CHAPTER 5: A SMARTPHONE-INTEGRATED PORTABLE ROTATING PLATFORM FOR ESTIMATION OF CONCENTRATION LEVEL OF PLASMA-CREATININE USING WHOLE HUMAN BLOOD

5.1 Chapter overview

This chapter describes the development of a simple, affordable, and portable spinning disc for measuring plasma-creatinine concentration with 10 μL of finger-pricked whole human blood. 5 μL of the alkaline picrate solution is loaded into the device and rotated at 1000 rpm to transport this solution to the periphery of the microchannel. Further, 10 μL whole blood is loaded in the same channel and spun at 1300 rpm for 10 minutes. The creatinine in the blood plasma reacts with alkaline picrate (Jaffe reaction), and the color of the mixture changes to yellow-orange color from its original yellow color. The resulting color is captured with a smartphone, and creatinine concentration is estimated using an in-house developed app (CREA-SESE). The value of creatinine measured with the present device and the gold standard device are highly correlated (R^2 = 0.998). The bias and standard deviation of the difference between the two measurements are 0.134 mg/dl and 0.143 mg/dl. This study demonstrates the feasibility of a simple, inexpensive, and portable rotating device for measuring creatinine concentration using 10 μL of finger-pricked whole human blood, which can easily be deployed to the underserved population in resource-constrained settings with the engagement of minimally skilled human resources to monitor renal diseases.

5.2 Introduction

Creatinine is an end-product of creatine and creatine phosphate as a result of muscle and protein metabolism (Chaiyo et al. 2018b; Shariati and Khayatian 2022). The creatinine

produced in the body is transported to the kidney via the blood circulation, continuously filtered out, and removed through the urine (Hu, Ding, and Tang 2022; Thompson and Joy 2022). The creatinine level in human blood and urine could indirectly indicate renal, muscular, and thyroid functions (Kashima et al. 2017; Pundir, Yadav, and Kumar 2013). The normal creatinine range in serum is 0.5 to 1.0 mg/dl for healthy adult women and 0.7 to 1.2 mg/dl for healthy adult men (Kashima et al. 2017; Prabhu, Mukhopadhyay, and Liu 2022). A decreased concentration level of creatinine in the blood signifies loss of muscle mass (Osei-Owusu et al. 2022), whereas an elevated level indicates renal dysfunctions (Parmar et al. 2016). Therefore, determining creatinine concentration in bodily fluids is considered as a valuable tool for kidney function.

Several methods have been reported in the literatures for creatinine detection, including spectrophotometry (Krishnegowda et al. 2017), colorimetry (Hall et al. 2017), voltammetry (Saidi et al. 2018), amperometry (Kumar et al. 2017), potentiometry (Erenas et al. 2019), fluorescent (Ellairaja, Shenbagavalli, and Vasantha 2017), molecular imprinted polymer (Diouf et al. 2017), chromatography (Tsikas et al. 2010), capillary electrophoresis (Grochocki, Markuszewski, and Quirino 2017), and mass spectrophotometry (Suzuki et al. 2016). These methods employ highly sensitive and accurate modern equipment. However, these instruments have limitations for their deployment in resource-poor settings due to high equipment cost and time-consuming analysis, the requirement of a large amount of samples as well as costly reagents, lesser portability, and the requirement of skilled human resources. Therefore, it is important to develop a simple, fast, disposable, affordable, and portable point-of-care device to estimate creatinine levels. This can be achieved using Lab-on-a-disc (LOAD) platform.

Over the last few decades, microfluidic LOAD devices have been proven their mettle in several laboratory operations such as blood separation, metering, aliquoting,

