Scientific paper

Electrochemical Determination of Acetaminophen in Different Pharmaceutical Forms with Gold Nanoparticles Carbon Paste Electrode

Sayed Mehdi Ghoreishi,* Mohsen Behpour, Somaye Sadeghzadeh and Mahshid Golestaneh

Department of Analytical Chemistry, Faculty of Chemistry, University of Kashan, Kashan, I. R. Iran

** Corresponding author: E-mail: s.m.ghoreishi@kashanu.ac.ir*
Tel. No.:+983615552930; Fax No.: +983615552935

Received: 03-08-2010

Abstract

The electrochemical behavior of acetaminophen on a gold nanoparticles (nAu) carbon paste electrode (CPE) was investigated. Cyclic voltammograms of acetaminophen with nAu-CPE exhibited two well defined redox waves for anodic and cathodic peak, respectively. The oxidation reaction of acetaminophen was studied with differential pulse voltammetry (DPV) and square wave voltammetry (SWV). The oxidation of acetaminophen occurs at 405 and 440 mV by DPV and SWV, respectively.

Under the optimum pH of 4.7 in 0.1 mol l^{-1} Britton–Robinson (B-R) buffer solution, the DPV anodic peak current showed a linear relation versus acetaminophen concentration in the range of $5.0 \times 10^{-8} - 1.0 \times 10^{-4}$ mol l^{-1} with a detection limit of 2.6×10^{-8} for DPV, and from $9.0 \times 10^{-7} - 1.0 \times 10^{-4}$ mol l^{-1} for SWV with a detection limit of 1.0×10^{-7}. The performance of this modified electrode was verified by the determination of acetaminophen in dosage form tablet. The result obtained in the recovery study was comparable to its labeled.

Keywords: Acetaminophen, Gold nanoparticles, Carbon paste electrode, Voltammetry, Determination

1. Introduction

Acetaminophen (N-acetyl-P-aminophenol or Paracetamol, AP) is a long-established substance being one of the most extensively employed drugs in the world. It is a valuable non-steroidal anti-inflammatory drug that is widely used for the management of pain and fever, as well as for simple headaches, in a variety of patients including children, the elderly and those with osteoarthritis. It is primary metabolic pathways involving the liver oxidation.[1,2]

The large scale therapeutic use of this drug generated the need for the development of fast, simple and accurate methodologies for the determination of AP; for quality control analysis (in pharmaceutical formulations) and for medical control (in biological fluids as urine, blood and plasma).[3]

CPE was introduced by Adams in 1958.[4] The ease and speed of preparation, obtaining a new producible surface and low cost of carbon paste are advantages of CPEs over other electrodes.[5] Carbon pastes undoubtedly represent one of the most convenient materials for the preparation of modified electrode.[6] Preparation of chemically modified carbon paste electrode (CMCPE) is very simple and fast.[7] By adding modifier materials in paste can improve the electrode selectivity and sensitivity.[8] CMCPEs mainly used in the field of voltammetric determination.[9–14]

Colloidal nAu have been extensively used for electrochemical labeling and are usually embedded or coimmobilized into the CPE.[15] Due to their physical characteristics, the hydrophilic nAu and much smaller nAu, may be possible leaked out and cause some problems such as sensor design, the wettability balance needs to be turned between the hydrophobic paraffin oil and hydrophilic nAu.[16] As it is well known, these modified carbon paste electrode can be prepared by mixing the metal nanoparticles with graphite powder and the binder. Many assays have been described for AP including titrimetry,[17] chromatography,[18] capillary electrophoresis,[19,20] colorimetry,[21] UV spectrophotometry[22–24] and various modes of electrochemistry.[2,25,26]

Electrochemical methods, specially the voltammetric and the amperometric methods give the opportunity to study the oxidation mechanism, the redox metabolites and their detection from pharmaceuticals and body fluids.[27]

The main objective of this work was to develop a simple but sensitive electrochemical sensor for the determination of AP. Based on the good electrocatalytic activity of nAu modified CPE toward the electrochemical oxidation of AP, a very sensitive and selective electrochemical method has been proposed for the determination of AP in pharmaceutical samples.

