

Carbon paste electrode modified molecularly imprinted polymer as a sensor for creatinine analysis by stripping voltammetry

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Preface: International Conference and Workshops on Basic and Applied Sciences (6th ICOWOBAS 2017)

Ladies and Gentleman,

It is an honor and great pleasure for me to welcome all participants and presenters to Kurdistan Region and Erbil the cradle of mankind and civilization. International Conference and Workshops on Basic and Applied Sciences (6th ICOWOBAS 2017) is organized as implementation of existing collaborations between Salahaddin University of Erbil, Kurdistan, Iraq, University Airlangga Surabaya, Indonesia and University Teknologi Malaysia, Malaysia in order to promote the development of sciences and their prospect of application in industry and medical devices. The programs of the conference are the presentation of the papers and posters in the area of chemistry, biology, physics, mathematics and their application. The meeting intends to bring together researchers, scientists and scholars to exchange and share their experiences, new ideas, research novelties in related fields and discuss the practical challenges encountered and the solutions adopted.

In total, we received 221 Abstract submissions for oral and 44 posters, and more than 200 paper submissions from 12 countries and 48 universities. The selected papers will be published on AIP Conference Proceedings (Scopus index).

ICOWOBAS is a lot of work. We could not have done it without help from many people. We especially like to thank the president of Salahaddin University for permitting and supporting us to conduct the conference; Scientific board of ICOWOBAS, for inviting us to chair the meeting; our colleagues in Salahaddin University, for their support in the conference; the local committee, for organizing and handling conference; the many reviewers, for providing professional reviews; our sponsor: Ministry of Higher Education and scientific research and fast link company for their timely support.

Thank you and enjoy the conference

Assist. Professor Dr. Hewa Y. Abdullah

Chair of 6th ICOWOBAS 2017



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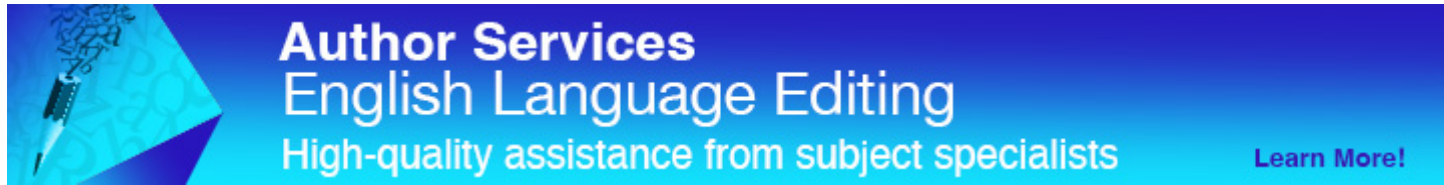
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Carbon Paste Electrode Modified Molecularly Imprinted Polymer as a Sensor for Creatinine Analysis by Stripping Voltammetry

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Abstract. Modification of carbon paste electrode with molecularly imprinted polymer (CP-MIP) as a voltammetric sensor for creatinine has been developed. MIP was synthesized by reacting melamine, chloranil and creatinine with a mole ratio of 1:1:0.1. Creatinine was extracted from polymer chain by using hot water to form a specific imprinted for creatinine molecule. Carbon paste-MIP electrode was prepared by mixing activated carbon, solid paraffin, and MIP in a 45:40:15(w/w %) ratio. The optimum conditions of creatinine analysis by differential pulse stripping voltammetry (DPSV) using the developed electrode were the accumulation potential -1000 mV during 90 s at pH 5. The precision of the method for 0.1-0.5 µg/L creatinine was 88.7–96.3%, while the detection limit of this method was 0.0315 µg/L. The accuracy compared by spectrophotometric method was 95.3–103.6%

INTRODUCTION

Creatinine is a form of creatine anhydride, mostly made in the muscle through a process of irreversible dehydration nanoenzymatic creatine phosphate [1]. Creatinine levels in the body can be used as an indicator of kidney function, thyroid and muscle. High creatinine levels in the body indicate diabetic nephropathy, eclampsia, glomerulonephritis and renal failure. Meanwhile if the creatinine level in the blood is too low it can cause muscular dystrophy [2]. A normal creatinine level in the serum of adults is ranged from 0.7 to 1.5 mg/dL [3].

The most commonly used methods for the analysis of creatinine are based on colorimetry using the Jaffé's reaction or the enzymatic colorimetric method [4]. However, colorimetric methods are affected by numerous metabolites and drugs found in biological samples [5]. In the recent decade, voltammetric method is widely applied in health and biomedicine fields for measuring few compounds levels in the body which should always be controlled like uric acid, creatine, and creatinine. This is because voltammetric method has low detection limit, high sensitivity, requires little sample preparation, requires small sample volume and less time consuming [6].

