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Simultaneous Determination Of BUN-Creatinine as Kidney Function Biomarkers in Blood using a Microfluidic Paper-based Analytical Devices

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Abstract. In this paper, we describe a Microfluidic Paper-based Analytical Devices (μ PADs) for the simultaneous quantification of two important biomarkers of kidney function in blood. This paper provides a simple, disposable, portable, and inexpensive colorimetric method for the quantification of Blood Urea Nitrogen (BUN) and serum creatinine (sCRE). BUN detection is based on the Berthelot reaction, in which urea is converted to ammonium by the use of urease, ammonium ion which then reacts with a mixture of salicylate, sodium nitroprusside, and hypochlorite to yield a blue-green chromophore. sCRE detection is based on the Jaffe reaction, in which sCRE under alkaline conditions react with picrate ions forming an orange complex. The intensity of the color formed is proportional to the BUN & sCRE concentration in the sample. Various experimental parameters were optimized to achieve the best performance of the μ PADs. There were no significant differences between the results obtained using the μ PADs and a comparative method.

Keyword. BUN, creatinine, kidney, microfluidic paper-based analytical devices.

1. Introduction

Chronic Kidney Disease (CKD) can originate from acute kidney injury (AKI) cases or is purely a CKD case. The paradigm of AKI cases has changed a lot, around 15-20% of cases of AKI will develop into advanced CKD in 24 months [1]. CKD is a public health problem throughout the world. The main results of chronic kidney disease, whatever the cause, develops kidney failure, complications of decreased kidney function, and cardiovascular disease. A lot of research shows this can be prevented or delayed by early detection and treatment. Unfortunately, CKD is underdiagnosed and treated early, in part because there is no agreement on the definition and classification of stages in the development of CKD and the availability of simple detection devices [2].

Nitrogen compounds that accumulate in the blood (BUN) and serum creatinine (sCRE) are routinely used as biomarkers for the initial stages of AKI [3]. These two biomarkers are also useful in the diagnosis of CKD where the blood BUN-sCRE ratio increases [4].

Metabolism gives the end result in the form of urea and creatinine. Dietary proteins and alteration of tissue proteins produce primary urea metabolites, whereas muscle creatin catabolism produces creatinine. Both are small molecules (60 and 113 Dalton each) which are distributed throughout the body fluid [5].

UV Vis Spectroscopy [6], High Performance Liquid Chromatography (HPLC) [7], Surface Enhanced Raman Spectroscopy (SERS) [8], Impedimetric Biosensor [9], Potentiometric Biosensors [10], Chronoamperometry [11], Slide Commercial Colorimetric Test on Smartphone Platforms [12],



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Microfluidic Systems [13], Graphene Quantum Dots (GQDs) - Enzymatic Determination based on Fluorescent Sensing [14], and Amperometric Biosensors [15] commonly used in BUN determination. UV Vis Spectroscopy [6], High Performance Liquid Chromatography [16], Potentiometric Biosensors [17], Surface Enhanced Raman Spectroscopy (SERS) [18], Liquid Chromatography-Mass Spectrophotometry (LCMS) [19], Use Microfluidic Chips [20], Chronoamperometry [11], Highly Sensitive TITP-CZE [21], Amperometric Biosensor [22], Non-Enzymatic Electrochemical Sensors [23], Sequential Injection in Valve Mixing (SI-VM) [24], Micro Paper-based 3D Analytical Devices (3D-mPADs) [25] commonly used in determining sCRE. These methods mostly require expensive tools with special skills, not disposable, not portable. Simple methods, a disposable and portable device are needed in areas with limited tools and limited skills. Those needs can be provided by microfluidic Paper-based Analytical Devices (μ PADs).

μ PAD is a single analytic system with all the components needed for analytical detection (immobilized reagents, sample transportation, and detection systems) on paper. Filter paper or paper chromatography with the main component of cellulose needed as an immobilization medium [26].

