

Poly (Patton and Reeder's) Modified Carbon Paste Electrode Sensor for Folic Acid

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Abstract

Poly (Patton and Reeder's) modified carbon paste electrode was fabricated and it was employed to study the electrochemical response of folic acid (FA) in phosphate buffer solution (PBS) of pH 7.4 by cyclic voltammetric (CV) technique. The results obtained at Poly (Patton and Reeder's) modified carbon paste electrode shows a good electrocatalytic activity towards the oxidation of FA. The lower limit of detection of FA was found to be 1.88×10^{-8} M by CV technique. The parameters like effect of concentration and scan rate reveal the electrode process was controlled by adsorption of an analytes. The poly (Patton and Reeder's) modified carbon paste electrode was used for the simultaneous determination of FA and uric acid (UA) at physiological pH.

Keywords: Poly (Patton and Reeder's); Folic acid; Uric acid; Carbon paste electrode; Electrocatalytic activity

Introduction

In recent years the development, design, fabrication and application of electrochemical sensors for the electroactive molecules has been of great interest in modern electroanalytical research [1-3]. Particularly in the development of voltammetric sensors for the quantification and determination of biologically important molecules such as folic acid has been received much more attention of young electroanalytical researchers [4,5].

Folic acid (FA) is a water-soluble vitamin-B that helps to build up the healthy cells. The deficiency of FA in human body leads to anaemia and is thought to increase the shells of heart attack and stroke [6]. FA deficiency causes failure to make the purines and thymine required for DNA synthesis and many studies suggests that diminished folate status is associated with enhanced carcinogenesis [7]. FA is a potential agent in preventing the growth of cancer cells by free radical scavenging and antioxidant property [8]. Per conceptual supplementation of FA has been demonstrated to significantly reduce the incidence and reoccurrence of neural tube defects, such as spina bifida of women [9,10]. The sensitive methods are required for the determination of FA in pharmaceutical and clinical diagnosis. There are so many research reports are there for the determination of FA such as, liquid chromatography [11], high performance liquid chromatography [12,13], flow-injection Chemiluminometry [14], Isotope Dilution-Liquid Chromatography/Tandem Mass Spectrometry [15] and Spectrophotometric method [16]. As FA is an electroactive component, some electrochemical methods have been reported for its determination [17,18].

Uric acid (UA) is the major nitrogenous compound in the urine and it is a primary product of purine metabolism in the human body [19]. Its deficiency in human body leads to many diseases, such as gout, hyperuricaemia and Lesch-Nyan disease [20-22]. Hence, monitoring the concentration of UA in biological fluids is very important. From the literature, various methods such as chemiluminescence [23-25], Chromatography [26], Capillary electrophoresis [27] are proposed. Compared with other techniques, the electrochemical method is more desirable because of the instrumental sensitivity, low cost, stability and quick response.

The problem is that at bare working electrode the voltammetric response of these biomolecules is less sensitive, least selective and

gives poor voltammetric response. There are so many endless efforts to overcome this challenge. The widely accepted and common route is to use a modified electrode [28,29]. There are several methods to modify the bare carbon paste electrode either by mechanically grinding the different type of modifiers with the carbon paste [30,31] or by electropolymerisation of the modifier [32,33]. The modifier chosen for the present work is the Patton and Reeder's reagent. It is a complexometric indicator extensively used in the field of analytical chemistry especially in the determination of the calcium ions. Swamy *et al.*, reported the detailed procedure for the fabrication of Poly (Patton and Reeder's) modified carbon paste electrode [34]. The intension of our work was to fabricate a stable working electrode by electropolymerising Patton and Reeder's reagent on the surface of carbon paste electrode and utilising its application as an electrochemical sensor for FA at physiological pH.

Experimental Section

Reagents

Graphite powder of 50 μm particle size was purchased from Merck and silicone oil from Himedia was used to prepare the carbon paste electrode (CPE). Folic acid (FA), Uric acid (UA) and Patton and Reeder's reagent was obtained from Himedia. The stock solution of 25×10^{-4} M FA and 25×10^{-4} M UA was prepared in 0.1 M NaOH solution. Buffer used was 0.2 M Phosphate buffer solution (PBS) of pH 7.4. All the chemicals mentioned were all of analytical grade used as received without any further purification.