The most important part of voltammetric method is the working electrode. A lot of studies have been conducted to increase the selectivity and sensitivity on creatinine analysis by developing the working electrode [7-10]. Electrode modification technique which is very interesting to study is a modified electrode by molecularly imprinted polymers (MIP) [11,12]. Its stability at high temperatures and extreme pH, low cost, and reuseable causing this technique has been developed. MIP is very easily adjusted in any form and shape to the target molecule (analyte). Analyte acts as a template to be surrounded by a polymer formed from a mixture of monomers and cross-linker. Then the template is removed from the polymer pores through the extraction process to form analyte molecular imprinted polymers [13].

In this research, the developments of creatinine sensor by modifying carbon paste electrodes with MIP (carbon paste-MIP) by differential pulse stripping voltammetry (DPSV) have been studied. The carbon paste electrode can be easily modified, has a wide measurement range, and low cost on its manufacture [14]. MIP was synthesized by reacting melamine, chloranil and creatinine with a molar ratio of 1:1:0.1 [15]. Carbon paste-MIP electrode was prepared by mixing activated carbon, paraffin, and MIP with ratio of 45:40:15 (w/w%) [16].

EXPERIMENTAL

Material and instrument

Chemicals used were creatinine, melamine, chloranil (Sigma), dimethyl formamida (DMF), acetic acid, sodium acetate, sodium hydrogenphosphate, sodium dihydrogenphosphate, hydrochloric acid, n-hexane, ethanol (Merck). All chemicals were analytical grade. Water used is UHP (*ultra high pure*) water. The stock solution of creatinine 1000 mg/L was prepared by dissolving 0.1000 g creatinine in about 10 mL water and diluted with water until 100 mL in volumetric flask. Working creatinine solutions under 1 mg/L were prepared daily by diluting appropriate working solutions, and their pH were adjusted with the addition of acetate or phosphate buffer. The samples used in this study were serum from patients of a local clinical laboratory.

The instruments used in this study were 797 Computrace Voltammetry (MVA system-1) which comprises a sample container, stirrer, processor units, PCs, working electrode of carbon paste-MIP, reference electrode of Ag/AgCl (KCl 3M) and Pt auxiliary electrodes. The other equipments were reflux apparatus, micropipette, pH meter, hot plate and glassware.

Synthesis of poly-melamine-co-chloranil, non imprinted polymer (NIP), and molecularly imprinted polymer (MIP)

Poly-melamine-co-chloranil was synthesized by dissolving 0.1261 g of melamine in 30 mL of dimethyl formamide and 0.2458 g chloranil in 10 mL of dimethyl formamide. The two solutions were mixed and heated for about 1 hour at temperature 160°C to evaporate the solvent [17]. Afterwards, the solution is cooled and refluxed for 5 hours at temperature 160°C [18]. The mixture was then heated to evaporate the solvent until there are only solids remained.

The NIP was synthesized by similar procedure by adding as much as 0.0113 g creatinine in 2 mL of hot water as the analyte. The mole ratio between the melamine, chloranil, and creatinine was 1: 1: 0.1 [5]. Furthermore NIP solids washed with DMF and ethanol (± 5 mL) to remove residual unreacted monomers. The remaining ethanol was evaporated and the solids are heated in an oven at $\pm 80^\circ\text{C}$. Creatinine was then extracted from the NIP by centrifugation using ± 5 mL hot water to form mold in the polymer chain which is specific for creatinine. The extraction was done three times and each during 5 minutes. Then the solid was dried in an oven at $\pm 105^\circ\text{C}$. Further, MIP, NIP and poly-melamine-co-chloranil characterized by using FTIR.

Fabrication of carbon paste-MIP electrode

This work was preceded by the activation of carbon powder chemically and physically. Chemical preparation was done by soaking the carbon in a solution of 4N HCl for 24 hours, then the powder was immersed in n-hexane. The physical preparation was done by heating the carbon in a furnace at a temperature of 500 °C. Carbon paste-MIP electrode was fabricated by mixing activated carbon, paraffin and MIP with mass ratio of 45: 40: 15 [16] and heated on hot plate until form a paste. The mixture was put into the micropipette tube which has Ag wire been inserted in the body to electrical contact with the instrument. The remaining space in micropipette tube was filled with melted paraffin. The electrode surface was polished using a paper to produce a smooth and flat surface [19].

Optimization of research condition

The research conditions optimized were accumulation potential, accumulation time, and pH solution. Optimization of accumulation potential was done by analyzing the solution of 20 mL creatinine 5 $\mu\text{g/L}$ by DPSV with stirring rate 2400 rpm and accumulation time 30 s. Variation of accumulation potential started -1.1 until -0.1 V

(vs Ag/AgCl). Variation of accumulation time started 30 s to 150 s which has 30 seconds intervals using the optimum accumulation potential. pH of creatinine solution was varied from 4 to 8 by adding buffer solution. The added buffer solution also functions as a supporting electrolyte.

