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Rapid Paper-Based System for Human Serum Creatinine Detection

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Received: 9 April 2018; Accepted: 21 May 2018; Published: 28 May 2018

Abstract: An integrated system consisting of a paper-based chip and a smart detection device is proposed for determining the human serum creatinine concentration based on Jaffé reaction theory. In the proposed approach, the reaction zone of the paper-based chip is implanted with picric acid and NaOH reagent and dried at 35 °C for 20 min. Human serum creatinine is dripped onto the reaction zone of the chip. A Jaffé reaction is induced by heating the chip at 37 °C for 5 min and the creatinine concentration is then derived by analyzing the RGB (red, green and blue) intensity of the resulting Janovsky complex using self-written analysis software installed on a smartphone. The validity of the proposed method is demonstrated using control samples with creatinine concentrations ranging from 0.2~8 mg/dL. The detection results obtained for 32 real-world creatinine samples are shown to be in excellent agreement with those obtained using a standard macroscale method (R^2 = 0.9994). Overall, the results show that the proposed system provides a compact, low-cost and reliable approach for human serum creatinine concentration detection.

Keywords: paper-based chip; smart detection device; creatinine; Jaffé reaction

1. Introduction

Creatinine is an end-product of creatine metabolism and is one of the most useful analytes for the assessment of renal function. In normal healthy individuals, the typical reference ranges for serum creatinine are 0.5 to 1.0 mg/dL for women and 0.7 to 1.2 mg/dL for men [1,2]. In healthy humans, creatinine is continuously excreted by the kidneys at a rate of around 1.6~1.7% per day [3]. Thus, increasing levels of creatinine in serum (or decreasing levels in urine) provide a possible indication of kidney failure. The creatinine concentration is also employed as a correction factor for fluctuations in the urine volume and is thus useful in accurately determining the micro-albumin/creatinine ratio. Consequently, sensitive and accurate assays for measuring the creatinine level in blood and urine samples are of significant interest in the clinical diagnostics field.

Numerous approaches for creatinine detection have been reported in the literature, including Jaffé kinetic assays [4–6], enzyme reactions [7], capillary electrophoresis [8], chemiluminescence [9], chromatography [10], molecular imprinted polymer (MIP) assays [11], spectrophotometry [12,13], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [14], potentiometric sensors [15,16], electrochemical sensors [17,18], colorimetry [19,20], pH sensors [21,22], amperometric sensors [23,24]

and nanoparticle-enhanced sensors [25–27]. Although the results of these common methods provide accurate and reliable results, while almost all these instruments are bulky, expansive and require complicated operations.

Recent years have seen a growing interest in micro-total analysis systems (μTAS) and lab-on-a-chip (LoC) devices. Microfluidic systems and microfluidic paper-based devices (μPADs) offer many advantages over traditional devices, including a reduced reagent consumption, a faster analysis time, a lower cost, an improved portability and better disposability. As a result, they are regarded as extremely promising candidates for point of care testing (POCT) and disease screening applications in the developing world [28–32]. Various microfluidic systems for the monitoring and diagnosis of disorders such as cardiovascular disease, diabetes, cancer and renal disease have been proposed in the literature [32–36]. Furthermore, several integrated microfluidic devices (or LoC devices) [37–41] and μPADs [42–45] have been presented for determining the creatinine concentration. For example, Dosso et al. [41] developed an integrated SIMPLE-based biosensor (named Creasensor) for the detection of creatinine in blood plasma samples. The platform combined fast bioassay on a self-powered SIMPLE microfluidic cartridge with an integrated signal processing unit and a simple but robust colorimetric read-out. The platform was validated on field in a single-blind study by measuring the creatinine levels in 16 creatinine-spiked plasma samples. An excellent agreement was observed between the measured concentration values and the spiked concentrations, over the creatinine range of 0.76~20 mg/dL. Sununta et al. [45] presented a μPAD for determining the creatinine concentration in urine samples by means of a Jaffé reaction process followed by colorimetric detection. The device was shown to achieve a wide linear range of 0.2–1 mM with a limit of detection of 0.08 mM.