Apparatus

The electrochemical experiments were carried out using a model

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CHI-660 c (CH Instrument-660 electrochemical workstation). A conventional three electrode cell was used with a saturated calomel electrode (SCE) as a reference, a platinum wire as a counter electrode, and bare or Poly (Patton and Reeder's) modified carbon paste electrode (MCPE) as a working electrode.

Preparation of bare carbon paste electrode

The bare carbon paste electrode (BCPE) was prepared by hand mixing of 70% graphite powder and 30% silicone oil in an agate mortar for about 45 min until a homogeneous paste was obtained. The paste was then packed into a cavity of PVC tube of 3 mm internal diameter and smoothed on a tissue paper. The electrical contact was provided by a copper wire connected to the end of the tube.

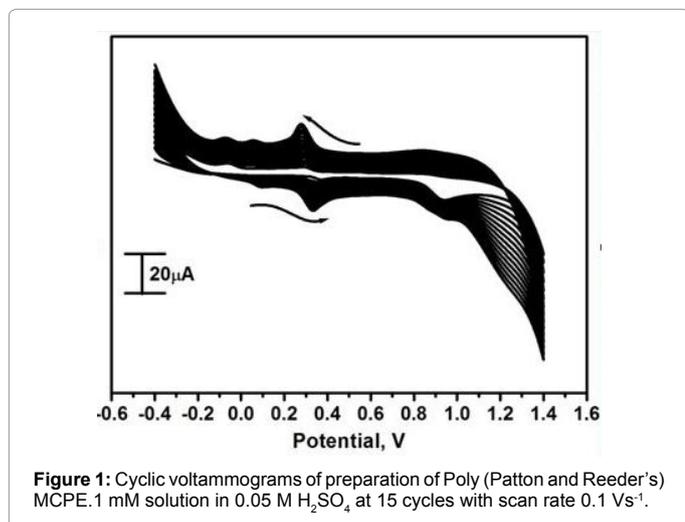
Preparation of poly (Patton and Reeder's) modified CPE

The paste packing procedure was same as that at BCPE. The electropolymerisation of Patton and Reeder's reagent on the surface of CPE was achieved by using cyclic voltammetric technique. The CPE was scanned for 15 multiple cycles in an electrochemical cell containing aqueous solution of 1.0 mM Patton and Reeder's reagent with 0.05 M H₂SO₄ with in the potential window of -0.4 V to +1.4 V at a scan rate of 0.1 Vs⁻¹. After this the electrode was rinsed thoroughly with double distilled water. The same electrode was used for the voltammetric measurements.

Result and Discussion

Electrochemical polymerization of Patton and Reeder's reagent on CPE

The Poly (Patton and Reeder's) MCPE was prepared by placing 1.0 mM Patton and Reeder's reagent along with 0.05 M H₂SO₄ in an electrochemical cell. The thin film that grew in between the potential window of -0.4 V to 1.4 V with scan rate 0.1 Vs⁻¹ for 15 multiple cycles as shown in the Figure 1. During the process of multiple cycles the voltammogram was increased at first and later becomes virtually constant. This suggests that the Poly (Patton and Reeder's) film was formed and deposited on the surface of CPE [34]. The oxidation mechanism of the Patton and Reeder's reagent was depicted in the Scheme 1 and Figure 1.



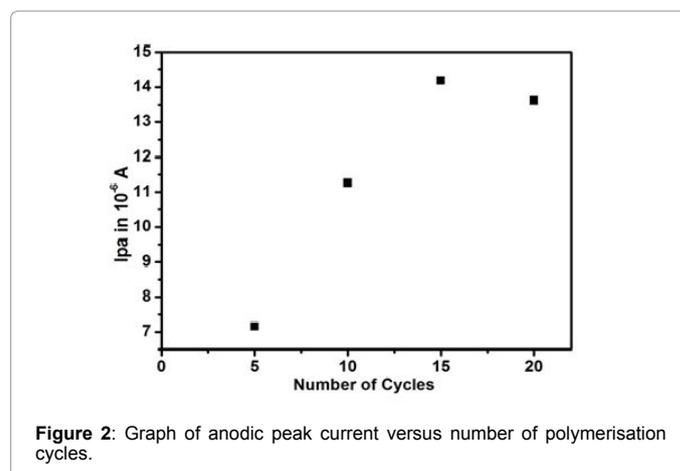
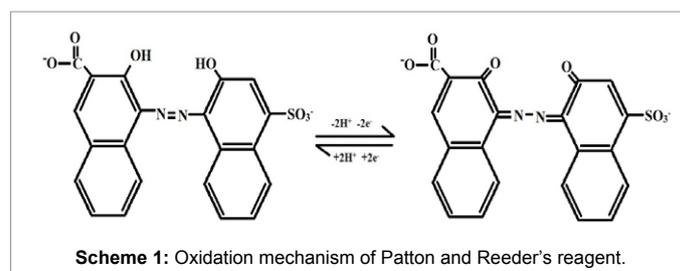
Effect of multiple cycles on the preparation of poly (Patton and Reeder's) MCPE

