

## The Electrochemical Properties of Acetaminophen on Bare Glassy Carbon Electrode

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We have reported the modification of acetaminophen polymer films with chitosan. In this paper, the electrochemical oxidation of acetaminophen in different pHs and conditions at the bare glassy carbon electrode. The bare glassy carbon electrode also exhibits a promising enhanced electrocatalytic activity towards the oxidation of acetaminophen. Different methods were used for the formation of poly-acetaminophen films and the deposition of chitosan. The presence of chitosan enhances the loaded and stability. Cyclic voltammograms (CVs) and electrochemical impedance spectroscopy (EIS) are used for the determination of acetaminophen and the apparent diffusion coefficient values for these compounds at different concentration as it gives some information about the kinetics of charge transfer during the redox reactions of these compounds. The effect of the method of poly-acetaminophen films formation on the synergism between the polymer film and the subsequently loaded chitosan is thoroughly studied. Finally, we have studied the surface morphology of the modified electrode using atomic force microscopy (AFM), which revealed that acetaminophen is coated on chitosan.

**Keywords:** Acetaminophen, Chitonsan, Modified electrodes, Drug Analysis, Electrochemical impedance spectroscopy (EIS), Chemical Sensors, Electrochemistry.

### 1. INTRODUCTION

Acetaminophen (paracetamol, N-acetyl-*p*-aminophenol) is a well-known drug which has extensive applications in pharmaceutical industries. It is an antipyretic, non-steroidal anti-inflammatory drug [1]. It is the preferred alternative to aspirin, particularly for patients who cannot tolerate aspirin [2] and its use is one of the most common causes of poisoning worldwide [3] and analgesic compound that has high therapeutic value. It is also used as a precursor in penicillin, and as stabilizer for hydrogen peroxide, photographic chemical, etc. It is a suitable alternative when the patients are sensitive to aspirin [4]. At the recommended dosage, there are no side effects. However,

overdoses of acetaminophen cause liver and kidney damage [5] and may lead to death. It is suspected that a metabolite of acetaminophen is the actual hepatotoxic agent [6]. It is reported, in therapeutic doses, 60–90% of the drug is metabolized by conjugation to form acetaminophen glucuronide and sulphate; 5–10% is oxidized by mixed-function oxidase enzymes such as cytochrome P-450 to form highly reactive N-acetyl-*p*-benzoquinone-imine, which is immediately conjugated with glutathione and subsequently excreted as cysteine and mercapturate conjugates. Only 1–4% of a therapeutic dose of acetaminophen is excreted unchanged in the urine [7-12]. Various studies were reported for the determination of acetaminophen in drug formulations using different techniques [13-20]. These techniques such as titrimetry, spectrophotometry, and liquid chromatography (LC) [21-22] have been applied for the determination of acetaminophen in pharmaceutical formulations and biological fluids. Titrimetric and spectrophotometric methods involve tedious extraction processes prior to the determination, and LC method is time-consuming. Among these several methods of determination techniques, the electrochemical methods have more advantages over the other in sensing acetaminophen. Due to the advantages of relatively low cost, fast response, simple instrumentation, high sensitivity, facile miniaturization, and low power requirement, numerous voltammetric methods have been developed for determination of acetaminophen [23-26]. Electrochemical methods are more and more widely used for the study of electroactive compounds in pharmaceutical forms and physiological fluids due to their simple, rapid, and economical properties [27]. As an electroactive substance, acetaminophen has also attracted much interest.

Such technologies therefore have the potential to enhance our understanding of disease and drug activity during preclinical and clinical drug development. Recently, many scientists and biologists focused on the preparation of newer nanocomposite with good biocompatibility that could be the promising matrices for drug immobilization which can enhance the selectivity and sensitivity of the biosensors. Chitosan is a polysaccharide derived by deacetylation of chitin. It has primary amino groups that have a  $pK_a$  value of approximately 6.3 [28]. As well as a high positive charge density. Due to its positive charge, it can easily form polyelectrolyte complexes with negatively charged drug by electrostatic interaction. Chitosan is a hydrophilic, biodegradable [29], high mechanical strength, fast metal complexation, susceptibility to chemical modification [30], non-antigenic biopolymer and has a low toxicity toward mammalian cells. Chitosan have remarkable characteristic such as exceptionally minute pore size with very outsized surface area-to-volume proportion, high porosity and diameters was in nanometer scale. These properties of chitosan hold fine drug immobilization scaffold and it was exploited for biosensor applications [31-33]. These interesting matrices provide high surface area for high drug loading and compatible micro-environment helping drug stability. Besides, chitosan provides direct contact between drug active site and electrode. Hence, it has great potential as a biomaterial because of its excellent biocompatibility. Conjugated to additional materials, chitosan composites result in a new class of biomaterials that possess mechanical, physicochemical and functional properties, which have potential for use in advanced biomedical imaging applications.

This paper discusses the electrochemical polymerization of acetaminophen films composed of acetaminophen and chitosan on various electrodes, and the enhancement of the electropolymerization by chitosan modification of the electrode surface. It was interesting to study the electrochemical oxidation of acetaminophen in different pHs and conditions. In addition, the observed behavior of

hydrolyses, hydroxylation and dimerization reactions from acetaminophen have been estimated by digital simulation of cyclic voltammograms (CVs) and electrochemical impedance spectroscopy (EIS).

## 2. EXPERIMENTAL

### 2.1. Materials

Acetaminophen (Aldrich) was used as received. Chitosan was obtained from Sigma. All other chemicals used were of analytical grade and used without further purification pH 7.0 (0.1 M Na<sub>2</sub>HPO<sub>4</sub> and 0.1 M NaH<sub>2</sub>PO<sub>4</sub>) Phosphate buffer solutions (PBS), pH 1.0 H<sub>2</sub>SO<sub>4</sub> solutions and pH 13 KOH buffer were used as supporting electrolyte. Aqueous solutions were prepared using doubly distilled deionized water and then deaerated by purging with high purity nitrogen gas for about 20 min before performing electrochemical experiments. Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements.

