International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 06 Issue: 07 | July 2019 www.irjet.net p-ISSN: 2395-0072

A Smartphone ALS based Syringe System for Colorimetric Detection of Creatinine at Point-of-Care Settings

Sanjay M[1]

[1]*Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India*

Abstract - *Creatinine is routinely tested in clinical settings, as it is a vital analyte that indicates the status of renal function. Jaffe's test is widely used for colorimetrically quantifying the amount of creatinine in both serum and urine samples. Over the years, several biosensors and assays have been reported for sensitive quantification of creatinine. However, Jaffe's test remains in use till date due to the simplicity and frugalness of the assay. In this paper, we have examined the feasibility of a smartphone platform for estimation of creatinine, using a syringe system to carry out the Jaffe's reaction and a detection module attached to a smartphone to detect creatinine, colorimetrically. The detection module contained a cuvette fixed over the smartphone's ambient light sensor (ALS) and 560nm LED light source fixed over the cuvette, all confined in a dark opaque enclosure. The cuvette had an inlet and outlet for the sample flow. The syringe contained 1% picric acid solution and the test sample mixed with 0.75N NaOH was aspired into it and agitated using the vibrating setup attached to the syringe to form the coloured creatinine picrate complex. The illuminance data was obtained through Phyphox android application in graph mode. Standards were made to plot the calibration graph and the sample concentration was found from the graph. Finally, the results obtained from the smartphone-based system were compared to the results obtained through a conventional spectrophotometric method to validate the efficacy of the smartphone platform.*

Key Words: Smartphone, Colorimetry, Spectrophotometry, Creatinine, Point-of-care testing, Ambient light sensor, Jaffe's assay

1. INTRODUCTION

Creatinine is excreted from the muscles after the breakdown of creatine phosphate after which it is eliminated directly from the blood by the kidneys in its unchanged form [1]. Hence, abnormal creatinine levels imply deterioration of renal function [2]. The rate at which the kidneys excrete creatinine is widely used to determine the glomerular filtration rate, which is basically calculated by comparing the serum and urine creatinine levels and it is corrected for the patient's body surface area [3]. Methods used for creatinine measurement include enzymatic assays, isotope dilution-mass spectrometry (IDMS) and High Performance Liquid Chromatography [4]. Nevertheless, creatinine has been historically measured using Jaffe's reaction, where in creatinine in the serum or urine sample reacts with picric acid under basic conditions to produce an orange-colored complex that can be quantified at 520nm [5]. Recent reports suggest that Jaffe's reaction is not an accurate measure of creatinine especially in serum due to intrusion from undesired interferents like ketones, proteins and bilirubin. However, it is still in practice in clinical settings due to the readily available nature of the reagents in this method, its convenience and the inexpensiveness of this technique [6]. Nowadays, these discrepancies have been offset by modifying the standard assay method with correction factors and even kinetic assays have been designed to improve the accuracy of the method [7][8].

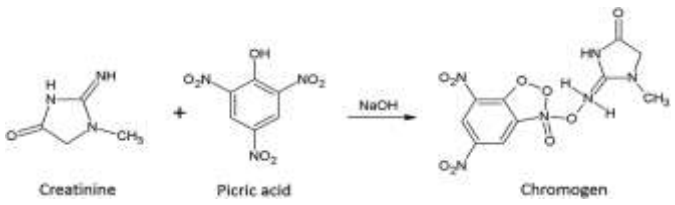


Fig. 1 Jaffe reaction for the determination of creatinine.[9]

The normal clinical range for serum creatinine in adult males, is 0.8 to 1.5 mg/dl. For the adult females, it lies between 0.5 to 1.1 mg/dl. However, it can exceed 1000 µM in certain pathological conditions. General urine creatinine values range between 40 and 300 mg/dL in males and 37-250 mg/dL in females. Some pathological conditions however result in constantly dilute urination, which leads to lesser creatinine concentrations [8].

Several creatinine biosensors have surfaced in recent years mainly and they come with their own set of advantages and disadvantages [10]. Some exhibit very low limit of detection values, far below the physiologically relevant ranges. However, they are not relevant in a clinical context. Further, the inexpensive nature and the inexpensive reagents in Jaffe's reaction, continues to fuel research in this direction.