The extent to which the modifier has adhered on the surface of the CPE in the form a thin film was also has a significant contribution in the electrocatalytic property of the electrode. The coating was controlled by varying the multiple cycles on the CPE (from 5 to 20 multiple cycles). And the corresponding electrocatalytic activity towards the oxidation of 1 mM FA in 0.2 M PBS of pH 7.4 was investigated. From the Figure 2 it was observed that the maximum enhancement of anodic peak current was occurred at 15 multiple cycles. Therefore, at this multiple cycle the Patton and Reeder's reagent can form a thin layer on the surface of CPE and facilitates the comfortable oxidation of FA.

With the polymerisation cycles increasing the electrocatalytic response of FA increased at first. But when the polymerizing cycles is more than 15, the peak currents begin to decrease. This was due to the increase in thickness of the film that would prevent the electron transfer process [28]. Therefore, 15 cycles was used for all electrochemical analysis (Figure 2).

Electrochemical oxidation FA at of poly (Patton and Reeder's) MCPE

The Figure 3 shows the cyclic voltammograms of 0.2 mM FA at BCPE and Poly (Patton and Reeder's) MCPE at scan rate of 0.05 Vs⁻¹ with supporting electrolyte 0.2 M PBS of pH 7.4. At BCPE the oxidation of FA showed poor sensitivity and the anodic peak potential was located at around 0.74 V (versus SCE). However, the voltammogram obtained for Poly (Patton and Reeder's)MCPE (solid line) in the same condition was with high current signal with slight shifting in the anodic peak potential towards the negative side comparing to BCPE. The anodic peak potential was located at 0.67 V (versus SCE). This result of maximum enhancement in current signal and minimisation of over potential confirms the electrocatalytic activity of the fabricated



Poly (Patton and Reeder's) MCPE for the detection of FA [35]. The oxidation mechanism of FA is shown in Scheme 2 and Figure 3.