The present study proposes an integrated system for rapid human serum creatinine concentration measurement consisting of a paper-based chip and a detection system comprising a heating module, a voltage regulator module, a cooling module, a CMOS camera, a USB connector and a smartphone. In the proposed detection process, a Jaffé reaction is induced between the creatinine and alkaline picrate reagent implanted in the detection zone of the chip by heating the chip at 37 °C for 5 min in the detection box. The color intensity of the resulting yellow-orange reaction complex is captured by the CMOS camera and transferred to the smartphone using the USB connector. Finally, the concentration of the creatinine sample is evaluated by analyzing the RGB (red, green and blue) color intensity of the reaction image using self-written software installed on the smartphone in the form of an app. Overall, the proposed system provides celerity, convenience, low-cost, miniaturization and automation for human serum creatinine concentration measurement.

2. Materials and Methods

2.1. Reagent Preparation

Creatinine powder and reagent (R1: sodium hydroxide; R2: picric acid) were purchased from Roche calibrator (Roche Diagnostics, Taipei, Taiwan) and Roche/Hitachi Creatinine plus (Roche Diagnostics, Germany). Creatinine detection was performed using a reagent solution consisting of 0.1 M sodium hydroxide (R1, pH > 13.5) and 50 mM picric acid (R2, pH = 6.5). Phosphate buffered saline (PBS) solution was prepared comprising 0.2 M sodium dihydrogen phosphate (NaH_2PO_4), de-ionized (DI) water and 0.15 M sodium chloride (NaCl). Finally, a standard 1000 mg/dL creatinine ($C_4H_7N_3O$) stock solution was prepared by mixing 0.1 g creatinine powder with 10 ml 0.1 M HCl. Control samples with creatinine concentrations in the range of 0.2~8 mg/dL were then prepared by diluting the stock solution with appropriate quantities of PBS solution.

2.2. Fabrication of Paper-Based Chip

Figure 1a–d show the main steps, including (a) pattern design, (b) wax printing, (c) heating wax process and (d) reagent and sample dropped, in the fabrication process used to prepare the paper-based chip. The chip was fabricated on Advantech qualitative filter paper (No. 1, Toyo Roshi Kaisha Ltd.,

Tokyo, Japan; pore size: 6 µm; thickness: 0.2 mm) and consisted of a circular reaction zone with a diameter of 3 mm bounded by an impermeable wax ring with an outer diameter of 5 mm. The chip was designed using CorelDrawR Graphics Suite X5 software and printed using a commercial wax printer (Fuji Xerox ColorQube 8750, Japan). After printing, the chip was heated for 3 min at 120 °C in a benchtop furnace (Vulcan A-550, Taiwan) to melt the wax and form a hydrophobic barrier through the thickness of the paper. Finally, the paper-based chip was bonded with a cardboard substrate using 3 M double-side tape (V1205, 3 M Comp., Taipei, Taiwan).

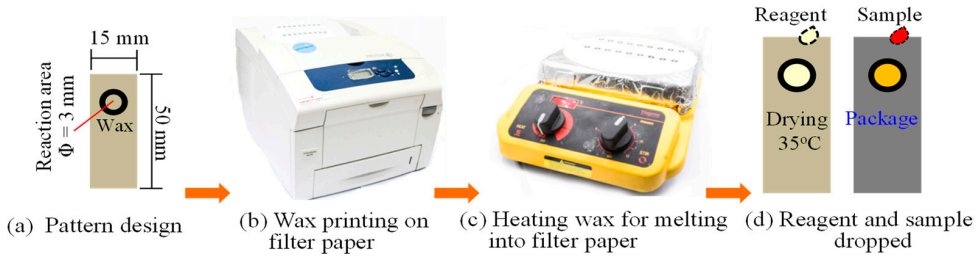


Figure 1. Schematic illustration showing main steps in fabrication procedure for paper-based chip.