Performance of modified electrode, method validity and application

Carbon paste electrode modified MIP was used to analyze the working solution creatinine 5 $\mu\text{g/L}$ as much as 20 mL by DPSV. Voltammogram and current peak value compared with the results from the analysis obtained by using bare carbon paste, carbon paste-NIP and carbon paste poly-melamine-co-chloranil electrodes. Further he determined the method validity includes the detection limit, precision and accuracy. The developed method was applied to measure creatinine levels in serum samples and compared with the spectrophotometric method which is commonly used in the medical field.

RESULTS AND DISCUSSION

Synthesis and characterization of poly-melamine-co-chloranil, non imprinted polymer (NIP), and molecularly imprinted polymer (MIP)

Monomers used to manufacture polymers are melamine and chloranil. Selection is based on the structure of monomers having $-\text{NH}_2$ functional groups on the melamine and the functional group $\text{C}=\text{O}$ at chloranil that can interact with a functional group of creatinine through hydrogen bonding [20]. Polymer formation was indicated by discoloration, increased viscosity and formation of solids. The liquid formed after the reflux process is blackish brown, increasing viscosity and there are solid of dark brown. The mixture is then evaporated to remove DMF residual solvent. Formation of poly-melamine-co-chloranil was classified on condensation polymerization, as in the formation of polymer molecules releasing HCl. NIP is synthesized by reacting melamine and chloranil as monomer and creatinine as templates with a mole ratio of 1: 1: 0.1 [15]. Estimates polymerization reaction and hydrogen bonds formed between creatinine with polymer chains can be seen in Fig. 1. Synthesis MIP was done by extracting creatinine which is trapped in the pores of NIP using hot water ($\pm 80^\circ\text{C}$).

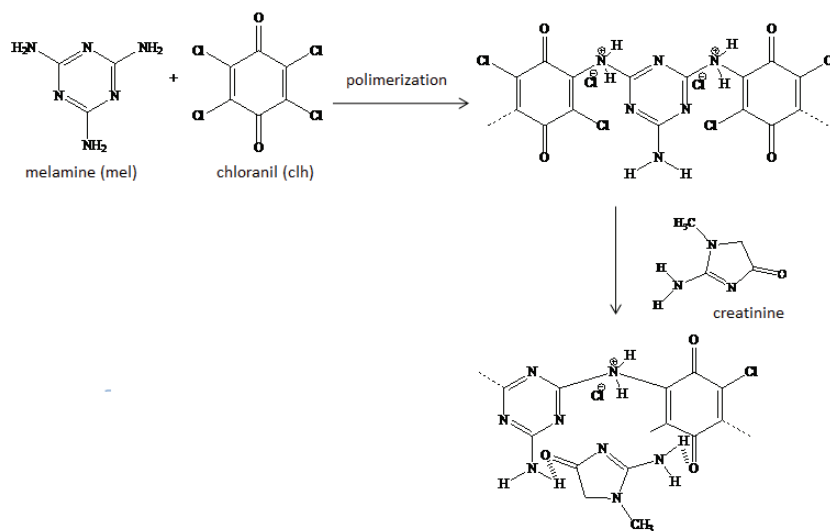


FIGURE 1. Binding of template (creatinine) on the poly-melamine-co-chloranilchain [20]

Characterization of the polymer, MIP and NIP was done using FTIR to determine and compare the results of polymeric melamine-co-chloranil, NIP and MIP. The spectra of poly-melamine-co-chloranil, NIP and MIP is shown in Fig. 2. While the data wave number of some specific functional groups on the poly-melamine-co-chloranil, NIP and MIP is shown in Table 1.

TABLE 1. Data of wave number of polymer, NIP and MIP spectra

Polymer	Wave number of functional grup (cm ⁻¹)		Remark
	NIP	MIP	
3398.34	3398.34	3336.62	Stretching -NH (melamine)
775.33	779.19	779.19	Stretching C-Cl (chloranil)
-	2850.59; 2923.88	-	Stretching C-H sp ³
1500.52	1531.37	1519.80	Stretching C=C (chloranil)
1303.79	1299.93	1296.08	Stretching C=N (melamine and creatinine)
1022.20	1022.20	1064.63	Stretching C-N (melamine and creatinine)

The difference of poly-melamine-co-chloranil, NIP and MIP spectra can be seen from the trapped and the released of creatinine from polymer network that was indicated by no peak of the stretching group -CH sp³ at 2850.59 and 2923,88 cm⁻¹, but the group appears in the NIP spectra.

