CHAPTER 2: REAGENT-FREE HEMOGLOBIN ESTIMATION ON A SPINNING DISC

2.1 Chapter overview

This chapter describes the development of a low-cost reagent-free method for hemoglobin estimation on a simple portable microfluidic device that can be implemented in under-served locations without sacrificing the fundamental principle of direct evaluation of hemoglobin extracted from the human blood sample. Though there are many existing microfluidic assays for determining hemoglobin concentration, such assays use low volume of reagents due to which storage and stability of such reagents as well as supply chain management would become very difficult especially for field application along with the elevated cost of the blood test kit due to the presence of such costly reagents. In sharp contrast with reported findings, our blood test kit is reagent free and it uses DI water only which can easily be stored in plastic bottles. Exploring the fundamental principle of osmotic hemolysis using DI water for extraction of 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. The efficacy of the device has been justified by validating with established pathological gold standards. These results are likely to pave the pathway of establishing the paradigm of a first-principle-based reagent-free evaluation of blood pathology in health and disease.

2.1.1 Introduction

Quantitative estimation of hemoglobin (Hb) as an essential biomarker is critical for several physiological disorders, especially untreated anemia that remains to be widely prevalent in the underserved population. The World Health Organization (WHO) defines the normal range of concentration of Hb, as 13.5 – 17.5 g/dl for males and 12.5 – 15.5 g/dl for females in

adults. In anemic patients, blood Hb concentration becomes less than 12 g/dl, whereas for severe anemia, it becomes less than 7 g/dl (Pagana and Pagana 2014). While Hb concentration is not a specific diagnostic marker, its value in an abnormal range may indicate different medical concerns and provides pointers for further examination. For instance, due to chronic blood loss, decreased red blood cell (RBC) production, or abnormal destruction of RBC (hemolysis), the concentration of Hb may decrease to a considerable extent, leading to severe anemia, which can be considered as a life-threatening condition (Aspuru et al. 2011; McLean et al. 2009; S. and G.E. 2012; Shander 2004). On the other hand, abnormally high concentrations of Hb levels may create different medical conditions such as congenital heart disease, polycythemia vera (Marchioli et al. 2013), severe chronic obstructive pulmonary diseases (COPD) (VICARI AURELIO M. et al. 1998), severe dehydration (Tefferi 2003), etc. Therefore, accurate quantification of Hb concentration turns out to be a critical proposition even for routine blood pathology examination.

The concentration of Hb in the blood is commonly measured by different methods such as automated hematology analysis, cyanmethemoglobin method, gravimetric copper sulfate method, and color code-based Hb estimation (Nkrumah et al. 2011; Sari et al. 2001). The hematology analyzers provide gold-standard estimations that are proven to be highly accurate. However, these are expensive and cannot be implemented at the extreme point of care in an effort to cater to the needs of the underserved population. The cyanmethemoglobin approach is cheaper compared to the automated hematology analysis but involves a laborious as well as a time-consuming approach. The gravimetric copper sulfate method and color code Hb estimation have been attributed with several shortcomings, challenging their accuracies in terms of quantitative predictive assessments (Paddle 2002). The direct spectrophotometric method, off late, has emerged as an alternative viable approach (Noe, Weedn, and Bell 1984). Electrochemical detection of Hb

using glassy carbon electrodes coated with Nafion layers has also been carried out recently (Toh et al. 2014). Carbon dots fluorescent probe has been used for conducting spectrofluorometric Hb detection from deionized (DI) water-diluted blood, offering an alternative principle (Barati, Shamsipur, and Abdollahi 2015).

The standard method for estimation of Hb is the measurement of venous whole blood obtained via venipuncture. Though this method has high sensitivity and specificity; it is invasive, time-consuming, as well as requires skilled technicians for the collection of blood. Hence, few non-invasive techniques are proposed for the estimation of Hb but those have some limitations. The non-invasive method is conducted at a single site, and accordingly, environmental effects, such as temperature differences or activity of the clinic and the composition of skin color base in terms of ethnicity might affect the accuracy of the device under an uncontrolled environment (Chattopadhyay et al. 2021a; Kim et al. 2013; Sümnig et al. 2015). Therefore, in spite of the fact that the non-invasive Hb measurement is a reasonable first-line approach; it is not clinically approved at present. To circumvent these limitations, the level of Hb in human blood as collected by minimally invasive technique may be potentially used for routine blood pathology examination and further downstream analysis. Commercially available point-of-care (POC) devices, thus, have emerged as potentially viable alternatives, as a compromise of gold standard accuracy and ease of deployment in resource-limited settings (Medina et al. 2005). Paper-based analytical platforms, premised on colorimetric analysis, have also been introduced in practice (Yang et al. 2013) as well as rotational microfluidic devices also have emerged as an effective substitute for centrifugation for pathological diagnostics (Ducrée et al. 2007; Foudeh et al. 2012; Ho et al. 2018; Kar, Dash, et al. 2015; Kar, Ghosh, et al. 2015; Kar, Maiti, and Chakraborty 2016; Kiran Raj et al. 2018; Lai et al. 2004; Marc Madou et al. 2006; Robert Gorkin et al. 2010; Sanjay et al. 2015; Smith et al. 2016; Taparia et al. 2017).

One compelling constraint, however, that limits their use in practice is not merely associated with the cost, but more essentially related to the extremely unstable nature of the reagents used for undertaking the tests.