2.3. Detection System and Jaffé Chemical Reaction Process

As shown in Figure 2, the main components of the detection system include a power source, a temperature controller, a voltage controller, a cooling module, a detection box and a smartphone (Xperia Z5, Sony Co., Tokyo, Japan) The detection box further contains a CMOS camera (1920 × 1080 pixels, Dmatek Co. Ltd., Taichung, Taiwan), two LED light sources, a microheater, a chip holder and a connector. A more detailed and the function of the module in the detection system can be referred to our previous study [46].

In performing the creatinine detection process, 3 µL of sample solution was dropped onto the reaction zone of the chip (reagent implanted with R1:R2 = 1 µL:2 µL and dried at a temperature of 35 °C for 10 min). The chip was then inserted into the chip holder of the detection box and heated at 37 °C for 5 min to induce a Jaffé reaction (i.e., Creatinine + Picric acid $\overset{alkaline}{\rightarrow}$ yellow-orange complex (see Scheme 1)). Images of the creatinine-picric complex were acquired by a built-in CMOS camera and transferred to a smartphone by means of the USB connector. Note that the smartphone was only applied to calculate the RGB values without catching images. Finally, the human serum creatinine concentration was derived using a self-written RGB color analysis app installed on the phone.

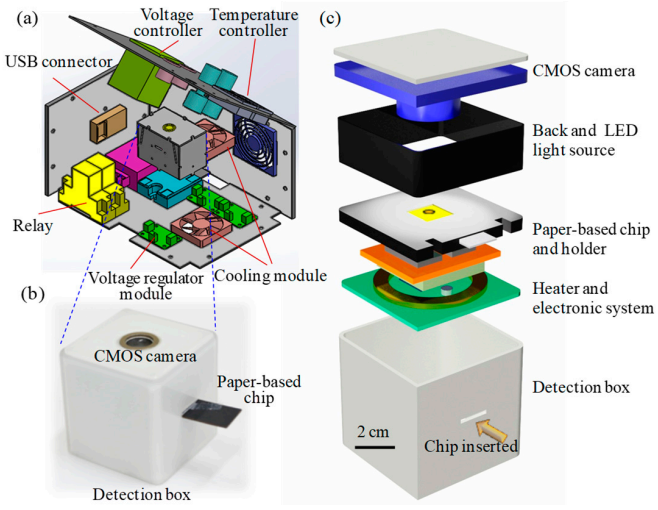
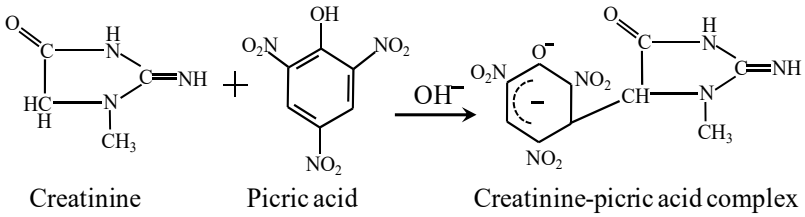


Figure 2. Photograph and schematic illustrations of: (**a**) main components of the smart detection device; (**b**,**c**) show detailed components of the detection box.



Scheme 1. Jaffé reaction between creatinine sample and picric acid/NaOH indicator.

3. Results and Discussion

3.1. Effects of Reaction Time and Reagent Concentration

A series of preliminary experiments were performed to investigate the effects of the Jaffé reaction time on the G (green) + B (blue) intensity of the reacted yellow-orange complex. Figure 3a presents the corresponding results obtained for creatinine control samples with concentrations of 0.2, 4.0 and 8.0 mg/dL given reaction times of 1~8 min and a constant reaction temperature of 37 °C. For reaction times of 1~3 min, the G (green) + B (blue) intensity steadily increases with an increasing reaction time. In other words, the reaction process between the reagent and the creatinine sample is incomplete. However, for reaction times of more than 5 min, the G (green) + B (blue) intensity remains stable for all three samples. In other words, the reaction process is complete. Thus, in performing all the remaining experiments, the reaction time was set as 5 min.

