Chapter 3 A low-cost and disposable capillary-based paper sensor for measuring blood-plasma viscosity using a smartphone app

3.1 Overview

In this chapter we will discuss about a low cost and disposable paper-based capillary viscometer device for plasma viscosity measurement. Plasma viscosity is a significant biomarker for the detection of several inflammatory diseases such as rheumatoid arthritis, cardiovascular disorders, autoimmune disease, and ischemic heart disorder. The traditional methods to measure viscosities are accurate and provide results rapidly, however, they are expensive, less transportable, and require a costly data acquisition system. Therefore, in this chapter we discuss about a simple, inexpensive, portable and disposable paper-based device for the measurement of plasma viscosity with the help of a smartphone app using only 50 µL blood plasma sample. The developed device works on the fact that the sample having higher viscosity will take longer time to travel a fixed distance on the porous substrate. A 50 µL of plasma sample is loaded onto the device. The time taken by the sample to travel a fixed distance of 30 mm is recorded, and the viscosity value is estimated with an in-house developed smartphone app. The detail measurement process is graphically represented in **Figure *3.1***. Further, the value of viscosity obtained with our device is benchmarked against the gold-standard results obtained from the Brookfield viscometer. An excellent correlation was found between the values measured by both methods with a coefficient of determination (R^2) of 0.9644. The proposed device covers the entire clinical range of blood plasma viscosity (0.9~4 cP) and can easily distinguish even a small difference of 0.13 cP in the viscosity value. Hence, our developed device renders not only a portable low-cost miniaturised platform but also a device with uncompromised accuracy which can easily be deployed to resource-poor settings as a point-of-care device.

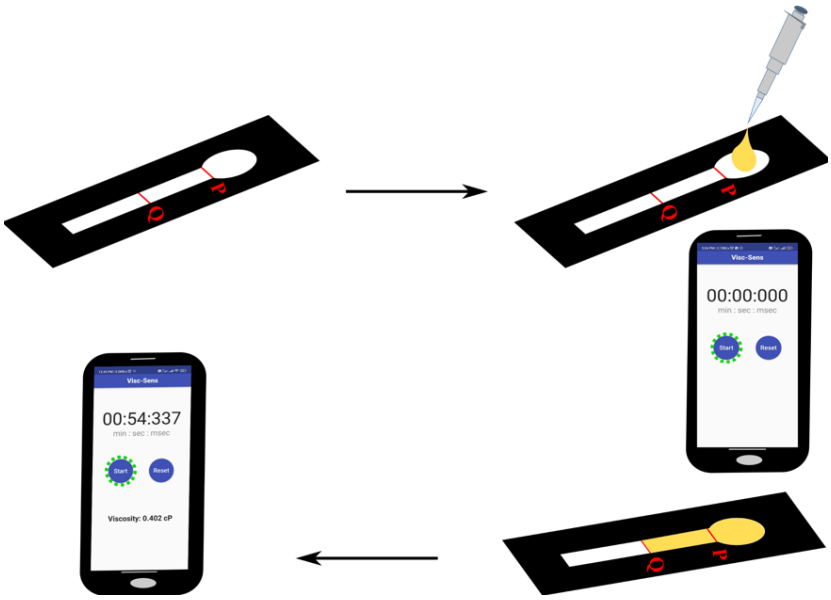

Figure 3.1 A schematic illustration depicts a paper-based chip designed for measuring plasma viscosity. In the initial step, plasma is pipetted into the inlet zone. Subsequently, the user opens the viscometer application on a mobile device and presses the start button as the liquid reaches point P. As the liquid arrives at point Q, the user then presses the stop button. The application calculates and displays the numerical value of the viscosity on the mobile screen.

In the next section a detail discussion has been covered on clinical background of plasma viscosity and various available measurement methods highlighted along with the shortcoming of the methods for implementation in resource limited setting.

3.2 Introduction

Human whole blood is a complex rheological fluid which constitutes namely two components cellular component and plasma. The cellular component consists of red blood cells (RBCs), white blood cells (WBCs), and platelets. Plasma is considered as a Newtonian fluid having a homogenous mixture of proteins [72]. The viscosity of plasma varies with the concentrations

of dissolve proteins. Therefore, plasma viscosity may be considered as a significant biomarker for the detection of several inflammatory diseases such as rheumatoid arthritis [73], cardiovascular disorders [74], autoimmune disease [75], ischemic heart disorder [76] etc. Though the normal value of plasma viscosity ranges from 1.3 to 1.7 cP at 25° C in healthy adults [72], in patients suffering from hyper viscosity syndrome it can even rise to 3 cP [77]. Patients suffering from hyper viscosity syndromes may indicate one or more symptoms including anaemia, high erythrocyte sedimentation rate (ESR), red blood cell aggregation in the smear, bone marrow infiltration, significant paraproteinemia, elevated serum and plasma viscosity, elevated whole blood viscosity, cryoglobulinemia, increased plasma and blood volume, prolonged bleeding time, decreased platelet adhesion, and renal disturbances [77], [78]. It is well-established fact that for patients suffering from hyper viscosity syndromes ESR and plasma viscosity rise simultaneously. The ESR value depends on several factors such as haematocrit, age, gender etc., however, plasma viscosity is independent of all these factors [76]. Therefore, for such patients measurement of plasma viscosity may provide more appropriate clinical information than the ESR measurement [79].

