Electrochemical Resolution of Ascorbic Acid from Dopamine and Uric Acid with Montmorillonite k10-Clay/Poly (Glycine) Electrode

Prabhakar Rao V.[1*], Jeevan Jyoti P.[2], Bhanuprakash P.[2] and Miditana Sankara Rao[3]
1. Department of Chemistry, Dr. YSR Government Degree College, Vedurukuppam-517569, Andhra Pradesh, INDIA
2. Department of Chemistry, PVKN Government College(A), Chittoor-517002, Andhra Pradesh, INDIA
3. Department of Chemistry, Government Degree College, Puttur-517583, Andhra Pradesh, INDIA
*vipparlaprabhakararao@gmail.com

Abstract

Ascorbic acid is a physiologically important biomolecule, acting as an antioxidant and controlling conditions like scurvy, cancer and AIDS. Hence, it is crucial to estimate its levels in body fluids to detect any disorders. In this study, a cost-effective cyclic voltametric method was used to determine ascorbic acid in the presence of uric acid and dopamine. A new modified carbon paste electrode, namely Montmorillonite K10-clay/Poly, was fabricated for this purpose. The obtained modified electrode exhibits excellent electrocatalytic activity for the selective detection of ascorbic acid, even in the presence of uric acid and dopamine. Its sensitivity for ascorbic acid is doubled compared to the bare carbon paste electrode.

The detection limit and quantification limit of this electrode for ascorbic acid are found to be 1.13×10^{-6} M and 3.77×10^{-5} M respectively. Additionally, the modified electrode recovers 96.5% of ascorbic acid in real sample analysis, such as the analysis of a vitamin C tablet (Celin-500mg). The selectivity, reproducibility and sensitivity of this modified electrode provide a promising approach for applying the technique in routine analysis of selected electroactive biomolecules in clinical and bioanalytical laboratories.

Keywords: Carbon paste electrode; Dopamine, Glycine, Montmorillonite K10- clay, Vitamin C, Voltammetry.

Introduction

Ascorbic acid (AA), dopamine (DA) and uric acid (UA) are physiologically important biomolecules that coexist in the extracellular fluids of the mammalian brain. AA, also known as vitamin C, is a water-soluble compound with antioxidant and pH-regulatory properties, which led to its inclusion in various food products and pharmaceuticals[13].

Its deficiency can result in scurvy, but it has also been found useful in managing conditions such as the common cold, mental illness, cancer, infertility and AIDS[23]. DA is a critical catecholamine neurotransmitter with applications in treating drug addiction, Parkinson's disease and Alzheimer's disease[28]. UA (2, 6, 8-trihydroxy purine) is a vital nitrogenous compound produced during purine metabolism and its abnormal levels in the blood can lead to various clinical disorders[16,24,32].

Elevated serum UA levels, known as hyperuricemia, have been linked to conditions like gout, kidney problems, Lesch-Nyhan syndrome and cardiovascular diseases[4,5]. Due to the significance of these biomolecules, it is crucial to resolve and quantify each of them individually to identify the disorders. However, resolving these compounds using cyclic voltammetry (CV) at bare carbon paste electrodes (BCPE) in real biological samples is challenging because all these compounds have similar reduction potentials, leading to overlapping voltametric peaks[26]. Additionally, BCPEs often suffer from fouling of the surface by the oxidation products of the biomolecules, resulting in poor reproducibility and sensitivity. Therefore, there is an urgent need to develop novel modified electrodes to improve the resolution of these biomolecules.

Chromatography techniques such as high-performance liquid chromatography with ultraviolet[6] or mass spectrometry detection[7,14], as well as capillary electrophoresis[22], are also useful for the simultaneous analysis of AA, DA and UA. However, these methods often require expensive equipment, well-equipped laboratories and trained personnel. Consequently, electrochemical sensors based on cyclic voltammetry have gained the attention of electrochemists as an alternative approach for the resolution of these biomolecules.

Modification of carbon paste electrodes can be achieved through three methods. The first method, called bulk modification, involves adding a modifier and a binding agent such as silicon oil to the carbon paste which is then ground using an agate mortar[3,11] to create a homogeneous mixture. The second and third methods are electro polymerization[19,29] and immobilization[12,15] respectively.

In the present study, the carbon paste electrode was first bulk modified with Montmorillonite K10-clay and then the surface was further modified with glycine through electropolymerization. The resulting Montmorillonite K10-clay/Poly(glycine) modified carbon paste electrode (M-clay/Pgly/CPE) was used to determine ascorbic acid in the presence of DA and UA.

Montmorillonite k10-clay as a modifier: The bulk modifier used in the present study is Montmorillonite K10-

clay[30] whose structure is shown in figure 1. This mineral belongs to the very soft phyllosilicate group and exists in the form of microscopic crystals known as clay. It is a 2:1 clay, meaning it has two tetrahedral sheets of silica sandwiching a central octahedral sheet of alumina. The particles of montmorillonite K10-clay are plate-shaped, with an average diameter of 1 µm and a thickness of $9.6x10^{-9}$ m. Chemically, it is a hydrated sodium, calcium, aluminum and magnesium silicate hydroxide [$(Na.\ Ca)_{0.33}.\ (Al.\ Mg)_2.\ (Si_4O_{10}).\ (OH)_2.\ nH_2O$]. Potassium, iron and other cations are also common substituents. This clay mineral has a high capacity to absorb water and to swell. It is extensively used as a catalyst in petroleum cracking and its powder is utilized as a flocculant in ponds. Montmorillonite K10-clay also exhibits remarkable adsorption capacity and ion exchange properties which contribute to its use as a modifier in cyclic voltammetry.

Glycine as a modifier: Glycine, the smallest of the 20 amino acids commonly found in proteins, is another modifier (surface modifier) used in the present work, whose structure is shown in the figure 2. Its molecular formula is $H_2N\text{-}CH_2\text{-}COOH$ and it is a colorless, sweet-tasting crystalline solid that is soluble in water. Glycine is capable of forming polymers through peptide bonds when electropolymerized, as shown in scheme 1.

Material and Methods

Chemicals and Reagents: The analytical grade chemicals used in this work including ascorbic acid, dopamine, uric acid, glycine, sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) and silicon oil used in the work were procured from Himedia chemicals. Fine graphite powder (particle size < 20 µm) and Montmorillonite K10-clay were supplied by Sigma-Aldrich Chemicals.

All chemicals were used without further purification. Stock solution of UA was prepared by dissolving it in the minimum amount of 0.1 M NaOH. DA was prepared by dissolving it in minimum amount of 0.1 M perchloric acid. AA and glycine were prepared by dissolving them in double distilled water. 0.1 M phosphate buffer solutions (PBS) of pH 7 and other pH were prepared by mixing the standard stock solutions in double distilled water of 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 in an appropriate quantity. All the experiments were conducted at room temperature.