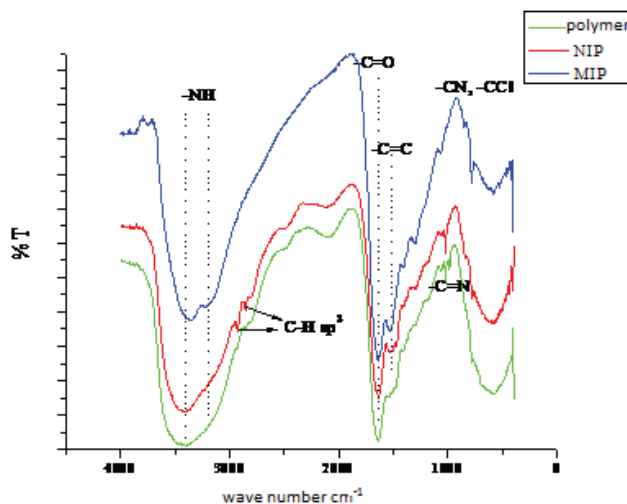


FIGURE 2. FTIR spectra of poly-melamine-co-chloranil, NIP, dan MIP

Optimization of pH, accumulation potential and accumulation time

The optimum pH was studied by analyzing 5 μg/L creatinine pH 4, 5, 6, 7 and 8, at deposition potential of -1.0 V and deposition time of 60 s. The relationship between pH of creatinine solution and measured current peak was shown in Fig. 3. The pH 5 was chosen as the optimum pH for analyzing the solution of creatinine based on high current and better voltammogram form among others.

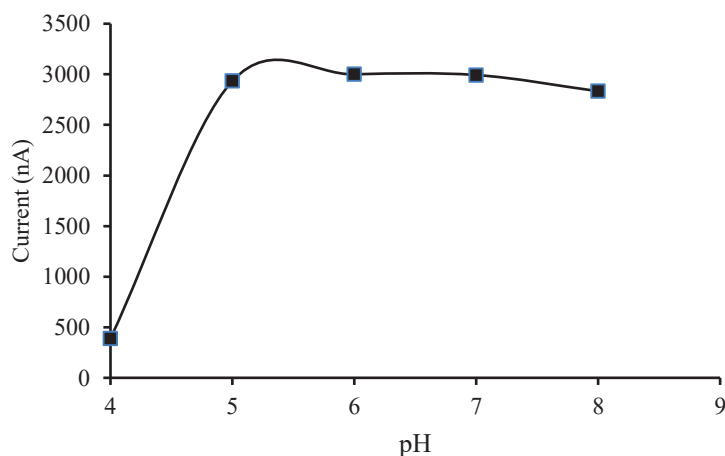


FIGURE 3. Curve relationship of peak current and the pH of creatinine 10 μg/L

The accumulation potential given to working electrode (carbon paste-MIP) causing the accumulated analyte to the electrode. Effect of accumulation potential on the peak current value is shown on Fig. 4. Selecting the optimum potential in this study is based on peak form of voltammogram and the resulting current value. The creatinine peak was detected at a stripping potential of 0.119 V on optimum accumulation potential range of -1.0 to -0.9 V.

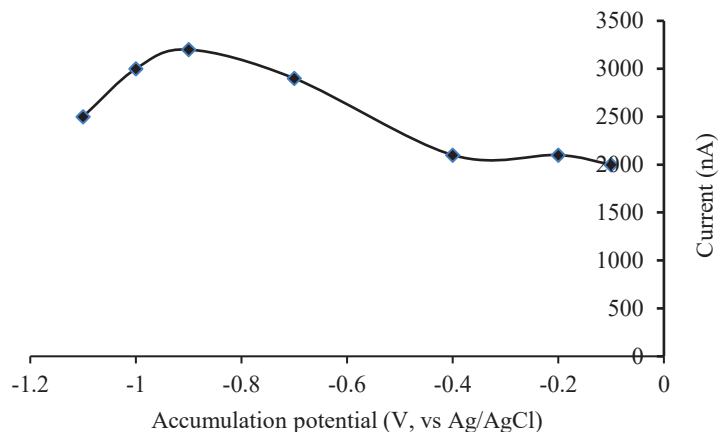


FIGURE 4. Curve of current on the variation of creatinin accumulation potential

Creatinine analysis in this study belong to the type of anodic stripping voltammetry. At the accumulation step, the working electrode is given a negative potential, so that the analyte is reduced on the surface of the electrode (electrode acts as the cathode), meanwhile when stripping step, analyte undergoes be oxidized and the working electrode acts as the anode [6]. Scheme of creatinine molecules rearrangement when trapped in the mold of MIP during analysis process is shown in Fig. 5.

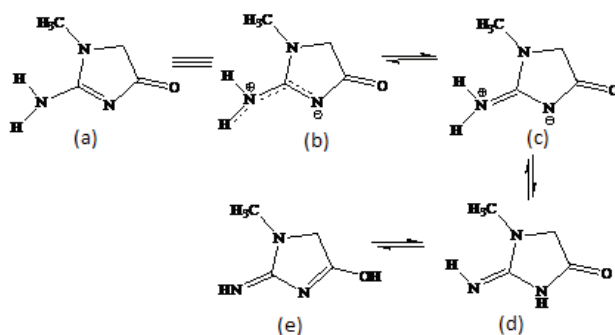


FIGURE 5. (a-e) Rearrangement of creatinine molecule, (c) polarisation (electron distribution) of creatinine on the polymer chain. (e) creatinine after electrochemically reduce [20].