Traditionally, plasma viscosity is measured either using a capillary tube viscometer or a cone and plate viscometer [80]. In a capillary tube viscometer, the fluid passes through a narrow orifice and the flow rate of the fluid is directly correlated with the viscosity of the passing fluid. However, in cone and plate viscometer, the rotational resistance of the fluid sample provides information of the viscosity. The traditional viscometers are suffering from one or more of the following shortcomings: (a) requirement of expensive instruments, large sample volume and highly skilled human resources, (b) non-transportability, (c) non-disposable nature of sample holder, and (d) chances of sample contamination due to manual cleaning process, and therefore increased health risk for operators [81].

To overcome the shortcomings of conventional viscometers, several alternative methods were developed to measure the viscosity of plasma. For example, Lee et al. [82] proposed an electro fluidics circuit-based viscometer for Newtonian and non-Newtonian fluids. This device exploits the flow resistance of the testing sample to measure the viscosity under various shear rates (3-500 s^{-1}) and temperature (4-70° C) with a small sample volume (less than 400 µL). However, this viscometer requires a syringe pump to transport the sample along the channel which makes the device unsuitable for its deployment as a POC device. Srivastava et al. [83] developed a self-calibrating, robust and simple viscometer to measure the viscosity of biological fluids such as urine, blood, and blood plasma. The device works on the principle of capillary pressure-driven flow inside the microchannel. Similarly, Han et al. [84] proposed a polydimethylsiloxane (PDMS) based microfluidic device for measuring the viscosity of Newtonian fluids, exploiting the travelled distance and flow velocity of sample and reference fluids. However, the requirement of a costly microscope for data acquisition makes them unsuitable for resource-limited settings. Some other literatures are also available which exploits flow rate [85], damping parameter [86], changes in flow pattern [87], [88], and particle diffusion pattern [89] to estimate the viscosity of the test sample. Microelectromechanical systems (MEMS)-based micro viscometers have also been developed using vibrational, thermally actuated, and rotational techniques to measure viscosity [90], [91], [92]. However, these techniques are not suitable for POC settings due to lack of repeatability, complicated operating procedure, and elevated cost due to the requirement of intricate and expensive equipment. Additionally, all the above-mentioned devices require sophisticated data acquisition systems as well as skilled technicians for operating such devices.

An innovative point-of-care (POC) platform known as a microfluidic paper-based analytical device (µPAD), first shown by the Whiteside group in 2007 [93], has gained significant interest as a potential replacement for traditional analytical devices [94], [95], [96], [97], [98]. Paper-

based devices have shown their popularity in several applications including chemical [99], biochemical [100], environmental, food [101], and clinical diagnostics [102] due to low-cost, transportability, easy-to-use and fabrication as well as self-pumping property [103]. These devices have provided several benefits due to the use of cellulose paper as a substrate, including extremely low-cost, ease of production, disposability, and biocompatibility [98], [104]. Furthermore, as the fluid is transported by capillary force in the paper substrate, there is no need for any external machinery for fluid transport [105], [106]. These unique characteristics of paper-based devices enabled them to serve as a portable technology for several applications such as serum protein [107], antigen [108], bacteria [109], metals [110], and enzymes [111]. Recently, some researchers have developed paper-based devices to measure the viscosity of the fluids [112], [113], [114]. They used the Lucas-Washburn equation [115] to track the imbibition length with respect to time for analysing the viscosity of the fluid. Li et al. and Elizalde et al. [112], [113] demonstrated that viscosity could be measured by tracking the flow rate of liquid on a paper substrate.

Jang et. al.[116] have demonstrated a fast flow microfluidic paper-based analytical device (μPAD) to measure the fluid viscosity using two papers and double-sided adhesives which ensures the requirement of a complicated fabrication technique. However, their devices require higher sample volume (100 μL) as well as complicated measurement and data acquisition technique, which makes them unsuitable for field application. Off late Rayaprolu et al. [114] have miniaturized the conventional capillary viscometer on paper-based platform to measure the viscosity of several fluids including saliva, glycerine, sucrose, bovine serum albumin, lysozyme, and dextran. However, this device needs the densities of the test liquid and a reference fluid to measure the viscosity, an additional instrument is required to measure the density of the liquid which make this device unsuitable for measurement of viscosity in a single step, especially in field-settings. Additionally, they had also not used real blood plasma

samples. On the other hand, our developed device obviates the need of density measurement and reference fluid and also requires lesser sample volume (50 μL). Here, we measure the viscosity of real plasma samples by measuring the time taken by the test liquid to travel a certain distance on the paper-based device. The accuracy of the developed device is improved by carefully optimizing the all-flow rate related parameters such as sample volume optimization, channel length and width. In the nutshell, we can say that our device measures the plasma viscosity using a smartphone app which makes it convenient to use in resource-limited settings and under-developing countries.

Herein, we develop a simple, inexpensive, portable and disposable microfluidic paper-based viscometer (μPV) for the measurement of plasma viscosity using only 50 μL blood plasma. First, we load a 50 μL of blood plasma onto the device, and record the time taken by the plasma to travel a distance of 30 mm on a 4 mm wide channel made of Whatman cellulose filter paper (Grade 4) and estimate the viscosity of the sample using Visc-Sens smartphone app. Further, the accuracy of the device was measured by comparing the viscosity values obtained with our device and the gold-standard data obtained from the Brookfield viscometer. An excellent correlation was found between the values measured by both methods with a coefficient of determination (R^2) of 0.9644. The proposed device covers the entire clinical range of blood plasma viscosity (0.9~4 cP) and can easily distinguish even a small difference of 0.13 cP in the viscosity value. Hence, our developed device renders not only a portable low-cost miniaturised platform but also a device with uncompromised accuracy which can easily be deployed for POC diagnostics among underserved population in resource-limited settings.