Instrumentation: Cyclic and differential pulse voltammetric experiments were conducted using a CH-instrument CHI610D electrochemical workstation, connected to a personal computer for electrochemical measurements and data analysis. A conventional three-electrode system was employed for the investigation, comprising of a standard calomel electrode as the reference electrode, a bare and M-clay/Pgly/CPE as the working electrodes and a platinum wire as the counter electrode. Additionally, a digital pH meter MKVI made by Systronics was utilized for pH measurements of various solutions.

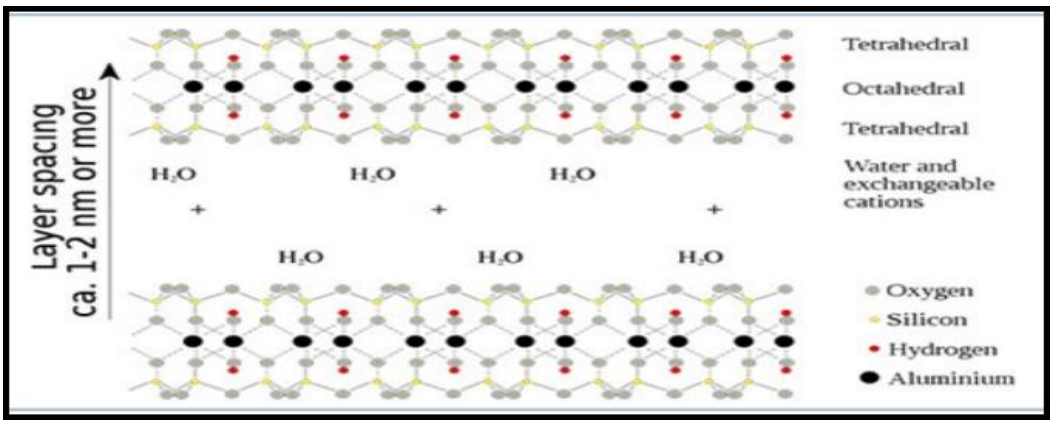

Fig. 1: Structure of Montmorillonite K10-clay

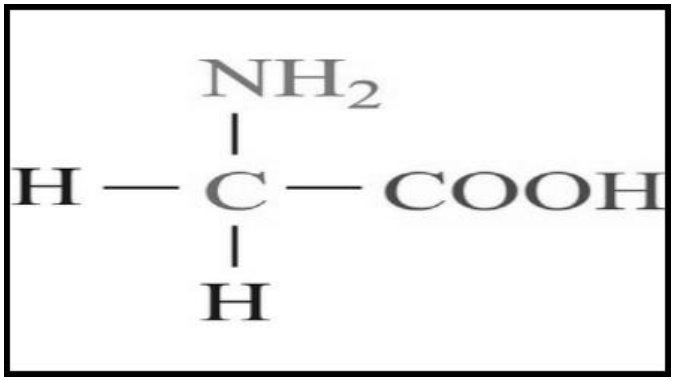

Fig. 2: Structure of glycine

Preparation of bare carbon paste electrode (BCPE): Graphite powder and silicon oil were hand-mixed in a 70:30 (W/W) ratio using an agate mortar to get a homogenous paste. This paste was tightly filled into a 3 mm diameter cavity in a teflon rod. Electrical contact to the paste was provided with a copper wire, resulting in the formation of BCPE. The surface of the BCPE was then smoothed by polishing on a weighing paper.

Preparation of montmorillonite K10-clay/ poly(glycine) modified carbon paste electrode: First, a bulk modified carbon paste electrode was prepared by thoroughly mixing 0.24g of graphite powder, 0.04 ml of silicon oil and 20 mg of montmorillonite K10-clay in an agate mortar for about 40 minutes. It was reported Jyothi et al[11] that 20 mg of montmorillonite K10-clay provided maximum anodic peak current and sensitivity for analytes. Next, this bulk modified carbon paste electrode was immersed in a 1 mM glycine solution in 0.1 M PBS at pH 7 and electropolymerized by running 15 cycles in the potential window of -0.5 V to 1.1 V at a scan rate of 50 mV/S using cyclic voltammetry to obtain the modified electrode M-clay/Pgly/CPE. The reason for electropolymerizing the CPE for 15 cycles was that this duration was found to give the best sensitivity and maximum anodic current output for the detection of ascorbic acid. The CV of glycine polymerization for 15 cycles is shown in figure 3(a).

Results and Discussion

Investigation of optimum number of cycles of polymerization for glycine on CPE: The Montmorillonite K10 clay bulk-modified CPE, prepared as discussed earlier, was immersed in a 1 mM glycine solution in 0.1 M PBS of pH 7 to investigate the optimum number of polymerization cycles within the potential range of -0.5 to 1.1 V. The CPE was polymerized for 5, 10, 15, 20, 25 and 30 cycles and the anodic peak currents for the detection of AA were noted. It was found that 15 cycles of polymerization provided the best sensitivity and maximum current output. The result is shown in figure 3 (b).

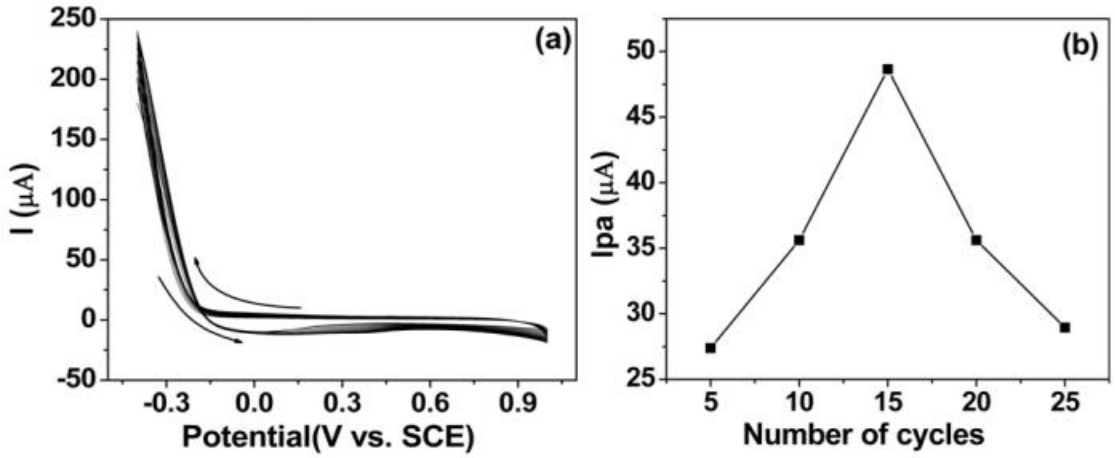

Fig. 3: a) Cyclic voltammogram for the electropolymerisation of glycine on bare carbon paste electrode for 15 cycles at the scan rate of 50 mV/S.
b) Dependence of anodic peak current on the number of cycles of polymerisation for ascorbic acid detection.