Smartphones are occupying an indispensable part in our lives in recent times and their potential for personalized and point of care diagnosis is gaining attention from the scientific community. Nowadays, smartphones are equipped with a lot of sensors like camera, accelerometer, gyroscope, magnetometer, ambient light sensor and microphone [11] some of which have been exploited for biosensing applications [12]. Efforts to utilize smartphones to monitor water quality [13][14] and health [15][16] have been previously explored. The ALS is responsible for adjusting the display brightness according to the variations of ambient light level in most modern electronic devices. This in turn improves the operating time of the devices [17] Although 59% of the total smartphones in 2017 were shipped with an ambient light sensor [18], ALS has not been widely exploited for colorimetry. Camera based colorimetry has been explored previously but the in-built image quality enhancement operations of the smartphone affects camera based colorimetry [19]. In this study, a syringe system was also put to use for conducting point-of-care assays for creatinine using a smartphone as a detector to determine the sample concentration. The sample in question mixed with 0.75N NaOH in and aspired into the syringe containing 1ml of 1% picric acid to create creatinine picrate, which was injected into the detection module. The illuminance data was obtained using Phyphox app. Creatinine in both serum and urine samples were assayed and compared with the results from a conventional UV-Vis spectrophotometer, to check for similarity between the platforms.

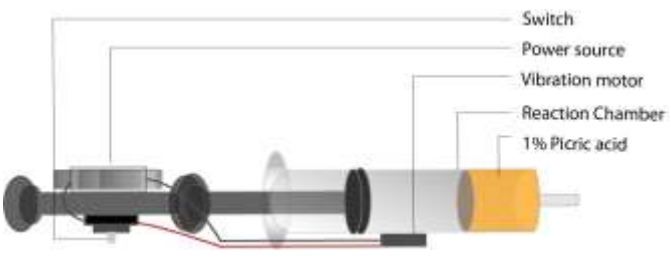


Fig-1: Design of the and PoC syringe platform

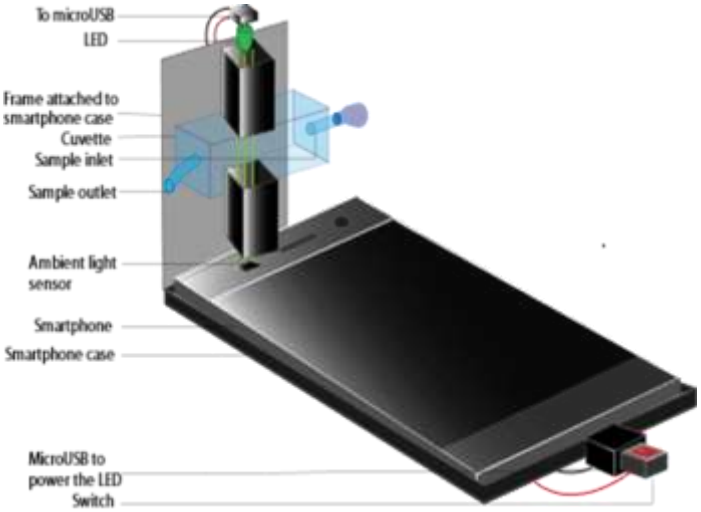


Fig-2: Design of the smartphone platform

2. MATERIALS AND METHODS

2.1 Fabrication of Smartphone Platform

A generic smartphone case was used and the detection module was attached over it. The detection module contained an acrylic cuvette fixed over the ambient light sensor and an LED was fitted over the cuvette overlooking the ALS. The light from the LED was made to propagate to the cuvette and then to the ALS using a black coated dark enclosure as shown in Fig.1. This entire setup was further enclosed in a dark chamber made from generic polyurethane sheets. The LED was of 3mm size and had an output maximum at 560nm. It was procured from Dongguan Hongqi Optoelectronics Ltd., Despite having an absorbance maximum at 520nm creatinine picrate exhibits significant absorbance at 560nm as well. Due to difficulties in obtaining a 520nm light source in a frugal manner an easily obtainable 560nm LED was opted as the light source. The acrylic cuvette was purchased from Fischer Scientific and cut into the required dimensions so as to fit into the detection module. This platform was tested on Xiaomi Redmi Note 5 Pro and the results of this device were compared with another smartphone Honor 7x (BND-AL10). The ALS in these smartphones were LiteOn LTR578ALSPS with a resolution of 0.015 lux and Avago ASPDS9922 with a resolution of 1 lux.

