International Journal of GEOMATE, Aug., 2016, Vol. 11, Issue 24, pp.2356-2362
Geotec., Const. Mat. & Env., ISSN: 2186-2982(Print), 2186-2990(Online), Japan

ATOMIC FORCE MICROSCOPIC AND ELECTROCHEMICAL CHARACTERIZATION OF THE MODIFIED SCREEN PRINTED CARBON ELECTRODE BY SELF ASSEMBLED DEPOSITION OF CHITOSAN AND ACTIVATED CARBON

Porntip Wongkaew[1,2] and Suta Poosittisak[1,3]

[1]Biosensing Technology for Sustainable Development Research Group, Khon Kaen University, Khon Kaen 40002, Thailand; [2]Division of Plant Pathology, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand; [3]Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

ABSTRACT: The surface topographic characteristics generated after a modification of screen printed carbon electrode (SPCE) with either chitosan or activated carbon blending chitosan were closely investigated by atomic force microscopy and a complementary electrochemical techniques. Eighty five percent deacetylation degree chitosan was used at 1% in 1% acetic acid while the activated carbon blending ratio used was 1:1 in chitosan solution. The modification was performed via self assembled deposition directly onto the working electrode surface area. Growth feature of each modified surface was obviously differentiated by all roughness parameters such as an average roughness, root mean square, height different, grain and fractal. The highest roughness value with the least grain was from chitosan modified SPCE. A good correlation result was obtained with electrochemical cyclic voltammetry that a great change in current response was also appeared. Both the modified SPCE by chitosan and activated carbon blending chitosan could produce comparable higher current sensitivity with up to around 0.5 slope value following by their regression coefficient of 0.99. Thus affordable and efficient platforms for a fabrication of desirable pesticide biosensor could be achieved with these modifications as expected.

Keywords: Atomic force microscopy, cyclic voltammetry, Modified SPCE, Chitosan, Activated carbon

1. INTRODUCTION

In the development of electrochemical appliances including sensors, catalytic implements and electronic devices, there are several types of materials introduced for active electrode fabrication such as metal oxides, clays, conducting polymers and carbon based elements [1]. Among these, however in our viewpoint, a natural biopolymer as chitosan and a high surface area activated carbon have attracted much attention by their excellent attributions which are not only a prompt availability and productivity but also environmental friendly and cheap. Chitosan, the second most abundant polymer in nature, is derived by deacetylation process from chitin which is the main exoskeleton component of crustaceans and cell wall of fungi. It has a broadening of being researched for applications in many fields because of many interesting properties related to both biological activity and electrochemical affinity [2]. It is indeed a natural cationic biopolymer and behaves as a polycation by its protonated amine function at multiple sites of the molecule chains. Another interesting category, the activated carbon, has been considered due to its impressive properties concerning a high magnitude in surface area, chemical stability and conductivity. Activated carbon is well known for its superfine nanoporous structure which provides superb simultaneous adsorptive activity that allows more chemical catalytic reaction and electrical charge built up. This material also enables superior energy storage performance and a processing for supercapacitors [3]. Recently it has become in use as a predominant electrode material for commercial electrochemical double-layer capacitance [4]. As the porous activated carbon can be coated or blended with a biocompatible polymer to give a smooth and permeable film without blocking the pores and its properties [5]. A homogenously dispersing of activated carbon in chitosan biopolymer is thus expected for the preparation of electroactive material in our modification of the basal screen printed electrode.

In this study, modified screen printed electrodes were formulated by surface incorporating with either chitosan or activated carbon blending chitosan to get an effective and affordable implement for electrochemical application. Their morphological characteristics were closely investigated using by

atomic force microscopic techniques in comparison to their original SPCE surface. Coexisting complementary electrochemical behavior of them were also determined by the use of voltammetric analysis.