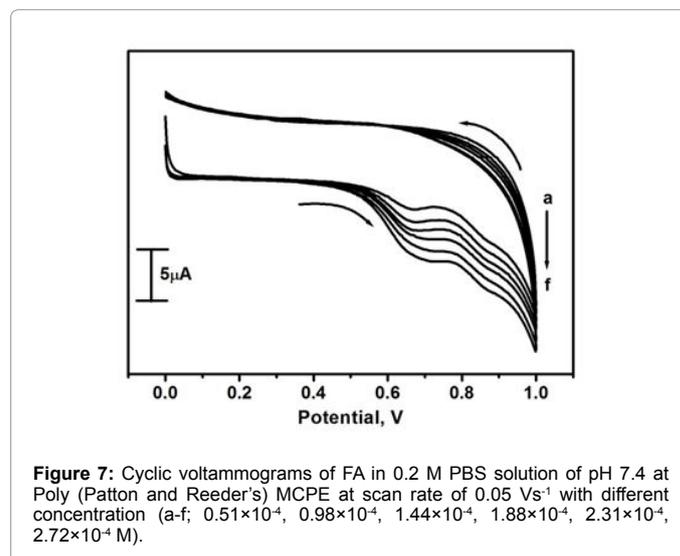
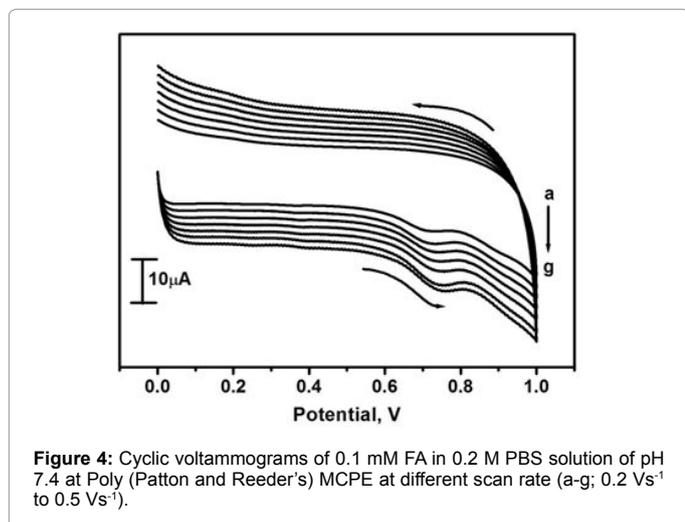
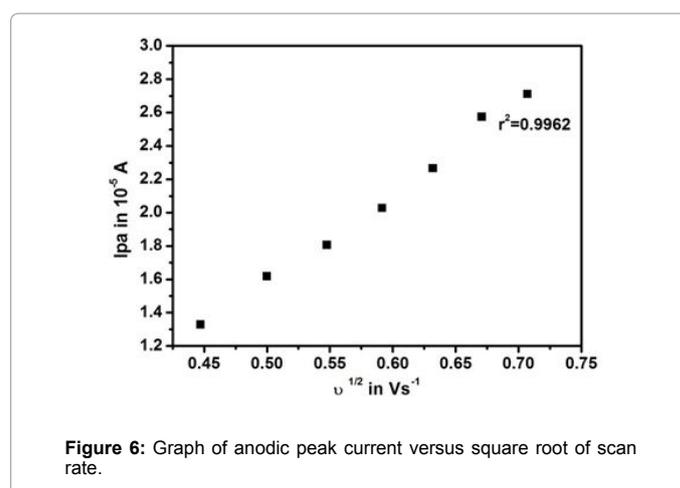
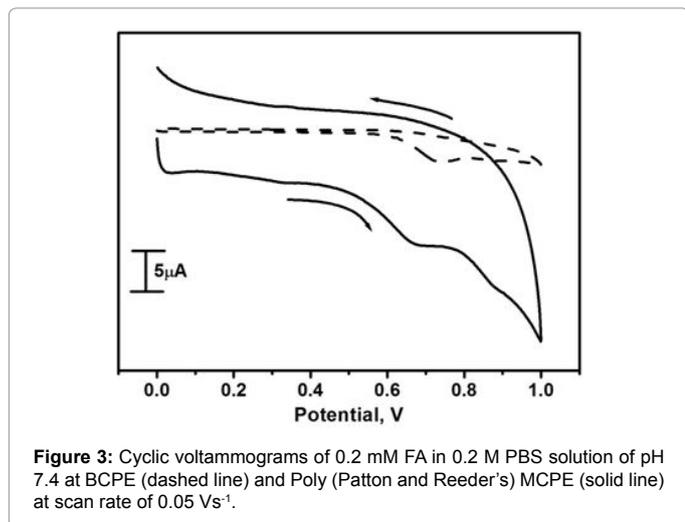
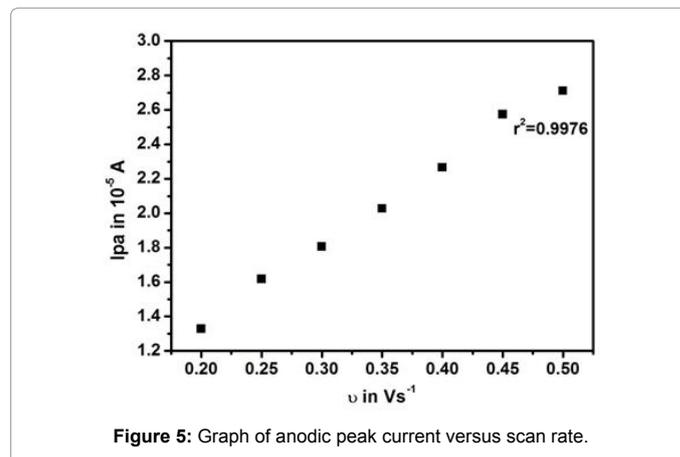
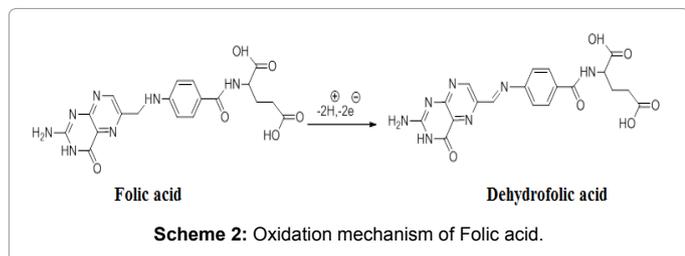
Effect of scan rate