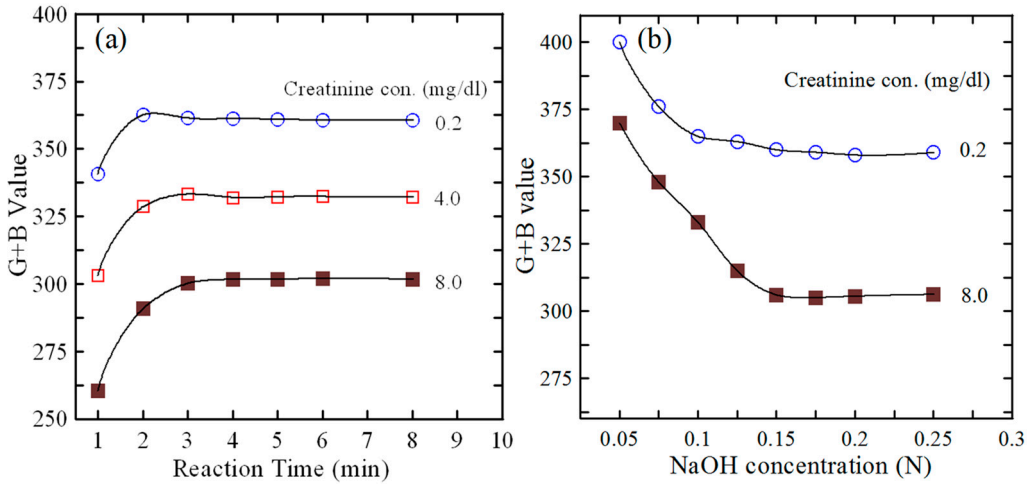


Figure 3. (**a**) Reaction time stability of samples with various creatinine concentrations given reaction temperature of 37 °C; (**b**) G + B values of creatinine samples with concentrations of 0.2 mg/dL and 8 mg/dL given different NaOH concentrations and constant reaction time of 5 min.

For RGB color analysis, a wide color distribution range is essential in improving the resolution and accuracy of the detection results. Figure 3b shows the G (green) + B (blue) intensity values of the reaction complexes obtained for 0.20 and 8 mg/dL creatinine control samples given NaOH reagent concentrations of 0.05~0.25 M, a reaction time of 5 min and a reaction temperature of 37 °C. For NaOH concentrations of less than 0.1 M, the G + B values of the two samples differ by only ~30 (G + B). However, as the NaOH concentration increases, the difference in the R+B values of the samples also increases. For example, given an NaOH concentration of 0.15 M, the interval between the G+B values of the two samples is equal to approximately 55 (G + B). In other words, the interval between the two values is more than 1.8 times higher than that obtained using 0.1 M NaOH. Consequently, the NaOH concentration was set as 0.15 M in all of the remaining detection tests.

3.2. Linearity of RGB Intensity Values and Calibration of Detection System

To determine the optimal RGB signal for detection purposes, the intensities of the R (red), G (green) and B (blue) signals were observed for a reaction temperature of 37 °C, a reaction time of 5 min and creatinine samples with concentrations in the range of 0.2~8 mg/dL. Figure 4a shows the corresponding results. It is seen that the R (red) intensity signal has a correlation coefficient of just $R^2 = 0.8603$ and is thus unsuitable for detection purposes. By contrast, the G (green) and B (blue) intensity signals both have correlation coefficients of more than 0.99. In other words, both signals are highly correlated with the creatinine concentration and thus provide a reliable basis for creatinine detection.