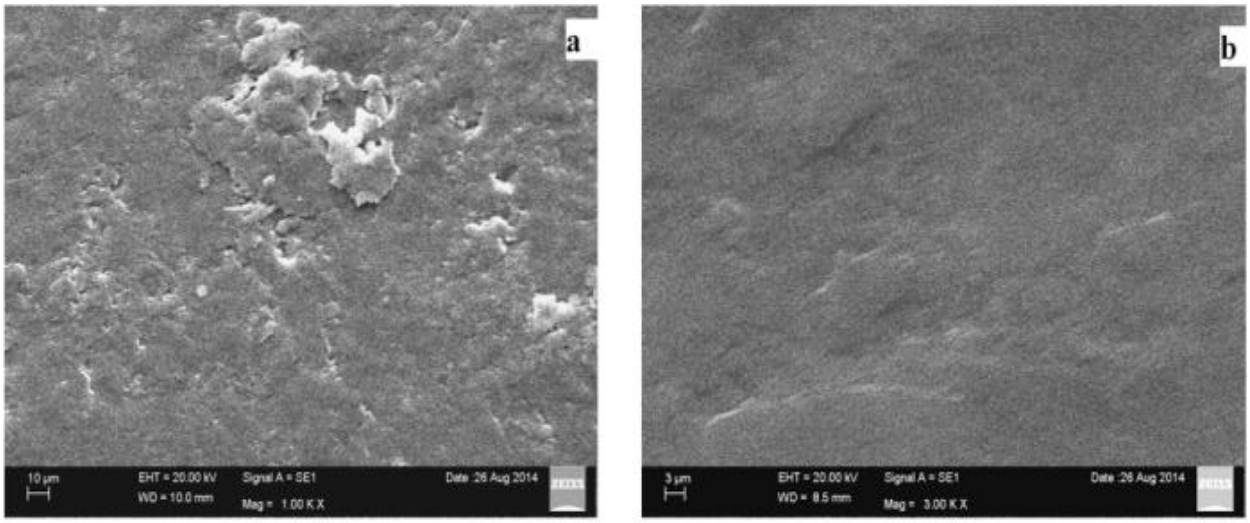

Fig. 4: Scanning Electron Microscopic images of (a) bare carbon paste electrode and (b) poly (glycine) modified carbon paste electrode.

It is inferred from figure 3(b) that the anodic peak current initially increased with up to 15 cycles of polymerization, reaching a maximum at 15 cycles and then decreased thereafter. The increase in current up to 15 cycles may be attributed to the gradual coverage of the CPE surface with the polymer film, leading to a gradual increase in the number of active sites. At 15 cycles, the entire surface is covered with the polymer film, corresponding to the maximum number of active sites and, consequently, the maximum current response. However, after 15 cycles, the current decreased as there was no further increase in the number of active sites and the thickening of the polymer film on the CPE surface might have decreased the permeability of the electrons[25].

In order to confirm the formation of a poly (glycine) layer on the CPE, a cyclic voltametric sweep was carried out in 0.1 M PBS of pH 7 across the range of -0.5 to 1.1 V at a scan rate of 50 mV/S. A broad cyclic voltammogram was obtained, indicating the formation of a polymer film on the CPE. The polymer film formation was also confirmed through Scanning electron microscopy. SEM images showed that the surface of the bare CPE was uneven as in figure 4(a). While the modified CPE surface was even confirming the presence of the polymer film as in figure 4(b).

The mechanism of glycine polymerization on the CPE is as follows: First, the N-H bond of the glycine molecule undergoes homolytic cleavage in the presence of the graphite paste, resulting in the formation of a nitrogen-based free radical. This radical then binds to the graphite carbon, which has an unpaired electron. This bound glycine serves as a template for the formation of the glycine polymer. The glycine molecules then add to one another through peptide bonds, leading to the formation of a polymer that covers the entire surface of the CPE. The mechanism of polymerization of glycine on the CPE is shown in figure 5.

Electrocatalytic oxidation of AA at M-clay/Pgly/CPE: The electrocatalytic activity of AA at BCPE, M-clay/CPE and M-clay/ Pgly/ CPE was investigated in the potential range of -0.1 to 0.75 V at 50 mV/S scan rate in 0.1 M PBS of pH 7. The result is shown in figure 6(a). The dotted line curve represents the CV of AA at BCPE, dashed line curve at M- clay/CPE and solid line curve at M-clay/Pgly/CPE. It is evident from the figure that Ipa at M-clay/Pgly/CPE is 2 folds and at M-clay/CPE it is 1.5 folds to that at BCPE. This result indicates that M-clay/Pgly/CPE has the best sensitivity. This may be attributed to
(i) more number of active sites at M-clay/Pgly/CPE compared to M-clay/CPE and BCPE. In M-clay/Pgly/CPE, the active sites are due to both montmorillonite K10-clay and polymer layer of glycine.
(ii) the 2:1 layered lattice structure of montmorillonite K10-clay has the property to incorporate the cations by ion exchange process[8], thus facilitating better current sensitivity.
(iii) interaction of AA with both montmorillonite K10-clay particles and poly (glycine) layer through hydrogen bonds as shown in figure 7. The hydrogens on two – OH groups of the five membered oxygen hetero cyclic ring of AA form hydrogen bonds with the amide oxygen atoms in the glycine polymer. This weakens the –O-H bond in AA and hence it is easily oxidized to C=O.