2.2 Fabrication of the syringe platform

The Jaffe's reaction was carried out in a syringe pre-filled with picric acid, the nozzle was fitted with a cap

so that the reagent does not flow out. The test sample was collected in a tube that already contained water and NaOH in required concentrations, to bring the sample to appropriate dilution. This in turn eases the point-of-care detection process.

The end of the plunger contained a 4.5V power source made using button cells for their compactness and replaceability. This power source was connected to a flat vibration motor through a switch. This constituted the agitation platform to facilitate mixing of reagents with the sample. The vibration motor was of 10mm diameter and 3.4mm height and provided a vibration amplitude of 0.75g. It was and obtained from Shantou Jiali Micro Motors Ltd., The syringes were of 5ml capacity and procured from Dispovan India.

2.3 Chemicals

Creatinine, Sodium hydroxide, HCl and all other chemicals used were of Analytical grade and acquired from Himedia Laboratory Products. The creatinine standard of 100 mg/dl concentration was prepared by dissolving it in 0.1M HCl. Then, the stock creatinine solution of 100 mg/dl was diluted 5x in 100 mM HCl to obtain a working solution of 20 mg/dl concentration. This working solution was used to prepare a series of creatinine standards between 1.25 to 20 mg/dl. The urine samples were 20x diluted in deionized water to eliminate matrix interference in the results and serum samples were made protein free using 10% sodium tungstate and 1 ml of 2/3N sulphuric acid as mentioned elsewhere [20].

2.4 Syringe platform operation

The prepared test samples were diluted 10x (urine) or made protein free (serum) and 1ml of sample was mixed with 1ml of 0.75N NaOH. Then 2ml of the NaOH mixed sample was aspirated into the syringe with picric acid followed by agitation for 10 mins to develop the color, after which it was injected into the detection module.

2.5 Detection module operation

The LED was switched on and initial intensity was obtained for a few seconds and then paused. Then the control was injected into the cuvette followed by the standards. After every injection the graph mode was paused and the cuvette was cleaned by passing deionized water.

2.6 Relative Illuminance measurement.

In this study, the Relative Illuminance (RI= Control Illuminance – Sample Illuminance) was calculated and plotted against the concentration of creatinine to obtain a calibration plot. The illuminance values were obtained using Phyphox app from RWTH Aachen. The data was obtained in graph mode and exported in excel format. The excel file was opened in MS Excel and a scatter plot was made, to find the Illuminance values of the control and various concentrations of creatinine following which their individual RI values were calculated. The control and the standards were passed into the detection module one after the other.

A total of six standards with concentrations of 1,2,5,10,20,40 mg/dl were used for spectrophotometer and ALS method. However, another 80 mg/dl standard was made to find the point of saturation of the ALS method. An Agilent Cary 60 UV-Vis spectrophotometer used to check and compare the results from the smartphone platform. Calibration plot for smartphone and spectrophotometer were made by using the same standards at the same time. Finally, five urine and serum samples obtained from healthy volunteers were used to compare the results of smartphone and spectrophotometer. LED is a variable intensity light source [22]. In order to determine if the variation of LED light intensity disturbs the reaction results the LED illuminance was measured with the smartphone over 95s, to check for inconsistencies in illuminance values. The same standards were used in another smartphone Honor 7x to check for universal utilization of this platform among all smartphones and the results from the two smartphones were compared.

2.7 Spectrophotometer operation

The same standards used in the smartphone platform were used in the spectrophotometer. Their ODs were found at 520nm and plotted against their concentrations to obtain the calibration plot for spectrophotometer[20].