3.3 Materials and instruments

Glycerol solution (99% Pure) was purchased from Thermo Fisher Scientific, India. Whatman cellulose filter paper (Grade 4, GE Healthcare, UK) was purchased from Sigma Aldrich. De-Ionized (DI) water was used to prepare glycerol solutions ranging from 0 to 40%. Digital Brookfield viscometer (DV1LV1.0.3, Spindle CPA422) was used to measure the viscosity of glycerol and blood plasma samples. A LaserJet printer (MFP M233 DW, HP) was used for preparing the channel on the filter paper. A hot plate (Digital Spinot, Tarsons India) was used for the post-heat treatment of the patterned channels.

3.3.1 Selection of cellulose paper, design and fabrication of the device

Paper substrate shows good wettability with biological samples in which the transportation of the fluid is governed by capillary action [117]. Various grades of filter papers are available for quantitative assay of biological samples including Whatman cellulose filter paper grade 1, grade 2, grade 3, grade 4, and so on. In the present study, we mainly focus on the flow behaviour of fluid for which grade 4 filter paper is the best choice [118] due to its relatively higher porosity compared to other filter papers. We used Whatman cellulose filter paper grade 4 for device fabrication. The channels were designed using Inkscape and printed on filter paper using a commercially available LaserJet printer. After printing the design, the pattern was heated on a hotplate at 150^0 C for 4-5 minutes [118]. At this temperature, toner particle melts and absorbs across the porous matrix of the paper and creates a strong hydrophobic barrier. Further, a piece of transparent tape was applied at the backside of the device to provide mechanical strength and to make it leak-proof. However, the same tape was also applied at the top of the device excluding the sample injection zone to minimize the evaporation effect of the fluid due to environmental conditions such as humidity. The design and specification of the device are

depicted in ***Figure 3.2***. The printing and heating are two crucial steps involved to fabricate reproducible devices. Therefore, we have optimized the heating process and analyzed the geometrical parameters such length, width, and area of circular inlet zone. We have designed the device in Inkscape, printed it. Further, we heated the device at 180 °C for a time interval of 4-5 minutes. The device was scanned after printing and heating process, respectively. The uncertainty in the dimensions of fabricated devices can be calculated in term of initial and final dimensions by Eq. 3.3.1. Six devices fabricated on different days were selected for estimating uncertainty. **Table *3-1*** shows the absolute value of uncertainty. The average value of uncertainty in length, width and circular area is found to be 0.30% and 2.75 %, and 0.48%, respectively.

$$|\% \in| = \left|\frac{FD-ID}{ID}\right| \times 100 \qquad 3.3.1$$

Table 3-1 Uncertainty analysis due to device fabrication process

Device	**Circle area (mm^2)**		**Absolute relative error (%)**	**Width (mm)**		**Absolute relative error (%)**	**Length (mm)**		**Absolute relative error (%)**
	Initial	**Final**		**Initial**	**Final**		**Initial**	**Final**	
1	113.09	112.24	0.73	4	3.94	1.60	30	30.12	0.40
2	113.09	113.29	0.18	4	4.12	3.02	30	30.15	0.52
3	113.09	113.59	0.44	4	4.13	3.25	30	30.13	0.45
4	113.09	112.81	0.25	4	3.89	2.75	30	29.97	0.09
5	113.09	112.46	0.56	4	3.90	2.47	30	29.94	0.18
6	113.09	112.25	0.74	4	4.14	3.42	30	29.96	0.14
		Mean	**0.48**			**2.75**			**0.30**