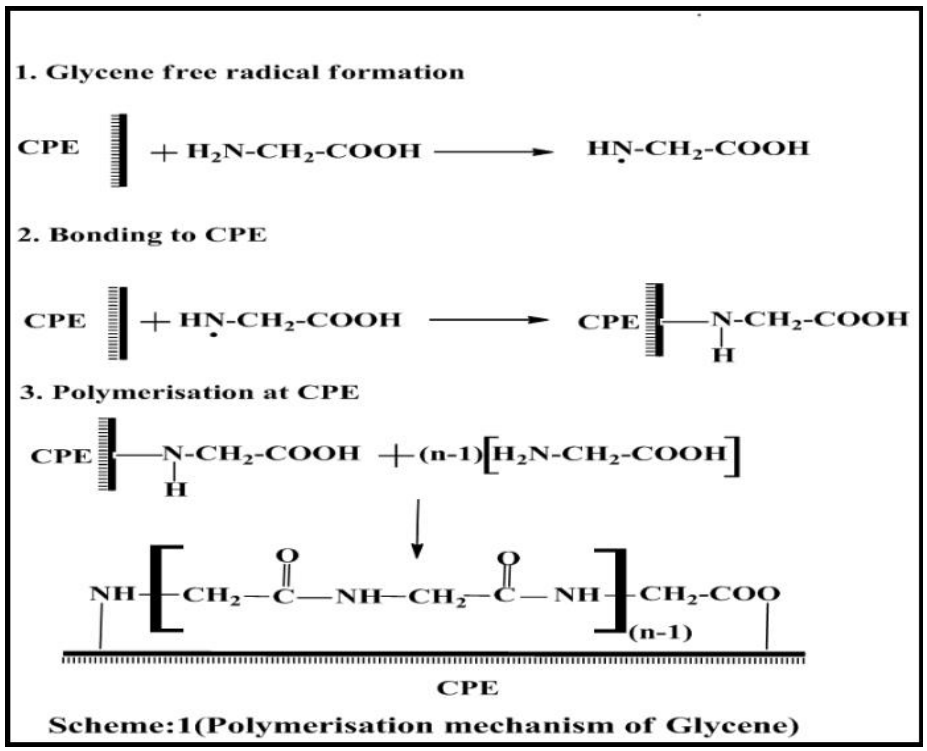

Fig. 5: Polymerisation mechanism of glycine

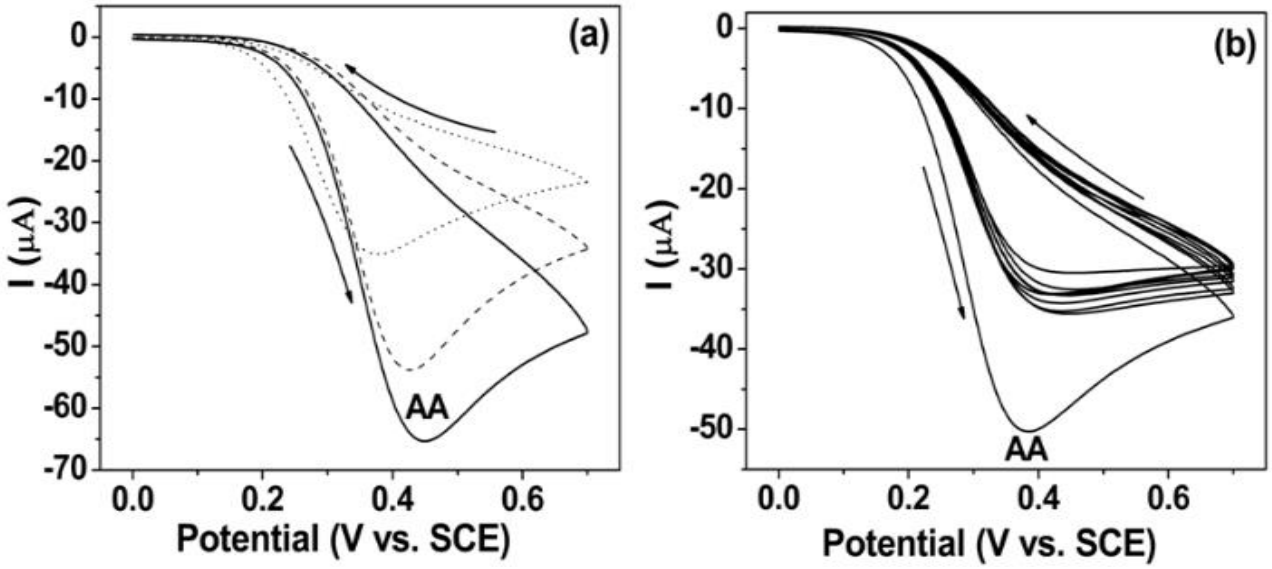

Fig. 6: a) Cyclic voltammogram of 1 mM ascorbic acid in 0.1 M PBS of pH 7 at BCPE (dotted line curve), M-clay/CPE (dashed line curve) and M-clay/Pgly/CPE (solid line curve) at 50 mV/S scan rate
b) Cyclic voltammograms of 1mM ascorbic acid for 15 multiple cycles on M-clay/Pgly /CPE at 50 mV/S scan rate to test the stability of the electrode.

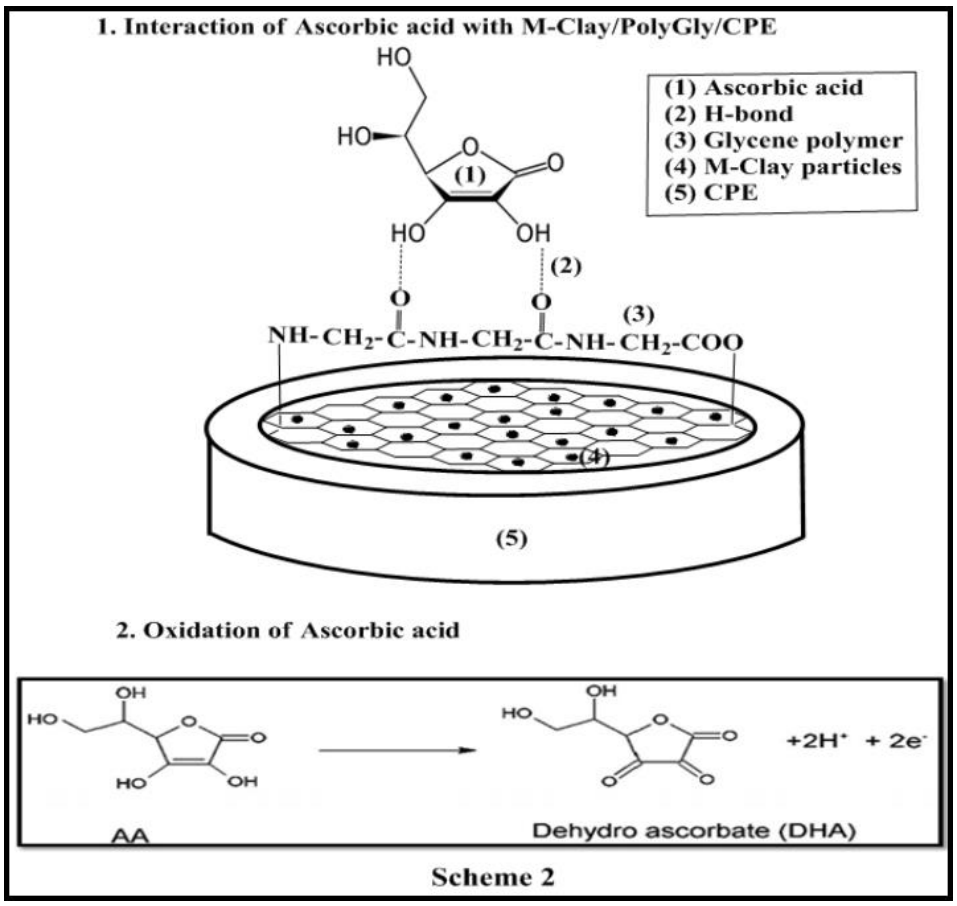

Fig. 7: Interaction of ascorbic acid with M-Clay/Pgly/CPE

Investigation of stability and reproducibility of M-clay/Pgly/CPE: Carbon paste electrodes can be prepared easily and rapidly using cheap materials. So, it is not important that whether the modified electrode prepared is stable for prolonged period or not. However, its stability and reproducibility were investigated for the determination of AA. It was investigated by running 15 cycles on 1 mM AA solution in 0.1 M PBS of pH 7. The cyclic voltametric stability curve is shown in figure 6(b). Almost all CV curves are overlapping (Ipa between 25 to 30 µA at 0.42V Epa). This confirms that the polymer layer formed on the CPE is fairly stable[1] and thus giving reproducible results.