### 2.2. Apparatus

Cyclic voltammetry (CVs) was performed in an analytical system model CHI-1205A potentiostat. A conventional three-electrode cell assembly consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode were used for the electrochemical measurements. The working electrode was glassy carbon electrode (GCE; area 0.07 cm<sup>2</sup>). In these experiments, all the potentials have been reported versus the Ag/AgCl reference electrode. The morphological characterizations of the films were examined by atomic force microscopy (AFM) (Being Nano-Instruments CSPM5000). Electrochemical impedance spectroscopy (EIS) measurements were performed using an IM6ex Zahner instrument (Kroanch, Germany). All the solutions were purged with high purity nitrogen gas for about 20 min before performing electrochemical experiments. Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements. All the experiments were carried out at room temperature ( $\approx 25^{\circ}\text{C}$ ).

### 2.3. Preparation of poly-acetaminophen/chitosan modified electrodes

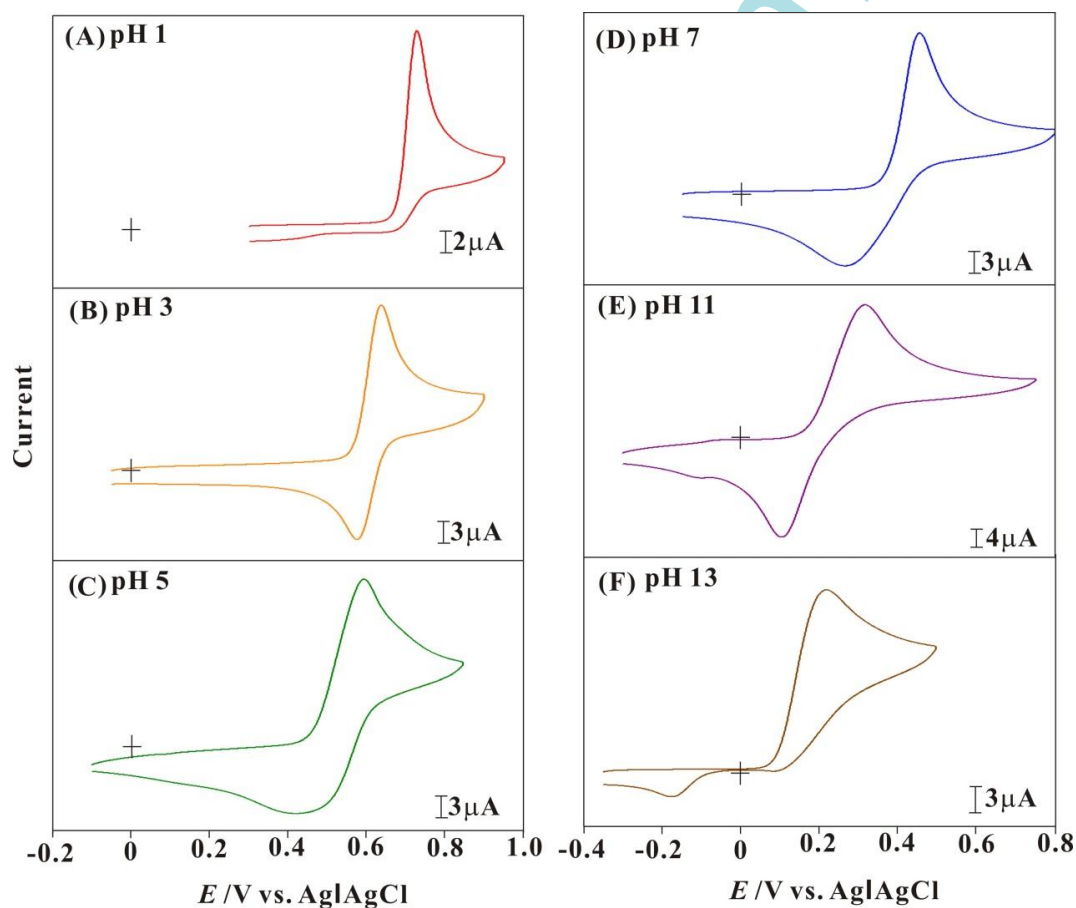
The produced 0.5% chitosan were suspended in pH 5.6 acetate buffer and sonicated in a sonication bath for 1 h. Thus obtained a uniform chitosan dispersion. Prior to modification, glassy carbon electrode (GCE) was polished with 0.05  $\mu\text{m}$  alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and dried at room temperature. The cleaned glassy carbon electrode was coated with 2  $\mu\text{L}$  of chitosan and the solvent allowed evaporating at room temperature. The electropolymerization of acetaminophen was done by electrochemical oxidation of acetaminophen ( $1 \times 10^{-3}$  M) on the chitosan modified glassy carbon electrode using pH 1.0 H<sub>2</sub>SO<sub>4</sub> buffer. It was performed by consecutive CVs over a suitable potential range of 0.1 to 0.8 V; scan rate = 100 mVs<sup>-1</sup>.

The optimization of poly-acetaminophen growth potential has been determined by various studies with different electropolymerization potentials.