2. MATERIALS AND METHODS

2.1 Materials

Screen printed carbon electrode DRP-150 that consisted of 4 mm spot carbon as working electrode surrounded with platinum line as counter electrode and silver line as reference electrode was provided by DropSens, S.L. Llanera (Asturias) Spain. Chitosan (85% degree of deacetylation) was obtained from Bioline Lab, Co., Thailand. An analytical grade of activated carbon (DARCO®) and other chemicals for reagent preparation were purchased from Sigma-Aldrich Co. LLC, USA and Ajax Finechem Pty, Ltd., Australia. High purity deionized water of 18.2 MΩ from Milli-Q RG system (Millipore Corporation, MA, USA) was used in all solutions.

2.2 Electrode modification

The Screen printed carbon electrode DRP-150 provided by DropSens, S.L. Llanera (Asturias) Spain was used as basal electrode for further modification. The working 4 mm spot surface area of SPCE was cleaned by 3 times rinsing with deionized water. The self assembled chitosan modified electrode was subsequently prepared by applying a 3.0 µl drop of 1% chitosan in 1% acetic acid solution onto this working carbon electrode surface area and naturally dried up. Then it was covered with a 5 µl drop of 0.1 M NaOH for 30 min to fix the film forming and let it dried again. In case of the activated carbon blending chitosan modified electrode, an activated carbon powder was added to 1% chitosan in 1% acetic acid solution at 1:1 ratio by weight and thoroughly mixed and degassed prior to use. Similarly, a 3 µl drop of this blending solution was applied to the working electrode surface spot area, dried up, and fixed by 5 µl drop of 0.1 M NaOH for 30 min. These modified electrodes were then ready after since a natural air dried process was finished.

2.3 AFM investigation

The working surface of each electrode was observed by a XE-120 Atomic force microscope (Park Systems Corp., Suwon, Korea) controlled with XEP software for data acquisition and XEI software for image processing and analysis. AFM images were obtained by 2×2 µm area of the sample surface (x-y accessible area) at 0.5 Hz scan rate. Measurements were based on true non contact mode with a PPP-NCHR silicon cantilever consisting of a < 10 nm tip radius and 42 N/m force constant (Nanosensors TM, Neuchâtel, Switzerland). Surface topography parameters such as average roughness *Ra* (nm), root mean square or standard deviation of the height value *Rq* (nm), height different or peak-to-valley (*Rpv*), ten point height (*Rz*), mean spacing average (*Rsm*), skewness (*Rsk*) and kurtosis (*Sku*) as well as fractal and grain analysis, all were inspected by the XEI software.

2.4 Electrochemical analysis

Electrochemical experiments were performed at room temperature using ECoChemie Autolab PGSTAT 302N controlled with the Autolab Nova software (Metrohm Autolab B.V., Utrecht, The Netherlands). Cyclic voltammetry (CV) was the principal method of electrochemical analyses in this observation. Configuration of the self assembled layer of chitosan and activated carbon blending chitosan on SPCE working area surface was testified by their CV performance in a 0.05 M phosphate buffer saline (pH 6.5) containing 0.05 M EDTA plus 0.1 M KCl and 1.0 mM K3Fe(CN)6 at a scan rate of 25, 50, 100, 150 and 200 m·Vs−1 with a scan from −0.6 to + 0.6 V.

3. RESULTS AND DISCUSSION

3.1 Morphological characterization by AFM

3.1.1 Surface roughness characteristics

Characterization of the investigated working electrode surfaces was achieved by measuring the roughness parameters extracted from AFM topography using instrument's XEI software. Quantitative morphological characteristics such as average roughness (*Ra*), root mean square roughness (*Rq*), peak-to-valley (*Rpv*), ten point height (*Rz*), mean spacing average (*Rsm*), skewness (*Rsk*),

kurtosis (*Sku*) and fractal were obviously perceived as compiled in Table 1. Different nanoscaled surface features were visualized as shown by 3D AFM images in Fig.1. An average and root mean square roughness of the chitosan modified SPCE were 1.2 fold higher but about 1.5 lesser in height and spacing parameter than the basal SPCE surface. These assessments provided a rougher but lower peak spacing and spike planar surface after chitosan covering on to basal SPCE, thus indicated a thick layer topping. While a covering with activated carbon blending chitosan yielded smoother surface with 1.6 to 2 fold smaller in roughness and height parameters. The fractal or self similarity value of this modified surface was increased up to 2.35 suggesting a thoroughly dispersion of activated carbon along the surface film layer.

