CHAPTER 1: INTRODUCTION

1.1 Motivation

Microfluidic technology has been one of the attractive research areas that couples multidisciplinary fields, including physics, chemistry, engineering, and biotechnology (Jia et al. 2017; Kulkarni and Goel 2022). It manipulates small amounts of fluids (10^{-9} - 10^{-18} L) in microchannels with dimensions of micro-scale (10 - 100 μm) (Bhatt et al. 2016; Carneiro et al. 2016). The primary focus of this technology is on chemical analysis, for which it provides several distinctive features such as decreased analysis time, reduced consumption of sample and reagents, low-cost of use and disposal, portability, and enhanced analytical performance (Ríos, Zougagh, and Avila 2012). Due to the advancement in microfabrication techniques, microfluidic technology has revolutionized the field of medical diagnostics by miniaturizing diagnostic devices which has true potential for its deployment in resource-constraint settings among underserved population (Fernandes, Gernaey, and Krühne 2018; Preetam et al. 2022).

Blood is the most common biofluid used for preliminary screening of health conditions of patients, even in resource-poor settings. This may be attributed to the fact that blood samples drawn from patients may provide valuable preliminary information that may guide healthcare experts in making an immediate clinical choice regarding the next course of actions. Blood is suspension in which blood cells (45% by volume of whole human blood) in plasma. The presence of these cells such as red blood cells (95% of total cells), white blood cells (0.13%) and platelets (4.5%) impart a non-Newtonian behavior to the blood. Red blood cells (RBCs) have a biconcave shape with diameter 6 to 8 μm and thickness 1.5 to 2 μm. Plasma is a straw colored liquid which consists of various proteins

such fibrinogen, globulin and albumin in water (95%) and is slightly viscous than water (Maria et al. 2017). The pathological examination with blood samples is traditionally dependent on a centralized laboratory equipped with sophisticated instruments and an uninterrupted power supply (Drancourt et al. 2016; Newman and Hardie 2021). The point-of-care (POC) technologies have been advanced to bring diagnostic benefits to patients living with extremely limited resources and does not have access to centralized healthcare facilities (Kozel and Burnham-Marusich 2017; Loubiere and Moatti 2010; Nayak et al. 2017). The POC technologies can be considered as disrupting technology which has the true potential to replace the standard laboratory-intensive approaches regarding healthcare screening due to the rapid, compact, portable, automatized nature of these POC devices which basically require micro-amount of blood sample for affordable healthcare diagnostics.

Microfluidics comprises several specialization such as paper-based, centrifugal, acoustophoresis, electrophoresis, droplet based, and so on (Banik et al. 2021; Foudeh et al. 2012; Kong et al. 2016; Sohrabi, Kassir, and Keshavarz Moraveji 2020; Zhou, Wang, and Lin 2012). All of them have a unique feature which is often characterized by relevant underlying forces. For example, in centrifugal microfluidics, a couple of forces such as centrifugal force, coriolis force, and Euler force play a crucial role to manipulate the fluid flow in microchannel. In case of acoustophoresis, an externally applied acoustic wave or simply a mechanical pressure wave causes a distinction based on the mechanical properties of the particles or fluid such as compressibility. Similarly, capillary microfluidics works on the principle of capillary action that allows the movement of the fluids in the microchannels without requirement of any external pressure or pumps. Further, in this case liquid flows in the capillaries due to surface tension and wetting properties of the capillary. It may be noted that these forces need not exist exclusively, however, a combination of

these forces are employed to accomplish the desired functionalities of the target microfluidic device.

1.2   Fundamental of centrifugal microfluidics

The fundamental principle behind centrifugal microfluidics is that fluid manipulation in this framework occurs under the action of three forces namely centrifugal, coriolis, and Euler forces. A combination of centrifugal and capillary forces is utilized to control fluids in this platform, however, coriolis and Euler forces allow to perform mixing and incubation operations. The relevant forces are shown in Fig. 1.1.
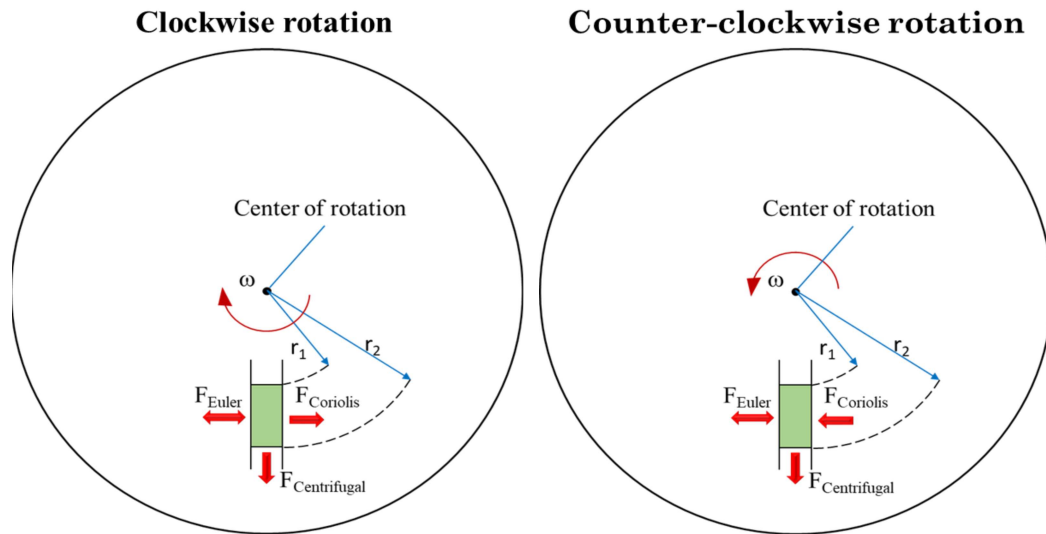