A simple, disposable, portable, and inexpensive colorimetric method for quantification of Blood Urea Nitrogen (BUN) and serum creatinine (sCRE) is designed simultaneously based on the Berthelot reaction and the Jaffe reaction. In the Berthelot reaction, urea is converted to ammonium using urease, ammonium ion which then reacts with a mixture of salicylate, sodium nitroprusside, and hypochlorite to produce blue-green chromophore. In the Jaffe reaction, sCRE in alkaline conditions reacts with picric ions to form an orange complex. The color intensity formed is proportional to the concentration of BUN & sCRE in the sample, measured using CorelDRAW software. The proposed analytic device can be used to evaluate kidney function and help prevent kidney failure.

2. Experimental

2.1. Chemicals and apparatus

Creatinine was purchased from Sigma-Aldrich, US. Creatinine colorimetric kit reagent was purchased from Glory Diagnostics, Spain. Urea was purchased from Merck, Germany. Urea colorimetric kit reagent was purchased from Biolabo, France. Whatman No.1 filter paper was purchased from GE Healthcare, UK. ColorQube 8570 Solid Ink Color Printer was purchased from Xerox, US. Micropipette was purchased from Thermo Fisher Scientific, US.

2.2. Design and fabrication of μ PADs

The μ PAD pattern was designed using CorelDraw, consist of two detection zones, BUN and sCRE simultaneously. Each detection zone had a diameter of 12 mm, with μ PADs dimensions of 30 x 16.5 mm. Wax was applied to the surface of Whatman No.1 filter paper with ColorQube 8570 Solid Ink Color Printer, heated on a hot plate at 150°C for 2 minutes. The wax that melts and penetrates to the filter paper becomes a hydrophobic barrier (Figure 1(a)). The backside of the μ PADs was coated with tape.

2.3. Colorimetric detection of BUN-sCRE

Simultaneous colorimetric detection procedure is schematically illustrated in Figure 1(b). The colorimetric kit reagents were used for BUN and sCRE quantification. First, 1 μ L R1 (urease 15 KUI/L, salicylate 31 mmol/L, and nitroprusside 1.67 mmol/L) was dropped into the BUN detection zone, followed by 1 μ L R2 (sodium hypochlorite 7 mmol/L and sodium hydroxide 62 mmol/L), forming a colorless side. On the other hand, 1 μ L R1 (25 mmol/L picric acid) was dropped into the sCRE detection zone, followed by 1 μ L R2 (300 mmol/L phosphate buffer, pH 12.7 and SDS 2.0 g/L), forming the yellow side. 1 μ L standard solutions or blood serum were dropped into each detection zone and filled the detection zone with capillary action, incubated for 45 minutes. The color of the reaction changes from colorless to blue-green for the determination of BUN and yellow to orange for the determination of sCRE. Finally, the μ PADs were captured using a scanner (HP Deskjet 2135 Ink Advantage).

2.4. Image processing for quantification

CorelDRAW-64 bit software was used to measure color intensity. The intensity of red, green, or blue is proportional to the concentration of BUN and sCRE.

2.5. Analysis of BUN-sCRE in serum samples

To analyze BUN-sCRE in serum samples, human serum samples were collected from Saiful Anwar General Hospital. Whole blood was collected in vacutainer tubes. After the collection, whole blood was centrifuged at 3000 rpm for 30 minutes. The resulting supernatant is designated serum. After centrifugation, it is important to immediately transfer the liquid component (serum) into a clean polypropylene tube using a Pasteur pipette. If the serum is not analyzed immediately, the serum should be apportioned into 0.5 mL aliquots, and stored at -20°C or lower.