Table 1 Surface profile parameters before and after modification of the screen printed carbon electrode with chitosan and activated carbon blending chitosan

Parameters	Bare SPCE	SPCE-Chi	SPCE-Chi-ActC
Ra (nm)	18.693	21.780	11.388
Rq (nm)	24.494	25.193	13.505
Rpv (nm)	157.231	109.762	77.546
Rz (nm)	155.236	107.966	76.862
Rsm (µm)	0.8303	0.4353	0.2398
Rsk	-0.191	-0.180	-0.521
Rku	3.316	1.950	2.623
Fractal	2.2095	2.213	2.348

Note: SPCE = screen printed carbon electrode, SPCE-Chi = chitosan modified screen printed carbon electrode, SPCE-Chi-ActC = activated carbon blending chitosan modified screen printed carbon electrode, *Ra* = roughness average, *Rq* = standard deviation of the height value, *Rpv* = peak-to-valley, *Rz* = ten point height, *Rsm* = mean spacing average, *Rsk* = skewness, *Rku* = kurtosis

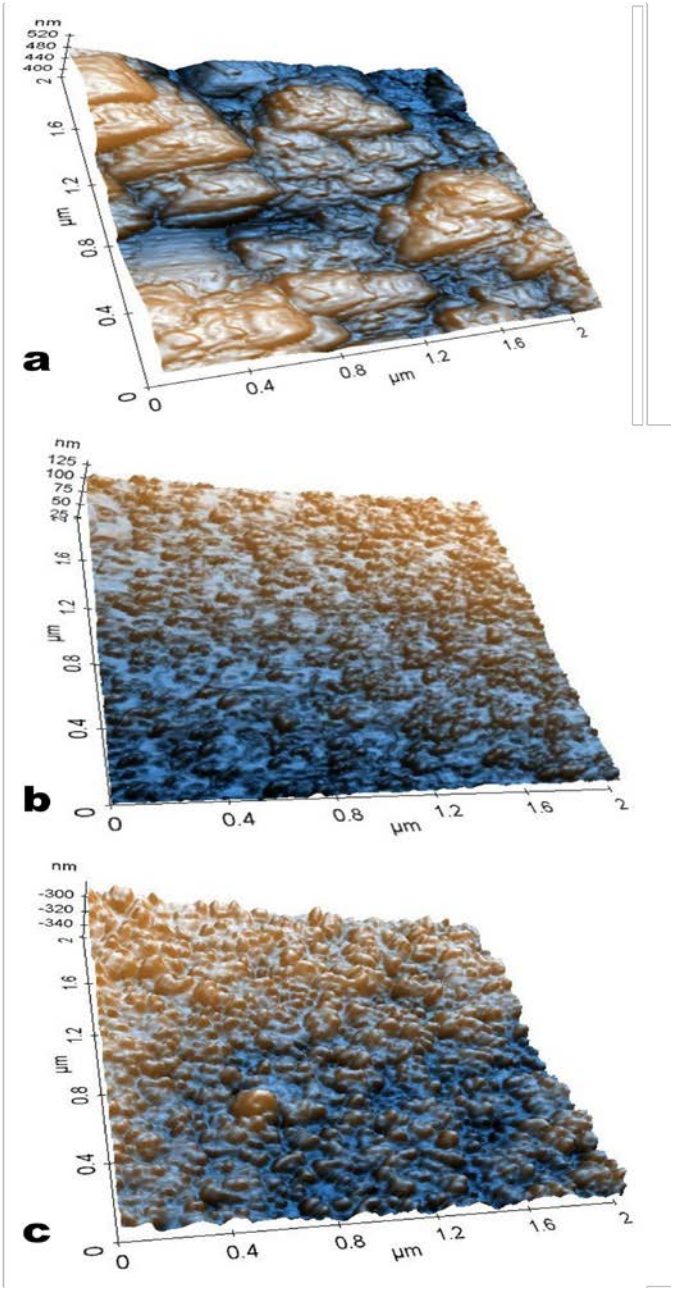


Fig. 1 Three-dimensional AFM images of (a) bare screen printed carbon electrode, (b) chitosan modified screen printed carbon electrode and (c) activated carbon blending chitosan modified screen printed carbon electrode.