Effect of scan rate: Studying the scan rate effect can provide insights into the electrode process. Various CVs were recorded by varying the scan rate from 50 mV/s to 400 mV/s on a 1 mM ascorbic acid solution at the M-clay/Pgly/CPE modified electrode, as shown in figure 8 (a). From the figure, it can be observed that the peak anodic current increased with the increase in the scan rate. The plots of Ipa Vs. ν (Ipa =$2.545x10^{-5}$ +$2.963x10^{-4}$.ν) and Ipa Vs. √ν (Ipa=-$2.449x10^{-5}$+$2.559x10^{-4}$.√ν) are shown in figure 8(b) [inset of figure 8(a)] and figure 8(c) respectively. These two plots are linear with correlation coefficients (R) of 0.9923 and 0.9831 respectively. These R-values confirm that the electrochemical oxidation of AA at the modified electrode is governed by both adsorption and diffusion-controlled processes.

Effect of pH of the solution: Investigations were conducted to determine the optimal pH for achieving the best sensitivity in the analysis of AA at the modified electrode. The pH of the 0.1M PBS was varied from 5.5 to 8.0 and the effects on Ipa and Epa were observed as shown in figure 9(a). As the pH increased, the Epa values gradually decreased, indicating the involvement of protons in the electrochemical oxidation process. The Ipa values initially increased from pH 5.5 to 7.0 but then decreased thereafter. The corresponding differential pulse voltametric curves are presented in figure 9(b). The slope of the Epa vs. pH plot was found to be 50 mV/pH which reveals that an equal number of electrons and protons participate in the oxidation process[10, 27].

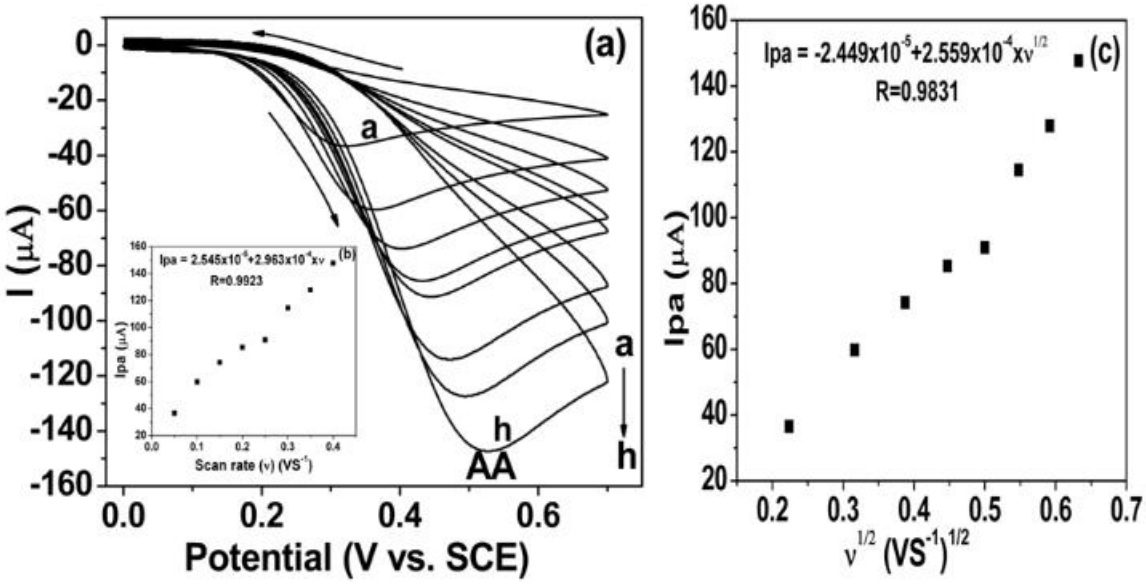

Fig. 8: a) Cyclic voltammograms of 1mM ascorbic acid on M-clay/Pgly/CPE at different scan rates (a-h: 50,100,150,200,250,300,350 and 400 mV/s) in 0.1 M PBS of pH 7
b) The plot of anodic peak current versus the scan rate (v) [inset of figure 8(a)].
c) The plot of anodic peak current versus the square root of scan rate (√v).

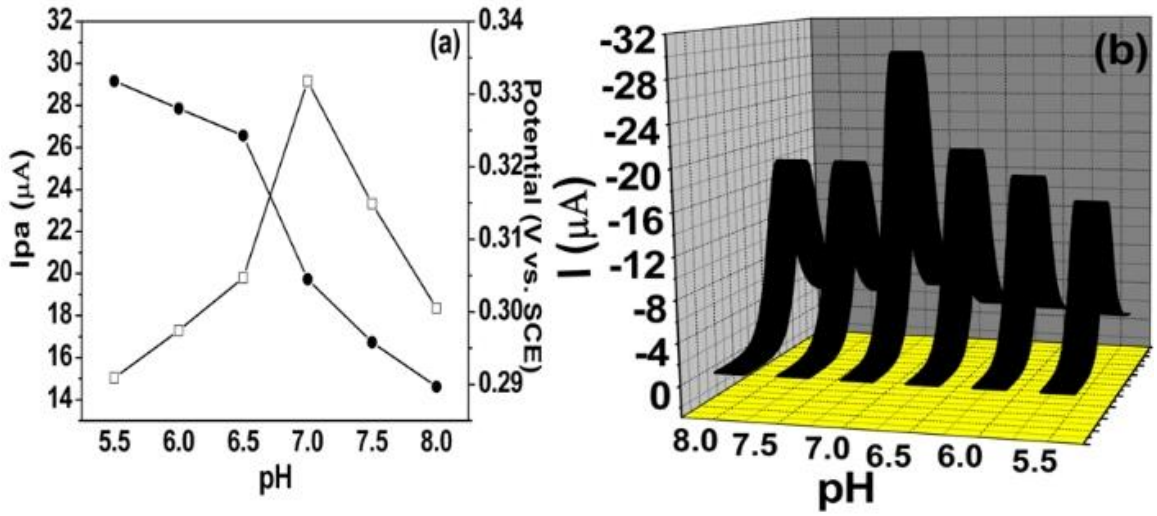

Fig. 9: (a) The plot of anodic peak current, peak potential versus pH for 1 mM ascorbic acid on M-clay/Pgly/CPE at different pH (5.5, 6, 6.5, 7, 7.5 and 8) at the scan rate of 50 mV/S (b) DPVs of ascorbic acid at different pH.