The effect of scan rate for 0.1 mM FA in 0.2 M PBS of pH 7.4 at Poly (Patton and Reeder's) MCPE was studied by cyclic voltammetric technique. According to Randles-Sevcik equation the peak current is directly proportional to scan rate. The Figure 4 shows the increase of anodic peak currents with increase in the scan rate from 0.2 Vs⁻¹ to 0.5 Vs⁻¹. The graph of anodic peak current (I_{pa}) versus scan rate (ν) and I_{pa} versus square root of scan rate (ν^{1/2}) were plotted as shown in Figure 5 and Figure 6 respectively. The graph obtained was with a good linearity

between the I_{pa} and scan rates in the range from 0.2 Vs⁻¹ to 0.5 Vs⁻¹. The correlation coefficient was r²=0.9976, which indicates the electrode process was controlled by adsorption of the analytes [17,30,32].

Effect of concentration on the peak currents of FA

The cyclic voltammograms were recorded for the oxidation of FA



with varying concentration in 0.2 M PBS of pH 7.4 at scan rate 0.05 Vs⁻¹ at Poly (Patton and Reeder's) MCPE. The cyclic voltammograms of different concentration of FA (0.51×10⁻³ M to 3.94×10⁻³ M) was shown in the Figure 7 and which shows the increase in anodic peak current due to increase in the concentration of FA. The plot shown in the Figure 8 shows the linear relationship between I_{pa} and the concentration of FA in the provided concentration range. The limit of detection was calculated according to the previous reported literature [3,32].

$$\text{LOD} = 3 \text{ S/M} \quad (1)$$

S is the standard deviation of six blank-solution measurements. M is the slope of the calibration graph. The LOD was calculated in the lower concentration range for FA and was found to be 18.8 nM for poly (Patton and Reeder's) MCPE [5,7,36-40]. The comparison of detection limit of the FA with other modified electrodes was shown in Table 1 and it shows relatively lower limit of detection with the previously reported literature.

Simultaneous determination of FA and UA

The Figure 8 shows the cyclic voltammograms recorded for the simultaneous determination of 0.1 mM UA and 0.2 mM FA in 0.2 M PBS of pH 7.4 at scan rate of 0.05 Vs⁻¹. At BCPE (dashed line) the voltammetric response of the oxidation of these two analytes is with a low current signal and exhibited the poor sensitivity. However, in the same identical condition the voltammogram obtained for the Poly (Patton and Reeder's) MCPE is with a higher current signal with improved sensitivity. The oxidation of UA and FA was appeared at 0.32 V and 0.67 V respectively. Hence, the Poly (Patton and Reeder's) MCPE can also be used for the simultaneous determination of UA and FA (Figure 9).

Conclusion

The Poly (Patton and Reeder's) MCPE was used for the cyclic voltammetric determination of FA. The modified electrode shows

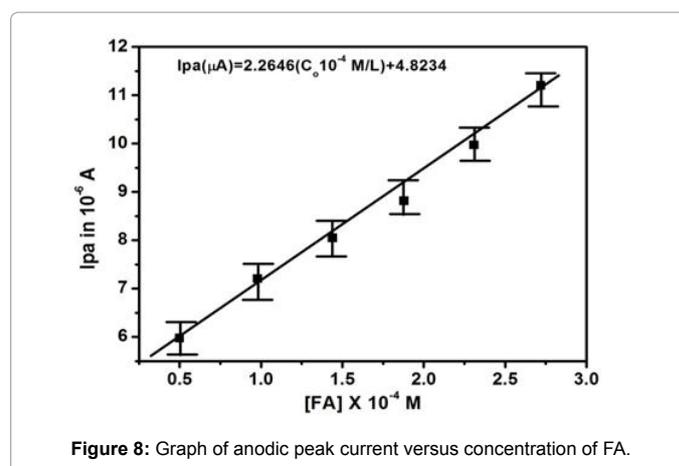


Figure 8: Graph of anodic peak current versus concentration of FA.

