

Research Article

Electrochemical Behavior of Poly (rutin) Modified Carbon Paste Electrode for the Determination of Uric Acid in the Presence of Ascorbic Acid and Dopamine

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Abstract

A poly rutin carbon paste electrode (CPE) was successfully fabricated by the electro polymerization process and characterized in this study by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). This modified carbon paste electrode has exhibited a good electro catalytic activity towards the determination of uric acid (UA) in 0.1M phosphate buffer solution (PBS) at pH 7.0. The effect of pH, scan rate and the concentration of UA on anodic peak current were investigated. The poly rutin modified electrode was successfully used as a sensor for the selective determination of UA in presence of dopamine and ascorbic acid without any interference. The modified electrode showed a good detection limit of 2.25 μM over the linear dynamic range of $1.24 \times 10^{-6}\text{M}$ to $2.43 \times 10^{-5}\text{M}$, which is superior to many reported methods in the literature.

The prepared modified electrode exhibited good stability, high sensitivity, better reproducibility, low detection limit towards the determination of uric acid (UA).

Keywords: Rutin, Uric acid, Dopamine, Ascorbic acid, cyclic voltammetry, Differential pulse voltammetry

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Introduction

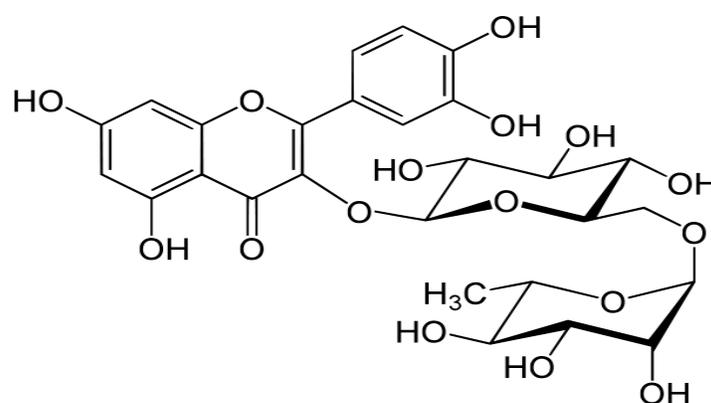
Presently chemically modified electrodes have attracted a lot of interest. The modified electrode surface includes composite generation and polymer coating techniques. Electro polymerization of carbon paste electrodes is an attractive research area in modern electro analytical chemistry especially due to various potential application possibilities of modified electrodes in electro analysis and chemical sensors due to the relative ease of electrode preparation and regeneration [1-5].

Uric acid (2, 6, and 8 – trihydroxy purine UA) is the final product of metabolic [6] breakdown of purine and is excreted in urine. In human blood plasma, the reference range of uric acid is typically 0.18-0.39 mM, in urine it is in between 1.49 mM to 4.46 mM [7]. Uric acid may be a marker of oxidative stress and may have a potential therapeutic role as an antioxidant. UA is a risk factor for cardio vascular disease, it is also affecting the circulatory system including the heart, lungs, brain, kidneys and other parts of the body [8-9] Therefore, the determination of UA concentration is helpful for the diagnosis of some diseases such as Lesch-Nyhan syndrome, gout, hyperuricaemia, hypouricemia, pneumonia, fatal poisoning, and toxemia of pregnancy when its level goes extremely abnormal [10], which makes determination of UA concentration in urine very important.

Dopamine (3,4-dihydroxyphenylethylamine) functions as a neurotransmitter in brain. In blood vessels, it inhibits norepinephrine release and acts as a vasodilator. Deficiency of DA can lead to several neurological disorders such as Parkinsonism (is a chronic and progressive movement disorder), Schizophrenia (is a mental disorder), Huntington's disease (is a neurodegenerative genetic disorder), Toilette's syndrome (inherited neuropsychiatric disorder), prone to drug addiction, HIV infection and senile dementia [11-13] DA act as a cholinergic drug, it is widely applied to the treatment of circulatory collapse syndrome caused by heart attack, trauma, renal failure, cardiac surgery.

Ascorbic acid [vitamin C] is a water-soluble cellular anti-oxidant. It plays a significant role in the growth and repair of tissues. Vitamin C is needed for repairing and for healing wounds and maintaining bones and teeth. It also helps the body to absorb iron. AA can inhibit viral infectivity by inactivating viruses and by affecting viral replication and it is used in the treatment and prevention of vitamin C deficiency (scurvy) that is characterized by bleeding and slow healing of wounds. Abnormal levels of AA causes depression, thinking problems, dementia (hardening of the arteries in the blockage in blood flow in brain.), Alzheimer's disease, physical and mental stress, stomach ulcers, skin infections, infections of the bladder and prostate [14, 15].