To calibrate the proposed system, the G (green) + B (blue) intensity values were measured for five standard creatinine samples with concentrations ranging from 0.2~8 mg/dL. For each sample, five separate measurements of the G (green) + B (blue) intensity were obtained. The measurements were then averaged in order to obtain a representative value for the sample. In accordance with the results described above, the reaction process was performed using an NaOH concentration of 0.15 M, a reaction temperature of 37 °C and a reaction time of 5 min. Figure 4b shows the intensity values obtained for the various control samples. Applying a regression analysis technique to the experimental results, the G (green) + B (blue) intensity value (Y) is found to be related to the creatinine concentration (X) as $Y = -9.4150X + 344.837$. Moreover, the correlation coefficient is equal to $R^2 = 0.9934$. In other words, the G (green) + B (blue) intensity varies linearly with the creatinine concentration over the normal creatinine range in human blood. Thus, the validity of the proposed system is confirmed.

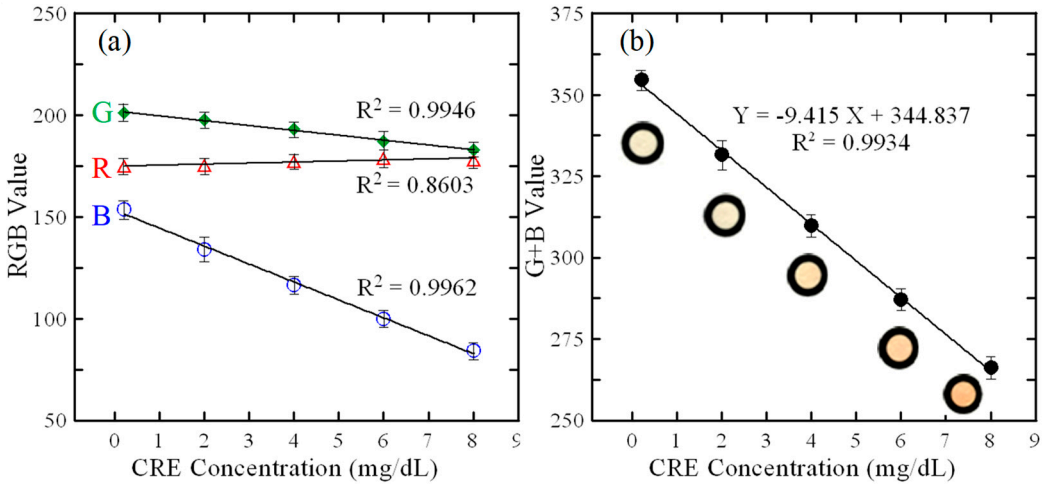


Figure 4. (**a**) RGB intensity values of creatinine samples and (**b**) variation of G (green) + B (blue) intensity (and associated color images) with creatinine concentration in the range of 0.2~8 mg/dL. (Note that the reaction temperature and reaction time are 37 °C and 5 min, respectively.).

3.3. Creatinine Detection in Real Samples

The applicability of the proposed platform for clinical diagnostic purposes was investigated using human serum creatinine samples obtained from 32 adult volunteers (patients at National Cheng Kung University Hospital in Taiwan). These human serum creatinine samples are obtained from whole blood samples via a separation and purification process at the hospital. The detection results were compared with those obtained using a macroscale human serum creatinine assay technique (a spectrophotometry method performed using an automatic biochemistry analyzer/integrated system 7600, Hitachi High-Technologies Co., Tokyo, Japan) at a major university hospital in Taiwan (National Cheng Kung University Hospital, Tainan, Taiwan). Figure 5a presents a scatter-plot of the results obtained for the 32 samples by the two methods. As shown in Figure 5b, the two sets of results are related as $Y = 0.9907X + 0.0001$, with a correlation coefficient of 0.9994. In other words, the ability of

the proposed paper-based human serum creatinine system to detect the creatinine concentration in real-world samples is confirmed.