3 RESULTS AND DISCUSSION

3.1 Description of the Smartphone and syringe platform

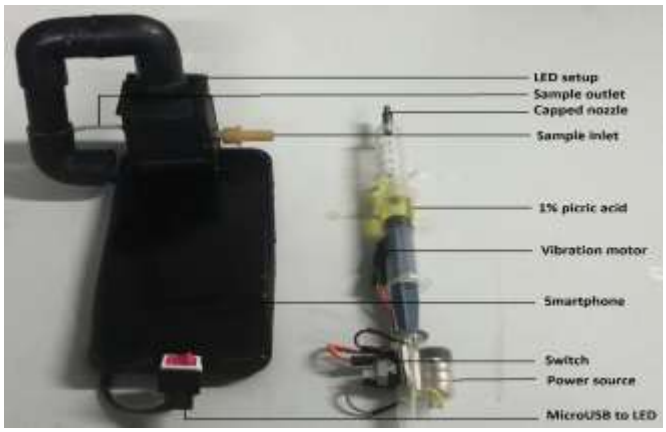


Fig-3: Picture of the fabricated ALS based detection platform (dorsal view) and syringe system

ALS measures the illuminance E, from a light source, using the formula, $E=\varphi/A$, where φ is the luminous flux and A is the area [21]. The detection module was fabricated over a generic smartphone case as mentioned earlier and fitted over the smartphone. The syringe filled with the reagents was used for estimating creatinine concentration from the urine and serum samples.

3.2 Measurement of LED Intensity

The LED illuminance was measured with the Phyphox app for a 95 s, to check for LED illuminance fluctuations. The changes in LED illuminance values were observed as shown in Fig.4. However, the inconsistencies were observed only in the third decimal digit. This error was mitigated by taking the mean of three readings

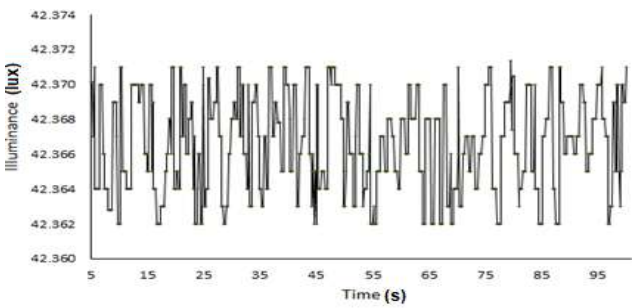
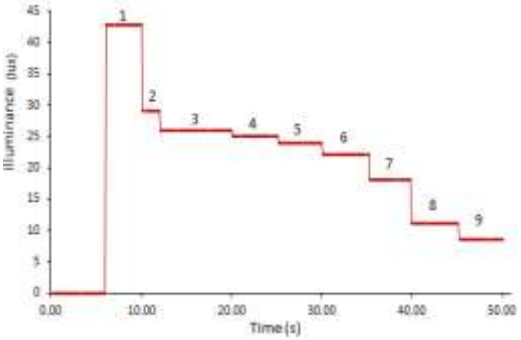


Fig-4: Illuminance data of the LED light source

3.3 Calibration plots

The blank and standards were injected and their illuminance data was obtained as shown in Fig.5. The numbering on the figure indicates the various injection stages in the detection module as shown in the table next to figure. The RI values were calculated and plotted against their concentrations to obtain the standard graph as revealed in Fig.4.



Stage	*Sample*
1	Initial LED intensity
2	Blank
3	1mg/dl creatinine
4	2mg/dl creatinine
5	5mg/dl creatinine
6	10mg/dl creatinine
7	20mg/dl creatinine
8	40mg/dl creatinine
9	80mg/dl creatinine

Fig-5: Illuminance data obtained from the Phyphox app with numbering to denote the time of injection of various standards

In order to compare the results obtained from the smartphone-based platform, the results from a spectrophotometer were used. The standard graph for the spectrophotometer was also obtained in a similar manner as depicted by Fig.6A

The standard deviations obtained from both the methods were within acceptable limits. Thus, the reliability of the detection systems under laboratory conditions was validated for standards. These calibration plots were used to detect the concentration of creatinine in urine and serum samples as mentioned previously. The comparative results are shown in Table.1. The sensitivity of this method using Redmi note 5 was calculated from the slope of the calibration plot as 0.377 RI/ (mg/dl of creatinine).

The limit of detection of creatinine through the smartphone platform was found using the formula (3× standard deviation of blank/slope of calibration curve) and calculated as 0.048 mg/dl. The dynamic range used for obtaining the calibration plot was between 1 to 80mg/dl. The platform exhibited linear response between 1 to 40mg/dl concentration of creatinine. These results obtained from Redmi note 5 platform were compared with the results of Honor 7X as shown

in Fig. 7B. Due to the poor resolution power of the Avago ASPDS9922 in Honor 7X, subtle decimal level changes in illuminance values were not perceived. Hence the values were typically higher than the actual values.