Earlier, electrochemical techniques have been extensively used for the simultaneous determination of UA, DA and AA including capillary electrophoresis [16], chromatography [17, 18], fluorescence [19], spectrofluorometry [20], enzymatic methods [21, 22] and chemiluminescence [23]. But, above techniques often require expensive instruments and more time for analysis. However, the key problems for monitoring UA and DA oxidation potentials always are close, so therefore, it is a significant attempt to separate the peak potentials of oxidation between DA and AA, or UA. Using the modified electrode, the oxidative potentials of DA and UA were separated successfully, thereby enabling the independent determination of DA in the presence of UA. The poly rutin modified electrode provides a clear advantage such as selectivity, sensitivity, and reproducibility.



Chemical Structure of Rutin

Rutin ($C_{27}H_{30}O_{16}$) is one of the most abundant bioactive flavonoid and it is a derivative of catechol. It is the main active ingredient in *Sophora japonica* L. Known as vitamin P, rutin has been widely used as a therapeutic agent and more than 130 preparations containing rutin are registered as drugs worldwide and has a broad range of physiological activities such as anti-inflammatory [24, 25] antiviral [26] anti-oxidant [27], hypertension [28] and anti-depression [29], and it has the potential to treat diabetes [30], commonly found in orange, grapefruit, lemon, berries and often present in human diet. Electrochemical behavior of rutin is characterized by two oxidative signals corresponding to two electron oxidation of $-OH$ groups at positions 3' and 4' forming an o-quinone. Poly rutin is used because of its electrochemical activity.

The focus of the work is to fabricate a stable and selective biosensor by the electro polymerizing of CPE modified with poly rutin using cyclic voltammetric technique. The Poly rutin not only exhibited strong electro catalytic activity for oxidation of UA, DA and AA, but also resolved their voltammetric responses into three well-defined peaks. This modified electrode showed an excellent sensitivity, selectivity, stability, and reproducibility of the developed method for the detection of UA at the poly rutin/CPE in the presence of DA and UA at the physiological pH of 7.0.

Experimental

Instrument

Voltammetric measurements were performed with CH-Instrument Model No. CHI610D Electrochemical work station is a conventional three electrode cell, which includes a working CPE, a platinum wire as counter electrode and saturated calomel electrode as a reference electrode. Bare CPE was prepared by grinding graphite powder of 70% and silicon oil of 30% in an agate mortar by hand mixing for about 30 min to get the homogenous graphite paste. A bare carbon paste electrode (3.0 mm diameter) / and poly (Rutin) MCPE was used as the working electrodes. The paste was packed into the cavity of CPE and smoothed on a weighing paper to obtain smooth surface.

Reagents

Rutin, Dopamine hydrochloride, ascorbic acid, uric acid, sodium dihydrogen phosphate (NaH_2PO_4) disodium hydrogen phosphate (Na_2HPO_4) and silicon oil was supplied by Himedia Chemicals. Fine graphite powder (particle size $<20\mu\text{m}$) were procured from Sigma-Aldrich Chemicals. All chemicals were of analytical grade and were used without further purification. Dopamine stock solution was prepared by dissolving in 0.1 M perchloric acid and ascorbic acid in double distilled water. The stock solution of uric acid (25 mM) was prepared by dissolving in 0.1 M sodium hydroxide solutions. The supporting electrolyte used was Phosphate buffer (pH 7.0) and was prepared with 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 . The stock solution of AA (25 mM) was prepared with water purified with a Millipore Milli-Q system the pH values were measured with Elico Li 120 pH meter. All measurements were carried out under room temperature.