Simultaneous determination of AA, DA and UA at M-clay/Pgly/CPE: The electrochemical behavior of the mixture of AA (4 mM), DA (0.2 mM) and UA (0.4 mM) was investigated using CV in the potential window of -0.2 V to 0.7 V at 50 mV/S scan rate at BCPE, M-clay/CPE and M-clay/Pgly/CPEs. The resulting CVs are shown in figure 10(a). It is evident from the figure that at BCPE (dotted line curve), the components of the mixture are not properly resolved whereas at M-clay/CPE (dashed line curve) and M-clay/Pgly/CPE (solid line curve), all the three components were clearly resolved. The fouling of the BCPE surface by the oxidation products of AA and DA may be the reason for unresolved peak at BCPE[18,27]. The modified electrodes gave the best sensitivity and selectivity.

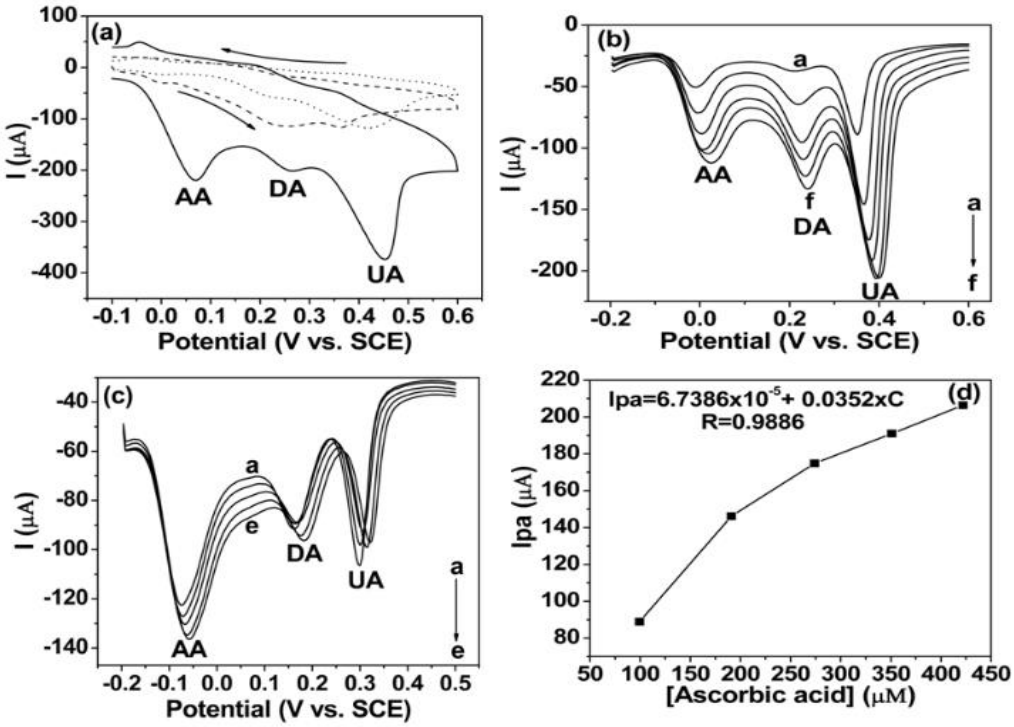

Fig. 10: a) Cyclic voltammogram for simultaneous determination of 0.4 mM UA, 0.2 mM DA and 4 mM AA mixture at BCPE (dotted line curve), M-clay/CPE (dashed line curve) and M-clay/Pgly/CPE (solid line curve) in 0.1 M PBS of pH 7 at the scan rate of 50 mV/S
b) DPVs of UA, DA and AA mixture by varying the concentrations of all the components (a-f): UA (0.1 to 0.6 M), DA (0.05 to 0.3 mM) and AA (1 to 6 mM)
c) DPVs of UA, DA and AA mixture by varying the concentration of AA (a-e: 1mM to 5 mM) keeping DA and UA concentrations fixed at 0.1 mM and 0.2 mM respectively
d) Anodic peak current versus AA concentration in the simultaneous determination of UA, DA and AA.

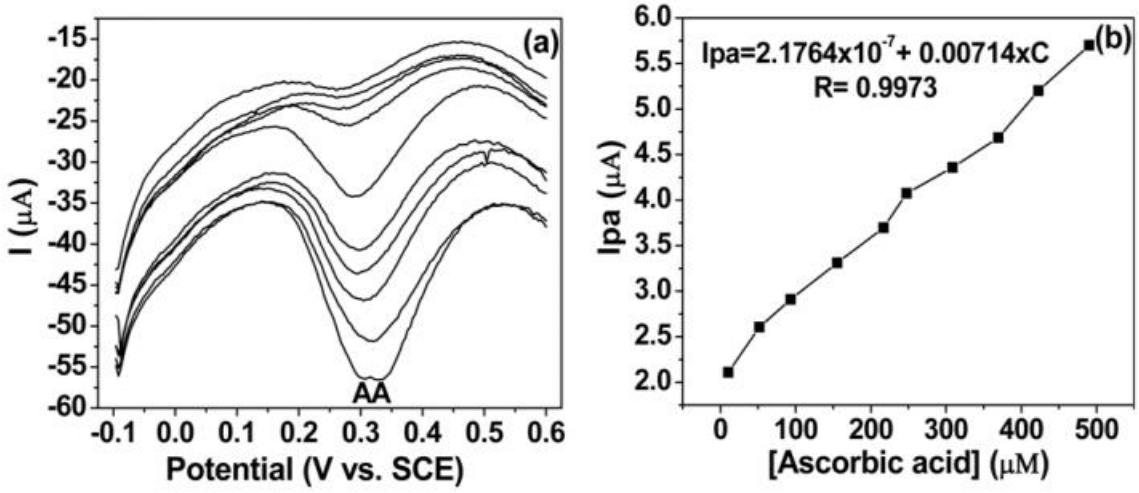

Fig. 11: (a) DPVs for various concentrations of AA (10 µM to 490 µM) at M- clay/Pgly/CPE in 0.1 M PBS of pH 7 at the scan rate of 50 mV/S
(b) The plot of anodic peak current versus concentration of AA.