3.1.2 Surface grain profile

The nanostructural differences among basal SPCE and its modifying surface were also defined by their grain profile determination by XEI software. Average grain size measured by area, volume, perimeter and peak-to-valley as well as an estimated grain numbers could be revealed as displayed in Table 2, notwithstanding their irregularity in shape and size. Chitosan modified SPCE yielded fewest amount of grain with 106.5 unit/μm^2 in their surface area while the highest amount of 307.5 unit/μm^2 were from activated carbon blending chitosan modified SPCE. The grain profile of basal SPCE was displayed as sizable and voluminous granule with reference to its predicated parameters. After a covering with chitosan, the surface became lessen in grain number in coupling a different form of its grain size and shape. These grains of chitosan modified surface were the largest in area and perimeter but very small peak-to-valley value and looked rather slightly wrinkle taper smear on top of basal SPCE. In case of a modification with activated carbon blending chitosan, the amount of the grain was increased to about 3 times more than the covering with chitosan only. The size and shape of the grains in this case was much smaller than both the basal SPCE and chitosan modified SPCE. Investigation on a distribution of different grain sizes along the 2×2 μm scanning surface layer of each inspected electrode was also done and its concordant results are as illustrated in Fig. 2.

Table 2 Grain parameters of electrode surface measured before and after modification of the screen printed carbon electrode with chitosan and activated carbon blending chitosan

Parameters	Bare SPCE	SPCE-Chi	SPCE-Chi-ActC
Area (μm^2)	0.0073	0.0177	0.0057
Volume (μm^3)	0.0031	0.0013	0.0019
Perimeter (μm)	0.378	0.493	0.328
Rpv(nm)	13.471	9. 365	9.759
Total Number/μm^2	143	106.5	307.5

Note: SPCE = screen printed carbon electrode, SPCE-Chi = chitosan modified screen printed carbon electrode, SPCE-Chi-ActC = activated carbon blending chitosan modified screen printed carbon electrode, *Rpv* = peak-to-valley

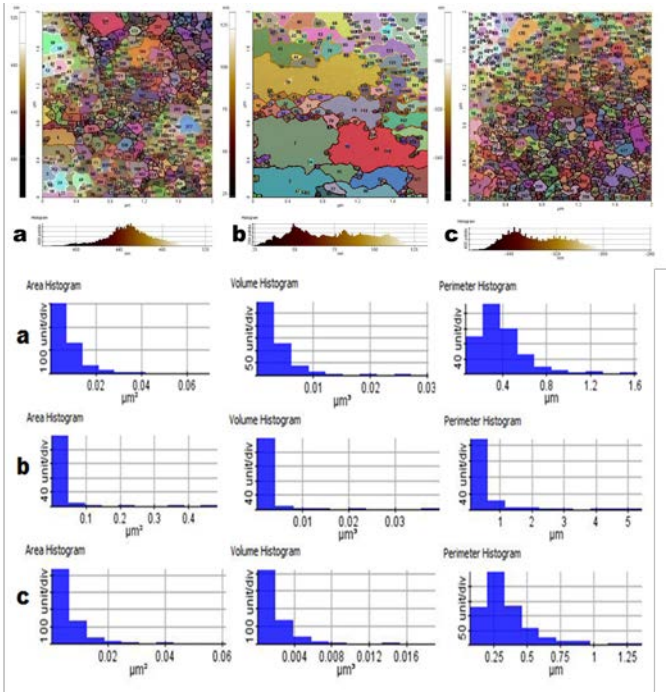


Fig. 2 Grain size distribution histogram of electrode surface by AFM analysis: (a) bare screen printed carbon electrode, (b) chitosan modified screen printed carbon electrode and (c) activated carbon blending chitosan modified screen printed carbon electrode.