Preparation of bare carbon paste electrode and Poly rutin modified carbon Paste electrode

The bare carbon paste electrode was prepared by adding the graphite powder and silicon oil in the ratio of (70:30%) in an agate mortar to obtain a homogenous carbon paste. The polymer film modified CPE was prepared by the electrochemical polymerization of Rutin on a carbon paste electrode in 0.1M phosphate buffer solution of pH 7.0 containing 1.0 mM of rutin with cyclic voltammetric sweeps in the potential range of -0.8V to 1.2V at the scan rate of 50 m V/s. Subsequently, the modified electrode was rinsed with water and placed in a buffer solution (pH 7.0) to test its electro catalytic behavior

Results and Discussion

Electrochemical polymerization of rutin on carbon paste electrode

To investigate the possible mechanism of rutin polymerization on the carbon paste electrode (CPE), the effect of the number of potential cycles during the preparation of the modified electrode in cyclic voltammetric responses was studied. For this propose, poly rutin was prepared from 1.0 mM solution of rutin at pH 7.0 using a different number of potential cycles. Electropolymerisation was carried out between the potential -100mV and 150 mV at a scan rate of 100 m V/s in 30 cycles and is shown in **Figure 1**. During the polymerization process, the cyclic voltammograms showed an anodic oxidation peak potential at 369 mV was obtained. The oxidation peak is directly proportional to the number of cyclic voltammetric scans, indicating that an electro-conductive polymer film was fabricated on the electrode surface.

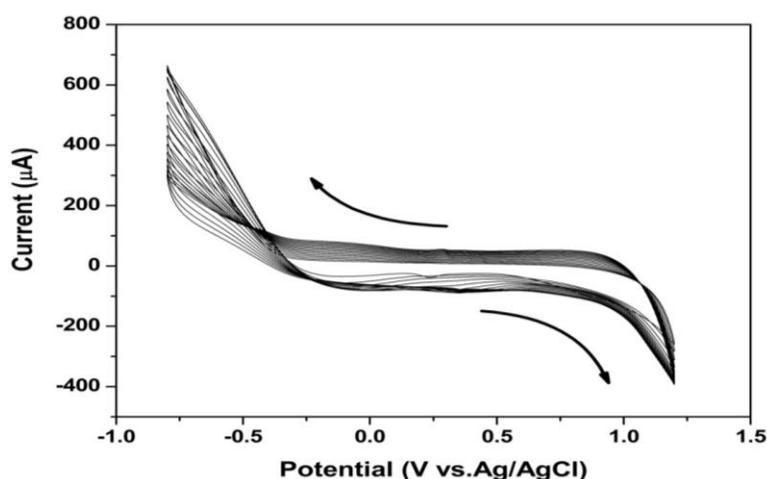


Figure 1 Cyclic voltammogramme for the electrochemical polymerization of 1.0 mM Poly rutin on a CPE at the scan rate 50 mV s^{-1}

Rutin was oxidized to form the free radical at the surface of CPE which combines with the surface of CPE quickly, indicating that the poly (rutin) undergoes addition polymerization with CPE. The electro polymerisation procedure was completed and the modified carbon paste electrode was rinsed with Millipore water.

Electrochemical oxidation of UA at the poly rutin

Figure 2 depicts the CV of a bare CPE (dashed curve 'a'), poly rutin (dotted curve 'b') in presence of 25 mM UA in the PBS of pH 7.0 with the scan rate of 50 mV/s. On bare CPE UA was oxidized at 276 mV with an anodic (oxidation) peak current of 60 μA , where as in the reverse scan no cathodic peak was observed. After the modification of CPE with poly rutin the peak current of UA increases to 146 μA . ΔE_{pa} was also increased from 272 mV to 352 mV for poly rutin/CPE respectively.

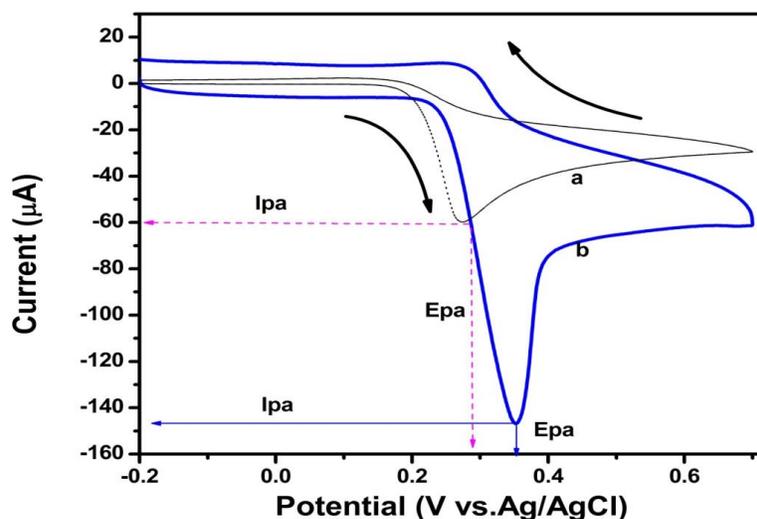


Figure 2 Cyclic voltammogram of bare (dotted line), poly rutin MCPE using 0.1 mM UA in 0.1 M PBS (pH=7.0) at the scan rate 50 mV s⁻¹

The peak potential difference (EP) 80 mV was obtained and indicates the same number of electrons and protons are involved in the reaction mechanism [31]. This concludes that the poly rutin modified carbon paste electrode (Solid line) exhibited an excellent sensitivity for the determination of UA.

