampton, D. Eichner, Quantification of insulin-like growth factor-1 in dried blood spots for detection of growth hormone abuse in sport, Analytical and Bioanalytical Chemistry 405(6) (2013) 1949-1958.
[126] P.E. Turpin, J.E. Burnett, L. Goodwin, A. Foster, M. Barfield, Application of the DBS methodology to a toxicokinetic study in rats and transferability of analysis between bioanalytical laboratories, Bioanalysis 2(8) (2010) 1489-1499.
[127] S.L. Parker, T. Dorofaeff, J. Lipman, D.E. Ballot, R.M. Bandini, S.C. Wallis, J.A. Roberts, Is there a role for microsampling in antibiotic pharmacokinetic studies?, Expert Opinion on Drug Metabolism & Toxicology 12(6) (2016) 601-614.
[128] T. Verhaeghe, L. Dillen, H. Stieltjes, L.d. Zwart, B. Feyen, L. Diels, A. Vroman, P. Timmerman, The application of capillary microsampling in GLP toxicology studies, Bioanalysis 9(7) (2017) 531-540.
[129] L.C. Martial, R.E. Aarnoutse, M. Mulder, A. Schellekens, R.J.M. Brüggemann, D.M. Burger, A.H. Schene, A. Batalla, Dried Blood Spot sampling in psychiatry: Perspectives for improving therapeutic drug monitoring, European Neuropsychopharmacology 27(3) (2017) 205-216.
[130] L. Patteet, K.E. Maudens, C.P. Stove, W.E. Lambert, M. Morrens, B. Sabbe, H. Neels, The use of dried blood spots for quantification of 15 antipsychotics and 7 metabolites with ultra-high performance liquid chromatography - tandem mass spectrometry, Drug Testing and Analysis 7(6) (2015) 502-511.
[131] R. Jantos, A. Vermeeren, D. Sabljic, J.G. Ramaekers, G. Skopp, Degradation of zopiclone during storage of spiked and authentic whole blood and matching dried blood spots, International Journal of Legal Medicine 127(1) (2013) 69-76.
[132] A.F. Lehner, W. Rumbeiha, A. Shlosberg, K. Stuart, M. Johnson, R. Domenech, H. Langner, Diagnostic Analysis of Veterinary Dried Blood Spots for Toxic Heavy Metals Exposure, Journal of Analytical Toxicology 37(7) (2013) 406-422.