mixing, reagent storage, and sequential reagent delivery (Ducrée et al. 2007; Duford, Xi, and Salin 2013; Honda et al. 2005; Van Oordt et al. 2011; Steigert et al. 2007) for applications in biomedical (Godino et al. 2013), food allergen (Tortajada-Genaro et al. 2012), pathogen detection (Golden et al. 2013) as well as environment monitoring (Czugala et al. 2012; Hwang et al. 2013). Numerous LOAD devices have been reported in the literature for detecting different pathological parameters. For example, Chattopadhyay et al. (Chattopadhyay et al. 2021b) estimated the hemoglobin level from finger-prinked blood using the colorimetry method. Lee et al. (Lee et al. 2009) developed a LOAD system for the detection of the hepatitis virus. Similarly, Agarwal et al. (Agarwal et al. 2020) have developed a rotating platform for estimating hematocrit, hemoglobin, red blood cell, white blood cell, and platelet counts with 95 % accuracy. Off late Chattopadhyay et al. (Chattopadhyay et al. 2022) estimated plasma creatinine on a simple paper strip. Though clinical assays in gold standard devices as well as miniaturized microfluidic platforms are available for the measurement of plasma creatinine, these assays suffer from one or more of the following major shortcomings: (i) devices are bulky and not portable, (ii) multiplexing is not facilitated in the device, (iii) clinical assays bear elevated cost, (iv) involvement of highly skilled technicians, and (v) non-availability of smartphone app for rapid measurement.

Circumventing the constraints mentioned above, herein, we have chosen a lab-on-a-disc device to facilitate multiplexing leading to the analysis of multiple samples in a single miniaturized platform. The gold standard clinical assay has been translated in this miniaturized platform without sacrificing its fundamental principle for determination of plasma-creatinine level from the whole human blood, aiming to deploy our spinning disc integrated with CREA-SENSE app as a perfect candidate for field measurement of plasma-creatinine which would reduce renal disease burden among the underserved population to

the greatest possible extent. For its operation, the device utilizes centrifugal forces to separate the plasma from blood samples. The creatinine in the plasma reacts with alkaline picrate, and forms an image with a yellow-orange complex. The image is captured with a smartphone camera inside a lightbox, and creatinine concentration is estimated with an in-house developed app (CREA-SENSE). The creatinine values obtained from our device are compared with gold-standard laboratory results. The results render an excellent correlation with the coefficient of determination (R^2) as 0.998. The results obtained by our proposed device indicate that our device has true potential for deployment in resource-constraint settings with uncompromised accuracy.

5.3 Materials and methods

5.3.1 Chemicals and materials

Creatinine (anhydrous, ≥ 98%), sodium hydroxide (NaOH), and picric acid (moistened with water, ≥ 98%) are purchased from Sigma-Aldrich chemicals private limited (Bangalore, India). Deionized (DI) water is used to prepare 10% (w/v) aqueous solution of NaOH. Alkaline picrate solution is prepared by mixing picric acid and NaOH in 5:1 ratio. Different concentrations of creatinine for the calibration curve were prepared using DI water. All the chemicals used in this study are of analytical grade and utilized as received without further purification.

5.3.2 Device design and fabrication

The proposed LOAD device is a three-layer compact disc (CD) (Fig. 5.1). The top (Fig. 5.1(a)) and bottom (Fig. 5.1(b)) layers are made of one mm thick Poly-methyl-methacrylate (PMMA) sheet (Lexan, GE), while the middle layer is made of 20 μm thick double-sided pressure-sensitive adhesive (PSA) tape (Flex mount DFM 200 clear V-95, 3 M, Flexcon,

Inc., Spencer, MA). The diameter of all three layers is 90 mm. There are holes (radius 1.75 mm) on the top layer for pressure balancing and sample loading. The five microchannels are engraved in the PSA layer. Each microchannel is cut in the form of a sector of 45° with an inner radius 10 mm and an outer radius of 35 mm. The bottom layer does not contain any holes or channels. The purpose of this layer is to provide base and stability to the device. The top and bottom layers are machined with a tabletop CNC (T-Tech Inc., QC 5000) with a carbide router tool of 1 mm diameter, whereas the PSA layer is engraved using a cutting plotter (Graphtec CE7000-60, Thailand). Finally, all the three discs are manually aligned and pressed together to form a composite structure (Fig. 5.1(d)). Then the composite structure is passed through a roller press laminator to prevent the leakage of the reagents and samples from the channels.