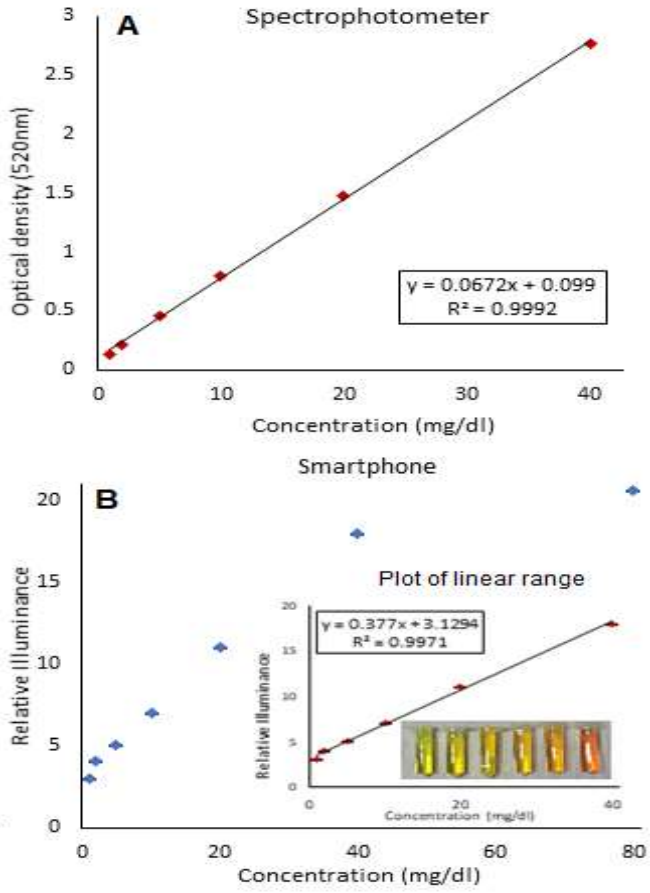
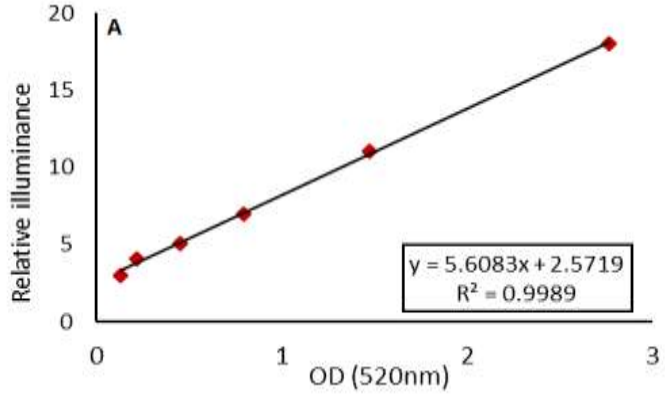


Fig-6: The calibration plots of Jaffe's assay using spectrophotometer (A) and smartphone (B) as detection platforms



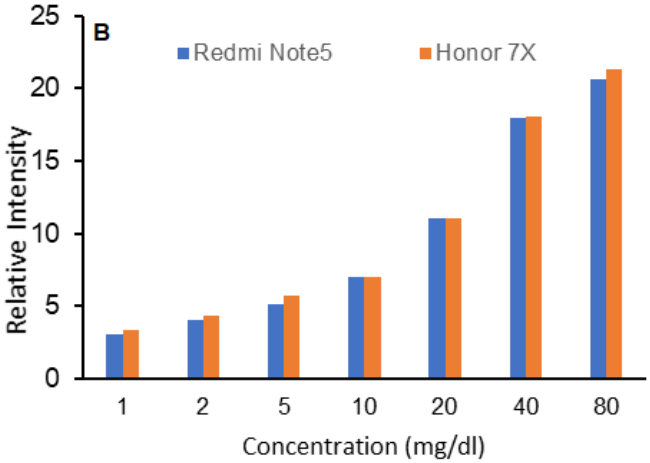


Fig-7: A) The linear correlation between spectrophotometer readings and the ALS platform readings B) Comparison of readings from Honor 7X and Redmi Note 5

3.4 Sample Analysis

Table.1: Concentrations of creatinine in serum and urine obtained using spectrophotometer (Spec) and smartphone (Phone). The coefficient of variation between the Spectrophotometer and smartphone-based method is also shown.