### 3. RESULTS AND DISCUSSIONS

#### 3.1. Electrochemical characterizations of acetaminophen in different pH

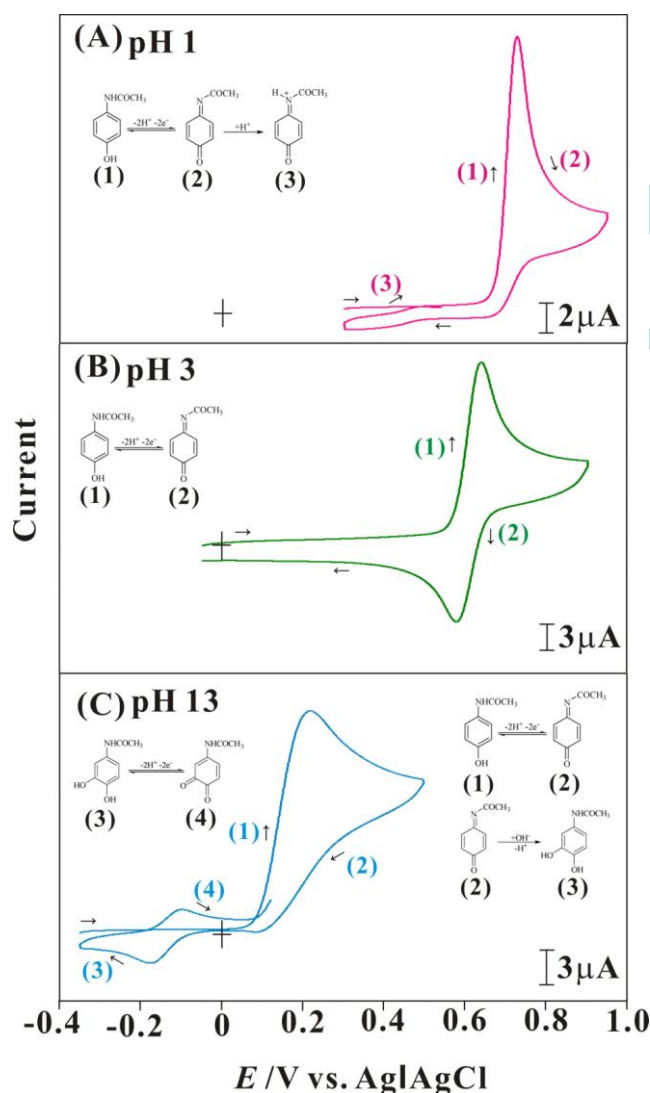
The electrochemical properties of acetaminophen at a glassy carbon electrode were investigated using cyclic voltammetry in aqueous solutions having pH values between 1 and 13. Figure 1 (A) to (F) showed the cyclic voltammogram of bare glassy carbon electrode obtained in various pH aqueous solution containing  $1 \times 10^{-3}$  M acetaminophen, scan rate =  $100 \text{ mVs}^{-1}$ .



**Figure 1.** Cyclic voltammograms of the bare glassy carbon electrode transferred to various pH solutions containing  $1 \times 10^{-3}$  M acetaminophen (A) 1; (B) 3; (C) 5; (D) 7; (E) 11; (F) 13. Scan rate  $100 \text{ mVs}^{-1}$ .

Figure (A) in low pH (pH 1.0) response of oxidation process resulted in irreversible oxidation peak about 725 mV. (B) to (E) in pH 3.0 to 11 showed that the peak potentials shifted to the negative potentials by increasing pH. Exhibited of oxidation process produced reversible redox peak. All

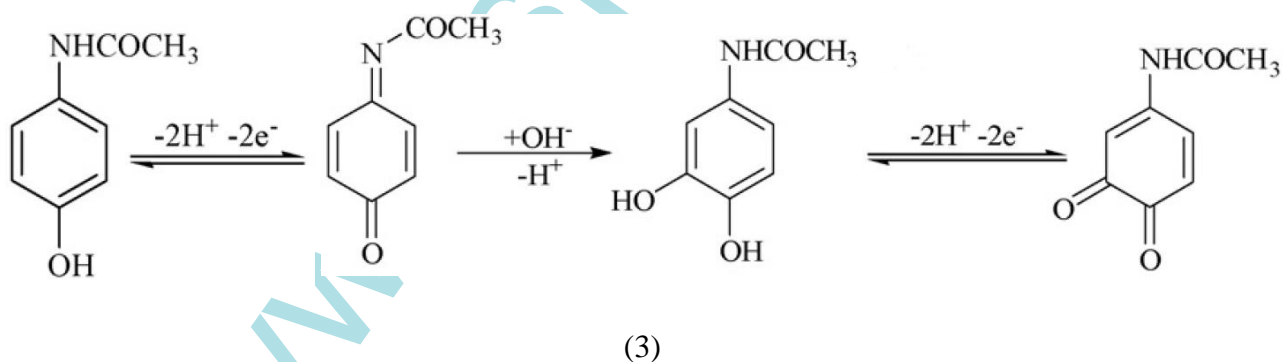
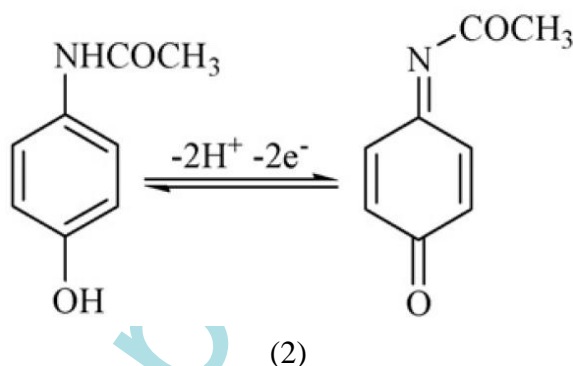
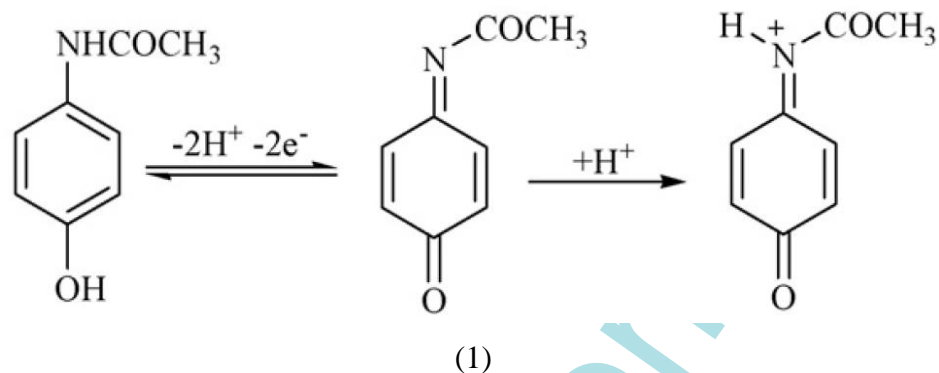
showed one reversible redox couple at potentials between 0.5 and 0.1 V (vs. Ag/AgCl). Figure (F) showed pH 13 results, the initial stage was similar irreversible oxidation process, followed appearance of another new reduction peak about -172 mV. In order to explain that arranged the follow-up experiment.