The electro catalytic activity of rutin is due to the presence of electron releasing groups such as -CH₃ and -OH on the surface of CPE. Due to the existence of electron rich groups, poly rutin shows a good affinity towards the UA and it can easily undergo oxidation by exchanging positive ions with the electron rich groups in Poly rutin MCPE.

Effect of scan rate

Figure 3a illustrates the CV response for the electro catalytic oxidation of UA in the presence of poly (rutin) at different scan rates between 50 and 500 mVs⁻¹. The scan rate depending on the peak current in 25mM of UA was studied. Figure 3b represents the Calibration plot of anodic peak current. A good linear relationship between the anodic peak current of UA and the square root of scan rate ($v^{1/2}$) with a correlation coefficient of $R = 0.9901$ was obtained. This indicates that the electrode reaction of UA is diffusion controlled process [32].

Effect pH of the solution

To analyze the effect of pH of PBS on the anodic peak current and the peak potential 0.1PBS containing 25 mM of UA was studied at poly (rutin) by employing CV technique. It is revealed from **Figure 4** that the experiment was conducted in the pH range from 5.0 to 8.0 the anodic peak current of UA increases with increase of pH and the anodic peak current gradually increase from 5.0 to 7.0 and then there was a decrease in the anodic peak current at the pH of more than 7.0. Furthermore, the anodic peak potential shifts towards more negative as the pH of the solution was increased from 5.0 to 8.0. This indicates the participation of protons in the electrochemical oxidation of UA at poly (rutin). The peak potential shows the linearity in the pH range of 5.5 to 8.0 with a slope of 50mV/pH and the linear regression equation is given by

$$E_{pa} \text{ (mV)} = 761.59 - 64.57 \text{ pH} \text{ (} R^2 = 0.9822 \text{)}$$

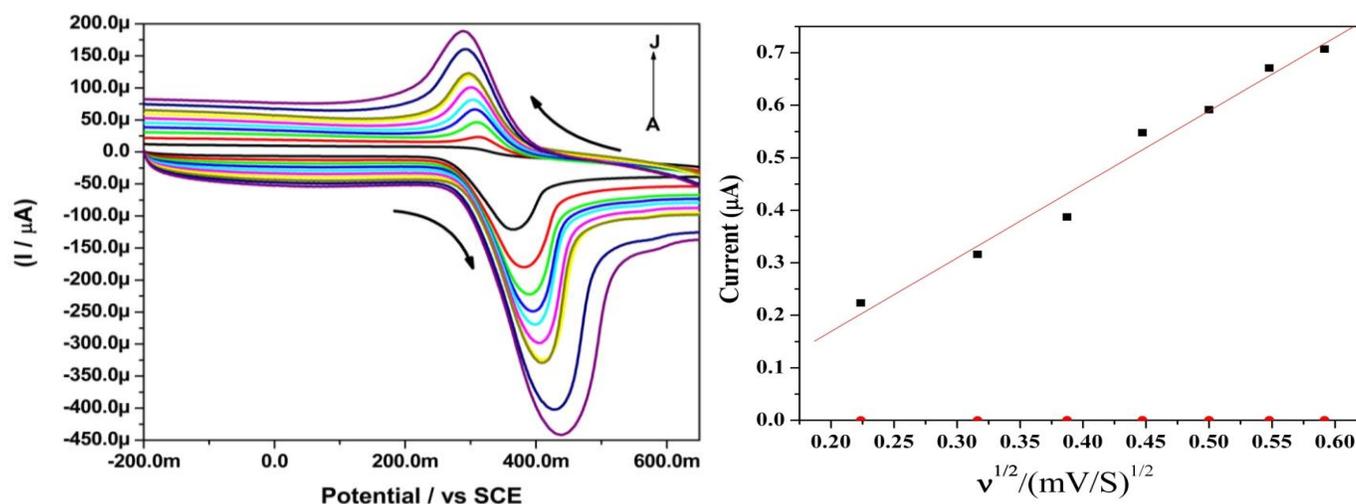


Figure 3 (a) Cyclic voltammogram of poly rutin CPE in the presence of 0.1 mM UA in 0.1 M PBS (pH=7.0) with various scan rates (50, 100, 150, 200, 250, 300, 350 mV s^{-1}), (b) Calibration plot of anodic peak current Vs square root of scan rate ($v^{1/2}$)