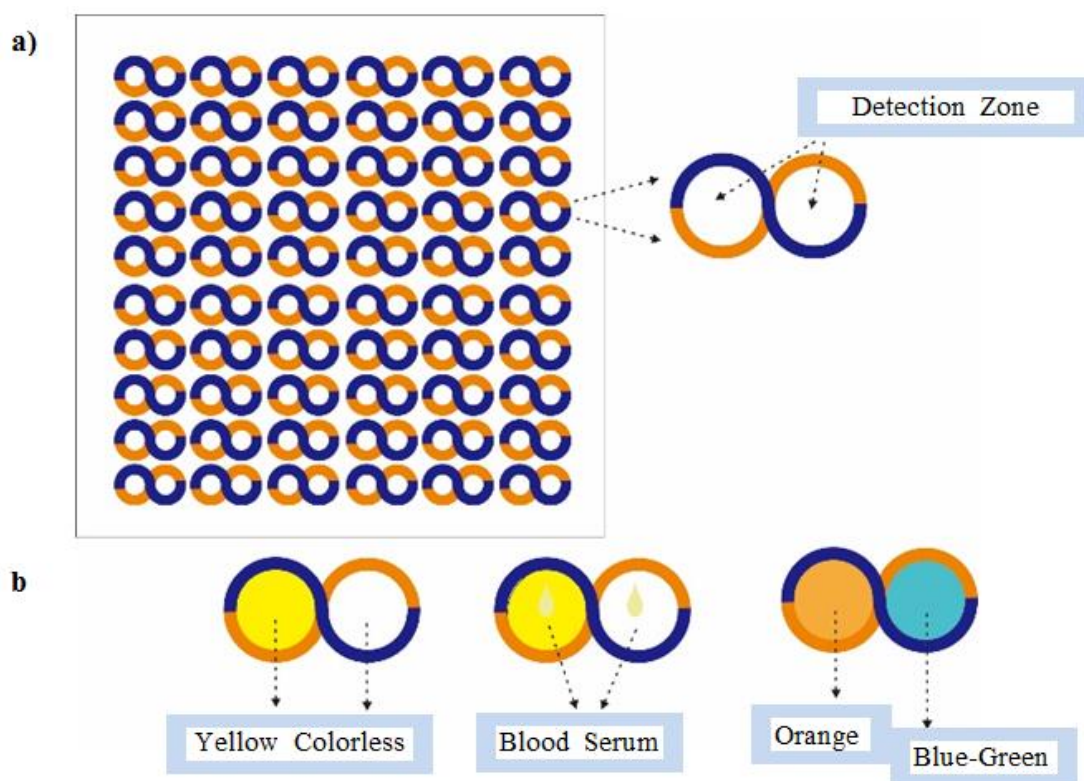


Figure 1. (a) The design of the μ PADs. (b) Simultaneous colorimetric detection procedure for BUN-sCRE. First, R1 (urease 15 KUI/L, salicylate 31 mmol/L, and nitroprusside 1.67 mmol/L) was dropped into the BUN detection zone, followed by R2 (sodium hypochlorite 7 mmol/L and sodium hydroxide 62 mmol/L), forming a colorless side. On the other hand, R1 (25 mmol/L picric acid) was dropped into the sCRE detection zone, followed by R2 (300 mmol/L phosphate buffer, pH 12.7 and SDS 2.0 g/L), forming the yellow side. Standard solutions or blood serum were dropped into each detection zone and filled the detection zone with capillary action, incubated for 45 minutes. Finally, the color of the reaction changes from colorless to blue-green for the determination of BUN and yellow to orange for the determination of sCRE.

3. Results an Discussion

3.1. Optimization of the penetration temperature and penetration time

The penetration temperature and penetration time of wax were investigated. Paper with the wax pattern was heated on the hot plate at 120°C, 130°C, 140°C, 150°C, 160°C for 30 seconds, 60 seconds, 90 seconds, and 120 seconds. The penetration time of 30 seconds, 60 seconds, and 90 seconds doesn't

produce perfect penetration. As shown in Figure 2, for temperature 120°C to 140°C, the inner diameter on the front side is always smaller than the inner diameter on the back side, which means that the penetration of the wax only takes place horizontally, not vertically, except on 150°C. The penetration temperature of 150°C and the penetration time of 120 s provided perfect wax penetration, where the inner diameter on the front side is equal to the inner diameter on the back side.