AA, DA and UA mixture was also used to find the sensitivity and selectivity of M-clay/Pgly/CPE by varying the concentrations of all the components. The resulting DPV is shown in figure 10(b). It was recorded in the potential interval of -0.2 V to 0.6 V at the scan rate of 50 mV/S. The oxidation peaks of the three analytes were well separated (AA-DA by 210 mV and DA-UA by 190 mV) and Ipa of each of them increased linearly with increase in the concentration of each component.

DPV for the mixture of AA, DA and UA was recorded by varying only the concentration of AA keeping the concentrations of DA and UA fixed. This is shown in figure 10(c). From this DPV, it can be concluded that varying the concentration of one of the components keeping the concentrations of remaining components fixed did not influence the resolution capacity of the modified electrode. The plot of Ipa Vs. concentration of AA for this mixture is shown in figure 10(d). It is linear (Ipa = 6.7386×10^{-5} + 0.0352xC) with R value of 0.9886.

Effect of AA concentration on the modified electrode: The DPV was used to estimate the linear range, detection limit (LOD) and quantification limit (LOQ) of the modified electrode for the determination of AA. The concentration of AA varied from 10 µM to 490 µM and the DPV was recorded as shown in the figure 11(a). As the concentration increased, Ipa also increased, with the linear regression equation of Ipa = 2.1764×10^{-7} + 0.00714xC and R value of 0.9973. This is shown in figure 11(b). From the figure 11(b) and by using the following equations[2,9,21,28], LOD and LOQ were calculated:

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

$$LOQ = 10S/M \quad (2)$$

where S is the standard deviation and M is the slope of the working curve. The determined LOD and LOQ were 1.13×10^{-6} M and 3.77×10^{-5} M respectively. These values are reasonably good for the modified electrode to be used in the field of medicine and analytical biochemistry for the estimation of biomolecules. Comparison of LOD of the electrode used in the present work with other electrodes quoted in the literature is shown in the table 1[17,20,31,33,34].

Real sample analysis – analysis of AA in the vitamin C tablet: To confirm the reliability and practical applicability of the fabricated modified electrode, an analysis of a vitamin C tablet was conducted. Vitamin C tablet was dissolved in 100 ml of Milli-Q water. This solution was then mixed with a supporting electrolyte of 0.1 M PBS at pH 7 in appropriate quantities to obtain solutions with concentrations of 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM and 0.5 mM. The average anodic peak currents of the five readings for each concentration were measured using DPV. The actual concentrations were estimated using the calibration curve shown in Figure 9(b), as presented in table 2. Based on these results, an average recovery of 96.5% was obtained with an RSD of 3.76%, indicating that the electrode is efficient and reliable for real-life applications.

Conclusion

M-clay/pgly/CPE was prepared through bulk modification using the Montmorillonite K10-clay modifier and surface modification by electro polymerization with glycine. This electrode demonstrated better sensitivity and selectivity compared to M-clay/CPE and BCPE.

Table 1
Comparison of detection limits

S.N.	Electrode	Detection limit (µM)
1.	Bi_2O_3/Li/MWCNT/GCE[33]	50.0
2.	OMC-Nafion/GCE[34]	20
3.	SWCNT/ZnO/CPE[17]	85.0
4.	WO_3/SWCNT/GCE[20]	80.0
5.	Plmox-GO/GCE[31]	18
6.	M-clay/Pgly/CPE*	11.3

***** Present work

Table 2
Real sample analysis of vitamin C tablet

Sample	Prepared AA concentration	Found AA concentration	Recovery %	RSD %
Vitamin C tablet	0.1	0.09	90	5.2
	0.2	0.18	90	5.2
	0.3	0.31	103	2.1
	0.4	0.39	97.5	4.4
	0.5	0.51	102	1.9
Average			**96.5**	**3.76**

This led to the conclusion that increased suitable modifications can improve the sensitivity and selectivity of the electrode. Optimizing the number of polymerization cycles and pH was crucial for the best results. The modified electrode could effectively resolve the three analytes in the mixture under various concentration variations. Its detection and quantification limits were quite good. Considering the ease of preparation and modification, low cost and enhanced properties, this electrode could be utilized in clinical and bioanalytical laboratories for determining various electroactive biomolecules.

References

1. Ates M., Castillo J., Sarac A.S. and Schuhmann W., Carbon fiber microelectrodes electrocoated with polycarbazole and poly (carbazole-co-p-tolylsulfonyl pyrrole) films for the detection of dopamine in presence of ascorbic acid, *Mikrochim. Acta*, **160**, 247-251 **(2007)**

2. Cai X., Kalcher K., Neuhold C. and Ogorevc B., An improved voltammetric method for the determination of trace amounts of uric acid with electrochemically pretreated carbon paste electrodes, *Talanta*, **41**, 407-413 **(1994)**

3. Chandra U., Swamy B.E.K., Gilbert O., Pandurangachar M. and Sherigara B.S., Voltammetric resolution of dopamine in presence of ascorbic acid at polyvinyl alcohol modified carbon paste electrode, *Int. J. Electrochem. Sci*., **4**, 1479-1488 **(2009)**

4. Chandrashekar B.N. and Swamy B.E.K., Simultaneous cyclic voltammetric determination of norepinephrine, ascorbic acid and uric acid using TX-100 modified carbon paste electrode, *Anal. Methods*, **4**, 849-854 **(2012)**

5. Chen J.C., Chung H.H., Hsu C.T., Tsai D.M., Kumar A.S. and Zen J.M., A disposable single-use electrochemical sensor for the detection of uric acid in human whole blood, *Sens. Actuators B Chem*., **110**, 364-369 **(2005)**

6. Cooper N., Khosravan R., Erdmann C., Fiene J. and Lee J.W., Quantification of uric acid, xanthine and hypoxanthine in human serum by HPLC for pharmacodynamic studies, *J. Chromatogr. B*, **837**, 1-10 **(2006)**

7. Dai X., Fang X., Zhang C., Xu R. and Xu B., 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**, 287-295 **(2007)**

8. Ghosh P.K., Bard A.J. Clay-modified electrodes, *J. Am. Chem. Soc*., **105**, 5691-5693 **(1983)**

9. Habibi B. and Pournaghi-Azar M.H., Simultaneous determination of ascorbic acid, dopamine and uric acid by use of a MWCNT modified carbon-ceramic electrode and differential pulse voltammetry, *Electrochim. Acta*, **55**, 5492-5498 **(2010)**

10. Huang P.F., Wang L., Bai J.Y., Wang H.J., Zhao Y.Q. and Di Fan S., Simultaneous electrochemical detection of dopamine and ascorbic acid at a poly (p-toluene sulfonic acid) modified electrode, *Mikrochim. Acta*, **157**, 41-47 **(2006)**

11. Jyothi P.J., Reddy K.G., Rao V.P., Lavanya M. and Madhavi G., Resolution of Dopamine in the Presence of Ascorbic Acid and Uric Acid by the Poly Oxalic Acid Modified Carbon Paste Electrode, *Chem. Sci. Trans*., **4(2)**, 541-549 **(2015)**