2. Experimental

2. 1. Apparatus

Voltammetric experiments were recorded with a M273 electrochemical workstation (EG&G Corporation USA) with a conventional three-electrode system. This equipment was equipped with a CPE (unmodified and modified) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire (Metrohm) as the counter electrode. The measurements of pH were made with a Metrohm 691 pH meter (Metrohm Ltd., Switzerland) using a combined glass electrode. Demineral water was formed with an ultrapure water system (smart 2 pure, TKA, Germany).

2. 2. Materials

AP was purchased from Farabi Pharmacy Company in Iran. nAu were prepared with hydrogen tetrachloroaurate ($HAuCl_4$) purchased from Merck. All other chemical reagents were purchased from Merck and were of analytical grade. These chemicals were used without further purification. All aqueous solutions were prepared in dimineral water and the experiments were carried out at room temperature. Stock solutions of AP was freshly prepared as required in 0.2 mol l^{-1} (B-R) buffer at pH 4.7 and were used as the supporting electrolyte. For the determination of AP in commercial vitamin preparations and tablets, each tablet was crushed with a mortar and pestle, and a suitable amount of powdered sample was dissolved in 100 ml of the buffer solution. The commercial samples were purchased from a local drugstore.

2. 3. Preparation of Gold Nanoparticles

Colloidal nAu were prepared, according to the literature,[17] by adding 0.5 ml of 1% sodium citrate solution to 50 ml of 0.01% $HAuCl_4$ (heated and stirred). The mixture was maintained at the boiling point for 15 min and stirred for another 15 min after removing the heating source to produce colloidal nAu with a diameter of 24 nm. The maximum UV-Vis absorption peak for the synthesized colloidal nAu was at 520 nm. The colloidal gold solution was stored in a refrigerator inside a dark-colored glass bottle until it was used.

2. 4. Preparation of CPEs

CPE was prepared according to the literature[28] by thoroughly mixing graphite powder and paraffin oil. (1g: 360 µl). The nAu- CPE was prepared according to the following procedure. One gram of graphite powder was thoroughly mixed with 3 ml of the 24 nm colloidal nAu solution, after the water was evaporated, 360 µl paraffin oil were added to the mixture. A portion of the resulting paste was packed into the end of an insulin syringe (i.d:5 mm). Electrical contact to the paste was established by inserting a copper wire down through the syringe and into the back of the mixture. The surface was smoothed on a piece of weighing paper. A new surface can be obtained by pushing an excess of paste out of the tube, removing this excess, and mechanically polishing the electrode surface.

2. 5. Analytical Procedure

A certain volume of AP stock solution was transferred into the cell containing 10.0 ml of 0.2 mol l^{-1} (B-R) buffer (pH 4.7), and then the three-electrode system was installed on it. Differential pulse voltammetric determination was performed in the potential range of 100–800 mV (vs. SCE) and the oxidation peak currents at 405 mV was recorded for oxidation of AP. The square wave voltammograms for AP was recorded in the same method with the frequency of 40.0 Hz. The cyclic voltammograms were obtained by scanning the potential from 200 to 800 mV (vs. SCE) at a scan rate 100 mVs^{-1}. All measurements were carried out at room temperature.

3. Results and Discussion

3. 1. Voltammetric Behavior of AP at nAu-CPE

The cyclic voltammograms for AP, with modified and unmodified CPE as the working electrodes are shown in Figure 1. The electrochemical reaction of AP is quasi-reversible at both electrodes. At the unmodified electrode (a) the AP peak potential separation is about 112 mV. Moreover, the cathodic peak current is very small compared to the anodic peak current, and the cathodic and anodic peaks were rather broad. These results indicate the slow electron transfer rates for the oxidation of this molecule at the CPE. In comparison to a CPE, the reversibility of AP at nAu-CPE (b) is improved, so that the anodic and cathodic peak potential separation becomes 100 mV, and the i_{pa}/i_{pc} ratio is near unity. The negative shift with an enhanced peak current and a sharper peak indicates an enhanced electron transfer rate at the surface of nAu- CPE.