All direct methods currently being used for Hb level estimation necessarily demand specific reagents for the test, thereby introducing several limitations inhibiting extreme-point-of-care applications. The most compelling constraints, in that regard, turn out to be the reagent storage and supply chain management in resource-limited settings. Circumventing the above constraints, we report here a direct method of first of its kind that does not use any reagent except DI water for performing the necessary analytical procedure. This is coupled with an inherent capability of portable rotating platforms (Agarwal et al. 2020) to perform the essential complementary aspects of density-driven segregation, to realize sample to result integration. A major specific innovation here is that the device exploits advantage of the rotationally-driven separation of RBC from whole blood followed by hemolysis through DI water and the subsequent release of Hb without the consumption of any commercial reagent. The DI water can be stored in plastic bottles, which can easily be transported for field trial even in an uncontrolled environment with the help of unskilled technicians. The process is followed by a simple image analysis rendering on spot detection of Hb, leading to the development of a POC device. Our detections of Hb show excellent agreement with the results obtained from the automated hematology analyzer as used in gold-standard clinical settings.

2.2 Experimental details

2.2.1 Device design and fabrication

The fundamental principle of transfer of gold standard technique for estimation of Hb from pathological laboratory conditions to POC applications via microfluidic approach is

already established (Gilmore, Islam, and Martinez-Duarte 2016; Kong et al. 2016). The principle behind this estimation is the separation of RBC from whole blood by centrifugation followed by lysis of RBC with the mixing of detergent or buffer to release Hb in solution, followed by a colorimetric assay. The overall process i.e. loading of whole blood and detergent separately, mixing them, and subsequent reaction requires the accomplishment of complicated channel design for on-disk Hb assay, as per the existing paradigm (Steigert et al. 2006). In sharp contrast, here we eliminate some of the complicated steps of the above-mentioned analytical procedure, by harnessing reagent-free osmotic hemolysis of RBC by DI water, simplifying the assay design to a large extent.

The overall experimental process on a spinning platform is illustrated in Fig. 2.1. Fig. 2.1(A) depicts the typical layered structure of the portable spinning disc device. The top layer, of the diameter of 130 mm, is made by a 1 mm thick Poly-methyl-methacrylate (PMMA) sheet (Lexan, GE) containing the loading and pressure balancing holes with a radius of 1.75 mm.

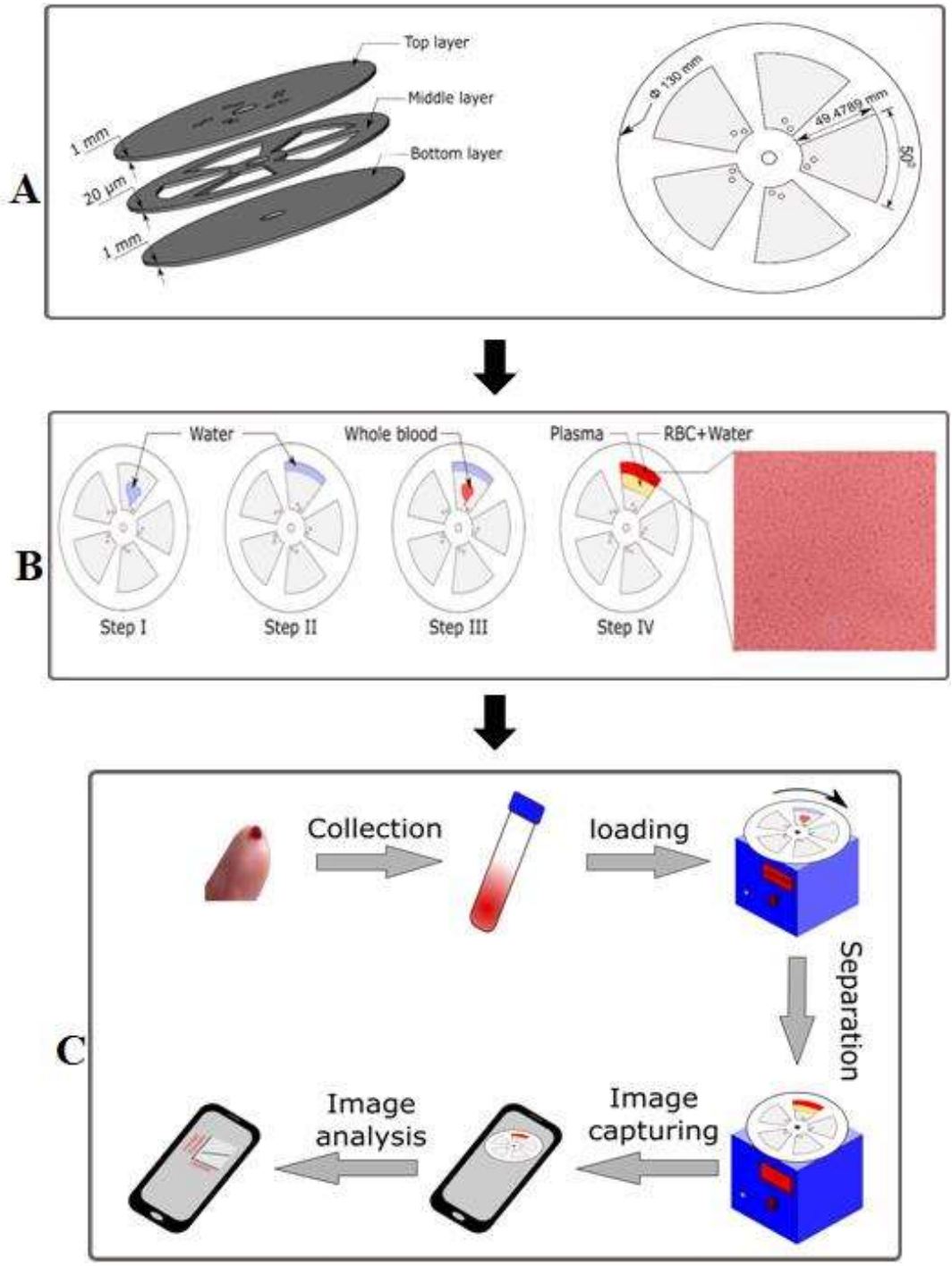

Fig. 2.1 Representation of the experimental process. (A) Design and specifications of the compact disc. Side view of the device showing thicknesses of the top, middle and bottom layers which are 1 mm, 20 µm, and 1 mm, respectively. The top view of the compact device showing the channel length which is 49.4789 mm, the angular span of each sector which is 50°, and the diameter of the disc which is 130 mm. (B) Stepwise procedure of sample processing in the spinning disc. Loading