Optimization of the accumulation time was performed to determine the required time of creatinine for optimally accumulate on the surface of the electrode. Accumulation is the electrochemical pre-concentration of the analyte on the electrode surface at the applied potential. This step involved the adsorptive accumulation or electron transfer mechanism of analyte on surface of modified electrode, depending on the interaction between analyte and the electrode. In the next step, analyte is stripped back into the solution. The current signal is observed during the stripping process. Based on Faraday's law, the longer the deposition time, the more accumulated analytes on the electrode surface and stripped back into the solution, thus produce the higher current signal. So, the longer the accumulation time, the higher the measured current. Curve of Fig.6 shows that at the accumulation time 0 minute, high current is obtained which is called as non-faradaic current [6] which is caused by the matrices in the sample. In this work, creatinine has been optimally accumulated during 90 s that been marked due better voltammogram shape and at the time the electrode has been able to distinguish changes in creatinine concentration. The results of the

accumulation time optimization of creatinine 1 $\mu\text{g/L}$ with pH 5 at the accumulation potential of -1.0 V is shown in Fig. 6.

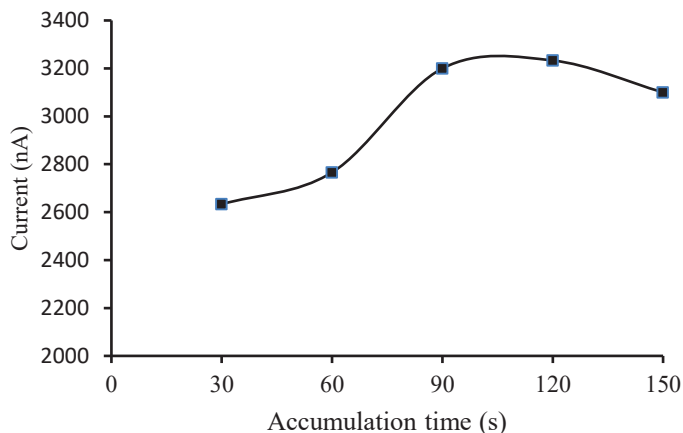


FIGURE 6. Curve of current on the variation of accumulation time of creatinine 1 $\mu\text{g/L}$

Performance of the electrode

Analysis of creatinine using bare carbon paste electrode generates a current equal to the creatinine current using an carbon paste-MIP electrode, because the bare carbon paste electrode has wider surface area so that the more creatinine can be contact directly with the electrode, while the carbon paste-MIP electrode contained specific mold so that the creatinine can be trapped optimally and generating a high current.

Analysis of creatinine using carbon paste modified poly-melamine-co-chloranil and carbon paste-NIP electrode generate peak current that were smaller than the carbon paste-MIP electrode (Table 2). This indicates that the carbon paste electrode modified poly-melamine-co-chloranil and carbon paste-NIP have not specific pore to creatinine. Molecule of creatinine that was still trapped in the NIP is not stripped into solution during the analysis, so it does not give increasing current compared with the peak obtained using the MIP modified electrode. Instead, creatinine molecule remained in the NIP and polymer network covers creatinine molecules in the solution for entry into the pores and reduces the area contact between creatinine and carbon paste. Analysis using carbon paste electrodes produces a large current enough and the stripping potential shifted from 0.119 to 0.27 V and generates not good peak shape. Voltammograms of creatinine using different types of electrode are shown in Fig. 7.

TABLE 2. Data of stripping potential and current of creatinine 5 $\mu\text{g/L}$ using various electrode

No.	Electrode	Peak potential (V, vs Ag/AgCl)	Peak current (nA)
1.	Bare carbon paste	0.27	2700
2.	Carbon paste-poly-melamine-co-chloranil	0.03	1900
3.	Carbon paste-NIP	0.00	1700
4.	Carbon paste-MIP	0.12	2700