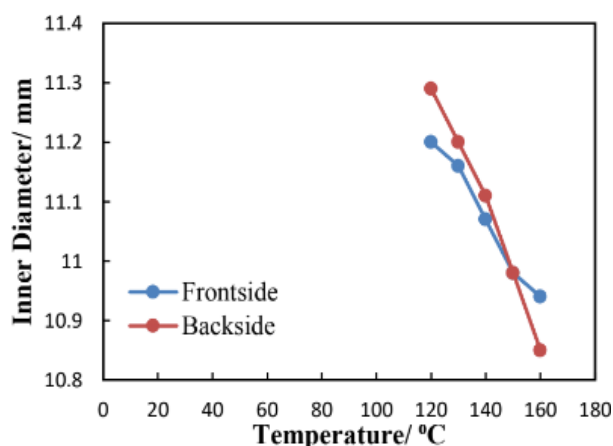


Figure 2. Optimization of the penetration temperature ($t=120$ seconds) ($n=3$).

3.2. Optimization of the order of addition of the reagents

The order of addition of the reagents and samples was investigated. As shown in Figure 3, for both, BUN and sCRE determination, the best spot color intensity was achieved when R1 was added first, followed by R2, and then blood serum sample.

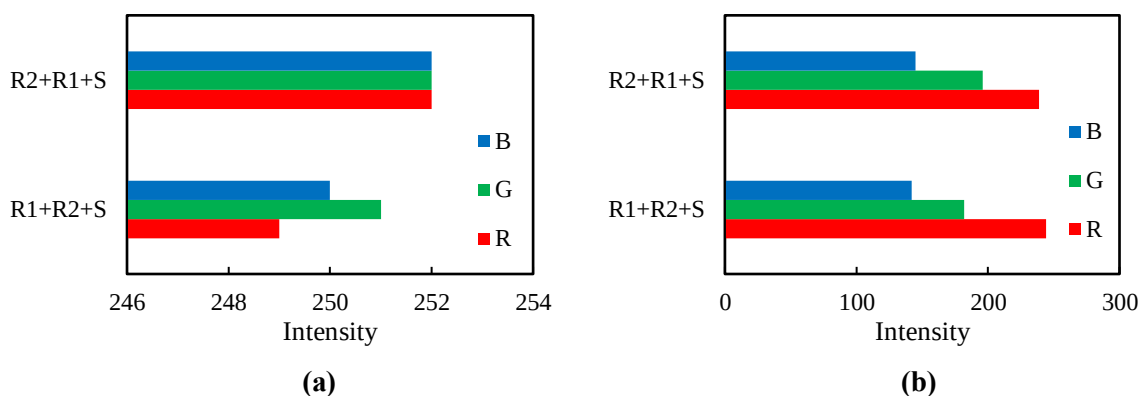


Figure 3. Optimization of the order of addition of the reagents (a) BUN and (b) CRE ($n=3$).

3.3. Optimization of reaction time

The reaction time was investigated. The reaction time is immediately recorded after the color change and the solution in the detection zone is completely dry. The μ PADs was captured using a scanner (HP Deskjet 2135 Ink Advantage) at 45 minutes, 60 minutes, 75 minutes, and 90 minutes. As shown in Figure 4, color intensity increases proportionally to 75 minutes. But the optimal reaction time is determined at 45 minutes for efficiency and the reason for the instability of the formation of the blue-green complex in the BUN colorimetric reaction. The color formed from the BUN colorimetric reaction gradually disappears.

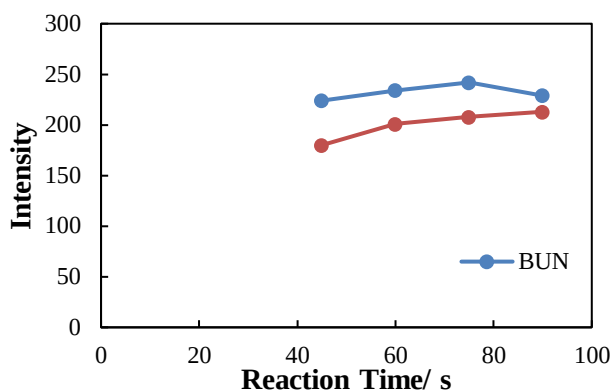


Figure 4. Optimization of the reaction time (n=3).

3.4. Linear range

The calibration curve was based on all optimizations (Figure 5). The linear range for determination of BUN is 10-100 mg/dL ($R^2 = 0.9905$). The linear range for determination of sCRE is 10-100 mg/dL ($R^2 = 0.9928$).