of DI water in the microchannel (Step I); rotating the disc (at 1000 rpm for 1 min) for transporting water to the outer periphery of the microchannel (Step II); loading of whole blood in the same channel (Step III); rotating the disc (at 2000 rpm for 13 min) to accomplish the separation of RBC from whole blood (Step IV) and mixing with DI water. A magnified view of the RBC lysis in a microchannel is shown as a blown-up figure. (C) Schematic illustration of the process flow of Hb estimation in the present POC device. The essential steps include a collection of finger pricked blood into a tube pre-coated with an anti-coagulant, loading of blood in the micro-channel of the disc, separation of RBC due to centrifugation, and mixing with DI water. The rotational speed is displayed using a display unit to attain a precise setting. After osmotic hemolysis leading to the release of Hb, the colored image is captured using a smartphone for rapid analysis.

The middle layer is fabricated by using a 20 μm thick sheet of double-sided pressure-sensitive adhesive (PSA) (Flex mount DFM 200 clear V-95, 3M, Flexcon, Inc., Spencer, MA) containing the microchannels. Each channel is cut in a sector of 50° having inner and outer radii of 10 mm and 59.4789 mm, respectively. The bottom layer is made by a 1 mm thick PMMA sheet which forms the base of the microchannel and provides structural stability to the overall device. All layers are designed using AutoCAD software and thereafter, they are exerted on PMMA discs using a computer numerical controlled tabletop CNC machine (T-Tech Inc., QC 5000) using a carbide router tool of 1 mm diameter, whereas the PSA is cut by a vinyl cutter machine (Graphtec CE6000-60 Plus). After cutting, the three layers are aligned manually and pressed together to form the composite structure (Fig. 2.1(A)). As the sector angle is 50^0, a maximum five number of microchannels can be accommodated in the spinning disc. After that, the compact disc is pressed by a roller press laminator under the action of centrifugal forces to seal the disc which provides constraints against leakages in the microchannels.

2.2.2 Blood sample collection and processing

In our experiments, capillary blood samples through finger-prick were collected using a standard protocol from the patients (ages between 10-60 years) from the in-house hospital of the parent institute of the authors. Ethical consent was obtained according to the study protocol approved by the Institutional Ethical Committee of the Indian Institute of Technology Kharagpur. The hands of the patients were placed into warm water (37–38°C) for approximately 5 minutes. The fingertip skin was then punctured using a safety lancet. The first drop of blood was discarded to reduce excess tissue fluids. Aliquots of blood from subsequent drops were then collected into tubes pre-coated with anti-coagulant dipotassium ethylenediaminetetraacetate (K_2-EDTA), giving a minimum volume (0.25 ml) blood. If necessary, the hand was gently massaged downwards towards the puncture site to obtain the required volume (Hollis et al. 2012).

After collection, the samples were processed immediately. Before any experimental run, the sample was shaken to make a homogeneous suspension. We have used blood samples having a wide range of Hb concentrations (from 5 g/dl to 16 g/dl). The Hb content was declared by the pathology unit of the hospital using an automated hematology analyzer (KX-21N, SYSMEX) which is generally considered a gold standard device.

Lyophilized human hemoglobin and phosphate-buffered saline (PBS) tablet (pH 7.0) were used (Sigma Aldrich, USA) to prepare different Hb concentrations for the sole sake of constructing the calibration curve of our device. Once prepared, the calibration curve could be used subsequently without any further need for these reagents, for testing the device on human patients. DI water was used for the preparation of the buffer. The analysis is chemical-free where no reagent was used.

2.2.3 Numerical modeling

For studying the flow behavior of blood on the rotational platform (Fig. 2.2(A)), phase-field method coupled with continuity and momentum conservation equations for fluid dynamics was used. A commercially available finite element-based COMSOL Multiphysics 4.3a was used to perform two-dimensional simulations. In the phase-field method, a phase-field function ϕ is defined over the entire domain to track the interface. Under this method, two separate phases (blood and DI water) are defined by two distinct values ϕ: $\phi = -1$ for blood and $\phi = 1$ for DI water. The phase-field function is governed by the following equation:

$$\frac{\partial \phi}{\partial t} + \vec{v} \cdot \nabla \phi = \gamma_1 \lambda_i \nabla^2 \cdot \left[\nabla^2 \phi + \frac{\phi\left(\phi^2 - 1\right)}{\varepsilon^2} \right] \tag{1}$$

Where ε is capillary width that determines the thickness of interface, λ_i is the mixing energy density and γ_1 is the mobility parameter. The governing equations for fluid flow are as follows:

$$\nabla \cdot \vec{v} = 0 \tag{2}$$

$$\rho\left(\frac{\partial u}{\partial t} + \vec{v} \cdot \nabla u \right) = -\frac{\partial p}{\partial r} + \nabla \cdot \left(\tau_{rr} + \tau_{rz} \right) + F_c \tag{3}$$

$$\rho\left(\frac{\partial v}{\partial t} + \vec{v} \cdot \nabla v \right) = -\frac{\partial p}{\partial z} + \nabla \cdot \left(\tau_{rr} + \tau_{rz} \right) \tag{4}$$