**Figure 2.** Cyclic voltammograms of bare glassy carbon electrode transferred to different pH solutions containing  $1 \times 10^{-3}$  M acetaminophen oxidation process, scan rate  $100 \text{ mVs}^{-1}$ , respectively. (A) pH 1; (B) pH 3; (C) pH 13.

Figure 2 (A) revealed in pH 1.0 oxidation of acetaminophen. In first segment, this is expected because of the participation of proton(s) in the oxidation reaction of acetaminophen to N-acetyl-p-benzoquinone-imine, and vice-versa within a quasi-reversible two-electron process of Eq. (1). From third segment appearance of another new oxidation peak about 473 mV. There is a direct relation between acidity and instability of N-acetyl-p-benzoquinone-imine in low pH. Figure 2 (B) showed acetaminophen in pH 3 oxidation process, the intrinsic stability of N-acetyl-p-benzoquinone-imine in

this range which can be due to dimerization (Eq. 2) of N-acetyl-*p*-benzoquinone-imine [34–37]. This verifies the occurrence of well-defined N-acetyl-*p*-benzoquinone-imine decomposition mechanism in our experiment condition. Figure 2 (C) showed in pH 13, there is a strong relation between basicity and instability of N-acetyl-*p*-benzoquinone-imine. This is expected because of the participation of hydroxide ions in reaction mechanism (EC mechanism or ECE mechanism)[38].

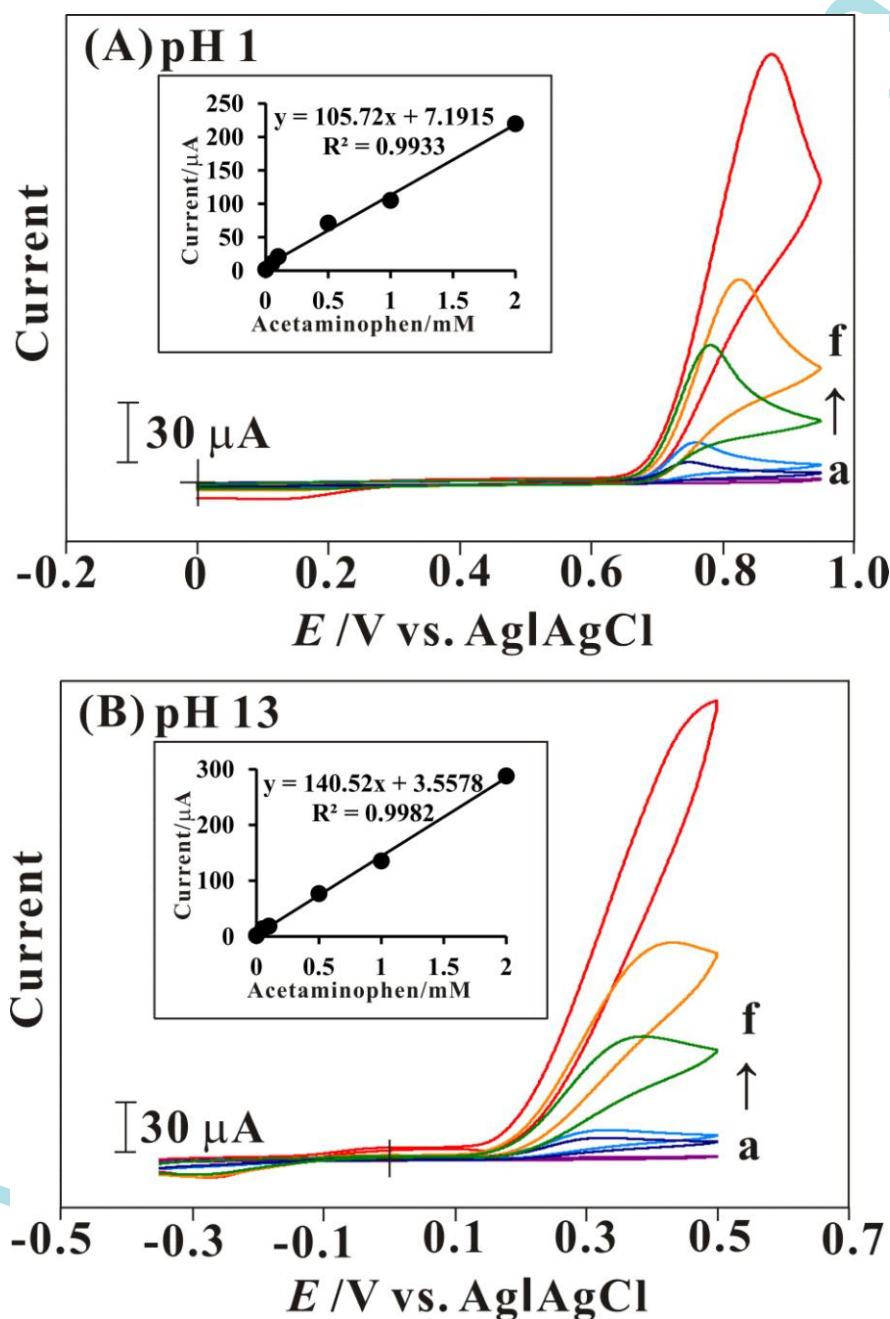


### 3.2. Electrocatalysis characterizations of acetaminophen in different pH

The electrocatalytic oxidation efficiency of baer glassy carbon electrode in the absence and presence of different concentration acetaminophen was investigated using cyclic voltammetry[39-40]. Figure 3 (A) showed the baer glassy carbon electrode deposition in pH 1.0 H<sub>2</sub>SO<sub>4</sub> aqueous solutions (curve a). Curve (b) to (f) showed that the growing current peak by increased concentration of acetaminophen. The response of sensitivity and correlation coefficient were 1.51 μA μM<sup>-1</sup> cm<sup>2</sup> and R<sup>2</sup>