3.2 Electrochemical characterization

3.2.1 Cyclic voltammetric behavior

Electrochemical characterization of both modified electrodes in comparison with the original SPCE was defined by cyclic votammetry using the ferri-ferro cyanide ($Fe(CN)6^{3-}/Fe(CN)6^{4-}$) response system indicator. As this performance usually involves a single electron transfer and produce an ideal quasi-reversible outer sphere kinetic behaviour at the electrode surface that can be used toward a quantitative analysis [6]. Comparison among voltammograms obtained from bare SPCE, chitosan modified SPCE and activated carbon blending chitosan modified electrode in 0.05 M phosphate buffer saline (pH 6.5) containing 0.05 M EDTA plus 0.1 M KCl and 1.0 mM $K_3Fe(CN)_6$ at 100 mVs-1 scan rate was as shown in Fig. 3. The voltammetric response could be detected in the potential ranged from −0.6 to + 0.6 V. A reversible process was appeared regarding a redox couple voltammogram with the peak current (I_p) of each at initial positive potential. The current signal responses of $K_3Fe(CN)_6$ were significantly enhanced at both modified electrode and hence indicated a prominent redox peak and peak current magnitude. The anodic

(I_{pa}) and cathodic (I_{pc}) peak current of chitosan modified SPCE was seen about +5e-5 A and -5e-5 A at the anodic (E_{pa}) and cathodic (E_{pc}) peak potential about 0.19 V and 0.07 V, respectively. Similar peak current pattern was also formed by the activated carbon blending chitosan modified electrode with the I_{pa} and I_{pc} about +4e-5 A and -4e-5 A at the E_{pa} and E_{pc} about 0.17 V and 0.07 V. These I_p values were almost 2-3 times higher with approximately 2 times lower in their corresponding E_p than that was yielded from the bare SPCE. The evaluated peak current and potential magnitude has indicated the consequent changes at the surface of basal SPCE after covering with the investigated materials. A higher peak current and a lower peak potential of the two modified electrodes could provided better flow of electron transfer and oxidative reaction that implied a more sensitivity and reactivity. Thus a significant electrochemical behaviour improvement could be achieved in the modification of SPCE with chitosan and activated carbon blending components.

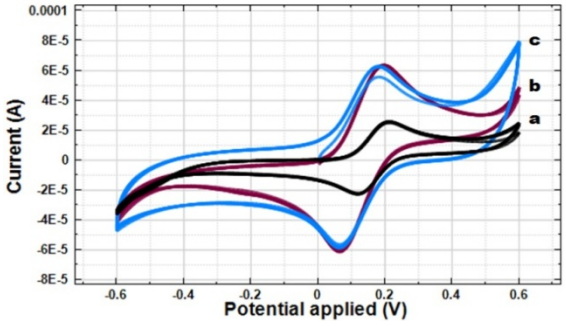


Fig. 3 Cyclic voltammograms of (a) bare screen printed carbon electrode, (b) chitosan-modified screen printed carbon electrode and (c) activated carbon blending chitosan-modified screen printed carbon electrode in 0.1 M PBE (pH 6.5) containing 1.0 mM $[K_3Fe(CN)_6]^{3-/4-}$ at 100 m·Vs−1 scan rate.

3.2.2 Scan rate effect determination

The scan rate effect from cyclic voltammetric performance of each electrode type was as demonstrated in Fig.4. Table 3 was allocated to represent the results concluded from this experiment on scan rate effect. Linear regression relationship could be constructed from the peak current versus square root of the scan rate variants. A proper linearity was produced and the slope magnitudes of both modified SPCE were approached to 0.5 with the regression coefficient around 0.99. The electrochemical property of these evidences is therefore belonged to diffusion controlled current and could be used for further study in quantitative determination.