The viscous stress $\left(\tau_{ij}\right)$ can be given as: $\tau_{rr} = \lambda\left(\nabla \cdot \vec{v}\right) + 2\mu \frac{\partial u}{\partial r}$, $\tau_{zz} = \lambda\left(\nabla \cdot \vec{v}\right) + 2\mu \frac{\partial v}{\partial z}$, $\tau_{rz} = \tau_{zr} = \mu\left(\frac{\partial v}{\partial r} + \frac{\partial u}{\partial z} \right)$; where u and v are the velocity components in the r and z directions, respectively, $\vec{v}$ is the velocity vector $\left(= u\hat{i} + v\hat{j}\right)$, λ is second viscosity

coefficient $\left(=-\left(2/3\right)\mu\right)$. The non-Newtonian behavior of the blood was modeled using power law relation (Chakraborty 2005) as $\mu = m\left(\partial u/\partial z\right)^{n-1}$ where m denotes the consistency coefficient and n is the flow behavior index. Further, on the rotating platform, the momentum equation contains an additional body force term $\mathrm{F_c}\left(=\rho_b r\omega^2\right)$, where F_c denotes the body force which is caused due to centrifugation of two fluids in this rotational microfluidic platform. Furthermore, the volume fraction of the second fluid (blood) was calculated as $V_f = \min\left(\max\left(\left(1+\phi\right)/2,0\right),1\right)$ and the effective density and viscosity were computed as $\rho_{eff} = \rho_1 + \left(\rho_2 - \rho_1\right)V_f$ and $\mu_{eff} = \mu_1 + \left(\mu_2 - \mu_1\right)V_f$, respectively. The subscripts 1 and 2 refer to fluid 1 (blood), and fluid 2 (DI water). For solving the above mentioned equations, we used the following boundary conditions: (i) open boundary conditions were assumed at inlet and outlet (ii) wetted wall conditions with no mass flux were imposed at the walls.

For optimizing the rotational speed and operational time, we have performed a series of simulations for different values of the disc-rotation speed, ω, ranging from 1000 to 3100 rpm. Initially, water is centrifuged till it reaches to the periphery of the channel. After that blood is loaded and due to centrifugation blood cells (RBCs) gradually diffuse into the water phase enabling the mixing of blood and water. When the diffusion of RBCs into the water phase would be complete, there will be complete mixing of blood and water facilitating osmotic hemolysis in a hypotonic solution. The time taken for blood to mix with DI water completely inside the channel has been considered as the separation time of RBCs due to density gradient. The mixing of DI water with blood at different time steps is illustrated in Fig. 2.2(B). Fig. 2.2(C) depicts the trend of the separation time with speed.

The decaying trend of normalized separation time with increasing values of ω reveals a saturation point at 2000 rpm (Fig. 2.2(D)), which indicates the optimum rotational speed.