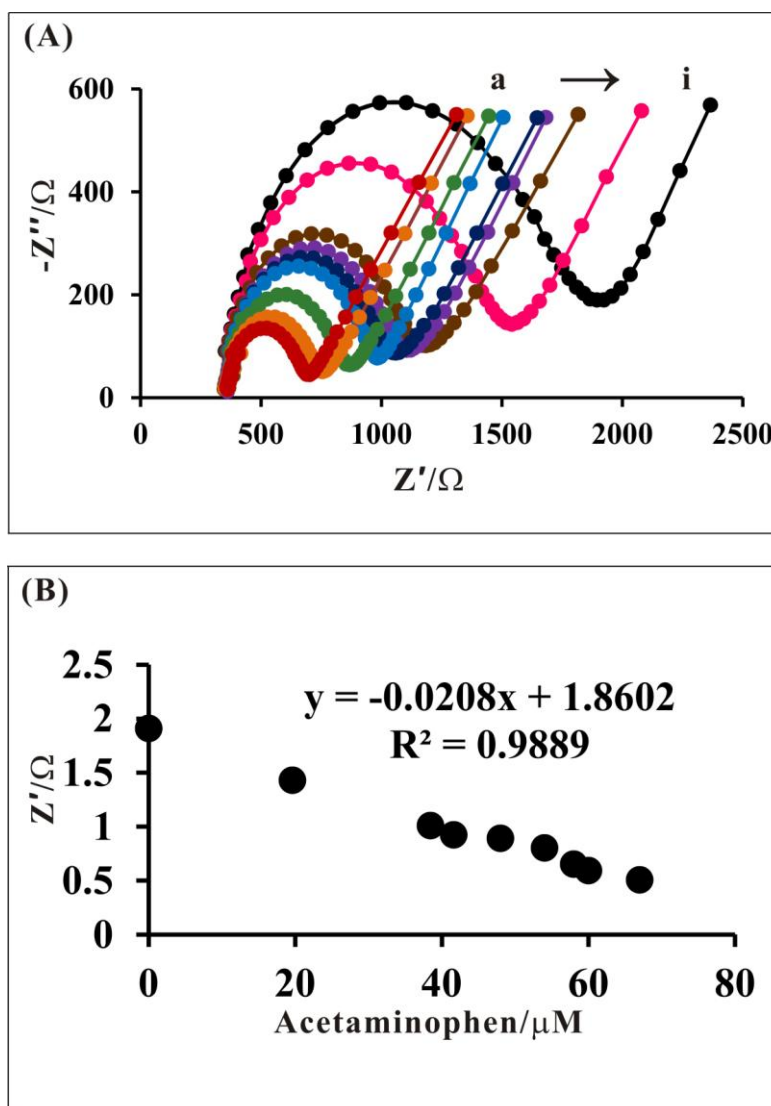


= 0.99. The inset showed the plot of current versus concentration of acetaminophen in pH 1.0. Figure 3 (B) showed in pH 13 KOH aqueous solutions at bare glassy carbon electrode (curve a). Different concentration of acetaminophen electrocatalytic oxidation showed in curve (b)  $5 \times 10^{-5}$  M; (c)  $1 \times 10^{-4}$  M; (d)  $5 \times 10^{-4}$  M; (e)  $1 \times 10^{-3}$  M and (f)  $2 \times 10^{-3}$  M. The response of sensitivity and correlation coefficient were  $2.01 \mu\text{A} \mu\text{M}^{-1} \text{cm}^2$  and  $R^2 = 0.99$ . The inset showed the plot of current versus concentration of acetaminophen in pH 1.3.



**Figure 3.** Cyclic voltammograms of bare glassy carbon electrode in (A) pH 1; (B) pH 13 with various concentrations of acetaminophen : (a) 0 M; (b)  $5 \times 10^{-5}$  M; (c)  $1 \times 10^{-4}$  M; (d)  $5 \times 10^{-4}$  M; (e)  $1 \times 10^{-3}$  M and (f)  $2 \times 10^{-3}$  M. The inset shows the plot of current versus concentration of acetaminophen.

## 3.3. Electrochemical impedance spectra (EIS) of acetaminophen



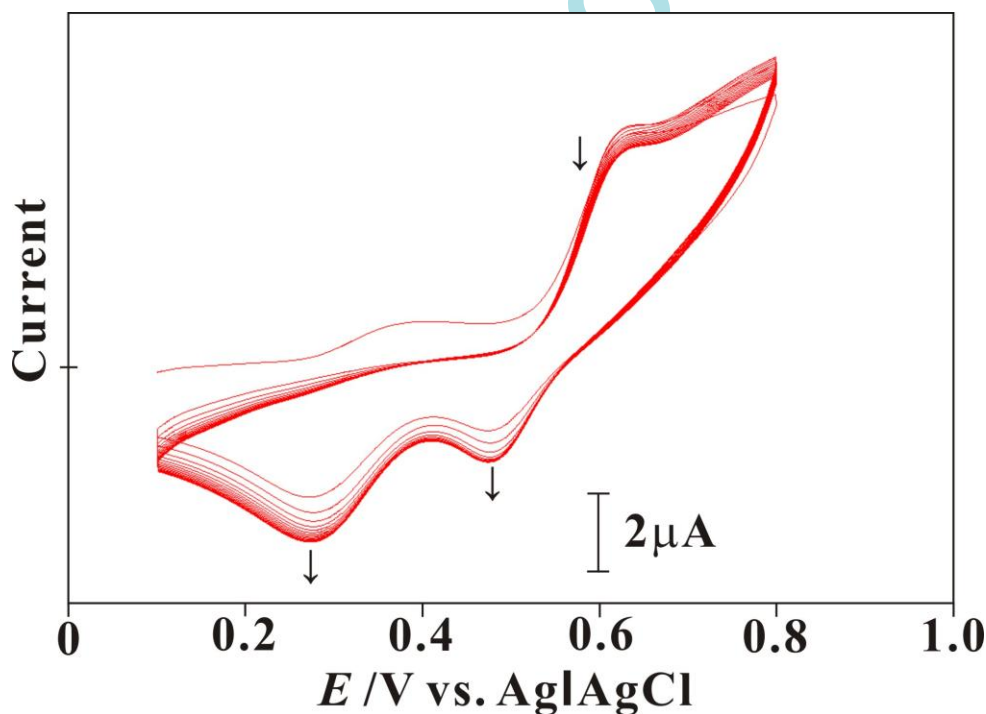
**Figure 4.** (A) Electrochemical impedance spectra (EIS) of bare glassy carbon electrode in pH 7.0 PBS containing  $5 \times 10^{-3}$  M  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ , Amplitude: 5 mV. (a) to (i) showed various concentrations of acetaminophen. (B) showed the plot of resistance versus concentration of acetaminophen.