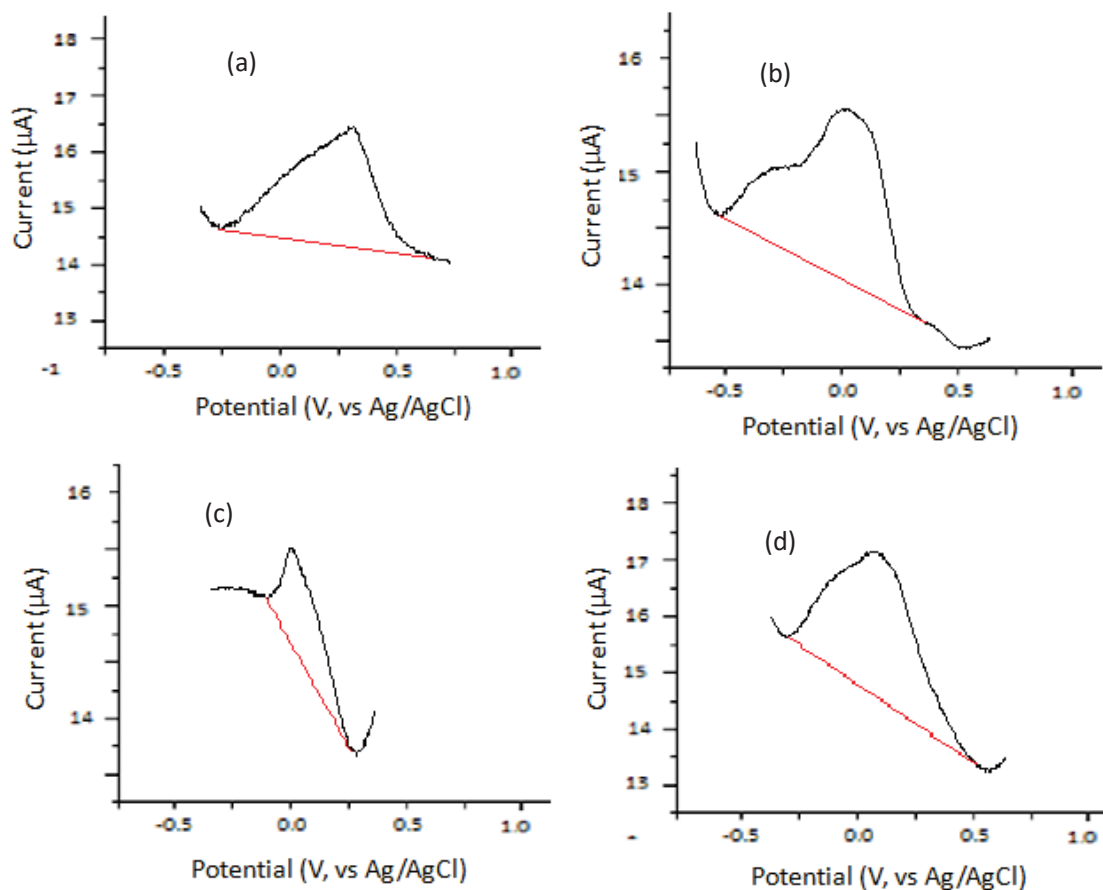


FIGURE 7. Voltammogram of creatinine analysis by DPSV using (a) bare carbon paste, (b) carbon paste-poly-melamine-chloranil, (c) carbon paste-NIP and (d) carbon paste-MIP electrode

Method validity and application

Creatinine standard solutions were made with concentration of 0.1; 0.2; 0.3; 0.4; and 0.5 $\mu\text{g/L}$. Each 20 mL of the solution was analyzed three times by DPSV at the optimum parameter. The resulting data were then used to create a calibration plot of creatinine as shown in Fig. 8.

In this study a calibration plot generated a linear regression of $y = 1448x + 1757$ with a correlation coefficient (r) of 0.9979. The value of the intercept of a graph represents the great non faradic current value. Non faradic current is generated by due the migration of electroactive matter on the electrode surface and the electrode material properties that enable adsorption and desorption of electroactive analyte from the solution to the electrode surface [21].

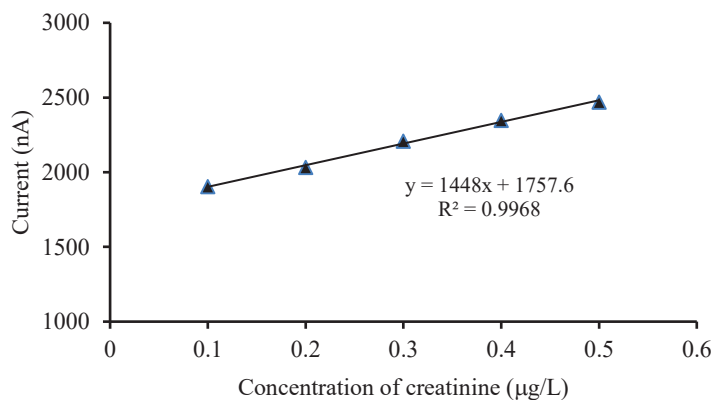


FIGURE 8. Calibration plot of creatinine

The calibration plot is then used to determine the method validity including precision, detection limit, and accuracy. In the measurement range of standard solution 0.1-0.5 µg/L generates precision of 88.7–96.3%, while according to Horwitz’s trumpet and the Association of Official Analytical Chemist (AOAC) the precision which is permitted for the µg/L level is 68-132% [22]. The detection limit of the method was 0.0315 µg/L, while the detection limit of the commonly used method to analyze creatinine in the medical field (spectrophotometric method) is in the level mg/L [4]. The detection limits in this study is also better than the results of previous studies [5, 12, 20, 23, 24]. The comparative test of DPSV method using the developed electrode with spectrophotometric methods for determination of five serum samples produces accuracy as shown in Table 3.