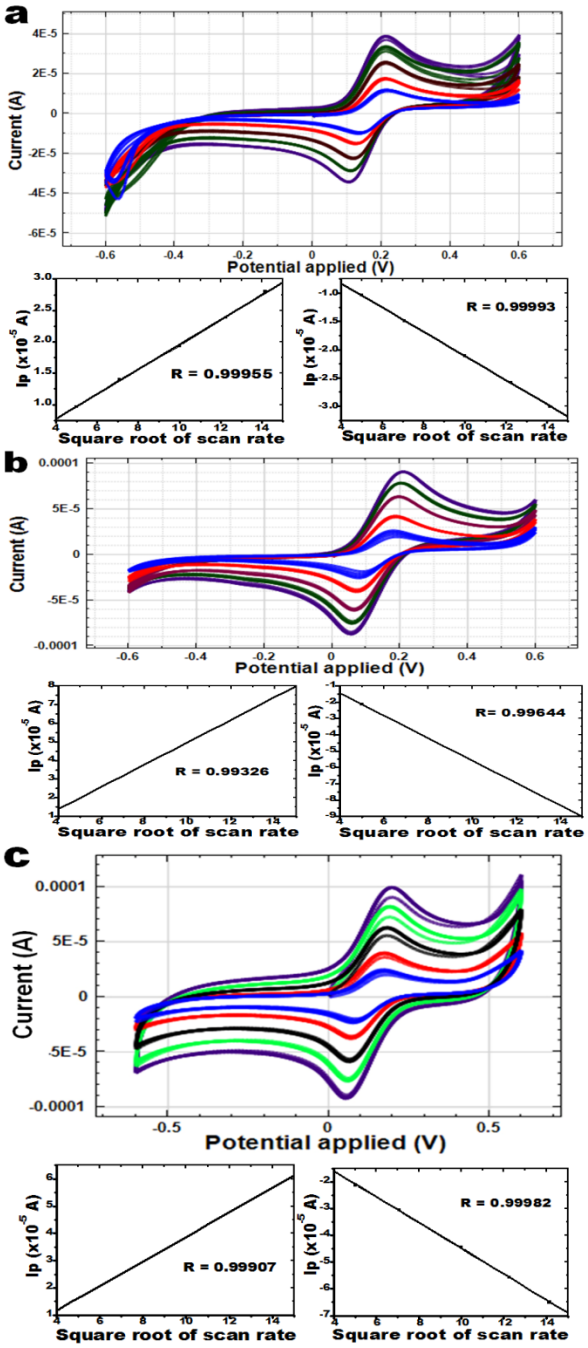


Fig. 4 Cyclic voltammograms of (a) bare screen printed carbon electrode, (b) chitosan-modified screen printed carbon electrode and (c) activated carbon blending chitosan-modified screen printed carbon electrode in 0.1 M PBE (pH 6.5) containing 1.0 mM [K3Fe(CN)6]3−/4− accounted by curves from inner to outer corresponding to 25, 50, 100, 150 and 200 m·Vs−1 scan rates, respectively. Plots located at the bottom of each CV show their corresponding linear relationship of anodic and cathodic peak current vs. square root of the scan rate.

Table 3 Linear relationship of anodic (*Ipa*) and cathodic(*Ipc*)cyclic voltammetric peak current vs. square root of the corresponding scan rate at 25, 50, 100, 150 and 200 m·Vs−1 among the investigated electrodes.