## 3.4. Electropolymerization of acetaminophen on chitosan modified glassy carbon electrode

Electrochemical impedance spectra (EIS) was applied to monitor the whole process of the electrode modification. EIS can give useful information of the impedance changes on the electrode surface between each step. Figure 4 showed the results of EIS for a bare glassy carbon electrode in the presence of equimolar 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  and different concentration of acetaminophen in pH 7.0 PBS. The EIS includes a semicircular part and a linear part. The semicircular part at higher frequencies corresponds to the electron transfer limited process and the diameter is equivalent to the electron transfer resistance ( $R_{ct}$ ). The linear part at lower frequencies corresponds to the diffusion process. During the fabrication, significant differences were observed. Impedimetric detection of



acetaminophen on bare glassy carbon electrode. Figure 4 showed the Nyquist curves obtained for oxidation of acetaminophen. The plot of the real component ( $Z'$ ) and the imaginary component  $Z''$  (imaginary) resulted in the formation of a semicircular Nyquist plot. This type of impedance spectrum is an analytic of a surface-modified electrode system in which the electron transfer is slow and the impedance is controlled by the interfacial electron transfer at high frequency. Open circuit potential was applied for this investigation. The concentrations of added acetaminophen were in the range of 0 to 67  $\mu\text{M}$ .  $R_{ct}$  changes from the baseline response for each addition of acetaminophen.  $R_{ct}$  of a bare glassy carbon electrode is 1.91  $\text{K}\Omega$  (curve i). When increased concentrations of acetaminophen (curve h to a) the  $R_{ct}$  value was decreased markedly, which enhanced the electron transfer kinetics at the bare glassy carbon electrode surface. The electron transfer resistance decreased with the increasing concentrations of acetaminophen, which gives rise to a linear-type detection response from 1.96 to 67  $\mu\text{M}$ . Up to 67  $\mu\text{M}$ , the experimental data fitted and the regression equation obtained was  $R_{ct} = 0.683 \text{ k}\Omega$  with a correlation coefficient of  $R^2 = 0.9889$  (inset of Figure 4 B). These results clearly explicate the impedimetric detection of acetaminophen using bare glassy carbon electrode. From these observations, we can conclude that the bare glassy carbon electrode were highly conductive and expected as a good platform for sensing applications.

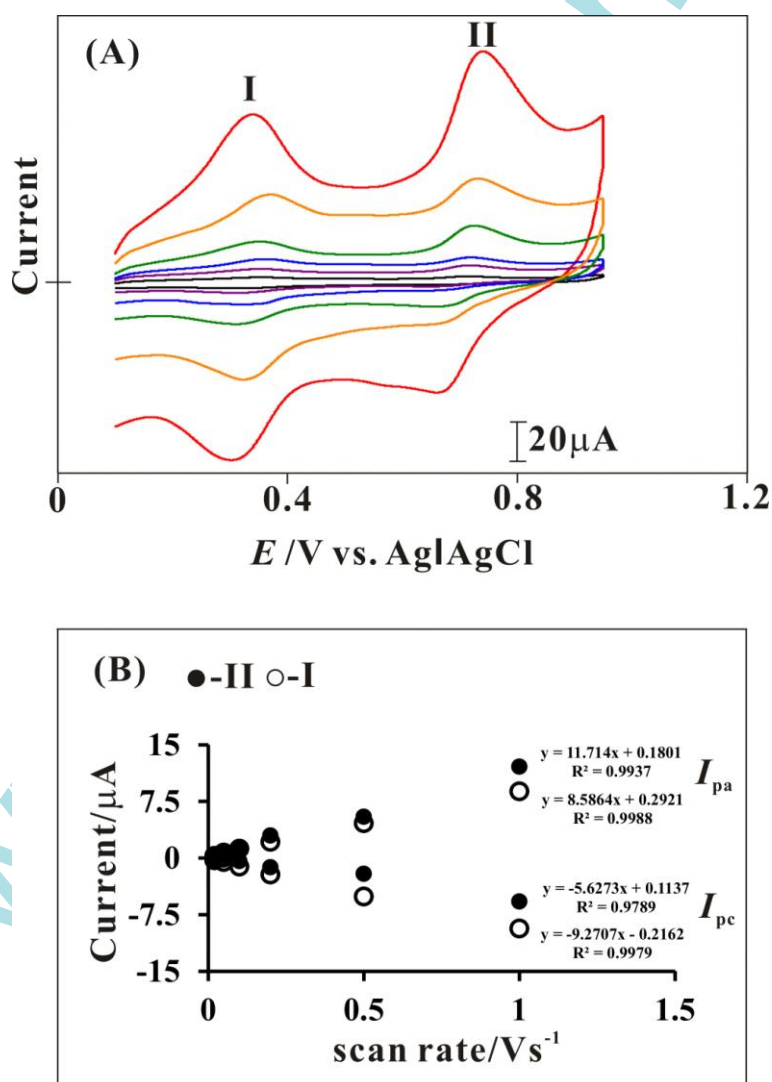


**Figure 5.** Cyclic voltammograms of chitosan modified electrode in pH 1.0  $\text{H}_2\text{SO}_4$  buffer containing  $1 \times 10^{-3} \text{ M}$  acetaminophen, scan rate at  $100 \text{ mVs}^{-1}$ .