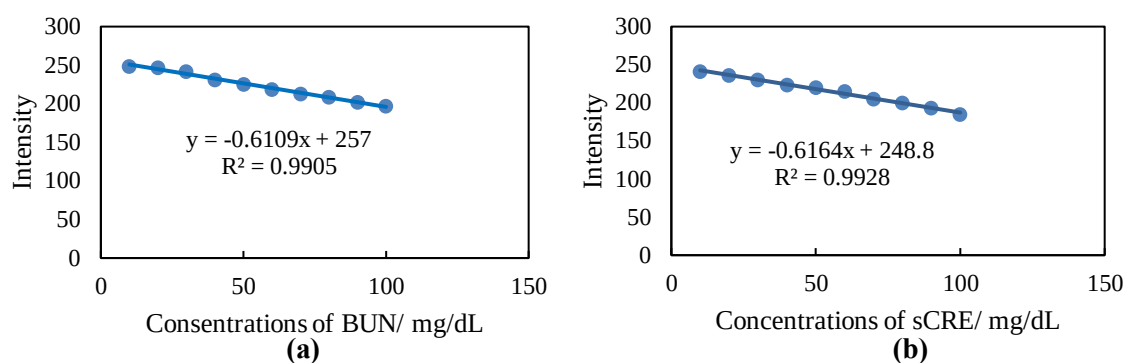


Figure 5. Calibration curve for (a) BUN determination and (b) sCRE determination (n=3).

3.5. Color charts

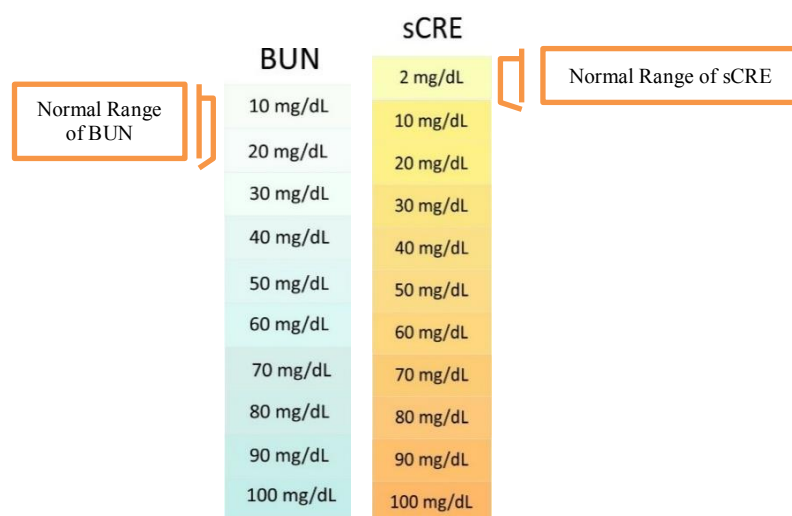


Figure 6. Color charts of (a) BUN and (b) sCRE.

Color charts were made to facilitate the use of μ PADs without other tools. The results obtained can be compared directly with the available color charts as shown in Figure 6. The normal range of BUN concentration in blood serum is 5 to 20 mg/dL. The normal range of sCRE concentration in blood serum is 0.8 to 1.5 mg/dL for male and 0.5 to 1.1 mg/dL for female.

3.6. Selectivity

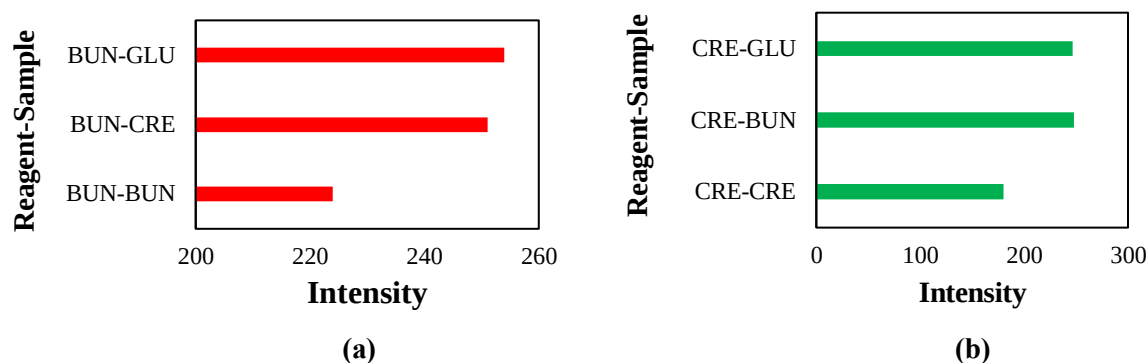


Figure 7. Selectivity of (a) BUN determination and (b) sCRE determination (n=3).