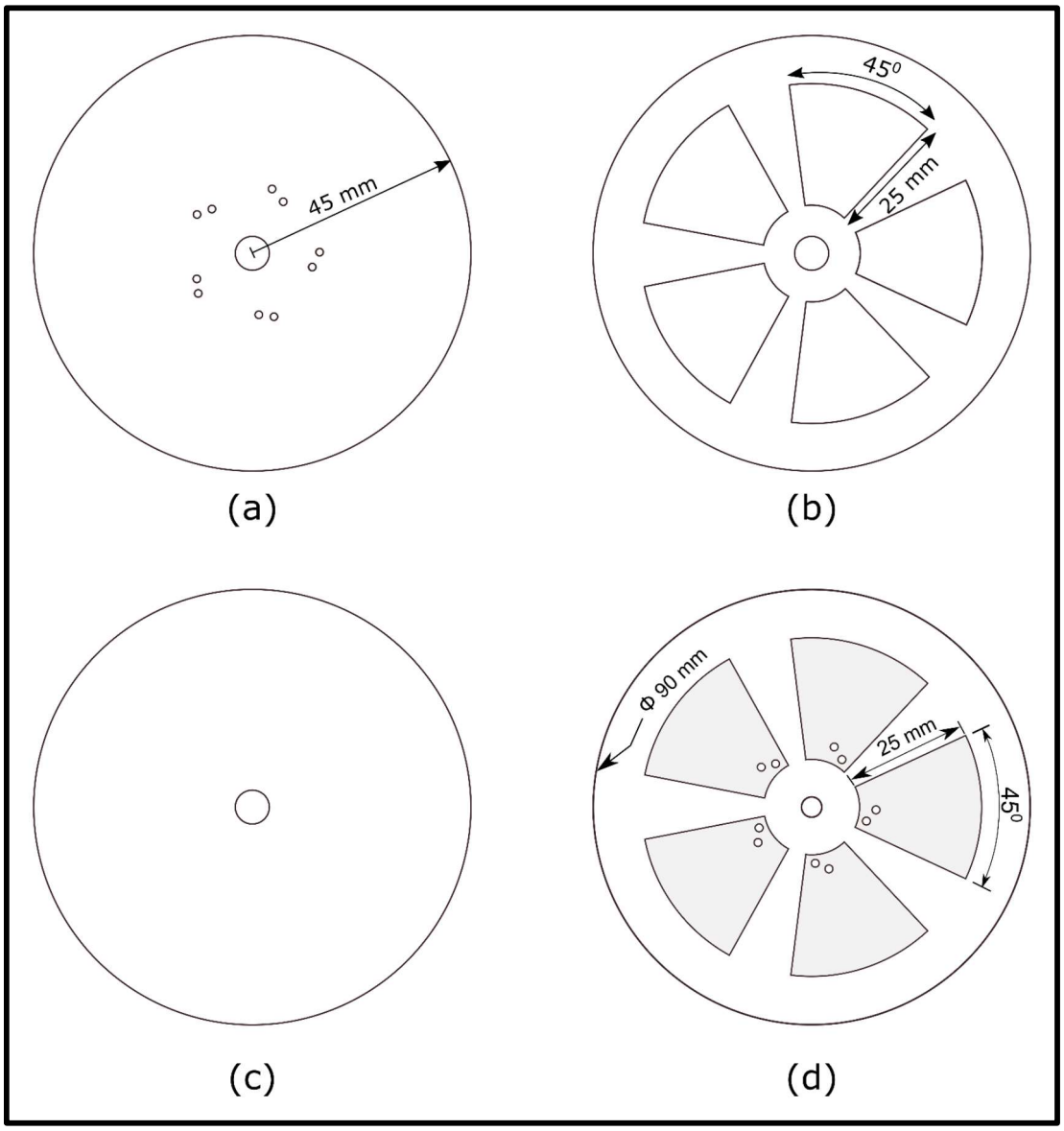

Fig. 5.1 Fabrication of the device: (a) Top layer is an one mm PMMA disc containing holes for sample loading and pressure balancing; (b) The middle layer is fabricated by 20 μL pressure-sensitive double-sided adhesives (PSA) incorporating the patterns for microfluidic channels in the form of a sector having a length of 25 mm and sector angle of 45°; (c) The bottom layer is also an one mm thick PMMA disc which provides base and structural support; (d) All three layers are manually aligned and compressed to obtain the final compact device.

5.3.3 Blood collection and processing

All the blood samples used in this experiment are collected in vacutainer tubes (K_2EDTA) from patients of Sir Sunderlal Hospital (Banaras Hindu University), Varanasi (India) through finger-pricked. Ethical approval is obtained from the Institutional ethics committee of the Institute of Medical Sciences (Banaras Hindu University), Varanasi (India). A lancet is used to puncture the skin of the fingertip, and the very first drop of the blood is discarded to minimize the contamination due to excess tissue, and subsequent drops are then collected. The blood samples with a wide range of creatinine are used for the experimental study. All the samples are shaken well to make them homogeneous for any experimental run. The creatinine levels of each sample is estimated using a fully automated Roche Cobas C 501 analyzer (Roche Diagnostic, Basel, Switzerland), and obtained results are taken as gold standard results.

