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Iron nanoparticles decorated multi-wall carbon nanotubes modified carbon paste electrode as an electrochemical sensor for the simultaneous determination of uric acid in the presence of ascorbic acid, dopamine and L-tyrosine

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A B S T R A C T
Iron nanoparticles decorated multi-wall carbon nanotubes modified carbon paste electrode (Fe-MWCNTs/MCPE) was prepared by bulk-modification method. The electrochemical impedance spectroscopy (EIS) suggests least charge transfer resistance at the modified electrode. The electrochemical behavior of UA was studied in 0.1 M phosphate buffer solution (PBS) of pH 3.0 using cyclic voltammetry (CV) while differential pulse voltammetry (DPV) was used for quantification. The spectreoelectrochemical study of oxidation of UA at Fe-MWCNTs/MCPE showed a decrease in the absorbance of two peaks with time, which are ascribed to n to n* and n to π* transitions. Under optimum condition, the DPV response offered two linear dynamic ranges for UA in the concentration range 7.0 × 10⁻⁶ M–1.0 × 10⁻⁶ M and 2.0 × 10⁻⁶ M–1.0 × 10⁻⁵ M with detection limit (4.80 ± 0.35) × 10⁻⁸ M (S/N = 3). The practical analytical application of this sensor was successfully evaluated by determination of spiked UA in clinical samples, such as human blood serum and urine with good percentage recovery. The proposed electrochemical sensor offers a simple, reliable, rapid, reproducible and cost effective analysis of a quaternary mixture of biomolecules containing AA, DA, UA and Tyr which was free from mutual interferences.

1. Introduction
Uric acid (UA) is the end product of the metabolism of purine, which is the nitrogen-containing component that occurs in nucleic acids. In the human body, UA is present in blood serum and in urine [1]. For a healthy human being, the normal concentration of UA in blood serum is in the range of 0.24–0.52 mM while that in urine is 1.49–4.46 mM [2]. Abnormalities in UA levels in the urine and in blood serum leads to various diseases such as gout, arthritis [3], cardiovascular diseases [4], neurological diseases [5], hypertension and renal insufficiency [6,7]. Moreover, studies have shown that a high level of uric acid has been found to directly inhibit insulin signaling and induce insulin resistance [8]. A recent study has revealed that excessive uric acid levels have a correlation to hyperthymic and irritable temperaments [9]. Determination of UA in body fluids, urine and blood may thus be used as powerful markers for early diagnosis of such diseases [10]. Therefore, there is a need for the development of a better sensor for the determination of UA in biological samples.

Though there are several diverse methods for the determination of UA such as chemiluminescence [11], ion exchange column chromatography [12], enzymatic [13], high performance liquid chromatography [14], spectrophotometry [15] and spectrofluorometry [16], these techniques have certain disadvantages such as their being often time consuming, expensive and complex. Electrochemical methods for UA determination have certain advantages such as simple, short detection time, reproducibility and ease of handling [17–21]. Moreover, when a reduction in cost is desired, especially for self monitoring, electrochemical techniques are useful and may be adopted in the construction of portable electrochemical devices.

Electrochemical methods constitute very useful techniques in the field of biological analysis, especially with regard to the determination of molecules such as norepinephrine, acetaminophen, n-acetylcysteine,
folic acid, epinephrine, uric acid, levodopa, carbidopa and isoproterenol while also being useful in environmental analysis — for example with regard to the determination of hydroxylamine in water samples [22–23].

L-Tyrosine (Tyr) is an important amino acid which is considered nonessential because the human body can make it from another amino acid called phenylalanine. In the human body, Tyr is used to make catecholamines. Tyr plays a crucial role in biological systems as it is a precursor of hormones as well as of neurotransmitters such as thyroxin and DA respectively, in addition to other physiologically essential biomolecules [31]. Tyr is often added to food products and to pharmaceutical formulations [32]. Tyr is critical to mental and physical health as it is needed to produce catecholamines through an iron-containing enzyme called tyrosine hydroxylase.

DA is a vital neurotransmitter which belongs to a group of catecholamines and abnormalities in the metabolism of DA may cause brain diseases such as schizophrenia and Parkinsonism [33]. AA is an essential vitamin which has been used for the treatment of common cold, mental illness, cancer and AIDS [34]. AA is present in the mammalian brain along with DA. It also participates in several biological reactions [35]. Therefore, the simultaneous determination of these molecules is definitely desirable since they play an important role in fields such as analytical chemistry, neuro-chemistry, biomedicine, and diagnostic research.

The major difficulty in the electrochemical detection of UA at an unmodified electrode is the presence of interferences from co-existing molecules such as AA and DA which have oxidation potentials similar to that of UA [36]. This problem is overcome by the modification of the electrode using materials such as polymers [37–39], carbon based materials [40–42] and noble metal nanoparticle [43,44].