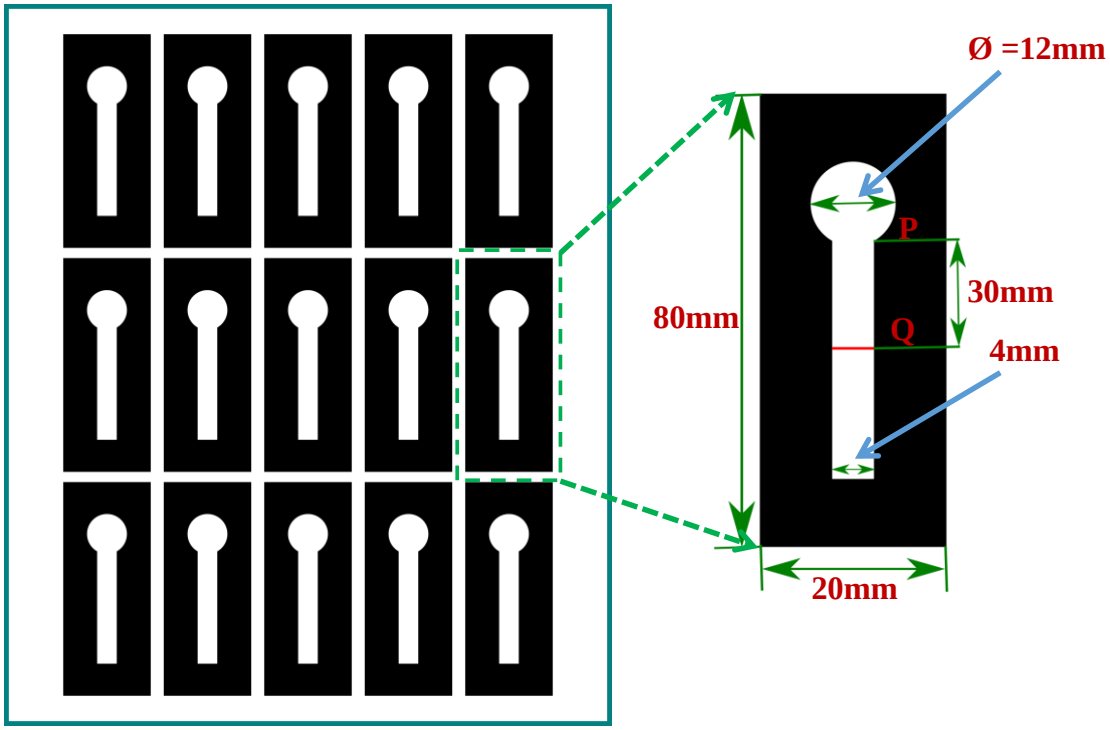

Figure 3.2 Schematic representation of µPAD. The design was printed on Whatman cellulose filter paper and heated at 150° C for 4-5 minutes to create a hydrophobic boundary. The µPAD is a rectangular chip with length and width of 80 mm and 20 mm respectively. The µPAD have a microchannel in the middle which is a combination of a rectangle of 4 mm in width and a circle of diameter of 12 mm.

3.3.2 Blood sample collection and processing

All blood samples were collected in vacutainer (K_2EDTA) tubes from the in-house hospital of Banaras Hindu University (BHU), Varanasi (India) after obtaining the requisite clearance from the ethical committee of the Institute of Medical Sciences (BHU), Varanasi (India). Further, we separated plasma from the collected whole human blood using a mini-centrifuge (6000 rpm, 5 minutes) and stored it at 4° C. The separated plasma samples were used for further experimental studies.

3.4 Experimental methodology

3.4.1 Measurement of viscosity with Brookfield viscometer

We used a range of glycerol solutions to cover the overall range of Plasma viscosity (1.06 - 3.02 cP). The viscosity of the glycerol solutions was measured using a Digital Brookfield viscometer. The viscosity of the glycerol solutions was listed in **Table *3-2***. Brookfield viscometer works on the principle of rotational viscometry, which consists of a spindle and a cup. The fluid whose viscosity has to be measured is placed inside the cup and the spindle is submerged in the fluid cup. A torque is applied on the spindle to measure the viscosity of the fluid. It is to be noted that one mL of sample is required for the measurement of the viscosity using this viscometer. All the measurements were taken thrice at 100 rpm and 25° C. The average value of all three measurements was taken to obtain the final value of viscosity. These measured values were considered with gold standard results and used for the comparison with the viscosity measured with our developed paper-based device.

Table 3-2 Values of viscosity of standard glycerol solutions obtained from Brookfield viscometer at 25 °C

Sl. No.	Glycerol %	Viscosity (cP)
1	0	0.82
2	5	0.95
3	10	1.11
4	15	1.31
5	20	1.56
6	25	1.88
7	30	2.29
8	35	2.83
9	40	3.55

3.4.2 Optimization of geometric parameters

The flow rate of fluid on the paper surface depends upon the viscosity of fluid, environmental conditions (relative humidity, temperature etc.) and design parameters such as sample volume, the width of the channel, length of the channel, and porosity of the substrate. Therefore, the design parameters playing a crucial role in developing any paper-based device are needed to be optimized to perform experiments.

3.4.2.1 Optimization of imbibition length

To optimize the imbibition length, three channels of dimensions 3 mm ×60 mm, 4 mm ×60 mm, and 5 mm×60 mm were used to measure the flow rate of 0% and 20% glycerol solutions and each set of experiment was performed five times. Initially, 45 µl of the sample was injected inside the circular reservoir and the phenomenon was recorded with help of a Camera (EOS 1500D, Canon). The images at each second were extracted from the video and imbibition length was measured with ImageJ software. It is very well-known fact that the fluid travels in porous media via capillary action, however, the wetted front velocity started to slow down as time passes due to resistance force (viscous drag) offered by interaction between the saturated wetted front and the porous substrate [119]. Same phenomenon has been depicted in ***Figure 3.3*** for 3 mm, 4 mm, and 5 mm wide channels, the wetted front velocity started to slow down after 15 mm length for different viscosity fluid samples (DI and 20% glycerol solution). The flow rate is almost constant before and after 30 mm length for both 3 mm, 4 mm, and 5 mm wide channels and travel time difference between various viscosity samples is also easily distinguishable. If a longer length is considered for the assay, the travel time increase drastically which may go beyond the time prescribed by the ASSURED criteria of World Health

Organization. Therefore, a 30 mm imbibition length was considered for channel width optimization as discussed in the next section.