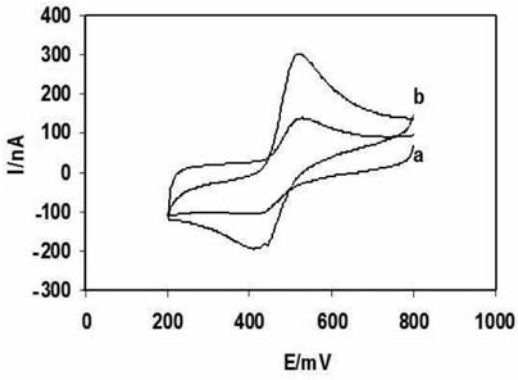


Figure 1. Cyclic voltammograms of 2.5×10^{-6} mol l^{-1} AP in 0.2 mol l^{-1} B-R buffer (pH 4.7) at the (a) unmodified CPE and (b) modified CPE. Scan rate = 100 mVs^{-1}.

3. 2. Effect of Scan Rate

The influence of scan rate (v) on the electrochemical behavior of AP on the nAu-CPE was investigated in the range of 20–500 mVs^{-1} (Figure 2A). The redox peak current increases linearly with the increase of square root of scan rate, demonstrating the diffusion-controlled process of AP on the nAu-CPE. The linear regression equation was i_{pa}= 489.5 + 382.0 $v^{1/2}$ (i_{pa}: nA, v: mVs^{-1}), i_{pc} = –827.6 – 182.4 $v^{1/2}$ (i_{pc}: nA, v: mVs^{-1}) with the correlation coefficient of 0.9958 and 0.9958, respectively (Figure 2B).

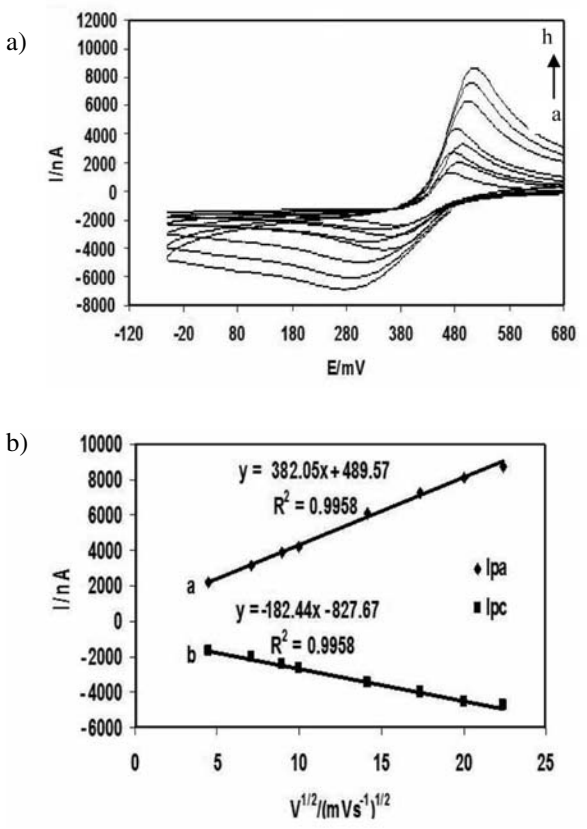
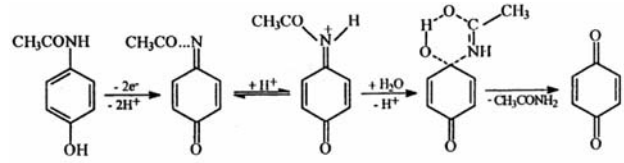


Figure 2. (a) Cyclic voltammograms of nAu-CPE in 0.2 mol l^{-1} B-R buffer (pH 4.7) for AP at different scan rates (a-h): 20, 40, 80, 100, 200, 300, 400 and 500 mV/s. (b) Plots of anodic and cathodic peak currents vs. $v^{1/2}$.