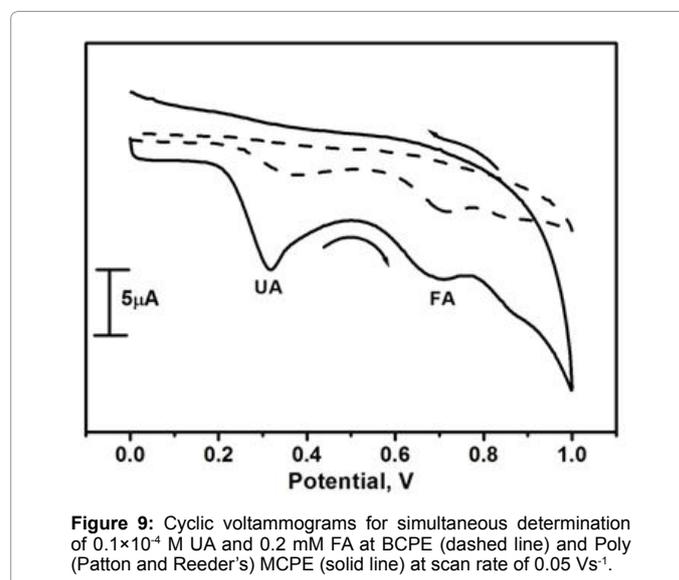


Figure 9: Cyclic voltammograms for simultaneous determination of 0.1×10⁻⁴ M UA and 0.2 mM FA at BCPE (dashed line) and Poly (Patton and Reeder's) MCPE (solid line) at scan rate of 0.05 Vs⁻¹.

Working Electrode	Limit of Detection (mol/L)	Method	References
Carbon nanotubes paste electrode modified with ferrocenedicarboxylic acid	1.1×10 ⁻⁶	DPV	[5]
Nanostructured polyaniline/tungstophosphoric acid CPE	3.0×10 ⁻⁷	DPV	[7]
Lead Film modified GCE	7.0×10 ⁻¹⁰	ASV	[36]
5-amino-2-mercapto-1,3,4-thiadiazole modified GCE	2.3×10 ⁻¹⁰	Amperometry	[37]
3-amino-5-mercapto-1,2,4-triazole modified GCE	2.5×10 ⁻⁷	DPV	[38]
2,2-[1,2-ethanediybis(nitriloethylidyne)]-bis-hydroquinonedouble-wall carbon nanotube paste electrode	1.1×10 ⁻⁷	Chronoamperometry	[39]
Multiwalled carbon nano tube/GCE	1.34×10 ⁻⁷	SVW	[40]
Poly (Patton and Reeder's) modified CPE	18.8×10 ⁻⁹	CV	This work

CPE- Carbon Paste Electrode

ASV- Adsorptive Stripping Voltammetry

CV- Cyclic Voltammetry

GCE- Glassy Carbon Electrode

PANI- Nanostructured Polyaniline

SVW- Square Wave Voltammetry

Table 1: Comparison of detection limit of FA with other modified electrodes.

improved sensitivity, selectivity, antifouling property and a good electrocatalytic activity towards the oxidation of FA when compared with BCPE. The limit of detection of FA is 18.8 nM by CV technique, which is comparatively much lower than other reported methods. The modified electrode can also be used for the simultaneous determination of UA and FA in a physiological pH of 7.4. A simple modification procedure was reported for the development of electrochemical sensor or biosensor for folic acid.

References

- He J, Li RWQ, Xing S, Song Z, Li Q, et al. (2014) A voltammetric sensor based on eosin Y film modified glassy carbon electrode for simultaneous determination of hydroquinone and catechol. Anal Methods 6: 6494-6503.
- Ardakani MM, Mohseni MAS, Alibeik MA, Benvidi A (2012) Electrochemical sensor for simultaneous determination of norepinephrine, paracetamol and folic acid by a nanostructured mesoporous material. Sens Actuators B chemical 171: 380-386.
- Ganesh PS, Swamy BEK (2015) Simultaneous electroanalysis of hydroquinone and catechol at poly (brilliant blue) modified carbon paste electrode: A voltammetric study. J Electroanal Chem 756: 193-200.
- Beitollahi H, Mohadesi A, Mohammadib S, Pahlavan A, Maleh HK, et al. (2012) New voltammetric strategy for determination of dopamine in the presence of high concentrations of acetaminophen, folic acid and N-acetylcysteine. J Mol Liquids 169: 130-135.