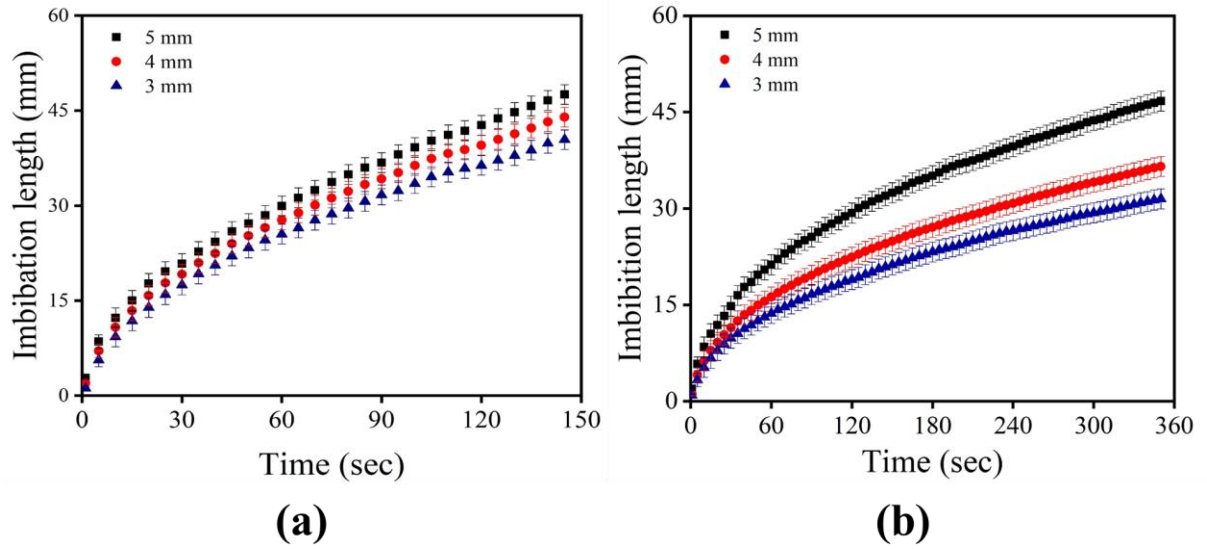

Figure 3.3 Variation of the flow rate for 3mm, 4mm, and 5 mm wide channels at 25 °C and 70 % RH for (a) DI water, (b) 20% glycerol solution. Each data set represents the mean value and error-bar shows the standard deviation of all five repetitive experiments

3.4.2.2 Optimization of channel width

In case of fluid flow in the paper-based microfluidic channels for constant length, the flow is primarily endeavored by two resistance forces – (1) internal resistance between fluid layers, and (2) drag force applied through hydrophobic boundary which is inversely proportional to channel width [120]. The combined effect of these two forces are used to decide the optimum width of the channel. In case of a 3 mm wide channel combined effect of resistance forces are significantly higher for highly viscous fluid and that is why it takes longer time to perform the assay. While in case of a 5 mm wide channel combined effect of resistance forces are lower for

low viscosity fluids, which results in a very less time difference and makes it difficult to predict the viscosity. However, in case of a 4 mm wide channel travel time difference from lower to higher viscous samples were easily distinguishable. Initially, a 45 µL sample was deposited onto the channels and time was recorded for 30 mm imbibition length and each set of experiment was repeated for five times. It was found that the time taken by the samples to travel the said distance reduces with increasing channel width ***Figure 3.4*** *(a)*. However, it can also be observed from ***Figure 3.4*** (b) that there is no significant difference in the travelled time for the fluids having viscosity values in the lower range (0.8 – 1.2 cP) for a 5 mm wide channel. Therefore, a 4 mm width is considered as an optimal width, despite 5 mm channel having a lower time to travel 30 mm length.

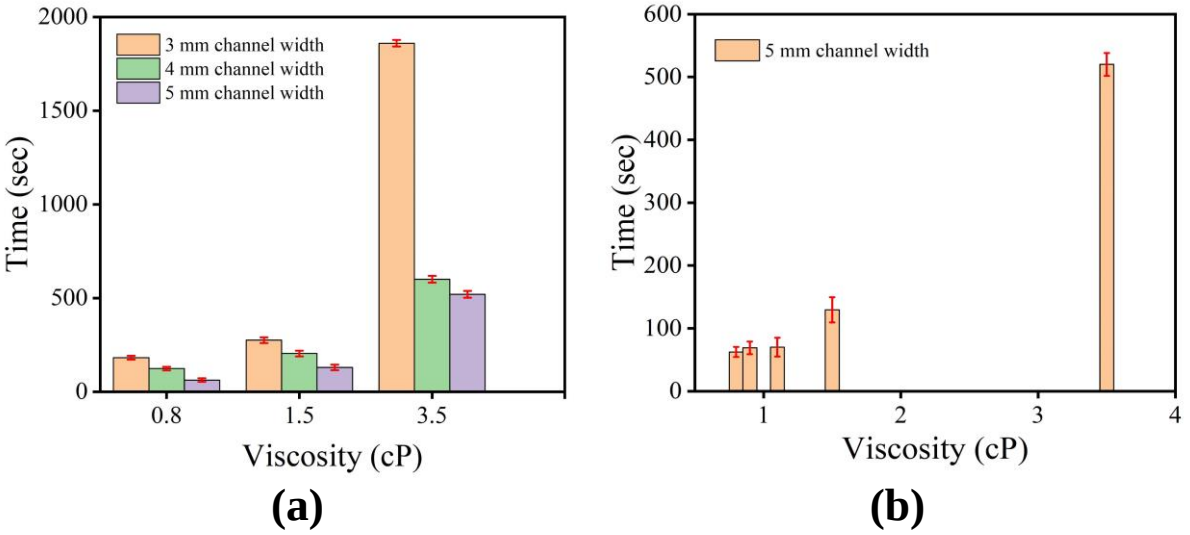

Figure 3.4 Variation of travel time with samples of different viscosities (DI, 20 and 40 % glycerol solution) to travel a fixed 30 mm imbibition length (25 °C, 70% RH) in (a) 3, 4, and 5 mm wide channels, and (b) 5 mm wide channel. Each data set represents the mean value and error-bar shows the standard deviation of all five repetitive experiments.