The cleaned glassy carbon electrode was coated with  $2 \mu\text{L}$  of chitosan and the solvent allowed evaporating at room temperature. Figure 5 showed the electropolymerization[41-43] of acetaminophen ( $1 \times 10^{-3} \text{ M}$ ) by electrochemical oxidation on the chitosan modified glassy carbon electrode using pH 1.0  $\text{H}_2\text{SO}_4$  buffer. It was performed by consecutive cyclic voltammogram over a suitable potential

range of 0.1 to 0.8 V; scan rate = 100 mVs<sup>-1</sup>. The chitosan included sufficient positive charge can adsorb more acetaminophen. The electrochemical formation of acetaminophen on a glassy carbon electrode along with enhanced electropolymerization by a chitosan modified electrode. The growth of the cyclic voltammogram current exhibiting a redox couple with a formal potential of  $E^{0'} = 0.45$  V and 0.64 V (vs. Ag|AgCl). The increase in peak current at the redox couple indicates that film formation occurred. Poly acetaminophen films could also be synthesized in strong acidic aqueous solutions using consecutive cyclic voltammetry on indium tin oxide (ITO) electrodes that had been modified by including chitosan on the electrode surface. In the following experiments, each newly prepared film on glassy carbon electrode has been washed carefully in deionized water to remove the loosely bounded acetaminophen on the modified glassy carbon electrode. It was then transferred to pH 1.0 aqueous H<sub>2</sub>SO<sub>4</sub> solution for the other electrochemical characterizations.

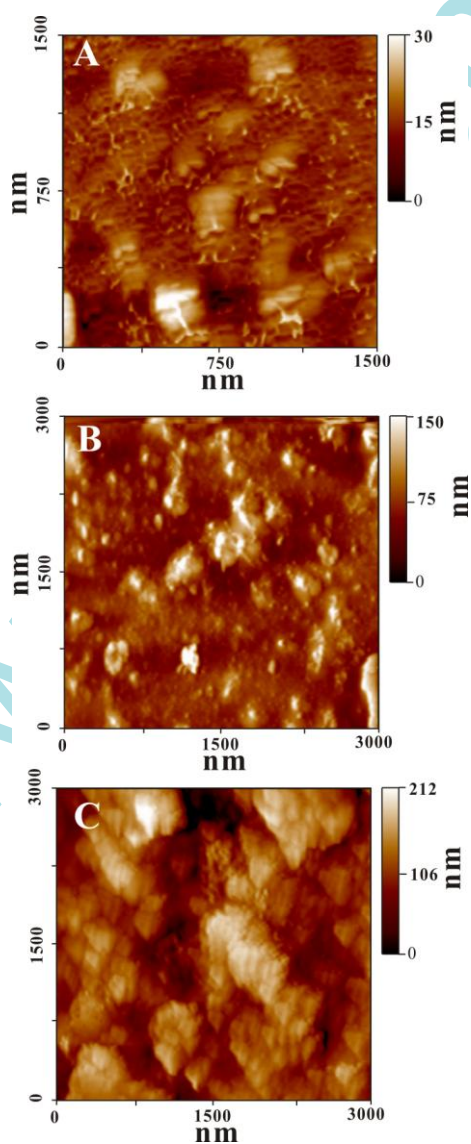
### 3.5. Different scan rate studies



**Figure 6.** (A) Cyclic voltammograms of poly-acetaminophen/chitosan modified electrode in pH 1.0 H<sub>2</sub>SO<sub>4</sub> buffer different scan rate from 20 mVs<sup>-1</sup> to 1000 mVs<sup>-1</sup>, respectively. (B) Calibration curve for data showed  $I_{pa}$  &  $I_{pc}$  vs. scan rate.

Figure 6 (A) showed that the Poly acetaminophen/chitosan film on a glassy carbon electrode had two chemically reversible redox couple at 0.35 and 0.71 V in the pH 1.0 aqueous  $\text{H}_2\text{SO}_4$  solution when cyclic voltammetry was performed at different scan rates (20 to  $1000 \text{ mVs}^{-1}$ ). The anodic and cathodic peak currents of both the film redox couples which have increased linearly with the increase of scan rates. The calibration curve for data in figure 6 (B) showed  $I_{pa}$  &  $I_{pc}$  vs. scan rate. The ratio of  $I_{pa}/I_{pc}$  from the figure (B) has demonstrated that the redox process has not been controlled by diffusion. This behavior perhaps occurs because of a reversible electron transfer process involving the poly-acetaminophen on the chitosan layer, with a proton exchange process occurring along with the electron transfer process. However, the  $\Delta E_p$  of each scan rate reveals that the peak separation of composite redox couple increases as the scan rate is increased.

### 3.6. Morphological characterization of acetaminophen



**Figure 7.** AFM images of (A) only acetaminophen, (B) chitosan and (C) poly-acetaminophen /chitosan on ITO electrode.

The surface morphology of poly acetaminophen/chitosan modified electrode has been examined using AFM. Here the AFM studies could furnish the comprehensive information about the surface morphology of nanostructure on the ITO surface. In prior to modification, ITO surfaces were cleaned and ultrasonicated in acetone–water mixture for 15 min and then dried. The AFM parameters have been evaluated for 1500×1500 nm and 3000×3000 nm surface area. Further, three different films; acetaminophen, chitosan and poly acetaminophen/chitosan modified electrodes have been prepared on ITO electrode were characterized using AFM.

From figure 7, it is significant that there are morphological differences between both the films. The top views of nanostructures (A) shows uniformly deposited homogeneously dispersed acetaminophen on this electrode. We can see the existence of nanostructures in obvious manner with the average size range of 27.3 nm. The other amplitude parameters such like roughness average (sa) for acetaminophen film (1500×1500 nm) was found as 3.14 nm. The root mean square roughness was found as 4.22 nm. The poly acetaminophen/chitosan film in figure 7 (C) reveals that the poly acetaminophen had covered the entire chitosan. Comparison of only chitosan (B) and poly acetaminophen/chitosan (C) reveals, these results in could be explained as the increase in deposition of acetaminophen presence of chitosan. We can clearly see that the immersed poly acetaminophen/chitosan have been gathered together.

#### 4. CONCLUSIONS

We have demonstrated application of the baer glassy carbon electrode for determination of acetaminophen. This feature provides a favorable clinical diagnosis for the electrocatalytic oxidation of acetaminophen at baer glassy carbon electrode. High sensitivity and stability together with very easy preparation baer glassy carbon electrode as promising candidate for constructing simple electrochemical sensor for acetaminophen determination. The experimental methods of CVs and EIS with biosensor integrated into the bare glassy carbon electrode (GCE) which are presented in this paper, provide an opportunity for qualitative and quantitative characterization, even at physiologically relevant conditions. Preparation of poly-acetaminophen/chitosan modified electrodes showed stable response. The AFM results have shown the difference between acetaminophen and poly-acetaminophen/chitosan films morphological data. Therefore, this work establishes and illustrates, in principle and potential, a simple and novel approach for the development of a voltammetric sensor which is based on the glassy carbon electrode and ITO electrodes.