3. 3. Effect of pH

The voltammetric, potentiometric or amperometric determination of AP is based on its electrochemical behavior. In CV studies, Kissinger et. al.[29, 30] described the electrochemical oxidation of AP. They suggested the following mechanism:



The first step is an electrochemical oxidation by two-electron, two-proton process. The result is N-acetyl-p-quinoneimine, which suffers some none electrochemical but pH dependent reactions, and the final product is a benzoquinone.

The electrochemical behavior of AP was investigated over a pH range of 2 to 11. It was observed that the oxidation potential decreased when the pH of the AP solution increased. This behavior was attributed to the increasing amount of hydrolysis that occurs as the pH is increased, leading to the formation of reducing compounds such as p-hydroxyaniline.[31] The negative shift in anodic peak potential (E_{pa}) with pH can be described by the following equation:

$$E_{pa}\,(\text{mV vs. SCE}) = 635.3 - 53.0\ \text{pH} \qquad (1)$$

The theoretical slope for the plot of E_{pa} versus pH for a classical Nernstian two-electron, two-proton process is –59 mV pH^{-1}. A slope of –53.0 mV pH^{-1} was obtained in these experiments. This value is close to that found by Gilmartin and Hart[32] who reported a slope –46.5 mV pH^{-1} over a similar range of pH to that utilized here. These authors suggest a more complex oxidation mechanism for AP. When the pH is increased above 10, oxidation becomes kinetically less favorable, and adsorption of analyte onto the electrode surface occurs more readily. This is presumably due to the presence of the phenoxide form. When the pH is more acidic, the stability of AP is maintained through the inhibition of hydrolysis.

The selection of a suitable pH involved a compromise between sensitivity and the electrochemical behavior of AP in acidic and alkaline solutions. The maximum anodic current was obtained at pH 4.7, using 0.2 mol l^{-1} B-R buffer. Therefore, pH 4.7 was selected for subsequent studies.

3. 4. Analytical Applications

3. 4. 1. The Linear Relation Range and the Relative Standard Deviation

Since DPV and SWV have a much higher current sensitivity than CV, these methods were used to estimate the lower range of detection limit and determination of AP. For determination of AP, DPV was carried out in the potential range of 100 to 800 mV. Samples with the concentration from 5.0×10^{-8} to 1.0×10^{-4} of AP were experimented under optimized conditions. Each determination was repeated six times. The results of these experiments were illustrated for DPV and SWV in Figures 3 and 4, respectively.

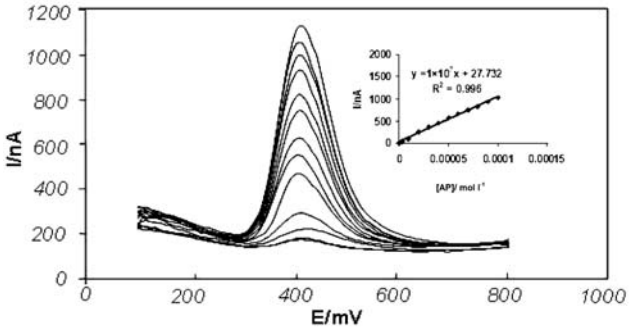


Figure 3. DPV of AP on nAu-CPE at different AP concentration from 5.0×10^{-8} –1.0×10^{-4} mol l^{-1}: (Inset) linear relationship between i_{pa} and AP concentration.

The calibration curve of AP in 0.2 mol l^{-1} B-R buffer solution (pH 4.7) at nAu-CPE was also established by DPV. The relationship between AP concentration and the oxidation peak current in the concentration range of 5.0×10^{-8} to 1.0×10^{-4} mol l^{-1} can be described with a regression equation of $i_{pa} = 1.0 \times 10^{-7}$ C + 27.73 (r = 0.996, C in mol L^{-1}, i_{pa} in nA) (inset in Figure 3).

Under the optimized experiment conditions described above, a detection limit of 2.6×10^{-8} mol l^{-1} AP was obtained with an accumulation for 4 minute at open circuit. The relative standard deviation (RSD) of 4.6 % for 6 times parallel detection of 1.0×10^{-7} mol l^{-1} AP, suggesting excellent reproducibility and repeatability of nAu-CPE.