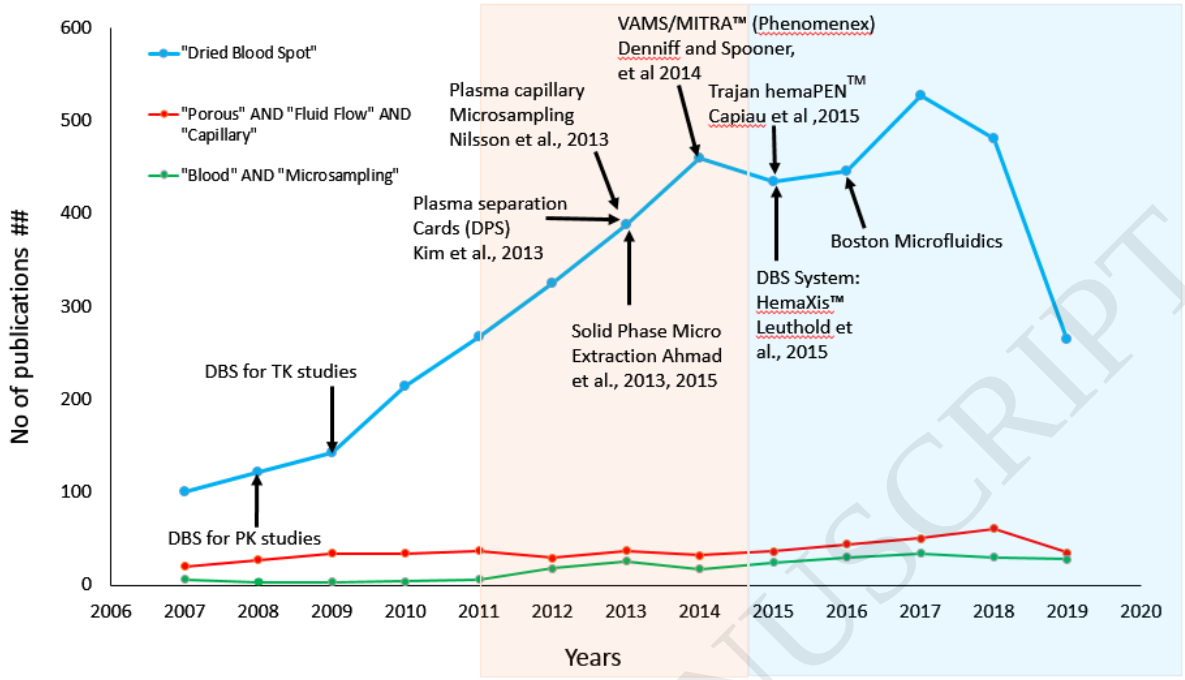


Figure 1: Graph showing the increasing trend in the scientific publications related to "Dried Blood Spot", "Blood microsampling" and "Fluid flow in porous media by capillary" in the last 12 years (Source: Scopus data till June, 2019). Major inventions in blood microsampling in the recent years have been highlighted.

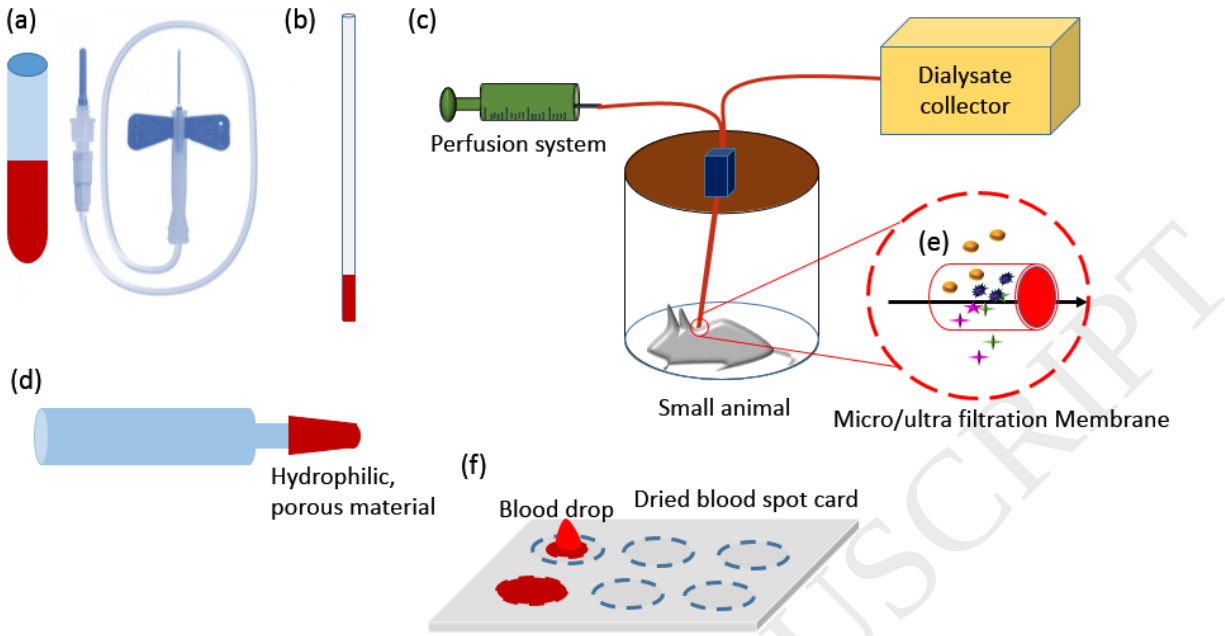


Figure 2: Schematic of blood microsampling techniques: (a) Vacutainers; (b) Capillary tube; (c) Automated microsampling technique; (d) Volumetric Absorptive Microsampler device; (e) Membranes used as microsampler and (f) Dried Blood Spot Card