5. Ensafi AA, Maleh HK (2010) Modified multiwall carbon nanotubes paste electrode as a sensor for simultaneous determination of 6-thioguanine and folic acid using ferrocenedicarboxylic acid as a mediator. *J Electroanal Chem* 640: 75-83.
6. Hoegger D, Morier P, Vollet C, Heini D, Reymond F, et al. (2007) Disposable micro fluidic ELISA for the rapid determination folic acid content in food products. *Anal Bioanal Chem* 387: 267-275.
7. Kim YI (1999) Folate and carcinogenesis: Evidence, mechanisms, and implications. *J Nutr Biochem* 10: 66-88.
8. Ardakani MM, Mohseni MAS, Alibeik MA (2013) Fabrication of an electrochemical sensor based on nanostructured polyaniline doped with tungstophosphoric acid for simultaneous determination of low concentrations of norepinephrine, acetaminophen and folic acid. *J Mol Liquids* 178: 63-69.
9. Mulinare J, Cordero JF, Erickson JD, Berry RJ (1988) Periconceptual Use of Multivitamins and the Occurrence of Neural Tube Defects. *J Am Med Assoc* 260: 3141-3145.
10. Milunsky A, Jick H, Jick SS, Bruell CL, MacLaughlin DS, et al. (1989) Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *J Am Med Assoc* 262: 2847-2852.
11. Holcomb IJ, Fusari SA (1981) Liquid chromatographic determination of folic acid in multivitamin mineral preparations. *Anal Chem* 53: 607-609.
12. Gregory JF, Day BPF, Ristow KA (1982) Comparison of High performance Liquid Chromatographic, Radiometric, and Lactobacilluscasei methods for the determination of folacin in selected foods. *J Food Sci* 47: 1568-1571.
13. Breithaupt DE (2001) Determination of folic acid by ion-pair RP-HPLC invitamin-fortified fruit juices after solid-phase extraction. *Food Chem* 74: 521-525.
14. Alwarthan AA (1994) Flow injection Chemiluminometric determination of Folic acid in pharmaceutical formulations. *Anal Sci* 10: 919-922.
15. Jung M, Kim B, Boo DW, So HY (2007) Development of isotope dilution-Liquid Chromatography/Tandem Mass Spectrometry as a candidate reference method for the determination of Folic acid in infant milk formula. *Bull Korean Chem Soc* 28: 745-750.
16. Buhl F, Hachula U (1991) Spectrophotometric determination of folic acid and other reductants using a coupled redox complexation reaction with cerium (IV) and arsenazo (III). *Chem Anal* 36: 27-34.
17. Ashoka NB, Swamy BEK, Jayadevappa H (2015) Synthesis, Characterization of Calcium Ferrite Nanoparticles and their Modified Carbon Paste Electrode for the Electrochemical Investigation of Dopamine in Presence of Uric Acid and Folic Acid. *Anal Bioanal Electrochem* 7: 197-209.
18. Majidi MR, Dastangoo H, Hasannejad M, Malakouti J (2011) Voltammetric determination of folic acid with an overoxidized polypyrrole film modified sol-gel carbon ceramic electrode. *International Journal of Polymer Anal Charact* 16: 486-495.
19. Premkumar J, Khoo SB (2005) Electrocatalytic Oxidations of Biological Molecules (Ascorbic Acid and Uric acids) at Highly Oxidized Electrodes. *J Electroanal Chem* 576: 105-112.
20. Harper HA (1977) Review of Physiological Chemistry. (16th edn), Lange Medical Publications, San Francisco, CA 6: 98-802.
21. Fox IH (1981) Metabolic basis for disorders of purin nucleotide degradation. *Metabolism* 30: 616-634.
22. Raj CR, Kitamura F, Ohsaka T (2002) Square wave voltammetric sensing of uric acid using the self-assembly of mercaptobenzimidazole. *Analyst* 127: 1155-1158.
23. Hong HC, Huang HJ (2003) Flow injection analysis of uric acid with an uricase and horseradish peroxidase-coupled sepharose column based luminol chemiluminescence system. *Anal Chim Acta* 499: 41-46.