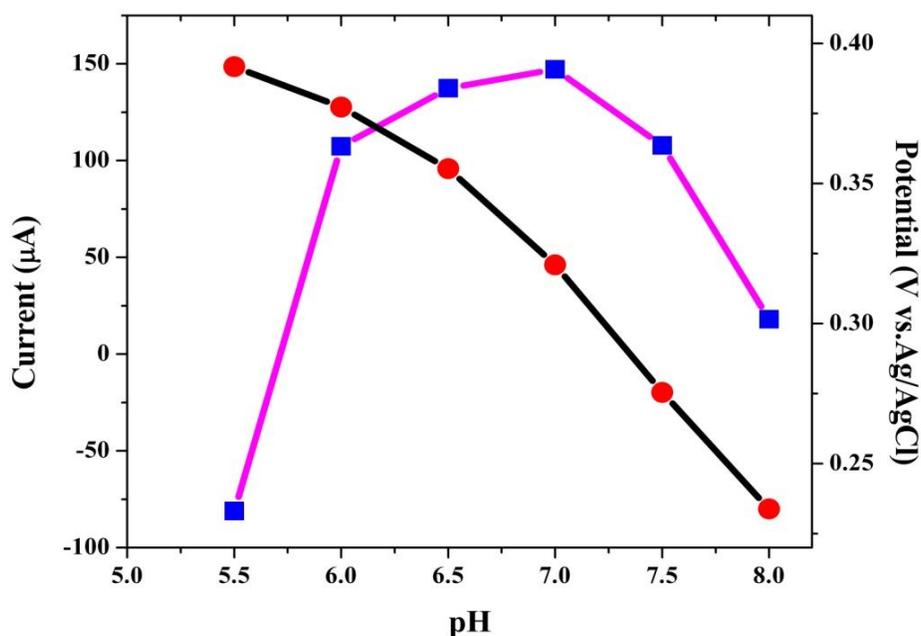


Figure 4 Effect of pH on anodic peak current (I_{pa}), anodic peak potential (E_{pa}) of 0.1 mM UA in 0.1 M PBS

Effect of UA concentration

It involves the determination of the detection limit of UA in the presence of poly rutin MCPE. From **Figure 5** it can be revealed that the anodic peak current observed at the modified CPE at various concentrations of UA by using DPV technique. The graph of I_{pa} Vs concentration of UA is shown in inset, showing the linear dynamic range from $1.24 \times 10^{-6} \text{M}$ to $2.43 \times 10^{-5} \text{M}$. The detection limit is $2.25 \mu\text{M}$ was calculated by using the following equation:

$$DL = 3 \times SD/B$$

Where SD= Standard deviation, B is the slope obtained from the calibration plots. The linear regression equation for UA is expressed as $I_{pa} (\mu\text{A}) = 0.1022 + 1.42(\mu\text{A}) (R^2 = 0.9944)$. The low detection limit were calculates here more sensitive than the values reported earlier.