TABLE 3. Accuracy of the DPSV compared by spectrophotometry method

Number of sample	Concentration (mg/dL)		Accuracy (%)
	DPSV	Spectrophotometry*)	
1	0.81	0.83	97.59
2	1.28	1.26	101.20
3	1.45	1.40	103.60
4	0.91	0.95	95.30
5	0.99	1.02	97.20

*) data from clinical laboratory

The developed electrode shows accuracy of 95.3-103.6%, while accuracy value that statistically acceptable for the concentration less than 1 µg/L is 40-120% [22]. According to the method validity, the carbon paste modified MIP electrode developed in this study can be used as alternative sensor on the creatinine analysis and applied in the medical field.

CONCLUSION

Carbon paste-MIP electrodes manufactured with ratio of carbon, paraffin, and MIP of 45:40:15 (w/w %) shows a good performance as a sensor on creatinine analysis by DPSV. The electrode exhibits the optimum performance at accumulation potential and time of -1000 mV and 90 s, respectively. The electrode has detection limit much lower than the spectrophotometric method, so voltammetric method using the developed sensor is potentially used to control of creatinine levels in a biological sample in the medical field.

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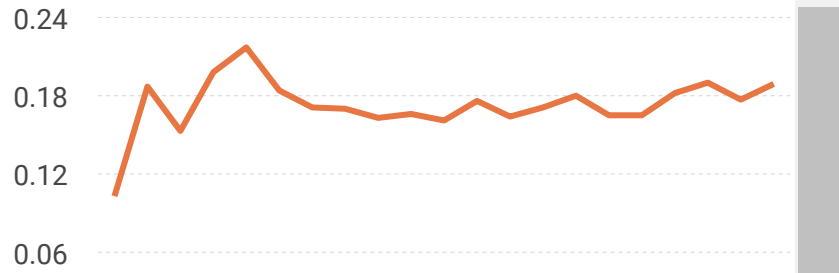
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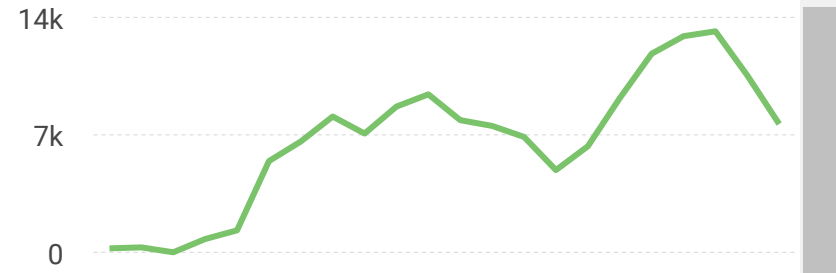
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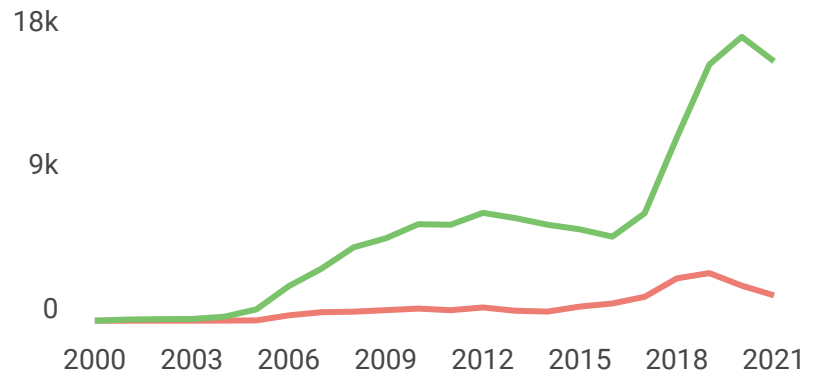