24. Nakashima K, Hayashida N, Kawaguchi S, Akiyama S, Tsukamoto Y, et al. (1991) Flow-injection analysis with chemiluminescence detection of glucose and uric acid using immobilised enzyme reactor. *Anal Sci* 7: 715-718.
25. Li Z, Feng M, Lu J (1998) KMnO₄-OctylphenylPolyglycol, Ether Chemiluminescence System for Flow Injection Analysis of Uric Acid in Urine. *Microchem J* 59: 278-283.
26. Inoue K, Namiki T, Iwasaki Y, Yoshimura Y, Nakazawa H (2003) Determination of uric acid in human saliva by high-performance liquid chromatography with amperometric electrochemical detection. *J Chromatogr B* 785: 57-63.
27. Guan Y, Chu Q, Ye J (2004) Determination of uric acid in human saliva by capillary electrophoresis with electrochemical detection: potential application in fast diagnosis of gout. *Anal Bioanal Chem* 380: 913-917.
28. Ganesh PS, Swamy BEK (2015) Simultaneous electro analysis of norepinephrine, ascorbic acid and uric acid using poly (glutamic acid) modified carbon paste electrode. *J Electroanal Chem* 752: 17-24.
29. Mahanthesha KR, Swamy BEK (2013) Pretreated/Carbon paste electrode based voltammetric sensors for the detection of dopamine in presence of ascorbic acid and uric acid. *J Electroanal Chem* 703: 1-8.
30. Ganesh PS, Swamy BEK (2014) Electrochemical Determination of Dopamine in Presence of Ascorbic Acid at Brilliant Blue Modified Carbon Paste Electrode: A Voltammetric Study. *Journal of Chemical Engineering and Research* 2: 113-120.
31. Reddy S, Swamy BEK, Jayadevappa H (2012) CuO nanoparticle sensor for the electrochemical determination of dopamine. *Electrochem Acta* 61: 78-86.
32. Ganesh PS, Swamy BEK (2014) Voltammetric Resolution of Dopamine in Presence of Ascorbic Acid and Uric Acid at Poly (Brilliant Blue) Modified Carbon Paste Electrode. *J Anal Bioanal Tech* 5: 229.
33. Shankar SS, Swamy BEK, Chandrashekar BN, Gururaj KJ (2012) Poly (xylenol orange) film modified carbon paste electrode as an electrochemical sensor for the determination of dopamine in the presence of ascorbic acid and uric acid: A voltammetric study. *Chemical Sensors* 2: 1-7.
34. Pandurangachar M, Swamy BEK, Chandra U, Gilbert O, Shergara BS (2009) Simultaneous Determination of Dopamine, Ascorbic Acid and Uric Acid at Poly (Patton and Reeder's) Modified Carbon Paste Electrode. *Int J Electrochem Sci* 4: 672-683.
35. Maiyalagan T, Sundaramurthy J, Kumar PS, Kannan P, Opallo M et al. (2013) Nanostructured α -Fe₂O₃ platform for the electrochemical sensing of folic acid. *Analyst* 138: 1779-1786.
36. Korolczuk M, Tyszczyk K (2007) Determination of Folic Acid by Adsorptive Stripping Voltammetry at a Lead Film Electrode. *Electroanalysis* 19: 1959-1962.
37. Kalimuthu P, John SA (2009) Selective electrochemical sensor for folic acid at physiological pH using ultrathin electro polymerized film of functionalized thiazole modified glassy carbon electrode. *Biosensors and Bioelectronics* 24: 3575-3580.
38. Revin SB, John SA (2012) Simultaneous determination of vitamins B₂, B₉ and C using a heterocyclic conducting polymer modified electrode. *Electrochimica Acta* 75: 35-41.
39. Beitollahi H, Ardakania MM, Ganjipour B, Naeimic H (2008) Novel 2,2-[1,2-ethanediybis(nitriloethylidene)]-bis-hydroquinone double-wall carbon nanotube paste electrode for simultaneous determination of epinephrine, uric acid and folic acid. *Biosensors and Bioelectronics* 24: 362-368.
40. Jiang X, Li R, Li J, He X (2009) Electrochemical Behavior and Analytical Determination of Folic Acid on Carbon Nanotube Modified Electrode. *Russian Journal of Electrochemistry* 45: 772-777.