The electrooxidation of AP under similar conditions was also studied using SWV technique. Typical square wave voltammograms are shown in Figure 4. The analytical curve is linear in the concentration range from 9.0×10^{-7} – 1.0×10^{-4} mol l^{-1} (inset in Figure 4).

The detection limit using SWV method was 1.0×10^{-7} mol l^{-1}. RSD of 5.3 % for 6 times parallel detection of 1.0×10^{-6} mol l^{-1} AP, was obtained. These results are comparable with values reported by other research groups (Table 1).

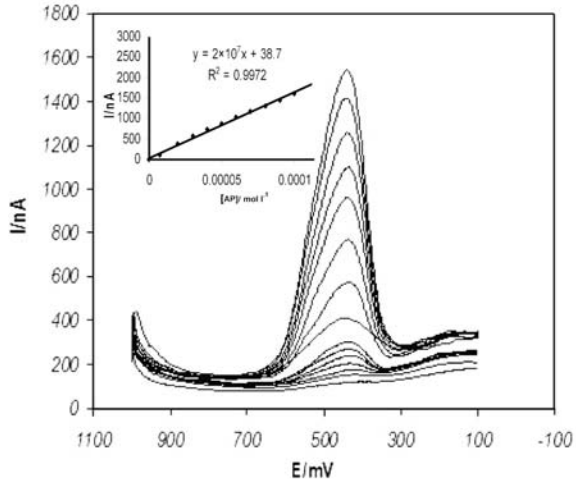


Figure 4. SWV of AP on nAu-CPE at different AP concentration from 9.0×10^{-7} – 1.0×10^{-4} mol l^{-1}: (Inset) linear relationship between i and AP concentration.

3. 5. Real Sample Analysis

DPV was applied for the quantitative determination of AP from dosage forms with the standard addition method. Tablet was weighed and then powdered. Suitable amount of powdered tablet was weighed accurately and placed into a backer with a magnet. AP samples were dissolved in dimineral water. After complete dissolution of samples, solution with different concentration prepared. For the standard addition method, 5.0 ml of unknown sample solutions that prepared in 0.2 mol l^{-1} B-R buffer (pH 4.7) was pipetted into the voltammetric cell. Five voltmmograms were recorded after adding different volumes of AP standard solution

Table 1. Comparison of the efficiency of some modified electrodes used in the electrocatalysis of AP

Electrode	Modifier	pH	Detection limit (μM)	Linear Range (μM)	Ref.
CPE	Thionine immobilized on multi-walled carbon nanotube	4.0	0.05	0.1– 100.0	3
CPE	in situ surfactant-modified multi-walled carbon nanotube	7.0	0.0258	0.291–62.7	33
CPE	multi-wall carbon nanotubes	5.5	0.043	0.15 –126	35
CPE	gold nanoparticle	4.7	0.33	0.66–530	35
CPE	gold nanoparticle	4.7	0.026	0.05–100.0	This work

under the same conditions as above. The results of comparison between observed and reported AP concentration in tablets/ vial are shown in Table 2. The results obtained in the study were comparable to its labeled. All the commercials samples of these drugs were produced in Iran.

Table 2. A comparison between observed and reported AP concentration in tablets/vial.

Tablet (syrup) name/ company name	Reported concentration (mol l^{-1})	Observed concentration (mol l^{-1})	Error (%)
Acetaminophen325mg (Chemidaru Pharmaceutical Co. Iran)	2.3×10^{-7}	2.2×10^{-7}	–4.3
cetaminophen codeine 500 mg A (Exir Pharmaceutical Co. Iran)	5.0×10^{-4}	4.7×10^{-4}	–6.0
Acetaminophen syrup 60 mL (Minoo Pharmaceutical Co.Tehran-Iran	8.5×10^{-5}	8.6×10^{-5}	1.18

3. 6. Recovery Test

Recovery tests of AP were carried out in the concentration range of 1.5×10^{-6}–8.5×10^{-5} mol l^{-1}. The results obtained are listed in Table. 3. Recoveries have been found to lie in the ranges of 94.0 to 103%.