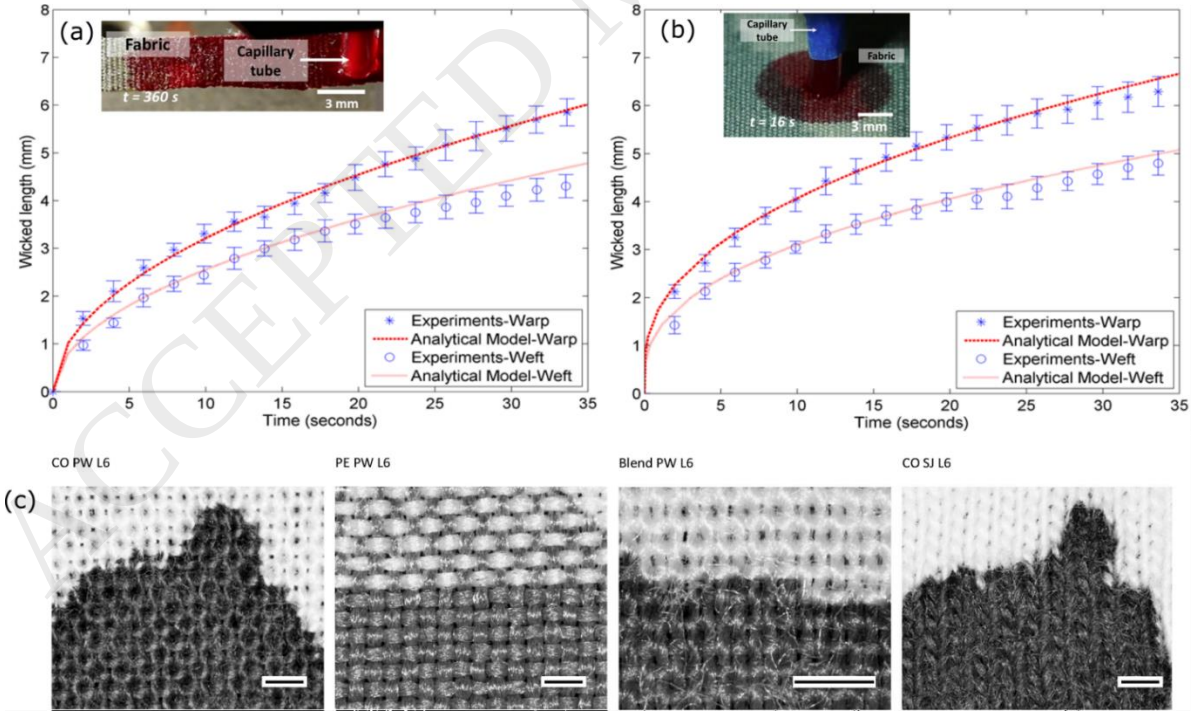


Figure 3: Blood flow properties: (a) Variation of the distance of blood wetting front from the source capillary tube (wicked length) with time for a linear imbibition case in a fabric strip (inset), for both warp and weft directions in the fabric[75]. The curves in red indicate the fit from

the analytical model for a Newtonian liquid flow [69]; (b) Variation of the distance of blood wetting front from the source capillary tube (wicked length) with time for a radial imbibition case on a fabric piece (inset) [75]. Spreading in the warp direction is measured from the radius in the horizontal direction in the image and spreading in the weft direction is measured from the radius in the vertical direction in the image. The curves in red indicate the fit from the analytical model for a Newtonian liquid flow [78]; (c) Circumference of a blood spot on different fabrics (from left to right): 100% cotton plain woven, 100% polyester plain woven, 65% polyester/35% cotton plain woven, 100% cotton knit, The black bars indicate 1 mm [77].