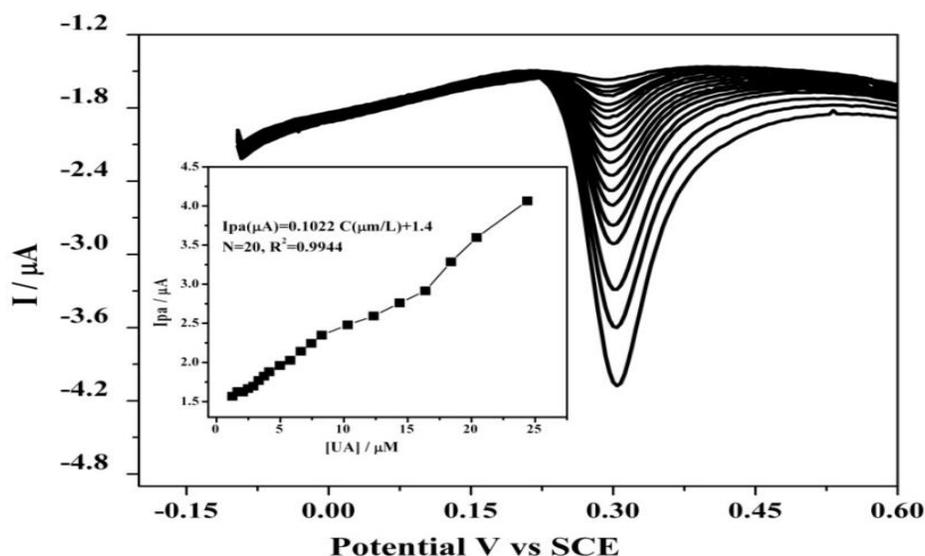


Figure 5 Cyclic voltammograms of UA at poly rutin MCPE at different concentration in 0.1M PBS at pH 7.0. Inset is the plot of the anodic peak current versus the concentration of UA.

Simultaneous determination of AA, DA and UA

In human body DA, AA and UA coexist in biological fluids. CV was carried out for the simultaneous determination of AA, DA and UA at a poly rutin/CPE in 0.1 M PBS (pH 7.0). The different concentrations of 2.5 mM of AA, 0.025 mM of DA and 0.25 mM of UA in the mixture at poly rutin/CPE, gave three well prominent oxidation peaks and all peak currents were increased linearly with increase in the concentration of AA, DA and UA. The concentration of UA was increased in the presence of high concentration of 5.0 mM of AA and 0.025 mM of DA, a good selectivity was observed by the poly rutin/CPE as there was no obvious change in the AA and DA peak current as a consequence of dismissing its tendency to interfere with AA and DA.

The cyclic voltammograms response of the bare CPE (dotted curve, a), and Poly rutin/CPE (Solid curve, b), toward DA, AA and UA are shown in **Figure 6**. The bare CPE failed to resolve the voltammetric response of DA and AA. Whereas poly rutin/CPE is succeeded in resolving the overlapped voltammetric response. Three distinct peaks at 70.5, 261 and 455 mV for AA, DA and UA were observed at poly rutin/CPE. Due to the Electrostatic attraction of AA, DA and UA are more effective surface area of modified electrodes poly rutin/CPE results in enhanced current sensitivity.

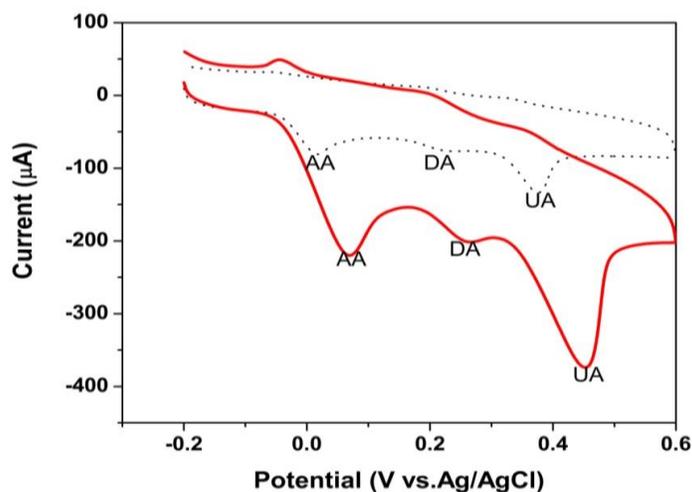


Figure 6 Cyclic voltammograms for simultaneous determination 2.5 mM of AA, 0.025 mM of DA and 0.25 mM of UA at bare carbon paste electrode (dotted line) and Poly(rutin)/CPE (solid line) in 0.1 M PBS of pH 7.0

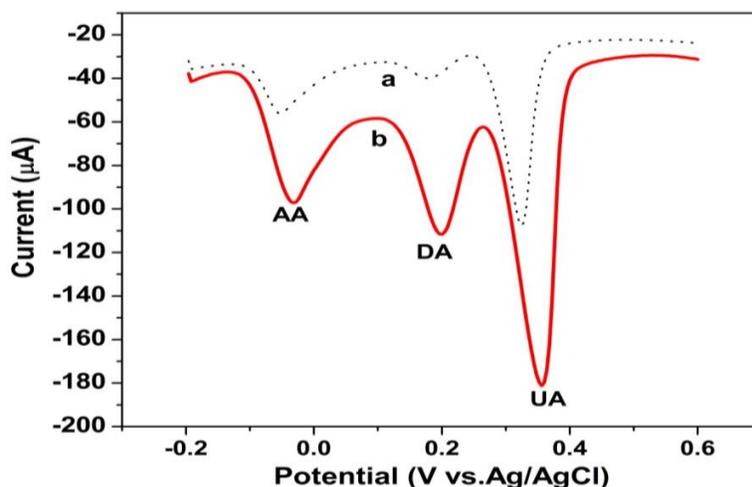


Figure 7 Differential pulse voltammograms for the simultaneous determination of 2.5 mM of AA, 0.025 mM of DA and 0.25 mM of UA at Bare carbon paste electrode (dotted line) and Poly(rutin)/CPE (solid line) in 0.1 M PBS of pH 7.0.