3.4.2.3 Sample volume optimization

Sample volume reduction is one of the crucial factors to minimise the uneasiness to the patients during testing.

However, a smaller sample volume may lead to more error in the measurement of the sample volume delivered to POC devices, while a larger sample volume may encourage bulk flow along the strip rather than capillary action, which could result in poor precision [121]. Therefore, the sample volumes that were examined here needed to be precisely optimised for measurement. From the preliminary study, the optimum sample volume seems to lie between 40 and 60 µL. **Table 3-3** lists the analytical parameters of the sample volumes of 30, 40, 50, 60, and 70 µL that were examined at three glycerol percentages (0, 20, and 40 %) for a 30 mm travel length. Each set of experiments was repeated 5 times. The sample volume is optimized on the basis of sensitivity (slope), linearity (R^2) and precision (% CV). A volume would be optimum corresponding to the higher magnitude of the slope (>1), high linearity (~ 1), and high precision (Low value of % CV). It is clear from the Table 3 that all sample volumes show excellent sensitivity. Therefore, best combination of linearity and precision are the concerning parameters in deciding the optimum sample volume. A 30 µL sample has demonstrated acceptable sensitivity (slope = 12.36) but comparatively inferior goodness of fit (R^2 = 0.9318) as well as less precision (CV = 13.0302%). However, 40, 60, and 70 µL samples achieved both acceptable sensitivity and precision but inferior goodness of fit when compared with 50 µL sample volume. Therefore, a 50 µL sample volume seems to be the best combination for sensitivity, the goodness of fit as well as precision.

Table 3-3 Sample volume optimisation data for a 4 mm wide channel at a 30 mm distance from the beginning of the rectangular section

Sample volume	Slope	R^2	% CV
30	12.80	0.932	13.030
40	12.81	0.931	9.678
50	8.45	0.999	4.932
60	12.36	0.918	5.345
70	12.06	0.867	8.825

On the basis of above studies, it can be concluded that 30 mm imbibition length, 4 mm wide channel, and 50 µL sample volume are the optimum parameters to perform further experiments for measuring plasma viscosity.

3.4.3 Measurement of viscosity using µPV

When a blood-plasma sample comes into contact with the paper surface (artificial surface), surface tension force supports the capillary action and drives the flow in the forward direction. The movement of fluid through porous media is primarily capillary driven flow. The imbibition of fluid into the pores structure occurs under the action of capillary pressure. The imbibition length of the fluid majorly depends on fluid property, pore size of paper substrate, interface tension and interaction between solid and fluid. Generally, lateral fluid flow in porous media is governed by two opposite forces - capillary and viscous forces. The variation of imbibition length is described Lucas-Washburn equation [122], [123], which is mathematically represented by (Eqn 3.4.1).

$$l = \sqrt{\frac{2\gamma cos\theta\ t}{4\mu}} \qquad 3.4.1$$

Where l the capillary length travel by the fluid, γ is surface tension of the fluid, θ is the contact angle of the fluid with porous surface, t is the travel time, and μ is the fluid viscosity. Accordingly, in the present work viscosity of the fluid is correlated with travel time of the fluid by keeping all other parameters constant.

First of all, we put our device (channel width 4 mm) inside a horizontally placed petri dish. The petri dish, containing a circular opening on the top surface, was kept on the hotplate to maintain a constant surface temperature (25° C). A 50 μL sample was injected into the circular reservoir of the device through the opening of the petri dish. The sample starts travelling along the rectangular section of the device and the time taken by the sample to travel a fixed distance of 30 mm between sections P and Q (***Figure 3.5***) was recorded and corresponding value of viscosity was displayed using an in-house developed smartphone app "Visc-Sens". The app records the time duration taken by the sample between point P and Q by pushing the start button to start the timer when sample reaches at point P and stop button to stop the time when sample reaches at point Q. Further, the app estimates the value of viscosity using calibration equation. The sequential operation of our developed μPV is illustrated in ***Figure 3.5***.

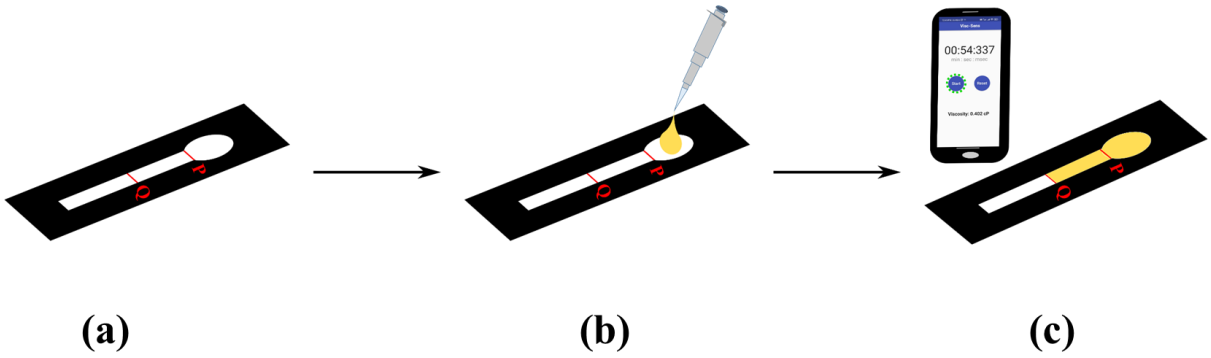

Figure 3.5 Sequential representation of experiment steps: (a) Fabricated device placed horizontally, (b) Injection of sample on the circular reservoir of the device, (c) Measurement of time travelled by the sample from point P to Q using Visc-Sens app.