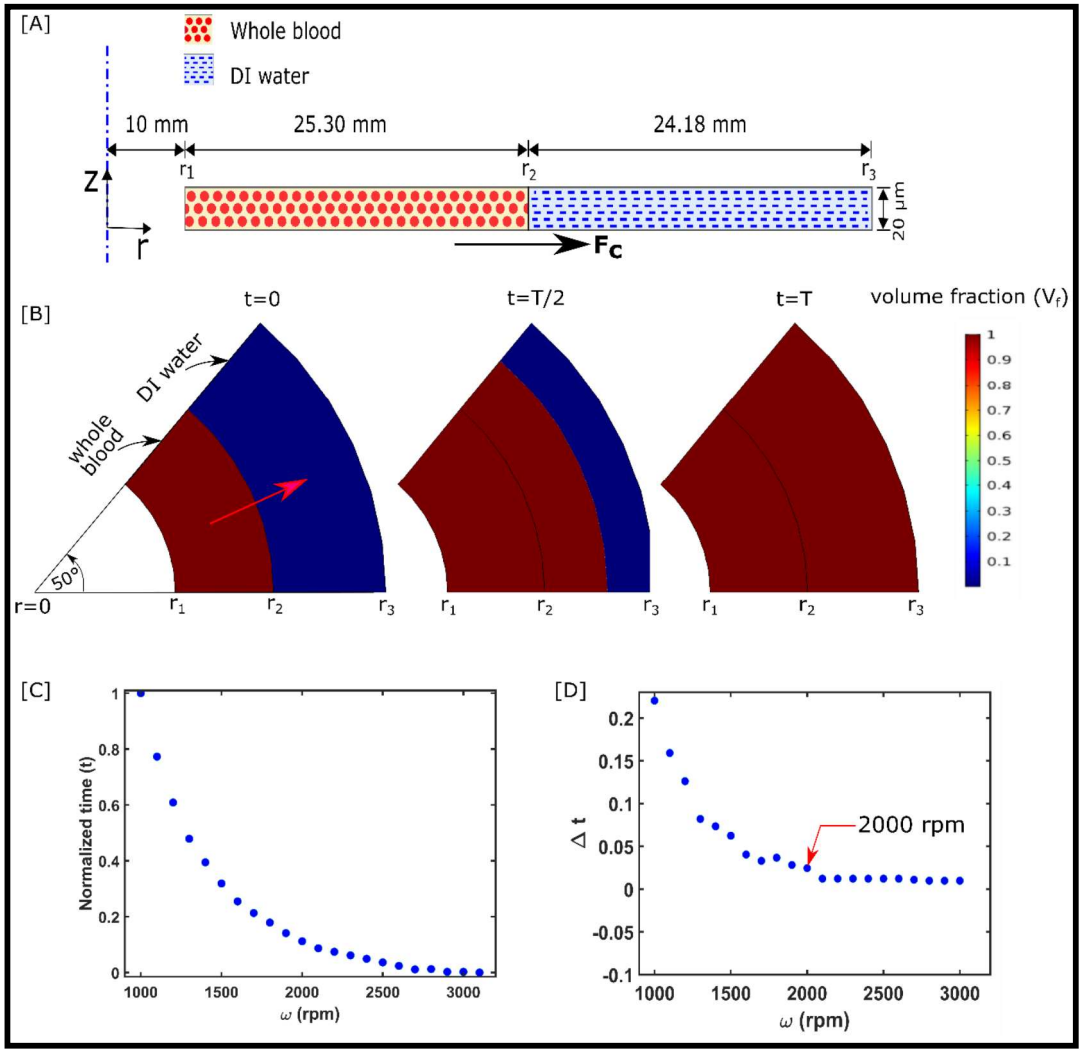

Fig. 2.2 Numerical simulation details. (A) Schematic representation of the computational domain used for the numerical simulation. The axis of rotation is along the z-axis at r = 0. The inlet of the micro-channel is at r_1=10. Initially, the whole blood is filled in the region ranges from r_1 to r_2 (25.30 mm in length), and DI water is filled in the region ranges from r_2 to r_3 (24.18 mm in length). The height of the entire micro-channel is 20 µm. (B) Mixing of blood with DI water inside the channel where T is the total time taken by the blood for complete mixing. (C) Variation of separation time

with angular speed. (D) Decaying trend of the normalized time with increasing value of rotational speed.

The time taken by the blood to mix with DI water completely inside the channel was found to be 7.25 minutes at 2000 rpm. However, it was observed experimentally that the process had taken another 5 minutes to push all RBCs to the channel end. Therefore, for the completion of the process, the device needs to rotate at optimum rotational speed and for a specific duration which was found to be 2000 rpm and 13 minutes, respectively.

2.2.4 Experimental setup and procedure

The fabricated disc was coupled to a rotational platform to spin at different speeds. The spinning unit was composed of a 360° rotational stage, driven by a DC motor which was controlled by microcontrollers. Subsequently, four simple steps were executed for separation and mixing (Fig. 1B). DI water was first loaded in one of the microchannels of the spinning disc (Step I). The disc was then rotated at 1000 rpm for 1 min for transporting water to the outer periphery of the microchannel (Step II). The whole blood was loaded on the same channel (Step III). The disc was rotated at 2000 rpm for 13 min to accomplish the separation of RBC from whole blood (Step IV) and subsequent mixing of the same with DI water.

The placing of RBC in DI water creates a hypotonic effect. Favoured by the selectively permeable membrane of RBC, water flows directly inside the RBC, passing through the cell membrane, which leads to cell swelling and rupture or lysis of RBC due to the resultant osmotic movement of water (Goodhead and MacMillan 2017). After osmotic hemolysis with DI water, Hb was extracted and images of the region-of-interest (ROI) were captured by a smartphone. Image processing by ImageJ software (version

1.51n) involved a series of image operations to enhance the quality of a digital image, by undergoing the process of distinguishing the object (ROI) from the background and producing quantitative information. The image was read by the ImageJ processor and converted to grayscale. Once the grayscale image was obtained, the spatial image filtering technique was applied for modifying or enhancing an image. Image intensity transformation was used to increase the contrast among certain intensities. Finally, the image was calibrated and analyzed with ImageJ for the estimation of Hb concentration (Li et al. 2020; Maejima, Hiruta, and Citterio 2020; Misawa et al. 2020). The overall clinical assay of Hb estimation on a portable spinning unit is illustrated in Fig. 2.1(c).

2.3 Results and Discussions

2.3.1 Extraction of Hb from RBC and optimization of the volume of DI Water

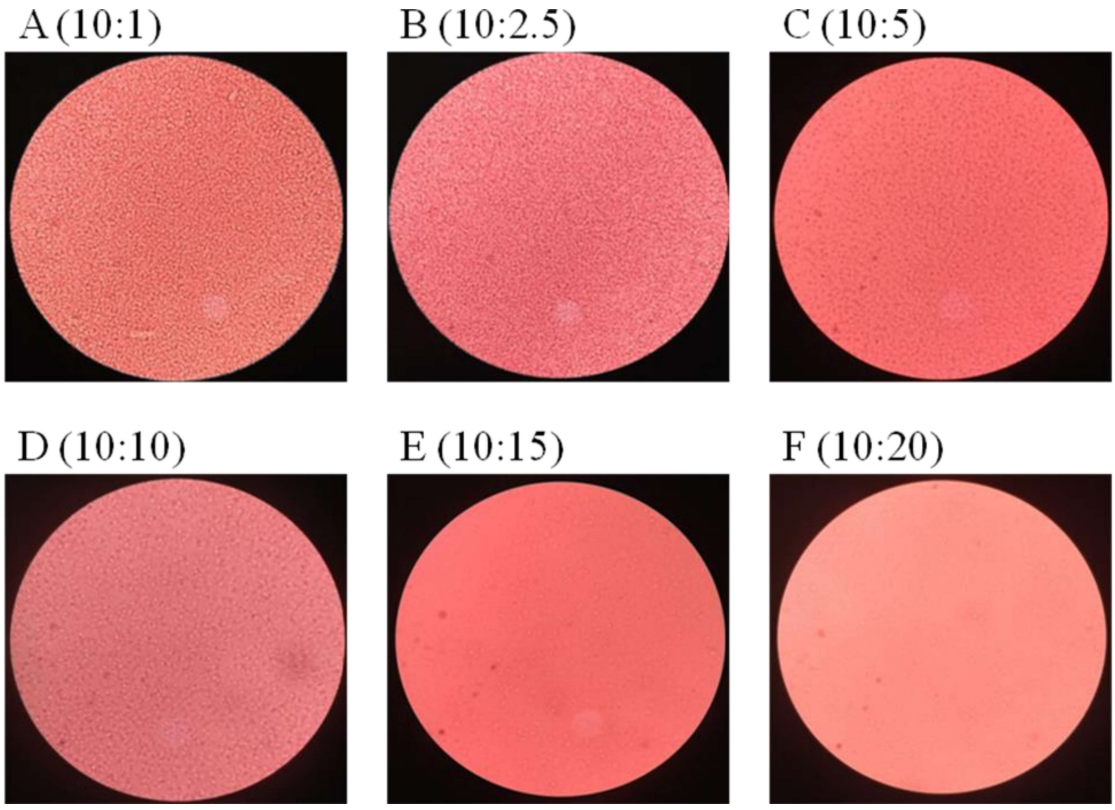

Fig. 2.3 Optimization of the volume of DI water for osmotic hemolysis of RBC. Blood has been mixed with DI water at 10:1, 10:2.5, 10:5, 10:10, 10:15, and 10:20 ratios (vol/vol) which are represented by images (A) to (F) respectively.