The selectivity of BUN-sCRE colorimetric determination was investigated, the specific color intensity between BUN reagents and standard BUN is shown in Figure 7(a) and the specific color intensity between CRE reagents and CRE standards is shown in Figure 7(b). Reagents used are specific to the analytes that determined.

3.7. Determination of BUN-sCRE in blood serum

The BUN-sCRE μ PADs were used for simultaneous determination of BUN-sCRE in human serum samples (Table 1).

Table 1. Concentration of BUN-sCRE in serum samples using the proposed method and the standard method RSSA* (n=3).

Blood Serum	BUN Concentration/ mg/dL		sCRE Concentration/ mg/dL	
	Proposed Method	Standard Method (RSSA* Lab Report)	Proposed Method	Standard Method (RSSA* Lab Report)
Sample 1	100	105.60	10	13.05
Sample 2	60	63.80	10	15.56

*RSSA (Saiful Anwar General Hospital)

4. Conclusions

A simple, disposable, portable, and inexpensive colorimetric method on μ PADs for the simultaneous quantification of Blood Urea Nitrogen (BUN) and serum Creatinine (sCRE) were successfully developed.

References

- [1] Chawla L S and Kimmel P L 2012 Acute kidney injury and chronic kidney disease: an integrated clinical syndrome *Kidney Int.* **82** 516–24
- [2] Levey A S, Coresh J, Balk E, Kausz A T, Levin A, Steffes M W, Hogg R J, Perrone R D, Lau J and Eknoyan G 2003 National Kidney Foundation Practice Guidelines for Chronic Kidney