3.5 Results and discussion

3.5.1 Performance analysis of the developed paper-based viscometer against the Brookfield viscometer

A calibration curve was constructed by plotting the viscosity of the glycerol samples on the horizontal axis and the average travelled time on the vertical axis (***Figure 3.6****(a)*). Average travelled time shows a high degree of correlation with the viscosity (Observed time =164.28 × (viscosity) – 12.117; $R^2 = 0.989$).20 blood plasma samples were used to check the accuracy of the developed device. The viscosity of the plasma samples were estimated using the developed paper-based device and compared with the viscosity measured by the Brookfield viscometer. The values of viscosity obtained from µPV show a high degree of correlation ($R^2 = 0.964$) with the values measured using the gold standard device (***Figure 3.6****(b),* red line) when the least-squares analysis was performed. The values of these two measurements are closely scattered around the y = x line (***Figure 3.6****(b),* inset) in the physiological range, indicating an excellent accuracy ($R^2 = 0.9644$) of viscosity values obtained from paper-based device with the

Brookfield value. The accuracy for blood plasma viscosity measurement using our developed device and gold standard data is shown in **Table *3-4***. The median of the accuracy obtained with our device is 97.23.

A Bland-Altman plot was constructed to assess the agreement between the developed device and the gold standard device (Brookfield viscometer) as shown in **Figure 3.6***(c)*. In the Bland-Altman plot, the mean of the two methods is plotted on the horizontal axis whereas the difference of both methods is plotted on the vertical axis. The bias and error in the measurement were estimated by calculating the mean and standard deviation of the difference of values measured by both methods. The bias and error were found to be - 0.0462 cP and 0.0553 cP, respectively. It can be observed from **Figure 3.6***(c)* that most of the observed data lie between limits of agreement (lower limit of agreement = - 0.1545, upper limit of agreement = 0.0622) for a 95 % clinical confidence interval. Therefore, our low-cost portable yet accurate µPV has the true potential to replace the expensive Brookfield viscometer.