Numerical modeling along with experimental studies were carried out to investigate optimal rotational speed and time to ensure complete mixing of red blood cells and water. At first, water was centrifuged to the periphery of the spinning disc which was demonstrated in step II of Fig. 2.1(B). Owing to higher density rather than other components of blood, RBC reaches the periphery and starts mixing with DI water. The time taken for complete mixing of RBC with DI water varies with rotational speed.

Accordingly, rotational speed and time were optimized using the Phase Field Model which was depicted in Fig. 2.2. In this process, the mechanism of osmotic hemolysis for the extraction of hemoglobin from red blood cells can also be interpreted. As deionized (DI) water is devoid of any ions, it is a very hypotonic medium, resulting in a net movement of water into the RBCs via osmosis, causing all of the cells to expand and therefore lose the integrities of their membranes and burst after a certain time, leading to hemolysis and releasing hemoglobin into the supernatant. Images of RBC lysis, captured by microscope (Olympus CX23) with 40X resolution, are delineated in Fig. 2.3. From Fig.2 3(F), it is evident that the required volume of DI water (20 µl) is approximately double the volume of blood (10 µl) for the complete lysis of RBC to extract Hb in comparison to other figures where lysis is rather partial (Fig.2 3(A)-(B)) and therefore volume combination of DI water and blood as depicted in Fig.2 3(F) is treated as an optimal combination.

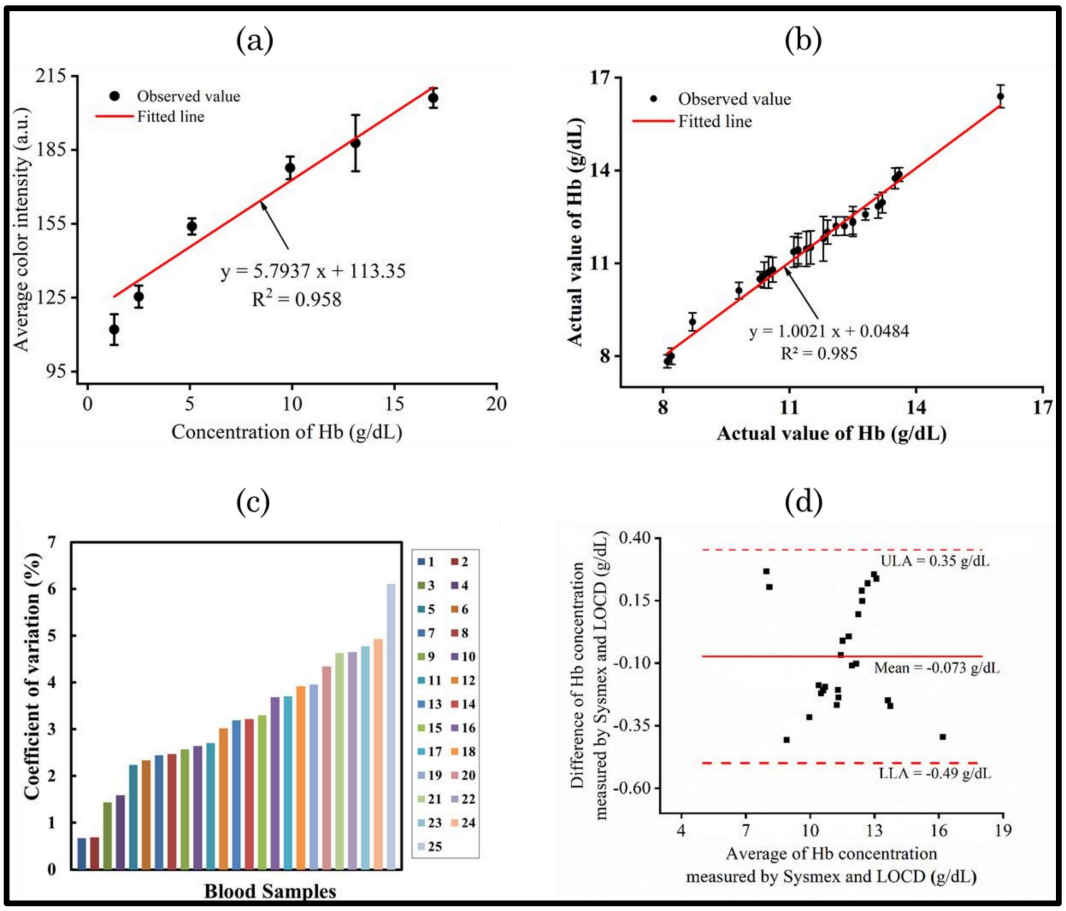

Fig. 2.4 Estimation of Hb on the proposed spinning disc. (a) Calibration curve showing the correlation between average color intensity measurements in our proposed device with the concentration of commercially available Hb standards. Every data point represents the mean ± SD. Three replicates of each concentration are used to generate the calibration curve. (b) Comparison of Hb measurements in the proposed portable device (Measured) with the standard results obtained using the automated hematology analyzer (Actual) for 25 different blood samples that are different from the ones used to construct the calibration curve. Error bars indicate the standard deviations among three independent experimental data. The experimental data with Hb concentrations ranging from 8 g/dl to 16 g/dl demonstrate a good agreement between the present and gold standard methods with a correlation coefficient of 0.992. (c) Coefficient of variation of measured data of Hb for 25 blood samples using the present device. The coefficient of variation for every sample is <7%, indicating good precision among the measurements. (d) The Bland–Altman analysis plot, comparing an average of the gold standard (Sysmex) and present (portable device) based

predictions of Hb concentrations, highlighting the difference between the gold standard and measured Hb concentration values, to evaluate the accuracy of the device. The red solid line shows a bias of -0.073 g/dl. Dotted red lines indicate 95% limits of agreement: 0.35 g/dl (upper limit) and -0.49 mg/dl (lower limit).