5.3.4 Experimental methodology

The fabricated device is attached to a spinning platform. The spinning unit consists of a stationary stage and a DC motor, a microcontroller to control the DC motor, and a display unit to show the rpm of the motor. As shown in Fig. 5.2, several simple steps are executed to estimate the creatinine level using finger-pricked whole human blood. First, 5 μL of alkaline picrate is loaded in one of the microchannels of the device. The disc is rotated at 1000 rpm for 1-2 minutes to transport the alkaline picrate to the periphery of the microchannel. Then 10 μL of finger-pricked blood is loaded in the same channel. The disc is rotated at 1300 rpm for 10 minutes; as a result, blood is mixed-up with alkaline picrate. After some time, RBCs and a mixture of plasma and alkaline picrate separate. The alkaline picrate reacts with creatinine present in plasma and produces a yellow-orange complex. The resulting color is digitalized using a smartphone (Redmi Note 11T 5G, India), and

finally, the concentration level of creatinine is evaluated using an in-house developed app (CREA-SENSE). A step-by-step clinical assay of creatinine estimation on the portable spinning disc platform is demonstrated in Fig. 5.2.

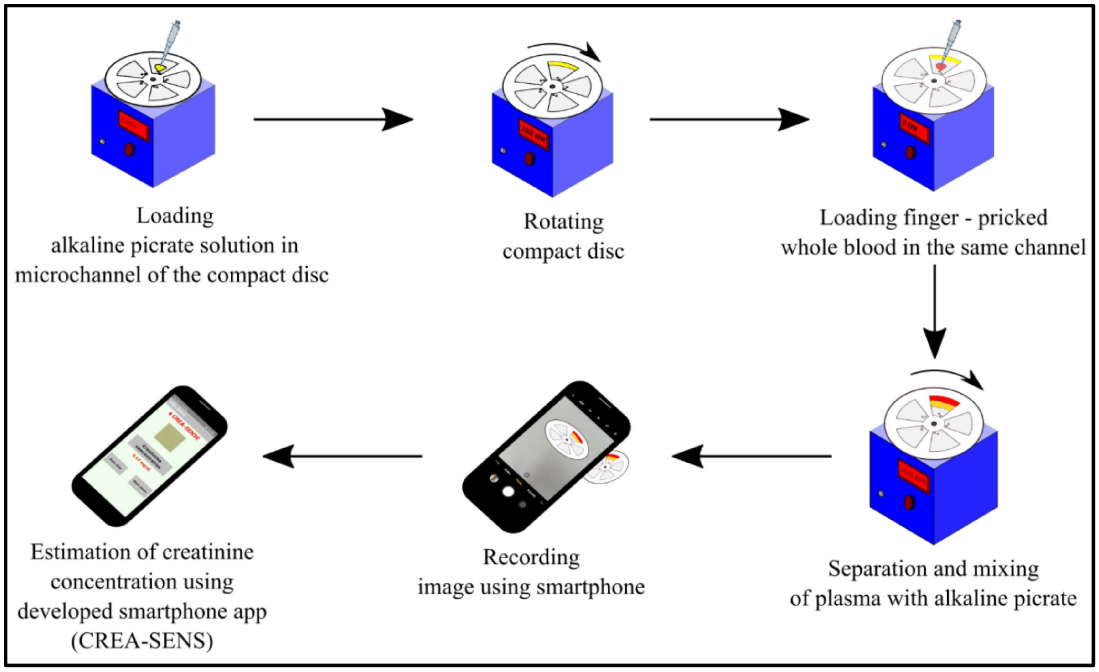

Fig. 5.2 Step-by-step clinical assay of estimation of creatinine level on the developed device: loading of 5 μL of alkaline picrate in the microchannel of the disc; rotating CD to transport the alkaline picrate to the periphery of the channel; loading of blood in the same microchannel of the CD; rotating CD for mixing of blood with alkaline picrate, and separation of RBCs and mixture of plasma and alkaline picrate; recording the image with smartphone camera inside a lightbox after the color of this mixture changes to yellow-orange; and quantification of creatinine level using CREA-SENSE app.