Linear regression relationship of	Equation (y= a+bx)	R^2
Bare SPCE		
Ipa	y = (-0.0217±0.0353) + (0.1981±0.0034)x	0.9988
Ipc	y = (0.0436±0.0889) + (-0.2202±0.0087)x	0.9938
SPCE-Chi		
Ipa	y = (-1.0207±0.4154) + (0.6016±0.0405)x	0.9933
Ipc	y = (1.2995±0.3442) + (-0.6879±0.0336)x	0.9964
SPCE-Chi-ActC		
Ipa	y = (-0.6349±0.1154) + (0.451±0.0113)x	0.9991
Ipc	y = (0.3236±0.0547) + (-0.4811±0.0053)x	0.9998

Note:R^2 = regression coefficient value

4. CONCLUSION

In this investigation, modification of screen printed carbon electrode (SPCE) with chitosan and activated carbon blending chitosan at the working carbon area surface was performed. Each modified SPCE and also its basal bare SPCE was intensely observed by atomic force microscope (AFM) to evaluate the consequent surface morphological developing. The surface roughness was found increasing but with much lower peak height and spacing in the chitosan modified SPCE. In case of the modification using activated carbon blending chitosan, however, the surface layer became smoother altogether with a higher fractal value which indicated an evenly dispersion of activated carbon particle in this surface layer. Similar assumption was noted from the grain profile analysis. The grain number per area was lesser in chitosan modified one and their size and shape tend to be tapering form in average. While more grain amount was obtained with the modification by activated carbon blending chitosan and the average grain was irregular nodule shape and much smaller in size than those of basal chitosan only. A good correlation between AFM and electrochemical determination could be achieved in this study. These topographic modifications of SPCE were strongly responded to electrochemistry surface as the voltammetric response was obviously improved. Remarkable better peak potential and peak current magnitude from the ferri-ferro cyanideredox activity was gained in both modified SPCE. The test in scan rate effect was also showed a diffusion controlled behavior with considerable high stability and sensitivity which capable for desirable quantitative inspection. Thus, an easy, simple and inexpensive fabrication of modified electrode can be presented for convenient applications in various fields of physicochemical and biological analysis.

5. ACKNOWLEDGEMENTS

The research work was carried out under the Biosensor Technology for Sustainable Development Research Group of Khon Kaen University. The authors acknowledge the University's Division of Research and Technology Transfer Affair for partial funding and also the Research Instrument Center for technical supporting through this program.

6. REFERENCES

[1] Ramachandran R , Mani V , Chen S-M, Gnana kumar G , Govindasamy M, "Recent Developments in Electrode materials and Methods for Pesticide Analysis - An overview", Int. J. Of Electrochemical Sciences, Vol. 10, Dec. 2015, pp. 859 – 869.

[2] Liu Y, Gaskell KJ, Chen Z, Yu LL, Pay GF, "Chitosan-coated electrodes for bimodal sensing: selective post-electrode film reaction for spectroelectrochemical analysis", Langmuir, Vol. 24, Jun. 2008, pp. 7223-7231.

[3] Wei L, Marta Sevilla M, Fuertes AB, Mokaya R, Yushin G, "Polypyrrole-Derived Activated Carbons for High-Performance Electrical Double-Layer Capacitors with Ionic Liquid Electrolyte", Advanced Functional Materials, Vol. 22, Feb.2012, pp. 827–834.

[4] Salinas-Torres D, Lozano-Castelló D, Magdalena-Titirici M, Zhao L, Yu L, Morallón E, Cazorla-Amoros D, "Electrochemical behaviour of activated

carbon obtained *via* hydrothermal carbonization", J. of Material Chemistry. A, Vol.3, Jun. 2015, pp. 15558-15567.

[5] Chang TM, "Coated charcoal haemoperfusion", Life Support System", Vol. 2, Apr-Jun1984, pp. 99-106.

[6] Nicholson RS, "Theory and Application of Cyclic Voltammetry for Measurement of Electrode Reaction Kinetics", Analytical Chemistry, Vol. 37, Apr.1965, pp.1351-1355.

International Journal of GEOMATE, Aug., 2016, Vol. 11, Issue 24, pp. 2356-2362
MS No. 5127j received on August 14, 2015 and reviewed under GEOMATE publication policies.
Copyright © 2016, Int. J. of GEOMATE. All rights reserved, including the making of copies unless permission is obtained from the copyright proprietors. Pertinent discussion including authors' closure, if any, will be published in April 2017 if the discussion is received by Oct. 2016.
Corresponding Author:Porntip Wongkaew