- Disease: Evaluation, Classification, and Stratification *Ann. Intern. Med.* **139** 137
- [3] Lisowska-Myjak B 2010 Serum and Urinary Biomarkers of Acute Kidney Injury *Blood Purif.* **29** 357–65
 - [4] Lopez-Giacoman S 2015 Biomarkers in chronic kidney disease, from kidney function to kidney damage *World J. Nephrol.* **4** 57
 - [5] Hosten A O 2006 BUN and creatinine In: Walker H K, Hall W D, Hurst J W, editors. Clinical methods: the history, physical, and laboratory examinations. 3rd ed. Boston: Butterworths; 1990. p. 874–8
 - [6] Upstone S L Ultraviolet/Visible Light Absorption Spectrophotometry in Clinical Chemistry *Encyclopedia of Analytical Chemistry* ed R A Meyers (Chichester, UK: John Wiley & Sons, Ltd)
 - [7] Czauderna M and Kowalczyk J Easy and Accurate Determination of Urea in Milk, Blood Plasma, Urine and Selected Diets of Mammals by High-Performance Liquid Chromatography with Photodiode Array Detection Preceded by Pre-column Derivatization *Chem. Anal. (Warsaw)*, **54** 919-937
 - [8] Han Y A, Ju J, Yoon Y and Kim S M 2014 Fabrication of Cost-Effective Surface Enhanced Raman Spectroscopy Substrate Using Glancing Angle Deposition for the Detection of Urea in Body Fluid *J. Nanosci. Nanotechnol.* **14** 3797–9
 - [9] Mozaffari S A, Rahmanian R, Abedi M and Amoli H S 2014 Urea impedimetric biosensor based on reactive RF magnetron sputtered zinc oxide nanoporous transducer *Electrochimica Acta* **146** 538–47
 - [10] Marchenko S V, Kucherenko I S, Hereshko A N, Panasiuk I V, Soldatkin O O, El'skaya A V and Soldatkin A P 2015 Application of potentiometric biosensor based on recombinant urease for urea determination in blood serum and hemodialyzate *Sens. Actuators B Chem.* **207** 981–6
 - [11] Kozitsina A N, Dedeneva S S, Shalygina Z V, Okhokhonin A V, Chizhov D L, Matern A I and Brainina K Z 2014 Determination of urea and creatinine by chronoamperometry *J. Anal. Chem.* **69** 758–62
 - [12] Wu Y, Boonloed A, Sleszynski N, Koesdjojo M, Armstrong C, Bracha S and Remcho V T 2015 Clinical chemistry measurements with commercially available test slides on a smartphone platform: Colorimetric determination of glucose and urea *Clin. Chim. Acta* **448** 133–8
 - [13] Yang Y A and Lin C H 2015 Multiple enzyme-doped thread-based microfluidic system for blood urea nitrogen and glucose detection in human whole blood *Biomicrofluidics* **9** 022402
 - [14] Shao T, Zhang P, Tang L, Zhuo S and Zhu C 2015 Highly sensitive enzymatic determination of urea based on the pH-dependence of the fluorescence of graphene quantum dots *Microchim. Acta* **182** 1431–7
 - [15] Dervisevic E, Dervisevic M, Nyangwebah J N and Şenel M 2017 Development of novel amperometric urea biosensor based on Fc-PAMAM and MWCNT bio-nanocomposite film *Sens. Actuators B Chem.* **246** 920–6
 - [16] Samanidou V F, Metaxa A S and Papadoyannis I N 2002 Direct simultaneous determination of uremic toxins: creatine, creatinine, uric acid, and xanthine in human biofluids by hplc *J. Liq. Chromatogr. Relat. Technol.* **25** 43–57
 - [17] Soldatkin A, Montoriol J, Sant W, Martelet C and Jaffrezic-Renault N 2002 Development of potentiometric creatinine-sensitive biosensor based on ISFET and creatinine deiminase immobilised in PVA/SbQ photopolymeric membrane *Mater. Sci. Eng. C* **21** 75–9
 - [18] Stosch R, Henrion A, Schiel D and Güttler B 2005 Surface-Enhanced Raman Scattering Based Approach for Quantitative Determination of Creatinine in Human Serum *Anal. Chem.* **77** 7386–92
 - [19] Carling R S, Hogg S L, Wood T C and Calvin J 2008 Simultaneous determination of guanidinoacetate, creatine and creatinine in urine and plasma by un-derivatized liquid chromatography-tandem mass spectrometry *Ann. Clin. Biochem.* **45** 575–84
 - [20] Ávila M, Floris A, Staal S, Ríos Á, Eijkel J and van den Berg A 2013 Point of care creatinine

- measurement for diagnosis of renal disease using a disposable microchip *electrophoresis* **34**, 2956–2961
- [21] Hattori T and Fukushi K 2016 Highly sensitive tITP-CZE determination of L -histidine and creatinine in human blood plasma using field-amplified sample injection with mobility-boost effect: ce and cec *electrophoresis* **37** 267–73
- [22] Kumar P, Jaiwal R and Pundir C S 2017 An improved amperometric creatinine biosensor based on nanoparticles of creatininase, creatinase and sarcosine oxidase *Anal. Biochem.* **537** 41–9
- [23] Raveendran J, P.E. R, T. R, G. Nair B and Satheesh Babu T G 2017 Fabrication of a disposable non-enzymatic electrochemical creatinine sensor *Sens. Actuators B Chem.* **243** 589–95
- [24] Sabarudin A 2018 Sequential Injection at Valve Mixing (SI-VM) for Determination of Albumin-Creatinine Ratio in Urine *Orient. J. Chem.* **34** 730–4
- [25] Sununta S, Rattanasat P, Chailapakul O and Praphairaksit N 2018 Microfluidic Paper-based Analytical Devices for Determination of Creatinine in Urine Samples *Anal. Sci.* **34** 109–13
- [26] Yamada K, Henares T G, Suzuki K and Citterio D 2015 Paper-Based Inkjet-Printed Microfluidic Analytical Devices *Angew. Chem. Int. Ed.* **54** 5294–310