5.3.5 Development of smartphone app and image analysis

To quantify the creatinine level in real-time, an android-based app, CREA-SENSE, is developed. The algorithm is developed in Python 3.7.9, whereas Android Studio 4.1 is used

for the software package. The algorithm and working of the developed app are shown in Fig. 5.3. After image acquisition, the quality of the digital image is improved, and quantitative information is generated. The image is analyzed in an automated digital environment, which obviates manual intervention. The developed app executes several stages for image analysis, including segmentation of region of interest (ROI), background correction, pre-processing, and extraction of image features (Fig. 5.3(a)). Images are recorded inside a lightbox (see sub-section 5.3.6) with the smartphone camera. The app reads the images and converts them to a grayscale image.

Further, the image quality is modified or enhanced with the spatial image filtering technique. A ROI centering the detection zone is identified, and mean gray color intensity is determined. The mean gray color intensity is calibrated with the known concentrations of creatinine. The concentration of the unknown sample is predicted using the calibration curve. The overall operation of the app is illustrated in Fig. 5.3(b).

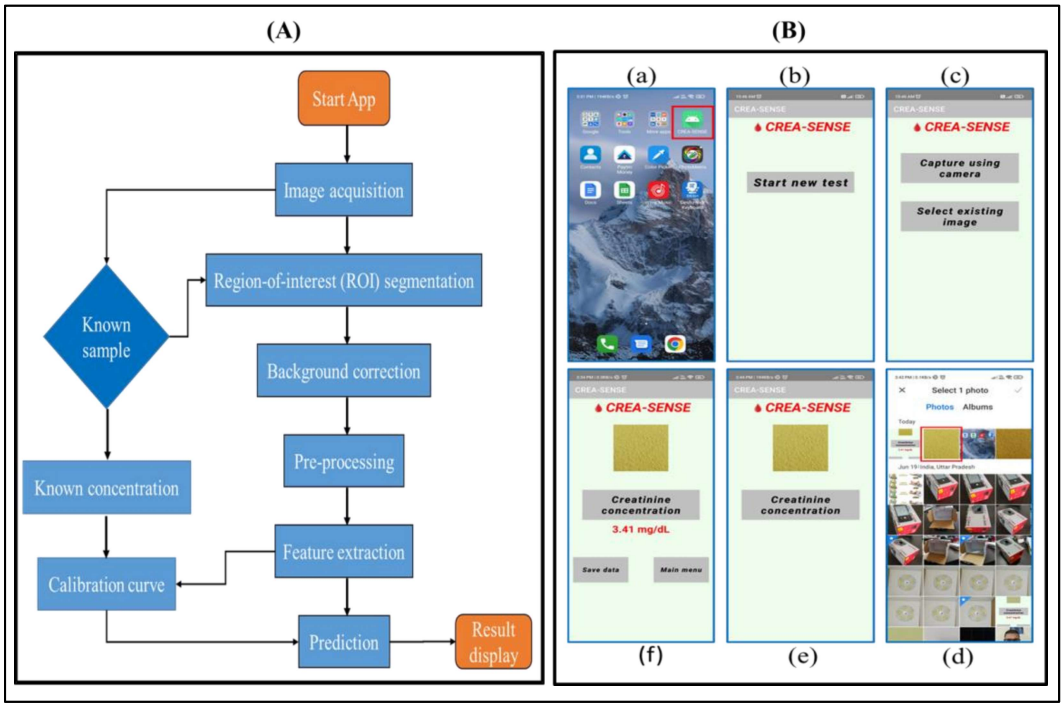

Fig. 5.3 Estimation of creatinine concentration using captured images inside the light box: (A) Algorithm implemented in the "CREA-SENSE" app for image processing; (B) Sequential operation of the app: (a) launching, (b) selecting new test, (c) image recording, (d) selecting image from smartphone gallery for analysis, (e) display of the selected image in the app and (f) displaying creatinine concentration.

5.3.6 Image recording process

A light box is constructed from a canon camera box to record all the images. The length, width, and height of the box are 220, 160, and 130 mm respectively. The four strands of LED lights are secure on the top surface with the help of double sided tape, and plugged-in to the power source. The lights are covered with several layers of printing paper to diffuse the light within the box. The smartphone camera lens is accommodated in the box by creating a hole in the top surface (Fig. 5.4).