DPV responses of the mixture of DA, AA and UA at bare CPE (dashed curve), and at the poly rutin (solid curve) are shown in **Figure 7**. The oxidation peaks of AA, DA and UA at the poly rutin/CPE are 30 mV, 199 mV and 357 mV respectively, shows three well defined peaks compared to bare CPE. The results demonstrate that the poly rutin/CPE have an excellent electro catalytic activities toward the oxidation of AA, DA and UA.

Conclusion

The reported, poly (rutin) modified CPE acts as an biosensor for the detection of Uric acid (UA). The poly (Rutin) film coated CPE had increased the anodic current remarkably. The poly (Rutin) film coated CPE showed an excellent selectivity and electro catalytic activity towards the oxidation of UA in the presence of DA and AA. The poly (Rutin) film coated CPE has showed satisfactory results with the detection limit of 2.25 μM . Hence, poly (rutin) modified CPE is acting as an excellent sensor for the simultaneous determination of UA, DA, and AA.

References

- [1] Beitollahi, H.; Raouf, J. B.; Hosseinzadeh, R. *Talanta* 2011, 85, 2128-2134.
- [2] Rodr'iguez-L'opez, A.; Torres-Torres, D.; Mojica-Gomez, J.; Estrada-Arteaga, C.; Anta~no-L'opez, R. *Electrochim. Acta* 2011, 56, 8078-8084.
- [3] Anik, U.; C, ubuk, cu, M. *Turk. J. Chem.* 2008, 32, 711-719.
- [4] Beitollahi, H.; Raouf, J. B.; Hosseinzadehc, R. *Electroanalysis* 2011, 23, 1934-1940.
- [5] Emamali Sabzi, R.; Minaie, E.; Farhadi, K.; Golzan, M. M. *Turk. J. Chem.* 2010, 34, 901-910.
- [6] 25. Devlin, T. M. *Textbook of Biochemistry with Clinical Correlations*, Wiley-Liss, New York, 1992.
- [7] F.Zhang, X.Wang, S.Ai,Z.Sun, Q.Wan, Z.Zhu, Y.Xian, L.Jin, K.Yamamoto, Immobilization of uricase on ZnO nanorods for a reagent less uric acid biosensor, *Anal. Chim. Acta* 519 (2004) 155-160.
- [8] B.F. Culleton, M.G. Larson, W.B. Kannel, D. Levy, Serum uric acid and risk for cardiovascular disease and death: the framing heart study, *Ann. Intern. Med.* 131 (1999) 7-13.
- [9] J. Fang, M.H. Alderman, Serum uric acid and cardiovascular mortality, *J. Am. Med. Assoc.* 283 (2000) 2404-2410.
- [10] V. V. S. E. Dutt and H. A. Mottola, *Anal Chem*, 46 (1974) 1777
- [11] A. Liu, I. Honma, H. Zhou, Simultaneous voltammetric detection of dopamine and uric acid at their physiological level in the presence of ascorbic acid using poly(acrylic acid)-multiwalled carbon-nanotube composite-covered glassy-carbon electrode, *Biosens. Bioelectron.* 23 (2007) 74-80.
- [12] S.K. Jaaskelainen, J.O. Rinne, H.Forsell, O.Tenovuo, V. Kaasinen, P. Sonninen, J. Bergman, Role of the dopaminergic system in chronic pain-a fluorodopa-PET study, *Pain* 90 (2001) 257-260.
- [13] G.S.H. Thien, A. Pandikumar, N.M. Huang, & H.N. Lim, highly exposed {001} facets of titanium dioxide modified with reduced graphene oxide for dopamine sensing, *Sci.Rep.* 4, 5044(2014). DOI: 10.1038/srep05044.