Table 3. Recovery data observed for AP at different concentrations

Added (mol l^{-1})	Detected (mol l^{-1})	Recovery (%)
1.5×10^{-6}	1.7×10^{-6}	103.0
7.5×10^{-5}	7.5×10^{-5}	100.0
1.5×10^{-5}	1.4×10^{-5}	93.3

3. 7. Interference Studies

The effect of possible Interferences was investigated by the addition of other compounds to a solution containing 2.0×10^{-5} mol l^{-1} AP in 0.2 mol l^{-1} B-R buffer solution. Each possible contaminant was first added to have the same concentration as that of AP and then another addition cause that the concentration of interfering was increased. When the peak current change exceeded 5%, indicating that the substance caused obvious interference, the corresponding concentration was defined as the tolerance level of the interferent and the results of presence of these foreign substances on the oxidation peak are shown in Table 4.

Table. 4. Interferences of foreign substances on the oxidation peak of 2.0×10^{-5} mol l^{-1} AP

Foreign substances	Tolerance level (mol l^{-1})
Ascorbic acid	40
Penicilline, Amoxicilline, Uric acid	20
Imidazole, Aspirin, Cloxacillin	10

4. Conclusion

The voltammetric methods were applied for the assay of trace amount of AP involving oxidation at a nAu-CPE. These methods were simple, requiring no separation stage, rapid and sufficiently precise for the routine assay of AP in AP tablets and vials. Finally, the methods proposed have a low, submicromolar detection limit with excellent reproducibility. The results demonstrated that the n-Au-CPE give several distinct advantages, including an extraordinary, stability, high surface charge transfer rate constant and good detection limit for AP. Also the modified electrode exhibited an attractive ability for AP determination in trace amount of concentration.

5. Acknowledgement

The authors express their appreciation to the University of Kashan research councils for their financial support of this work.

6. References

1. F. S. Felix, C. M. A. Brett, L. Angnes, *J. Pharm. Biomed. Anal*. **2007**, 43, 1622–1627.
2. J. Polson , F. H. Wians Jr, P. Orsulak , D. Fuller , N. G. Murray, J. M. Koff , A. I. Khan , J. A. Balko , L. S. Hynan , W. M. Lee, *Clin. Chimica Acta* **2008**, 391, 24–30.
3. S. Shahrokhian, E. Asadian, *Electrochim Acta* **2010**, 55, 666–672.
4. A. Safavi, N. Maleki, O. Moradlou, F. Tajabadi, *Anal. Biochem*. **2006**, 359, 224–229.
5. H. R. Zare, N. Nasirizadeh, M. Mazloum-Ardakani, M. Namazian, *Sens. Actuators B* **2006**, 120, 288–294.
6. H. R. Zare, N. Nasirizadeh, S. Golabi, M. Namazian, M. Mazloum-Ardakani, D. Nematollahi, *Sens. Actuators B* **2006**, 114, 610–617.
7. M. Mazloum Ardakani, Z. Akrami, H. Kazemian, H. R. Zare, *J. Electroanal. Chem*. **2006**, 586, 31–38.
8. M. Kooshki, E. Shams, *Anal. Chim. Acta* **2007**, 587, 110–115.
9 E. Niranjana1, R. Raghavendra Naik, B. E. Kumara Swamy, B. S. Sherigara, H. Jayadevappa, *Int. J. Electrochem. Sci*. **2007**, 2, 923–934.