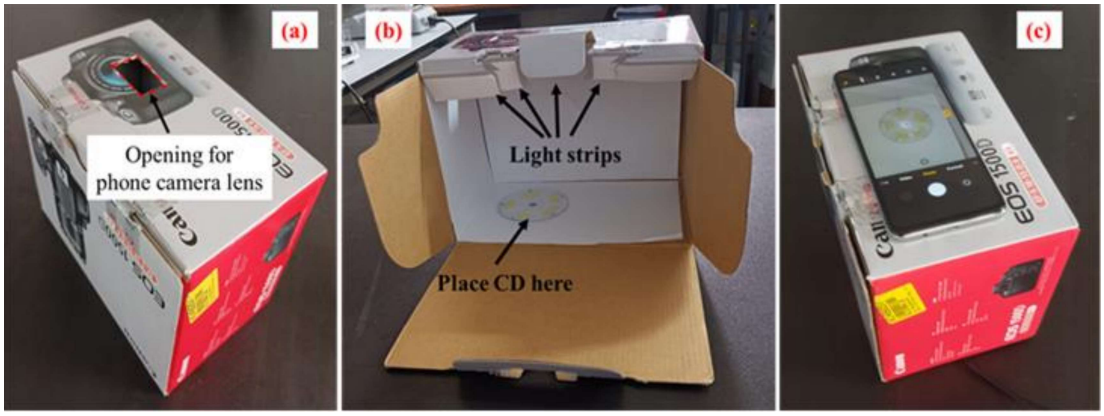

Fig. 5.4 Lightbox setup for image capture: (a) An opening is created in the top surface of the box to accommodate the smartphone camera lens; (b) Four LED light strips covered with paper are secured on the top surface with the help of double-sided tape, and after the development of the color, the CD is placed inside the light box at indicated placed; (c) All sides of the lightbox are closed with the lights on. The phone camera lens is placed over the opening, and the image is captured for analysis.

5.4 Results and discussion

5.4.1 Translation of modified Jaffe reaction for point-of-care settings

It is one of the critical and challenging tasks to transfer the lab-based standard pathological methods to extreme point-of-care settings for estimating creatinine levels. In traditional laboratories, the plasma is first separated from the whole blood using a centrifuge and the separated plasma is mixed with NaOH solution in a test tube. Further, the resulting solution is mixed with the picric acid solution. The final solution is mixed thoroughly and incubated at room temperature (20-25 ^{0}C) to complete the Jaffe reaction. After 15 minutes, the absorbance of the solution is observed to estimate the creatinine concentration.

Previously, Tseng et al. (Fu et al. 2018; Tseng et al. 2018) transferred the traditional lab-based technique for POC settings, which requires mandatory heating at 37 ^{0}C for 5 minutes to obtain a noticeable color signal as a result of the Jaffe reaction. However, in the

present context, the modified Jaffe reaction could be executed in normal ambient conditions in a shorter time span due to applying a premixed solution of picric acid and NaOH instead of using them separately. The eliminated heat step, along with the simple plasma separation method, renders the present assay user-friendly and promotes the deployment of unskilled healthcare workers in the field and in POC settings.

5.4.2 Performance evaluation of present device against the conventional approach

The calibration curve is constructed by plotting, the mean color intensity against the known concentrations of commercially available creatinine (mg/dl) as shown in Fig. 5.5(a). Average gray color intensities are shown a good linearity with creatinine concentrations (mean gray color intensity = 1.025 × creatinine concentration + 2.2427, $R^2 = 0.973$).

The accuracy of the proposed device is performed with 30 blood samples collected from different patients. The creatinine concentrations are calculated using the standard curve and compared with the creatinine value from the gold-standard device. All samples are measured three times. The linear least-squares regression analysis (Fig. 5.5(b)) showed an excellent correlation between the two measurements ($y = 1.0015\ x\ -\ 0.0178$, $R^2 = 0.998$).

The agreement between the developed method and gold-standard result is evaluated by constructing a Bland-Altman plot (Fig. 5.5(c)). The bias (mean) and standard deviation (SD) of the differences between both methods were 0.0134 mg/dl and 0.143 mg/dl, respectively. The lower and upper limits of agreement with a 95% confidence interval are - 0.27 and 0.30 mg/dl, respectively.