	Serum			Urine		
	Spec	Phone	CV(%)	Spec	Phone	CV(%)
Concentration of creatinine (mg/dl)	1.51	1.50	0.6	101.79	106.11	2.9
	0.66	0.67	1.1	194.38	188.50	2.2
	1.53	1.52	0.6	67.98	70.06	2.1
	0.80	0.82	1.7	143.75	144.82	0.5
	0.75	0.77	1.7	84.82	83.89	0.8

The use of smartphone-based creatinine detection is validated in this section. Same samples of urine or serum were used in both platforms and their creatinine concentrations were estimated. The concentrations determined from the smartphone showed some variability from the spectrophotometer values as indicated through the coefficient of variation. The variation in results can be attributed to the inconsistencies of the LED light source. This can be circumvented by utilizing a circuitry that facilitates constant light intensity output source or by using a high-performance light source. The observed coefficient of variations (CV%) in the 5 urine and 5 serum samples were between 2.9 to 0.67, which is an indicator of fairly reliable

results Further, through timed collection of urine and serum samples the creatinine clearance can also be calculated.

4. Conclusion

In brief, this study has experimented the use of ambient light sensor in a smartphone for estimation of creatinine in both urine and serum samples. The limitations of the LED light source prevent it from reporting accurate creatinine concentrations. Despite that, currently this smartphone colorimeter can be used for rough estimation of creatinine concentrations and was close in sensitivity to spectrophotometer in most cases. A platform like this will be useful for point-of-care settings or in resource limited laboratories, because it sidesteps the need for an expensive spectrophotometer and an operating computer. Further, the presence of the syringe system speeds up the result obtaining process, enables use by untrained personnel and eliminates the need for glassware and laborious assay preparation methods. Through the collection of a fixed amount of urine or serum in a container prefilled with NaOH and adequate water for dilution, sample processing, PoC utility and result acquisition were accelerated immensely. Through improvements in software, this setup can be used to obtain calibration plot and test sample concentrations from the smartphone itself and hence act as a self-sufficient ecosystem for colorimetry. Further, this platform can be capable of colorimetric quantification of any analyte of clinical or environmental significance, provided appropriate reagents are pre-filled in the reaction syringe. The absorption wavelength can also be changed according to the chemical reaction, by plugging an LED of required wavelength in the detection module. It can be used with any smartphone that has an in-built ALS but some dated smartphones have ALS with lesser resolution which consecutively affects the quality of the results and the universal applicability of this platform. Nevertheless, considering the ever-growing consumer needs in the smartphone market, more smartphones will sport an ultrasensitive ALS which will in turn expedite sensitive smartphone-based colorimetry.

5. Acknowledgements

I would like to thank the DBT- Centre of Excellence, IIT-Guwahati, Department of Biosciences and Bioengineering , IIT-Guwahati Department of Chemistry, IIT-Guwahati and members of the Biosensors Lab for providing the requirements and lab space for this project.

6. References

[1] "Creatinine," Clin. Vet. Advis., p. 615, Jan. 2013.

[2] M. E. Salive, C. A. Jones, J. M. Guralnik, L. Y. Agodoa, M. Pahor, and R. B. Wallace, "Serum Creatinine Levels in Older Adults: Relationship with Health Status and Medications," Age Ageing, vol. 24, no. 2, pp. 142–150, Mar. 1995.

[3] C. Couchoud, N. Pozet, M. Labeeuw, and C. Pouteil-Noble, "Screening early renal failure: Cut-off values for serum creatinine as an indicator of renal impairment," Kidney Int., vol. 55, no. 5, pp. 1878–1884, May 1999.

[4] T. Küme, B. Sağlam, C. Ergon, and A. R. Sisman, "Evaluation and comparison of Abbott Jaffe and enzymatic creatinine methods: Could the old method meet the new requirements?," J. Clin. Lab. Anal., vol. 32, no. 1, p. e22168, Jan. 2018.