12. Kachoosangi R.T. and Compton R.G., A simple electroanalytical methodology for the simultaneous determination of dopamine, serotonin and ascorbic acid using an unmodified edge plane pyrolytic graphite electrode, *Anal. Bioanal. Chem*., **387**, 2793-2800 **(2007)**

13. Kemppainen N., Marjamäki P., Röyttä M. and Rinne J.O., Different pattern of reduction of striatal dopamine reuptake sites in Alzheimer's disease and ageing, *J. Neural Transm*., **108**, 827-836 **(2001)**

14. Kim K.M., Henderson G.N., Ouyang X., Frye R.F., Sautin Y.Y., Feig D.I. and Johnson R.J., A sensitive and specific liquid chromatography–tandem mass spectrometry method for the determination of intracellular and extracellular uric acid, *J. Chromatogr. B*, **877**, 2032-2038 **(2009)**

15. Kleszczewski T. and Kleszczewska E., Flow injection spectrophotometric determination of l-ascorbic acid in biological matters, *J. Pharm. Biomed. Anal.*, **29**, 755-759 **(2002)**

16. Lavanya M., Reddy Y.V.M., Rao V.P. and Madhavi G., Electrochemical investigation of dopamine, ascorbic acid and uric acid at poly (Spands Reagent) modified carbon paste electrode, *Chem. Sci. Trans*., **3**, 1404-1420 **(2014)**

17. Jeevanjyothi P., Gangadhara Reddy K., Prabhakar Rao V. and Madhavi G., Resolution of Dopamine in the Presence of Ascorbic Acid and Uric Acid by the Poly Oxalic Acid Modified Carbon Paste Electrode, *Chem. Sci. Trans.*, **3**, 341-349 **(2016)**

18. Madhavi G., Damodar J., Mohan S.K. and Reddy S.J., Electrochemical behavior of dopamine and uric acid at poly (naphthol green B) modified carbon paste electrode, *Bull. Electrochem*., **15**, 535-541 **(1999)**

19. Naik R.R., Swamy B.E.K., Chandra U., Niranjana E., Sherigara B.S. and Jayadevappa H., Separation of ascorbic acid, dopamine and uric acid by acetone/water modified carbon paste electrode: a cyclic voltammetric study, *Int. J. Electrochem. Sci*., **4**, 855-862 **(2009)**

20. Ngai K.S., Tan W.T., Zainal Z., Zawawi R.B.M. and Zidan M., Electrochemical oxidation of ascorbic acid mediated by single-walled carbon nanotube/tungsten oxide nanoparticles modified glassy carbon electrode, *Int. J. Electrochem. Sci*., **7**, 4210-4222 **(2012)**

21. Ngai K.S., Tan W.T., Zainal Z., Zawawi R.M. and Zidan M., Voltammetry detection of ascorbic acid at glassy carbon electrode modified by single-walled carbon nanotube/zinc oxide, *Int. J. Electrochem. Sci*., **8**, 10557-10567 **(2013)**

22. Pormsila W., Krähenbühl S. and Hauser P.C., Capillary electrophoresis with contactless conductivity detection for uric acid determination in biological fluids, *Anal. Chim. Acta*, **636**, 224-228 **(2009)**

23. Pournaghi-Azar M.H. and Ojani R., Catalytic oxidation of ascorbic acid by some ferrocene derivative mediators at the glassy

carbon electrode: application to the voltammetric resolution of ascorbic acid and dopamine in the same sample, *Talanta*, **42**, 1839-1848 **(1995)**

24. Rao V.P., Reddy Y.V.M., Jyothi P.J., Kiranmai S. and Madhavi G., Electrochemical detection of dopamine in presence of ascorbic acid and uric acid at poly (Spands Reagent) modified carbon paste electrode, *Chem. Sci. Trans*., **5**, 94-99 **(2016)**

25. Rao V.P., Reddy Y.V.M., Lavanya M., Venu M. and Madhavi G., Zirconia/poly (oxalic acid) modified carbon paste electrode for electrochemical investigation of uric acid in presence of dopamine and ascorbic acid, *Asian J. Chem*., **28**, 1828-1834 **(2016)**

26. Reddy K.G., Madhavi G., Swamy B.E.K., Reddy S., Reddy A.V.B. and Madhavi V., Electrochemical investigations of lipase enzyme activity inhibition by methyl parathion pesticide: voltammetric studies, *J. Mol. Liq.*, **180**, 26-30 **(2013)**

27. Reddy S., Swamy B.E.K., Chandra U., Sherigara B.S. and Jayadevappa H., Synthesis of CdO nanoparticles and their modified carbon paste electrode for determination of dopamine and ascorbic acid by using cyclic voltammetry technique, *Int. J. Electrochem. Sci*., **5**, P. 10-17 **(2010)**

28. Reddy Y.V.M., Rao V.P., Reddy A.V.B., Lavanya M., Venu M., Lavanya M. and Madhavi G., Determination of dopamine in presence of ascorbic acid and uric acid using poly (Spands Reagent) modified carbon paste electrode, *Mater. Sci. Eng. C Biomim. Mater. Sens. Syst.*, **57**, 378-386 **(2015)**

29. Shankar S.S., Swamy B.E.K., Chandra U., Manjunatha J.G. and Sherigara B.S., Simultaneous determination of dopamine, uric acid and ascorbic acid with CTAB modified carbon paste electrode, *Int. J. Electrochem. Sci*., **4**, 592-601 **(2009)**

30. Terzi F. and Dossi N., Ti as an electrode material: how to make the future better than the present, *Anal. Bioanal. Chem.*, **407**, 7257-7261 **(2015)**

31. Thangaraj R. and Kumar A.S., Simultaneous detection of guanine and adenine in DNA and meat samples using graphitized mesoporous carbon modified electrode, *J. Solid State Electrochem.*, **17**, 583-590 **(2012)**

32. Volkov A., Tourillon G., Lacaze P.C. and Dubois J.E., Electrochemical polymerization of aromatic amines, *J. Electroanal. Chem. Interfac. Electrochem.*, **115**, 279-291 **(1980)**

33. Zidan M., Tan W., Zainal Z., Abdullah A.H. and Goh J.K., Electrocatalytic oxidation of ascorbic acid mediated by lithium doped microparticles Bi2O3/MWCNT modified glassy carbon electrode, *Int. J. Electrochem. Sci.*, **5**, 501-508 **(2010)**

34. Zheng D., Ye J., Zhou L., Zhang Y. and Yu C., Simultaneous determination of dopamine, ascorbic acid and uric acid on ordered mesoporous carbon/Nafion composite film, *J. Electroanal. Chem*., **625**, 82-87 **(2009)**.

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