- [14] E. Miland, A.J.M. Ordieres, P.T. Blnaco, M.R. Smyth, C.O. Fagian, Poly(o-aminophenol)-modified bienzyme carbon paste electrode for the detection of uric acid, *Talanta* 43 (1996) 785–796.
- [15] Z. Dursun, L. Pelit, I. Taniguchi, Voltammetric determination of ascorbic acid and dopamine simultaneously at a single crystal Au(111) electrode, *Turk. J.Chem.* 33 (2009) 223–231
- [16] V. Vuorensoola, H. Siren, U. Karjalainen, Determination of Dopamine and methoxy catecholamines in patient urine by liquid chromatography with electro chemical detection and capillary electrophoresis coupled with spectrophotometry and mass spectrometry, *J.chromatography B*,768(2003)277-28.
- [17] X. Dai, X. Fang, C. Zhang, R. Xu, B. Xu Determination of serum uric acid using high-performance liquid chromatography (HPLC)/isotope dilution mass spectrometry (ID-MS) as a candidate reference method, *J. Chromatogr. B* 857 (2007)287–295.
- [18] S.K. George, M.T. Dipu, U.R. Mehra, P. Singh, A.K. Verma, J.S. Ramgaokar, Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in urine, *J. Chromatography. B* 832 (2006) 134–137.
- [19] H-X Zhao, H.Mu, Y-H.Bai, H.Yu, Y-M.Hu, A rapid method for the determination of dopamine in porcine muscle by pre-column derivatization and HPLC with fluorescence detection, *J. Pharm. Analysis.* 1 (2011) 208 – 212.
- [20] R.R. Marquardt, A simple spectrophotometric method for the direct determination of uric acid in avian excreta, *Poult. Sci.* 62 (1983) 2106–2108.
- [21] S. Gjørup, H. Poulsen, E. Prætorius, The uric acid concentration in serum determined by enzymatic spectrophotometry, *Scand. J. Clin. Lab. Invest.* 7 (1955)201–203.
- [22] E. Prætorius, H. Poulsen, Enzymatic determination of uric acid with detailed directions, *Scand. J. Clin. Lab. Invest.* 5 (1953) 273–280.
- [23] J. H. Yu, S. M. Wang, L. Ge, S. G. Ge, A novel chemiluminescence paper microfluidic biosensor based on enzymatic reaction for uric acid determination, *Biosens. Bioelectron.* 26 (2011) 3284–3289.
- [24] L. Selloum, H. Bouriche, C. Tigrine, et al Anti-inflammatory effect of rutin on rat paw oedema, and on neutrophils chemotaxis and degranulation, *Exp.Toxicol.pathol.*54 (2003)313-318.
- [25] J. Tian, S. G. Song, Relationship between protective effect and antioxidative action of rutosid on experimental acute pancreatitis in rats, *Chin .J. Clin. Pharmacol.Ther.*04 (2004) 455-458.
- [26] J. Tao, Q. X. Hu, J. Yan, et al, Invitro anti HIV and HSV activity and safety of sodium rutin sulfate as a microbicide candidate, *Antiviral Res.*75(2007)227-233.
- [27] J. X. Yang, J. Guo, J. F. Yuan, Invitro oxidant properties of rutin, *Food Sci.Technol.*41(2008)1060-1066.
- [28] H.K.Hellerstein, J.L Orbison, S.Rodbard, et al., The effect of rutin in experimental malignanth hypertension, *Am Heart J.*42(1951)271-283.
- [29] G. M. D Aniele, E. B. B. Luis, P. C. Mauricio, Anti depressant like effect of rutin isolated from the ethanolic extract from *Schinus molle* L.in mice: evidence for the involvement of the serotonergic and noradrenergic systems, *Eur.J.Pharmacol.*587(2008)163-168.
- [30] B.H.Jan, H.D.Su, F.Xu, Prevention of rutin for diabetic nephropathy, *Heilongjiang, Med.J.*12(2005)899-901.
- [31] Manimangalam. Lavanya, Yenugu Veera Manohara Reddy, Manthrapudi. Venu, Gajulapalle Madhavi, Zerovalent Nano Iron Electrochemical Sensor for the Determination of Ascorbic Acid in Presence of Dopamine using Sodium Dodecyl Sulphate, *Anal. Bioanal. Electrochem.* 7 (2015) 22-34.
- [32] B.D. Jones, J.D. Ingle Jr. Evaluation of immobilized redox indicators as reversible, in situ redox sensors for determining Fe(III)-reducing conditions in environmental samples, *Talanta* 55 (2001) 699–714.

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