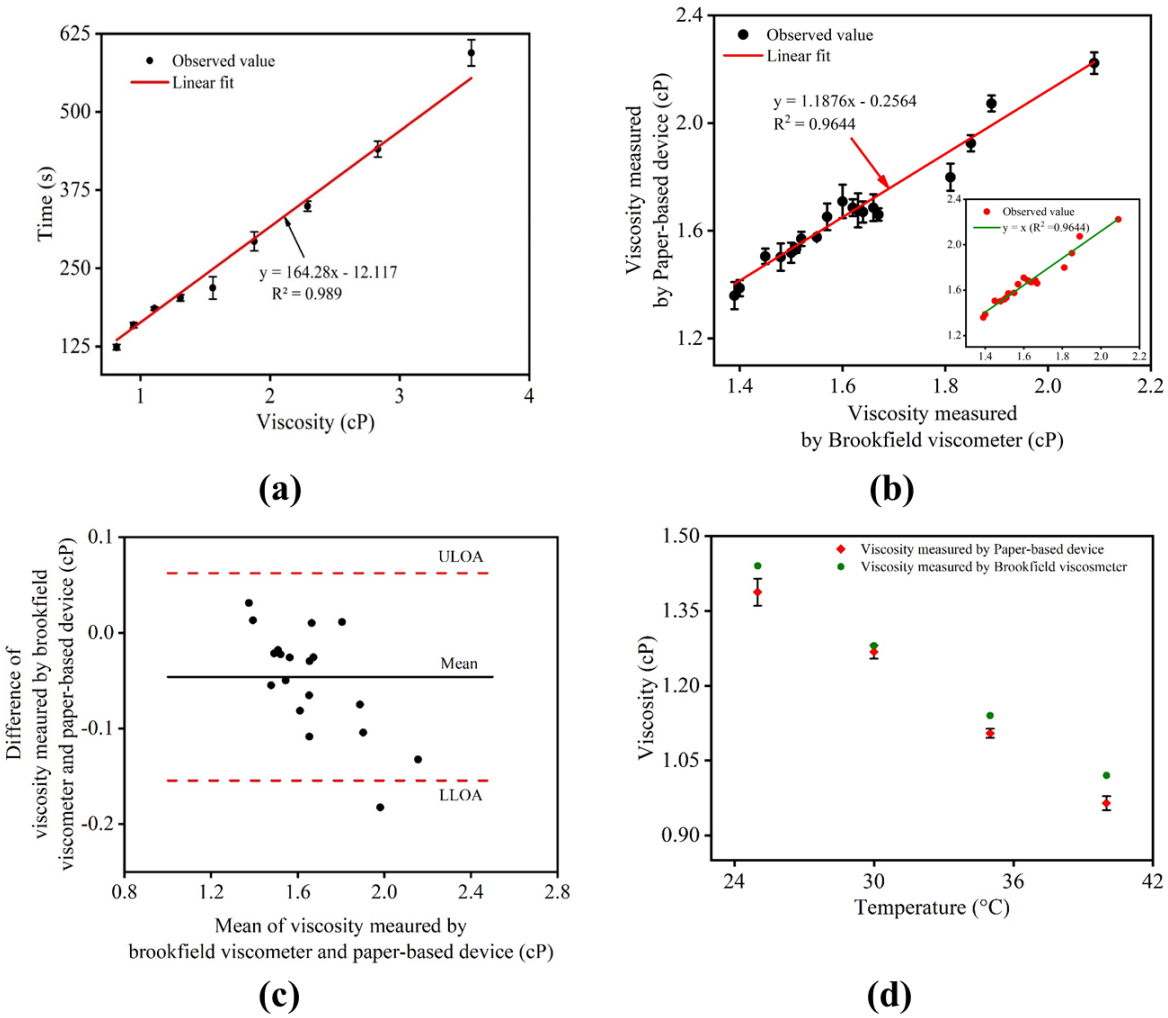

Figure 3.6 (a) A calibration curve was constructed by plotting observed time against the viscosity of standard Glycerol solution at 25° C. Each set of experiments was repeated five times. The average value was used for the calibration curve and the error was shown by standard deviation. Comparison of the viscosity measured by µPV with viscosity reported by Brookfield viscometer (n = 20 samples): (b) Linear least-square regression analysis of the correlation between the viscosities values measured by both the methods, (c) Bland-Altman plot was constructed by plotting difference of the two methods against the mean of the two methods. The bias (mean) and standard deviation of the differences are - 0.0462 cP and 0.0553 cP. The lower limit of agreement (LLOA) and upper limit of agreement (ULOA) are - 0.1545 and 0.0622, respectively. (d) The viscosity of the 15 % glycerol solution was measured at 25,

30, 35, and 40° C using both a standard Brookfield viscometer and our proposed device. Each measurement was repeated five times. The error bar is shown for five data points of every sample.

Table 3-4 Accuracy table for blood plasma viscosity measurement using our developed device and gold standard data

S.N.	Measured plasma viscosity value using the developed device (cP)	Plasma viscosity measurement using the gold standard device (cP)	Accuracy of our developed method	Median
	1.36	1.39	97.76	
2	1.39	1.4	99.07	
3	1.50	1.45	96.20	
4	1.51	1.48	98.52	
5	1.52	1.5	98.79	
6	1.53	1.51	98.51	
7	1.57	1.52	96.71	
8	1.58	1.55	98.32	
9	1.65	1.57	94.81	
10	1.71	1.6	93.20	97.23
11	1.68	1.62	95.95	
12	1.68	1.62	95.95	
13	1.67	1.64	98.18	
14	1.69	1.66	98.46	
15	1.65	1.67	99.40	
16	1.79	1.81	99.38	
17	1.92	1.85	95.94	

18	1.95	1.85	94.35
19	2.07	1.89	90.33
20	2.22	2.09	93.66

A chi-square test was used to assess the agreement between the measured value and the gold-standard value for 20 data points. Here, the agreement between both methods was considered as a null hypothesis. For 5% level of significance, the critical chi-square value observed from the chi-square distribution table is 30.144 which is much lesser than the calculated value (0.0588), indicating that the null hypothesis may be accepted at this level of significance.

3.5.2 Effect of temperature on Plasma viscosity

Inside the human body, the fluid flows at 37° C, hence, it is required to observe the effect of temperature on the viscosity of the sample. It is a well-known fact that the viscosity of liquid decreases with a rise in temperature which leads to a fast flow of fluid over the paper surface and subsequently reduces the travel time. The standard viscosity of 15% glycerol solution was measured at 25, 30, 35, and 40° C using a Brookfield viscometer. The viscosity of the same solutions was also measured with our paper-based viscometer at the same temperatures. The specific temperature was maintained using a hot plate. The variation of the viscosities with temperature is shown in ***Figure 3.6****(d)*. The values of the viscosity obtained from both devices are shown in **Table *3-5***. The viscosity measured with the paper viscometer is slightly lower than the value obtained from the Brookfield viscometer. This variation may be contributed due to the non-uniform temperature gain of the sample while moving forward in the channel. However, the variation in viscosity value obtained from our device is within the limits of agreement between the temperature range of 25° C and 40° C.

Table 3-5 Viscosities of 15 % Glycerol solution at different temperatures measured by both Brookfield viscometer and paper-based viscometer

Temperature (° C)	Viscosity measured by Brookfield viscometer (cP)	Standard deviation (cP)	Viscosity measured by the developed device (cP)	Standard deviation (cP)	% difference
25	1.38	0.0369	1.44	0.0047	3.648
30	1.27	0.02330	1.28	0.0082	0.97
35	1.11	0.0549	1.14	0.0124	3.11
40	0.96	0.0440	1.02	0.0082	5.44

3.6 Summary

We develop a low-cost and disposable paper-based device integrated with a simple smartphone app to measure the viscosity of blood plasma using a 50 µL sample volume. This device can measure the difference of 0.13 cP in viscosity value between 0.85 cP to 4 cP which covers the whole clinical blood plasma viscosity range. The effect of temperature on the viscosity of the 15 % glycerol sample is measured using both µPV and Brookfield viscometer. Our device renders similar values of viscosity against the Brookfield viscometer values with a difference of less than 6 %. Moreover, being a disposable device, it eliminates the manual cleaning process and chances of contamination while using the same for the next patient.

In short in in developed viscometer device there is no need of reference fluid, conventional viscometer, cleaning process, large sample requirement, and skilled operator as well as 98% accuracy as compare to gold standard method which makes it suitable option at resource constraint settings for viscosity measurement. For validation purpose we have used real sample

and clinical test required for acceptance of a new alternative device has been performed such as Bland Altman analysis and t test chi square test.

In a nutshell, our developed device measures the plasma viscosity with uncompromised accuracy with the engagement of minimally trained human resources in resource-poor settings.