2.3.2 Performance evaluation vis a vis gold standard

We compare the performance of the present device vis-à-vis laboratory gold standards mentioned earlier, as depicted in Fig.2 4. The calibration curve was constructed by plotting the average color intensity versus concentration of commercially available hemoglobin standards (Sigma Aldrich, USA), Hb (g/dl), and is shown in Fig. 2.4(a). Average color intensities obtained from our proposed device showed a strong linear correlation with commercial Hb concentrations (R^2 = 0.958), with a correlation coefficient of 0.978. To evaluate the predictive accuracy of the present platform, we performed tests with 25 blood samples collected from human patients. Calculations of the Hb concentrations were carried out using the standard curve and are compared with the hematology analyzer (Sysmex) data which are given in Fig. 2.4(b). All samples were measured three times and the average values and standard deviations are calculated. The gold standard values (Sysmex data) show good agreement with the Hb concentrations of the measured samples on the present platform. It shows the comparison of these two methods, achieving a correlation coefficient of 0.992.

To evaluate the repeatability of the acquired data, we calculated the percentage of the coefficient of variation (CV) as $CV = \frac{\sigma}{\mu} \times 100$, where σ is the standard deviation and μ is the mean of three different observations of each sample. Fig. 2.4(c) provides CV values of Hb of 25 samples for the present device. The analysis reveals a small deviation

among the three repetitions of each sample (i.e., CV < 7%), thereby, indicating good precision among the measurements. Fig. 4(d) shows the Bland–Altman analysis for these two methods, revealing a bias of -0.073 g/dl with 95% limits of agreement between - 0.49 g/dl and 0.35 g/dl.

A hypothesis testing is conducted where the null hypothesis is considered as an agreement between the gold standard and the present devices to determine the concentration of Hb. The level of significance (p-value) is considered as 0.05 and the degree of freedom (DOF) for this test is found to be 24. The value of chi-square (χ^2) for 25 observations is determined as 0.114 which is significantly less than χ^2 from the table (36.415) with this p-value and DOF. Hence, with this level of significance, the null hypothesis can be considered true.