Fig. 1.1 Schematic presentation of the forces acting on a centrifugal microfluidic platform

1.2.1   Centrifugal force

In a rotating microfluidic platform, the centrifugal force plays a significant role in the control of fluids (Lutz 2011). The centrifugal force density of fluid depends on the mass density (ρ), radial location $\left(\bar{r}\right),$ and an angular velocity $(\bar{\omega})$ as given by Eq. (1.1).

$$\overline{F}_{ce} = -\rho\overline{\omega}\times\left(\overline{\omega}\times\overline{r}\right) \qquad (1.1)$$

The centrifugal pressure due to a liquid column of height (r_2-r_1) in a rotating platform can be calculated by Eq. (1.2).

$$p_{ce} = \rho\omega^2\overline{R}\left(r_2 - r_1\right) \qquad (1.2)$$

1.2.2   Coriolis force

In a rotating reference system, the coriolis pseudo-force affects moving masses and resulting in a relative deflection of this mass perpendicular to the axis of rotation and the direction of motion (Lutz 2011). The coriolis force density $\left(\overline{f}_{co}\right)$ acing on a mass with a density and moving with a velocity $\left(\overline{v}\right)$ is given by Eq. (1.3).

$$\overline{f}_{co} = -2\rho\overline{\omega}\times\overline{v} \qquad (1.3)$$

1.2.3   Euler force

The Euler force acts on moving masses in an accelerated rotational reference system perpendicular to the rotational axis and the direction of acceleration (Lutz 2011). For a change in the angular velocity $\left(\dfrac{d\overline{\omega}}{dt}\right)$ the Euler force density $\left(\overline{f}_e\right)$ is calculated by Eq. (1.4).

$$\overline{f}_e = \rho\overline{r}\times\left(\frac{d\overline{\omega}}{dt}\right) \qquad (1.4)$$

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1.3   Fundamental of paper-based microfluidics

Paper-based microfluidic systems have emerged as one of the most promising technologies for usage in a wide range of possible applications, including point-of-care diagnostics, flexible electronics, energy storage, and so on (Kemal, Safwan, and R 2013; Vashist et al. 2015). Paper-based technology has been widely accepted in academic research labs as well as in industries for several decades (Cate et al. 2015). Owing to their distinctive qualities such as rapid, inexpensive, easy-to-use and fabricate, biodegradable, biocompatible and chemical inertness, paper-based microfluidic devices have become a prominent alternative for existing POC technologies (Rasmi et al. 2021).

1.3.1   Mechanism of fluid transportation on paper substrate

Paper microfluidics is concerned with fluid flow without the need of external force. The driving factor for the passive passage of fluid through the paper substrate is capillary action. The interaction between the paper and the contacting surface is normally regulated by two opposing forces, namely the cohesive force and the adhesive force. When fluid comes into contact with paper, an intermolecular interaction occurs between liquid molecules at the liquid-air interface (cohesion) as well as between solid-liquid interfaces (adhesion). The adhesive force is responsible of spreading the liquid onto the porous substrate, whereas the cohesive force, such as surface tension, is responsible for reducing the area of the liquid-air interface. As a result, fluid flow occurs only when the impact of adhesion exceeds that of cohesion. Wicking is affected by the physical and geometrical features of porous media such as paper materials, paper structure, pore size, permeability, paper size and shape, and the physical properties of the liquid. In general, fluid transport may be divided into two types: wet-out and fully wetted flows. The fluid front wicks along the dry porous material in the first kind of flow, which may be modelled using the basic Lucas-Washburn equation.

The fluid movement in the second scenario happens along the wetted porous medium and is regulated by Darcy's law (Rasmi et al. 2021).

1.4 Outline of the thesis

This section presents a roadmap to the organization of the thesis, which addresses the research gaps highlighted in the preceding section. In this thesis, we develop a multiplexed microfluidic platform for determining different analytes in blood through colorimetric assays.

Starting off with chapter 2, the development of a low-cost reagent-free method for hemoglobin estimation on a simple portable unit from the human blood sample is described. The device exploits the fundamental principle of osmotic hemolysis to extract hemoglobin from red blood cells. The method harnesses the dynamics of a blood drop on a rotating platform and simple imaging to come up with results in a turn-around time of about 13 minutes.

Chapter 3 presents a low-cost and simply-fabricated microfluidic paper-based analytical device (μPAD) to estimate plasma creatinine from the finger-pricked whole human blood. The device accurately measures the creatinine concentration using just 10 μL of figure-pricked blood. The proposed device mainly involves three steps: plasma separation, plasma transportation, and quantification of the creatinine level using an in-house developed app.

In chapter 4, a paper-based analytical device is developed to simultaneously determine the hematocrit and hemoglobin concentration. A 20 μL of the whole human blood and a 20 μL droplet of the mixture of blood and deionized water are deposited in the respective detection zone. The mean area of the stain is used to quantify the hematocrit level, while the mean grey color intensity is used to quantify hemoglobin concentration.

In chapter 5, a simple, affordable, and portable spinning disc for measuring plasma-creatinine concentration with 10 µL of whole human blood is presented. A well-known colorimetric detection method is used here to estimate the creatinine concentration. The creatinine in the blood plasma reacts with alkaline picrate (Jaffe reaction), and the color of the mixture changes to yellow-orange color. The resulting color is captured with a smartphone, and creatinine concentration is estimated using an in-house developed app (CREA-SESE).

Finally, the conclusion of the thesis is presented in chapter 6. The novel contributions from the preceding chapters are summarized, and some possible extensions are also discussed.