[5] L. C. Clark and H. L. Thompson, "Determination of Creatine and Creatinine in Urine," Anal. Chem., vol. 21, no. 10, pp. 1218–1221, Oct. 1949.

[6] J. R. Delanghe and M. M. Speeckaert, "Creatinine determination according to Jaffe-what does it stand for?," NDT Plus, vol. 4, no. 2, pp. 83–6, Apr. 2011.

[7] B. Wuyts, D. Bernard, N. Van Den Noortgate , J. Van De Walle, B. Van Vlem , R. De Smet, F. De Geeter, , R. Vanholder, and J.R. Delanghe, , "Reevaluation of formulas for predicting creatinine clearance in adults and children, using compensated creatinine methods.," Clin. Chem., vol. 49, no. 6 Pt 1, pp. 1011–4, Jun. 2003.

[8] A. O. Hosten, BUN and Creatinine. Butterworths, 1990.

[9] C. de Lelis Medeiros de Morais and K. M. Gomes de Lima, "Determination and analytical validation of creatinine content in serum using image analysis by multivariate transfer calibration procedures," Anal. Methods, vol. 7, no. 16, pp. 6904–6910, Aug. 2015.

[10] A. J. Killard and M. R. Smyth, "Creatinine biosensors: principles and designs," Trends Biotechnol., vol. 18, no. 10, pp. 433–437, Oct. 2000.

[11] C. Pei, H. Guo, X. Yang, Y. Wang, X. Zhang, and H. Ye, "Sensors in Smart Phone," 2011, pp. 491–495.

[12] A. Roda, E. Michelini, M. Zangheri, M. Di Fusco, D. Calabria, and P. Simoni, "Smartphone-based biosensors: A critical review and perspectives," TrAC - Trends Anal. Chem., vol. 79, pp. 317–325, 2016.

[13] Q. Wei , R. Nagi, K. Sadeghi, S. Feng, E. Yan, S.J. Ki, R. Caire , D. Tseng, and A. Ozcan, "Detection and spatial mapping of mercury contamination in water samples using a smart-phone.," ACS Nano, vol. 8, no. 2, pp. 1121–9, Feb. 2014.

[14] S. Schaefer, "Colorimetric water quality sensing with mobile smart phones," 2014.

[15] Y.G. Lee, W. S. Jeong, and G. Yoon, "Smartphone-Based Mobile Health Monitoring," Telemed. e-Health, vol. 18, no. 8, pp. 585–590, Oct. 2012.

[16] S. Choi, S. Kim, J.S. Yang, J.-H. Lee, C. Joo, and H.-I. Jung, "Real-time measurement of human salivary cortisol for the assessment of psychological stress using a smartphone," Sens. Bio-Sensing Res., vol. 2, pp. 8–11, Dec. 2014.

[17] R. Spreitzer and Raphael, "PIN Skimming," in Proceedings of the 4th ACM Workshop on Security and Privacy in Smartphones & Mobile Devices - SPSM '14, 2014, pp. 51–62.

[18] "Sensors in Smartphones to Top 10 Billion Unit Shipments in 2020 - Counterpoint Research." [Online]. Available: https://www.counterpointresearch.com/sensors-smartphones-top-10-billion-unit-shipments-2020/. [Accessed: 06-Jun-2019].

[19] L. Shen, J. A. Hagen, and I. Papautsky, "Point-of-care colorimetric detection with a smartphone," Lab Chip, vol. 12, no. 21, p. 4240, Oct. 2012.

[20] B. D. Toora and G. Rajagopal, "Measurement of creatinine by Jaffe's reaction--determination of concentration of sodium hydroxide required for maximum color development in standard, urine and protein free filtrate of serum.," Indian J. Exp. Biol., vol. 40, no. 3, pp. 352–4, Mar. 2002.

[21] I. Salinas, M. H. Giménez, J. A. Monsoriu, and J. C. Castro-Palacio, "Characterization of linear light sources with the smartphone's ambient light sensor," Phys. Teach., vol. 56, no. 8, pp. 562–563, Nov. 2018.

[22] Y. Liu, Q. Liu, S. Chen, F. Cheng, H. Wang, and W. Peng, "Surface plasmon resonance biosensor based on smart phone platforms," Sci. Rep., vol. 5, pp. 1–9, 2015.