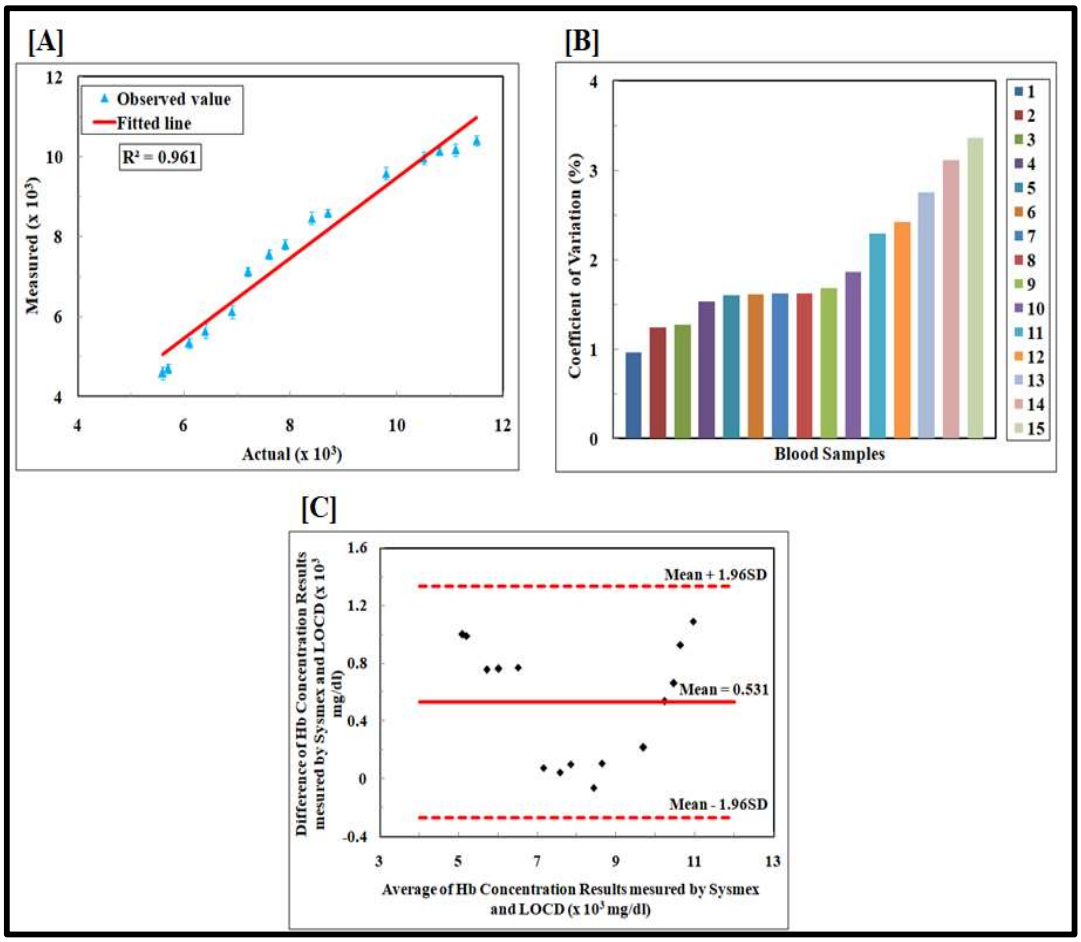

Fig. 2.5 Clinical evaluation of the present Hb sensor in anemia detection. (A) Correlation between Hb concentration measurement results of 15 blood samples in the proposed portable device (Measured) and automated hematology analyzer (Actual). Each data point represents the mean ± SD of three separate measurements. The experimental data with Hb concentrations ranging from 5 g/dl to 11.5 g/dl (in anemic range) demonstrates a good agreement between the two methods with a correlation coefficient of 0.98. (B) Coefficient of variation of measured data of Hb for 15 samples using the portable device. The coefficient of variation for each sample ranged from 0.95-3.36%, indicating very good precision among the measurements. (C) The Bland–Altman analysis results, comparing an average of the gold standard (Sysmex) and measured (present device) values of the Hb concentration, with the difference between actual and measured Hb concentrations to evaluate the accuracy of the device. The red solid line shows a bias of 0.531 g/dl. Dotted red lines indicate 95% limits of agreement: 1.33 g/dl (upper limit) and -0.26 g/dl (lower limit).

2.3.3 Detection of anemia

A quantitative comparison of results from the automated hematology analyzer and the present device was carried out, with a pinpointed effort of assessing the quantitative capability over low Hb concentration ranges ([Hb]<12 g/dl). Assessment of the performance of the hemoglobin sensor in anemia detection, which is an important criterion for judging the efficacy of the present device towards screening out high-risk patients, is shown in Fig.2 5.

We further considered Hb concentration values ranging from 5 g/dl to 11.5 mg/dl to evaluate the accuracy of our present device in detecting anemia for patients at high risk. The corresponding quantitative assessment with respect to gold standard pathology data is represented in Fig.2 5(A). All samples were measured thrice and thereafter, the average values, as well as standard deviations, are calculated. The gold standard values exhibit favorable linearity with the measured samples. The measurements both from Sysmex and the present device show a good correlation coefficient of 0.98. From Fig.2 5(B), it is also evident that the coefficient of variation for each sample ranges from 0.95-3.36% which indicates good precision among the measurements of 15 blood samples. We further perform Bland–Altman analysis on our results (Fig.2 5(C)), which shows a bias of 0.531 g/dl with 95% limits of agreement from - 0.26 g/dl to 1.33 g/dl.

A hypothesis testing is carried out for the exclusive set of data for the relatively high-risk patients, where the null hypothesis is considered as the acceptance of the present device for anemia detection in place of the traditional gold standard device. In this testing, the p-value is considered as 0.05 and the degree of freedom (DOF) for this test is found as 14. The value of chi-square (χ^2) from the table with this p-value and DOF is 23.68. For the data presented in the lower ranges of hemoglobin concentration, the same is determined as

0.879 which is sufficiently below the threshold value obtained from the table. Hence, with this level of significance, the null hypothesis can be considered true.

2.3.4 Comparison with other Hb measuring devices

Finally, we summarize some of the key favorable features of the present device as compared to the other reported methods. Table 1 presents an overall comparison of various methods of determining Hb concentration as developed in the recent past. The present work has established improvements over the reported methods in different accounts such as the infrastructural setting (laboratory or field), the detection range of Hb concentration with acceptable standards of accuracy, required volume of blood, the need of reagent, accuracy, and sufficiently low cost, which underpins its potential to be used as an extreme POC device, catering the needs of the underserved.

2.4 Conclusions

In this chapter, we have presented a new reagent-free yet direct method for a rapid and accurate Hb assay by deploying a simple and easy-to-fabricate portable rotating disc. Simple fluidic procedure and the bio-analytical procedure has been essentially coupled with a straightforward method of image analytics for rapid and accurate dissemination of the test results. Our measurements have yielded excellent agreement with results obtained from established gold standard pathological data. Further, the method has been proven to be capable of detecting the Hb levels in high-risk anemia with an extremely low coefficient of variation (≈3%), in tandem with a high level of sensitivity and specificity. With an obviation of deploying sensitive reagents, the methodology is therefore poised to be ideal for being functional with extremely minimal resources including laboratory infrastructure and technicians, resulting in obvious benefits to the under-served.

Table 2.1 Comparison of performance of present work with reported methods for estimation of Hb concentration

Technique	Setting	Hb detection range (x 10^3 mg/dl)	Reported accuracy (95% limits of agreement) (x 10^3 mg/dl)	Cost per test (USD)	Reagent used	Required whole blood volume (µl)	References
Spectrophotometric measurement of blood spotted on chromatography paper	Laboratory	7 to 16	-2.07 to 1.86	0.006	sodium deoxycholate	10	(Bond et al. 2013)
Cell phone-based analysis	Field	11 to 16	-0.54 to 0.63	0.2	RBC lysing buffer solution	10	(Zhu et al. 2013)
Electrochemical sensor	Laboratory	2.3 to 14	-	<0.02	Reagent-free	5	(Choudhary, Rajamanickam, and Dendukuri 2015)
Smartphone dongle based colorimetric analysis	Field	0.0 to 21.2	-1.08 to 1.22	1.0	CHAPS detergent	-	(Guo et al. 2015)
Luminescent terbium complexes sensor	Laboratory	0.001 to 0.4	-	-	Luminescent terbium complexes	20	(Morgner et al. 2015)
Curcumin nanoparticles based colorimetric chemosensor	Laboratory	0.0001 to 0.12	-	-	curcumin nanoparticles	1000	(Pourreza and Golmohammadi 2015)
Smartphone-based colorimetric sensor	Field	6 to 18	-0.62 to 0.75	0.15	Hemocor-D	5	(Pourreza and Golmohammadi 2015)
Portable rotating disc coupled with image analysis	Field	1 to 17	-0.49 to 1.33	0.006	Reagent-free	10	Present Work