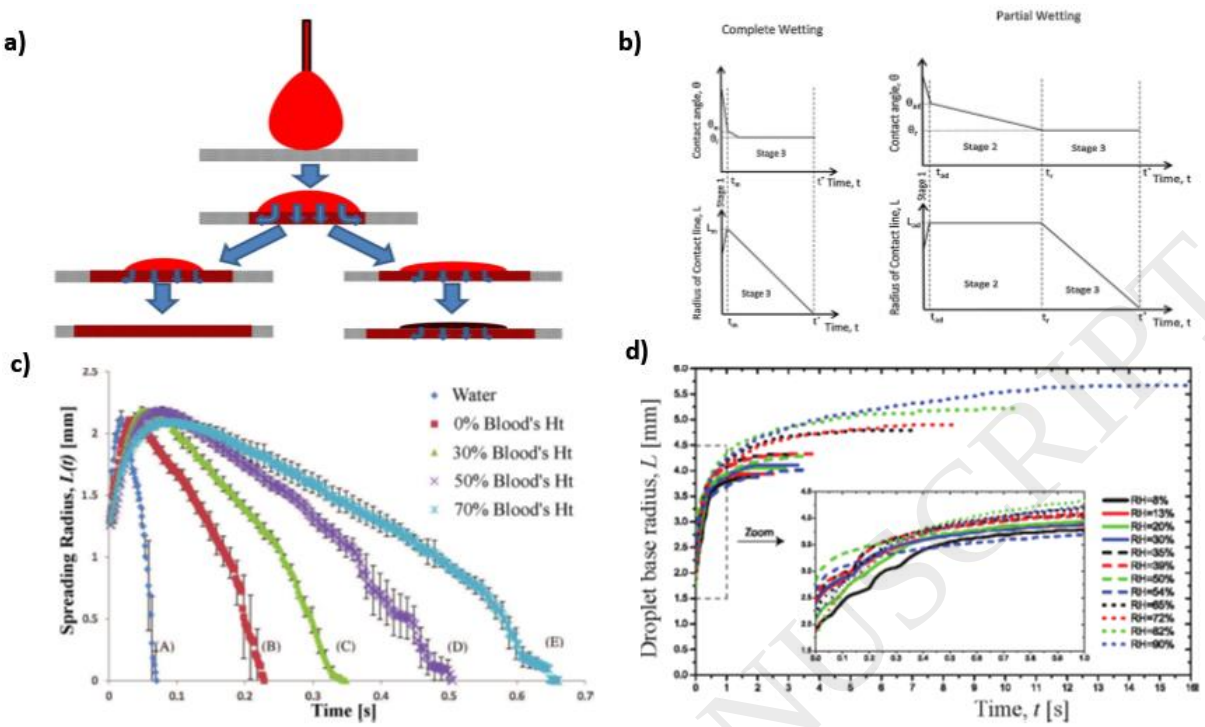


Figure 4: (a) Schematic representation of partial wetting (right panel) and complete wetting (left panel) cases of blood spreading on a porous sheet. In partial wetting there is a dried blood drop on the porous sheet, while in the complete wetting scenario, the complete blood drop imbibes into the porous sheet [17]; (b) Depiction of the variation in contact angle and drop radius with time in the complete wetting and partial wetting scenarios of drop spreading on a porous sheet[17]; (c) Variation of drop radius with time for blood with different hematocrit for spreading on a Whatman filter paper in a partial wetting scenario[17]; (d) Spreading of a blood drop on a glass substrate over time at different relative humidity (increasing from top (8%) to bottom (90%) in the legend) [82].