10. B. S. Sherigara, Y. Shivaraj , R. J. Mascarenhas, A. K. Satpati, *Electroch. Acta* **2007**, 52, 3137–3142.
11. H. Lin, G. Li, K. Wu, *Food Chem*. **2008**, 107, 531–536.
12. S. Young Ly, *Bioelectrochem*. **2006**, 68**,** 227 – 231.
13. Zh. Li, S. Junfeng, *Talanta* **2007**, 73, 943–947.
14 I. Svancara, M. Galyk, K. Vyt¡ras, *Talanta* **2007**, 72, 512–518.
15. L. Agy, J. Manso, P. Ynez-Sedeno, J. M. Pingarron, *Sens. Actuators B* **2006**, 113**,** 272–280.
16. L. Agy, C. Pena-Farfal, P. Ynez-Sedeno, J. M. Pingarron, *Talanta* **2007**, 74, 412–420.
17. M. K. Srivastava, S. Ahmed, D. Singh, I. C. Shukla, *Analyst* **1985**, 110, 735–737.
18. S. Ravisankar, M. Vasudevan, M. Gandhimathi, *Talanta* **1998**, 46, 1577–1581.
19. F. Y. He, A. L. Liu, X. H. Xia, *Anal. Biochem*. **2004**, 379, 1062–1067.
20. A. Kunkel, S. Gunter, H. Watzig, J. Chromatogr. A **1997**, 768, 125–133.
21. M. Knochen, J. Giglio, B. F. Reis, *J. Pharm. Biomed. Anal*. **2003,** 33, 191–197.
22. J. Hanaee, *Pharm. Acta Helv*. **1997,** 72, 239–241.
23. F. A. Mohamed, M. A. Abdallah, S. M. Shammat, *Talanta* **1997,** 44, 61–68.
24. Z. Bouhsain, S. Garrigues, M. de la Guardia, Fresenius, J. Anal. Chem. **1997,** 357, 973–976.
25. M. Kamberi., C. M. Riley, X. Ma, C. C. Huang, *J. Pharm. Biomed. Anal*. **2004,** 34, 123–128.
26. F. S. Felix, C. M. A. Brett, L. Angnes, *J. Pharm. Biomed. Anal*. **2007,** 43, 1622–1627.
27. R. Sacndulescu, S. Mirel, R. Oprean, *J. Pharm. Biomed. Anal*. **2000,** 23, 77–87.
28. D. Du, S.Liu, J. Chen, H. Ju, H. Lian, J. Lia, Biomaterials **2005**, 26, 6487–6495.
29. P. T. Kissinger, D.A. Roston, J. J. Van Benschoten, J. Y.Lewis, W.R. Heineman, *J. Chem. Edu.* **1983,** 60, 772–775.
30. D. J. Miner, J. R. Rice, R. M. Riggin, P.T. Kissinger, *Anal. Chem*. **1981,** 53, 22–58.
31. N. Wangfuengkanagul, O. Chailapakul, *J. Pharm. Biomed. Anal*. **2002,** 28, 841–847.
32. M. A. T. Gilmartin, J. P. Hart, *Analyst* **1994**, 119, 2431–2437.
33. B. J. Sanghavi, A. K. Srivastava, *Electrochimica Acta* **2010**, 55, 8638–8648.
34. H. Xiong, H. Xu, L. Wang, S. Wang, *Microchim Acta* **2009**, 167, 129–133.
35. Z. Xu, Q. Yue, Z. Zhuang, D. Xiao, *Microchim Acta* **2009**, 164, 387–393.

Povzetek

Raziskano je bilo elektrokemijsko obnašanje acetaminofena na elektrodi z ogljikovo pasto z dodatkom nanodelcev iz zlata. Ciklični voltamogrami kažejo dobro definirana katodna in anodna vrhova. Pri uporabi diferenčne pulzne voltametrije (DPV) opazimo oksidacijo pri 405 mV, pri uporabi »square wave« voltametrije (SWV) pa pri 440 mV. V 0,1 mol l^{-1} Britton–Robinsonovem (B-R) pufru je pri pH 4,7 zveza med anodnim tokom linearna v območju med $5{,}0 \times 10^{-8}$ in $1{,}0 \times 10^{-4}$ mol l^{-1} z mejo zaznave $2{,}6 \times 10^{-8}$ (DPP) in od $9{,}0 \times 10^{-7}$ do $1{,}0 \times 10^{-4}$ mol l^{-1} z mejo zaznave $1{,}0 \times 10^{-7}$ (SWV). Uporabnost modificirane elektrode je bila preverjena z določanjem acetaminofena v tabletah. Rezultati meritev so bili primerljivi z vrednostmi na oznakah.