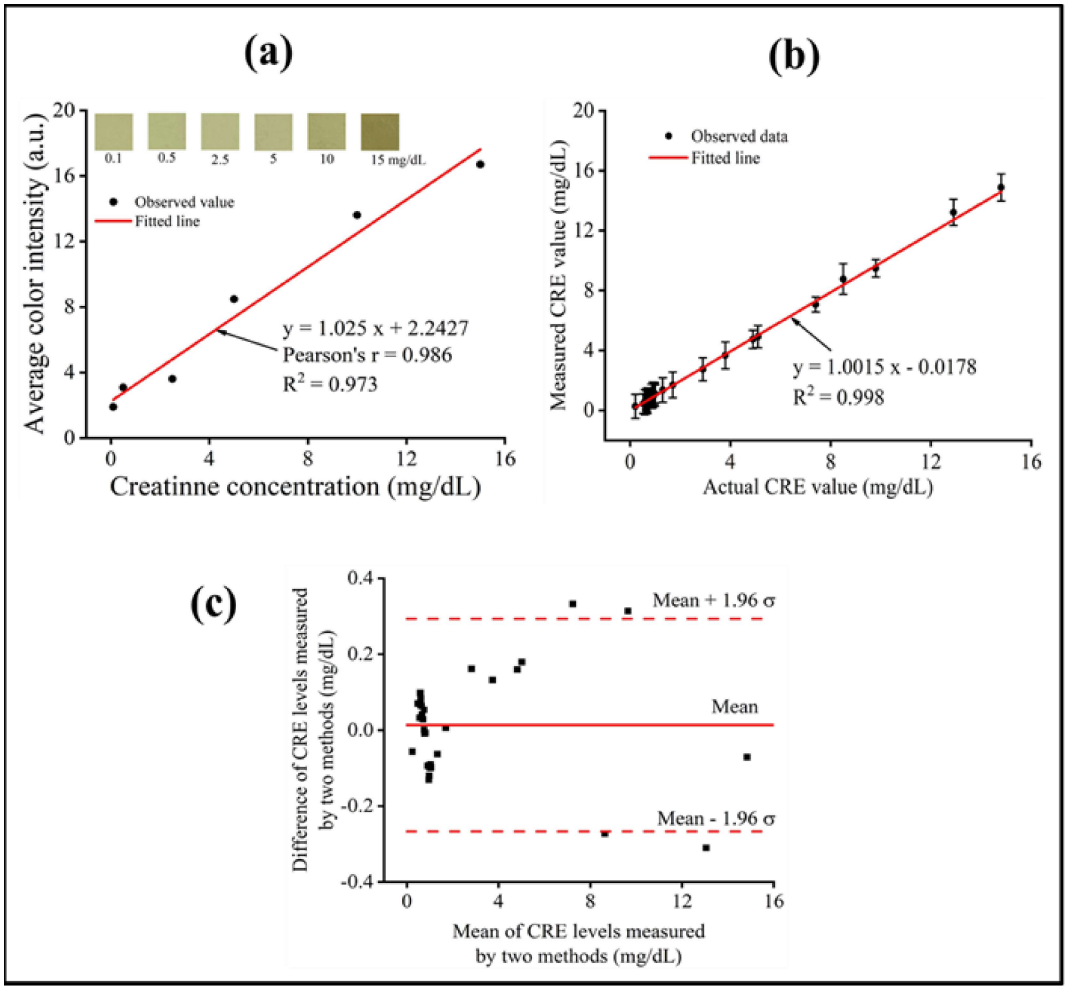

Fig. 5.5 (a) Calibration curve which is a plot of average color intensity against the known concentrations of commercially available creatinine (0.1 to 15 mg/dl); (b) Comparison between the creatinine values obtained from our device (Measured value) and biochemical auto-analyzer (Actual value) for 30 blood samples; (c) The Bland–Altman plot shows the bias in the values of the creatinine concentration is 0.0135 mg/dl. The lower and upper limits with 95% limits of agreement are - 0.27 and 0.30 mg/dl, respectively.