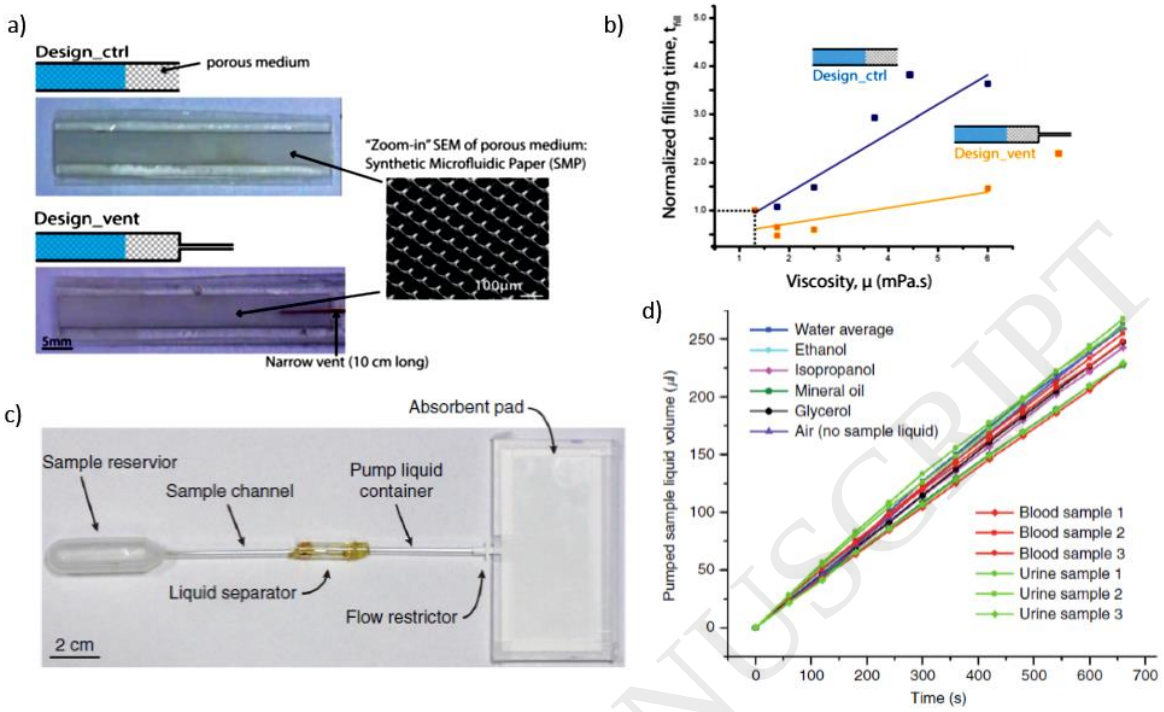


Figure 5: (a) Image of a device with a nanocapillary connected to porous substrate (synthetic microfluidic paper) to pump fluid independent of viscosity[104]; (b) Graph showing the normalized filling time of fluid having varying viscosity in a porous substrate associated with two different designs[104]; (c) Image of a device with a dropper connected to an absorbent pad via a fluid connector to pump fluid contained in a sample reservoir through suction pressure created by capillary flow of pump liquid in an absorbent pad[103]; (d) Graph showing the volume of different liquid samples of different density and viscosity pumped in a sample channel of the device shown in Figure 5c[103].

Table 1: Comparison of various blood microsampling techniques[50]

Microsampling Techniques	Vacutainers	Capillary Tube	Membrane Micro Sampling	Automated Micro Sampling	Volumetric Absorptive Micro Sampling	Dried Blood Spot
Sample volume collected	++	-	-	-	- -	- -
Effect of hematocrit on sample volume collection	--	++	+	+	- -	+
Possibility of sample transport by mail at a room temperature	- -	- -	- -	- -	++	++
Stability of samples during storage at a room temperature	- -	- -	- -	- -	++	++
Speed sample preparation	+	++	- -	-	++	++
Easy of sample analysis	+	+	-	-	- -	- -
Involvement of automation in sample preparation and analysis	- -	- -	++	++	++	+
Compliance with humans	+	++	- -	- -	++	++
Wastage of sample	++	+	-	- -	- -	- -
Expense of sampling (collection, storage and transportation)	+	+	++	++	+	- -

Note: ++ very high; + high; -- very low and – low

Table 2 Summary of modifications in DBS to overcome its current challenges

	Proposed Modifications	Role in overcoming challenges	References
Design of DBS device	Separation of blood components	Separates plasma and blood cells to facilitate quick wicking of plasma	Ref [79, 80]
	Perforation in the DBS paper	Reduces hematocrit effect	Ref [84]
	Pre-cut/punched DBS paper	Finite size of DBS paper and non-contact mode of DBS paper with an underlying substrate while wicking reduces hematocrit effect	Ref [81-83]
	Capillary assisted suction pressure	Design of device facilitate pumping of high density and viscosity fluid through porous media	Ref [92-94]
	Shape of the filter paper	Reduces hematocrit effect	Ref [85, 86]
	Novel microfluidic design	Microfluidics integrated with filter paper leads to quick wicking and spreading, overcomes hematocrit issue	Ref [87, 88]
New materials for microsampling	Whatman paper (31 ET CHR, FTA DMPK-C, 903), Perkin Elmer 226 and Agilent Bond Elut DMS DBS cards	Different paper demonstrated different performance at extreme hematocrit values	Ref [96]
	Woven polyester material	Hydrophilic, multi-layered oriented fibers	Ref [78, 98]
	Glass fiber membrane	High hydrophilicity, non-swelling and non-absorbent to plasma of blood	Ref [69]
	Nitro-cellulose membrane	Hydrophilic, Controlled pore-size, assist in blood and plasma separation	Ref [17]
	Superabsorbant polymers like Polyurethane sheet, cellulose based hydrogels, synthetic paper	Super absorbent to blood	Ref [101-103]