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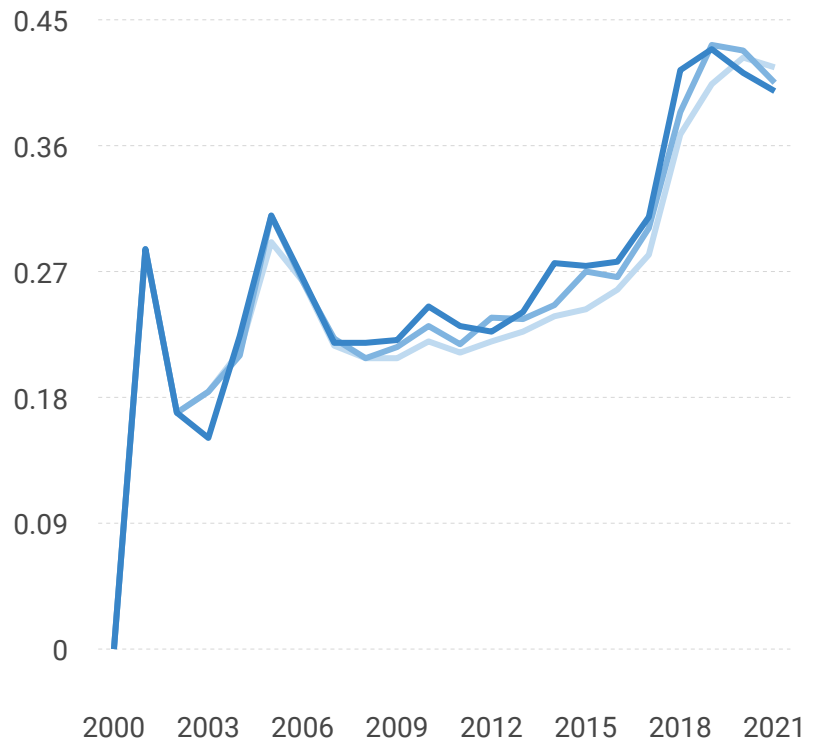
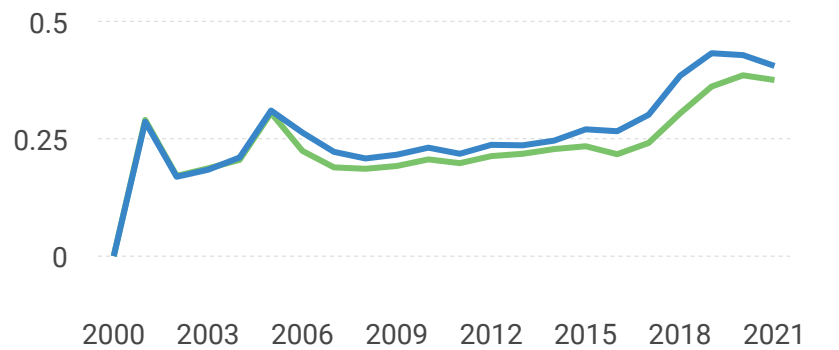


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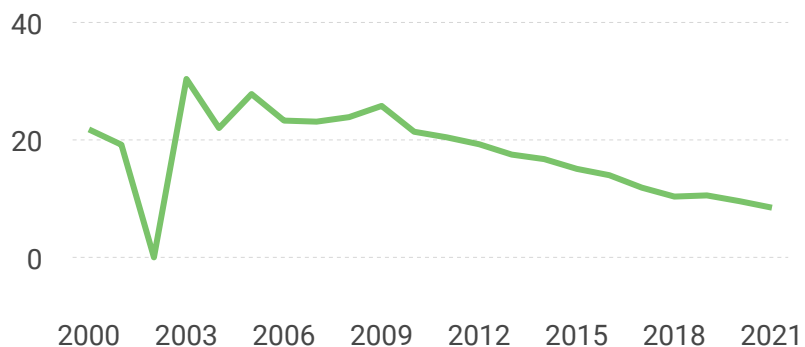
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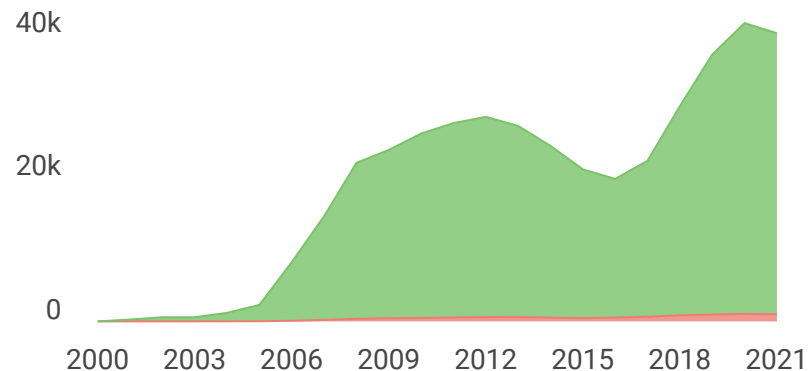
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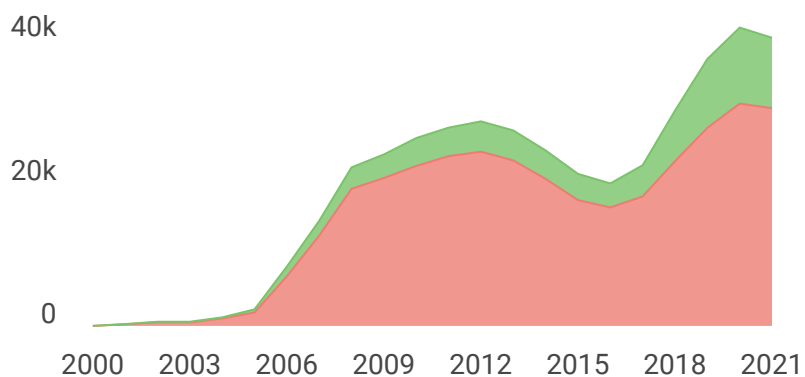
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