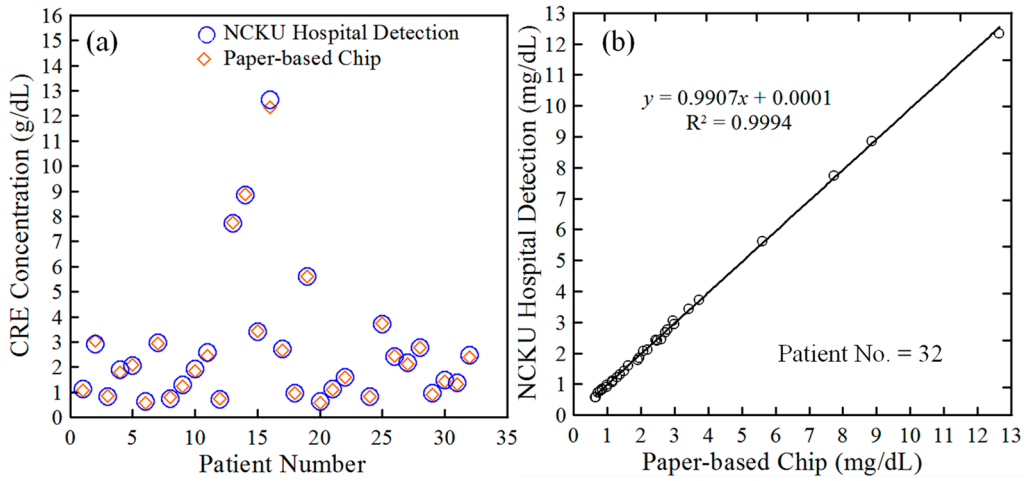


Figure 5. Comparison of human serum creatinine detection results obtained using proposed paper-based system and traditional macroscale system (**a**) a scatter-plot and (**b**) a related-plot by the two methods.

4. Conclusions

This study has presented a simple method based on Jaffé reaction theory for performing creatinine concentration detection using a paper-based chip and a smart detection system comprising a detection box, a temperature controller, a voltage regulator, a power source, a USB connector and a smartphone. The detection box further comprises a CMOS camera, two LED light sources, a chip holder and a hotplate. In the proposed system, the reaction region of the paper chip is implanted with 50 mM picric acid reagent and 0.15 M NaOH and the chip is then dried at 35 °C for 20 min. In the detection process, the serum creatinine sample is dripped onto the reaction zone of the chip and the chip is then transferred to the hotplate in the detection system, where a Jaffé reaction is induced by heating at 37 °C for 5 min. The experimental results have shown that the creatinine concentration measurements obtained by the proposed platform for 32 adult volunteers are in excellent agreement with those obtained using a conventional macroscale technique (R^2 = 0.9994). In other words, the feasibility of the proposed paper-based system for practical clinical purposes is confirmed. Overall, the proposed method provides a simple, portable and low-cost approach for human serum creatinine concentration detection. Thus, it represents an ideal solution for a wide range of diagnostic and POCT applications.

Author Contributions: L.-M.F., C.-C.T. and W.-J.J. conceived and designed the experiments; W.-J.J. performed the experiments; L.-M.F. and R.-J.Y. analyzed the data; W.-J.J. contributed reagents/materials/analysis tools; L.-M.F. wrote the paper.

Acknowledgments: The authors would like to thank the Ministry of Science and Technology of Taiwan for the financial support of this study under Grant Nos. MOST-104-2221-E-006-154-MY3, MOST 106-2314-B-020-002-MY3, MOST 106-2221-E-020-019-MY3, MOST 106-2622-B-020-001-CC2, MOST 107-2622-B-020-003-CC2 and 107TFDA-A-106.

Conflicts of Interest: The authors declare no conflict of interest.

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