5.4.3 Analytical performance of the proposed method

The precision of the developed device is tested by evaluating repeatability and reproducibility. The repeatability is tested by measuring repeatedly (n = 3) the creatinine

concentration for a series of blood samples 0.52, 0.68, 0.80, 1, 4.9, 8.8, 14.8 mg/dl. The repeatability is measured as the percentage of the relative standard deviation and found to be 6.34 %, 1.77 %, 1.97 %, 3.08 %, 0.97 %, 0.99 %, and 1.29 % respectively. The reproducibility of the device is evaluated as the percentage of relative standard deviation. The device-to-device performance is evaluated by repeatedly measuring the creatinine concentration for the blood sample with a creatinine concentration of 3.8 mg/dl in the devices fabricated on the different days, while the day-to-day performance of the device is evaluated by repeatedly measuring the creatinine concentration for the blood sample with creatinine concentration 3.8 mg/dl in the devices on different days. The device-to-device and day-to-day performances are 0.57% and 0.32%, respectively.

5.4.4 Selectivity of creatinine

There are several substances present in the plasma samples. The selectivity of the method is evaluated by testing the aqueous solution of interfering substances. Some interfering substances, including creatine, bilirubin, Albumin, urea, glucose, NaCl, KNO_3, and KBr, are investigated, and corresponding results are shown in Fig. 5.6. All the experiments are carried out in an aqueous solution in the absence and presence of other interferences and 5

μL of alkaline picrate solution. As it is evident from Fig. 5.6, the creatinine can be selectively determined in the presence of other interfering substances.

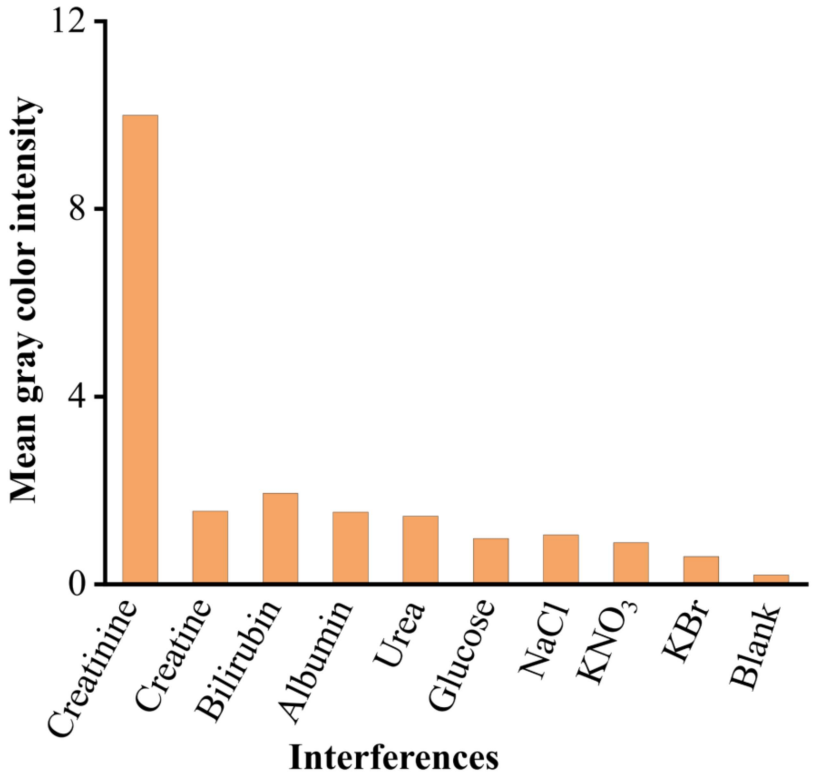

Fig. 5.6 Selectivity test of the device in the presence of creatinine and other interfering substances

5.5 Conclusion

We have developed a simple, rapid, and cost-effective yet accurate smartphone-integrated portable spinning disc platform using finger-pricked blood for estimation of plasma creatinine concentration. This developed platform utilizes a light box that can easily attached to the smartphone camera to record images of various blood samples inside a disc containing several microchannels. The recorded images are rapidly analyzed using an in-house developed smartphone app (CREA-SENSE) and creatinine concentration is automatically determined. We have evaluated the performance of our platform using thirty

blood samples from different patients. An excellent correlation with correlation coefficient (R^2 = 0.998) have been found between the results obtained from our platform and gold-standard pathological data. The repeatability of the device ranges 0.97-6.34%. The device-to-device and day-to-day performance are found to be 0.57%, and 0.32%, respectively. With its simple plasma separation method with smaller blood requirement, our developed platform is well-suited for under-served population.