#### ACKNOWLEDGEMENT

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#### References

1. L.J. Roberts, J.D. Morrow, in: J.G. Hardman, L.E. Limbird, A. Goodman Gilman (Eds.), *The Pharmacological Basis of Therapeutics*, 10th ed., McGraw-Hill, London, 2001.

2. R.V. Blanke, W.J. Decker, in: N.W. Tietz (Ed.), Textbook of Clinical Chemistry, W.B. Saunders, Philadelphia, 1986.
3. D. Gunnell, V. Murray, K. Hawton, *Suicide Life Threat. Behav.*, 2000, 30, 313.
4. W.A. Martindale, The Extra Pharmacopoeia, 27th ed. The Pharmaceutical Press, London, 1979.
5. B.D. Clayton, Y.N. Stock, *Bioelectrochemistry*, 2001, 74, 223–226.
6. N. Wangfuengkanagul, O. Chailapakul, *J. Pharm. Biomed. Anal.*, 2002, 28, 841.
7. P.I. Dargan, A.L. Jones, *Drug Saf.*, 2002, 25, 625.
8. R. Keys, P.M. Harrison, *J.A. Wendon BMJ*, 1991, 303, 1026.
9. J.G. O'Grady, G.J.M. Alexander, K.M. Hayllar, *Gastroenterology*, 1989, 97, 439.
10. J.A. Vale, A.T. Proudfoot, *Lancet*, 1995, 346, 547.
11. D.C. Dahlin, G.T. Miwa, A.Y.H. Lu, S.D. Nelson, *Proc. Natl. Acad. Sci.*, 1984, 81, 1327.
12. H. Dong, R.L. Haining, K.E. Thummel, A.E. Rettie, S.D. Nelson, *Drug Metab. Dispos.*, 2000, 28, 1397.
13. J.E. Wallace, *Anal. Chem.*, 1967, 39, 531.
14. F.M. Plakogiannis, A.M. Saad, *J. Pharm. Sci.*, 1975, 64, 1547.
15. K.K. Verma, A.K. Gulati, S. Palod, P. Tyagi, *Analyst*, 1984, 109, 735.
16. S.M. Sultan, I.Z. Alzamil, A.M. Aziz Alrahman, S.A. Altamrah, Y. Asha, *Analyst*, 1986, 111, 919.
17. F.A. Mohamed, M.A. AbdAllah, S.M. Shammat, *Talanta*, 1997, 44, 61.
18. J.F. van Staden, M. Tsanwani, *Talanta*, 2002, 58, 1095.
19. M. Oliva, R.A. Olsina, A.N. Masi, *Talanta*, 2005, 66, 229.
20. M.K. Srivastava, S. Ahmad, D. Singh, I.C. Shukla, *Analyst*, 1985, 110, 735.
21. N. Erk, *J. Pharm., Biomed. Anal.*, 1999, 21, 429–437.
22. Y. Ishii, M. Iijima, T. Umemura, A. Nishikawa, Y. Iwasaki, R. Ito, K. Saito, M. Hirose, H. Nakazawa, *J. Pharm. Biomed. Anal.*, 2006, 41, 1325–1331.
23. Z.A. Alothman, N. Bukhari, S.M. Wabaidur, S. Haider, *Sens. Actuators B*, 2010, 146, 314–320.
24. F.G. Bidkorbeh, S. Shahrokhian, A. Mohammadi, R. Dinarvand, *Electrochim. Acta*, 2010, 55, 2752–2759.
25. N.F. Atta, M.F. El-Kady, *Talanta*, 2009, 79, 639–647.
26. F.S. Felix, C.M.A. Brett, L. Angnes, *J. Pharm. Biomed. Anal.*, 2007, 43, 1622–1627.
27. S.A. Kumar, C.F. Tang, S.M. Chen, *Talanta*, 2008, 76, 997–1005.
28. M. Ebadi, *Electrochim. Acta*, 2003, 48, 4233.
29. P. Sorlier, A. Denuziere, C. Viton, A. Domard, *Biomacromolecules*, 2001, 2, 765–772.
30. A. Lahiji, A. Sohrabi, D.S. Hungerford, C.G. Frondoza, *J. Biomed. Mater. Res.*, 2000, 51, 586–595.
31. X. He, R. Yuan, Y. Chai, Y. Shi, *J. Biochem. Biophys. Methods*, 2007, 70, 823–829.
32. E. Khor, *Curr. Opin. Solid State Mater. Sci.*, 2002, 6, 313–317.
33. M. Rinaudo, *Polym. Int.*, 2008, 57, 397–430.
34. A. Yang, R. Wu, *J. Mater. Sci. Lett.*, 2001, 20, 977–979.
35. D.W. Potter, D.W. Miller, J.A. Hinson, *J. Biol. Chem.*, 1985, 260, 12174.
36. V. Fischer, P.R. West, L.S. Harman, R.P. Mason, *Environ. Health Perspect.*, 1985, 64, 127.
37. J. Van Steveninck, J.F. Kostert, T.M.A.R. Dubbelman, *J. Biochem.*, 1989, 259, 633.
38. S.M. Chen, K. T. Peng, *J. Electroanal. Chem.* 547 (2003) 179–189.
39. Y. Umasankar, B. Unnikrishnan, S.M. Chen, T.W. Ting, *Int. J. Electrochem. Sci.* 7(2012)484–498.
40. T.H. Tsai, T.W. Chen, S.M. Chen, K.C. Lin, *Int. J. Electrochem. Sci.* 6(2011)2058–2071
41. K.C. Lin, S.M. Chen, *J. Electroanal. Chem.*, 578(2005) 213–222.
42. S.M. Chen, K.C. Lin, *J. Electroanal. Chem.* 523 (2002) 93.
43. V.S. Vasantha and S.M. Chen, *J. Electroanal. Chem.*, 592 (2006) 77–87.