Carbon nanotubes are nanomaterials with properties such as extraordinary tensile strength, excellent electrical conductivity and high chemical stability [45]. These properties help carbon nanotubes find potential applications in the field of chemical and biological sensors [46–49]. Carbon nanotubes are basically categorized into two types: multi-wall carbon nanotubes (MWCNTs) [50] and single wall carbon nanotubes (SWCNTs) [51]. The unique electronic properties of carbon nanotubes show better electron transfer rates when used as electrode material and offer excellent prospects for development of miniaturized electronic devices. Metal nanoparticles possessing broad range of dimensions are expected to be endowed with size-dependent optical, magnetic, electronic and chemical properties appropriate for catalysts, optoelectronic devices, as well as for chemical and biosensor applications [52–54]. Iron nanoparticle/clusters have important properties — primarily, mechanical and magnetic properties — which have several applications such as in the domains of data storage, catalysis, magnetic fluids, biomedical applications, magnetic recording and media. They are also endowed with a high ratio of surface-atoms, which results in high catalytic activity [55–58].

CPE has been used as a working electrode for many biosensor applications because of its simple method of preparation, easy renewability of the surface, compatibility with various types of modifiers, cost effectiveness and, more importantly, biocompatibility [59–61]. In our earlier studies, we have demonstrated how different electrode materials influence the electrochemical reaction rate, as well as the selectivity of electroactive species, by developing sensors using different modifiers in CPE matrix [62–69]. To the best of our knowledge, iron nanoparticles decorated MWCNTs have so far not been exploited for their potential applications as sensors. Therefore, in this paper, our focus was directed towards the preparation of iron nanoparticles decorated MWCNTs modified CPE so as to utilize the beneficial characteristics of the modifier for the sensitive, simultaneous determination of UA in the presence of interfering molecules such as AA, DA and coexisting biologically important molecule Tyr which is the precursor of DA. The spectroelectrochemical study of UA using MCPE is not reported in the extant literature. Hence Fe–MWCNTs/MCPE is employed to study the electrochemical reaction mechanism of UA at the solution electrode interface. Ultimately, driven by the need to make it relevant as well as reliable for practical applications, the sensor was applied in the sensitive determination of UA in real samples such as blood serum and urine without subjecting it to any preliminary treatment.

2. Experimental

2.1. Reagents

UA, Tyr (SRL), DA (Sigma), AA, HClO₄, H₃PO₄, KH₂PO₄ and NaOH pellets (all of analytical grade) were purchased from Merck and were used as such. All chemicals were purchased from Labsupplies (India) Pvt. Ltd., Bangalore. All aqueous solutions were prepared using ultra pure water (> 18.2 MΩ·cm) from Milli-Q Plus system (Millipore India Pvt. Ltd., Bangalore). Stock solutions of UA, AA, DA and Tyr (25 mM) were prepared using 0.1 M NaOH, ultra pure water, 0.1 M perchloric acid and 0.1 M NaOH respectively. 0.1 M Phosphate buffer solution of
pH 3.0 was prepared from 0.1 M KH₂PO₄ and 0.1 M NaOH. The pH of solutions was adjusted using H₃PO₄ or NaOH solution. Graphite powder was purchased from Graphite India Ltd. The Iron nanoparticle decorated MWCNTs were obtained from Nanocyl S.A. (Belgium).

2.2. Apparatus

All electrochemical experiments were carried out using Chemilink model EA-201 Electro Analyzer (Chemilink, Navi Mumbai, Maharashtra, India). All electrochemical experiments were carried out using a conventional three electrode system comprising a bare or modified CPE as a working electrode, a platinum wire as auxiliary or counter electrode and saturated calomel electrode (SCE) as a reference electrode. The tip of the Luggin capillary was set approximately to the maximum closest distance from the surface of the working electrode in order to minimize error due to IR drop in the electrolyte. The electrochemical experiments were performed in quiescent solution and voltammograms were recorded at room temperature (~300 K). The surface morphology of the electrodes was studied using field emission scanning electron microscopy (FE-SEM) using Quanta 200, FEI, Germany; SUPRA 40 VP, Gemini, Zeiss, Germany, and elemental composition was obtained using EDX. The charge transfer resistance value of the electrode was obtained by performing electrochemical impedance spectroscopy using a CHI 660E electrochemical workstation (CH Instruments, Inc., USA). The spectroelectrochemical experiment was performed by coupling 760E potentiostat (CH Instruments, Inc., USA), Ocean Optics spectrophotometer and QE 65000 detector with dip probe assembly. The pH of the prepared phosphate buffer solution was measured during using digital pH/mV meter (ELICO LI 614, Elico Limited, Hyderabad, India). The potentiostat was calibrated by running a dummy cell (a network of capacitors/resistors that give a known result) as per the instructions in the user manual provided by ChemiLink Systems. Further calibration of the software and the performance of the instrument were performed using 6 mM potassium ferricyanide solution in 1.0 M KCl solution, which is a well known electrochemically reversible system. The cyclic voltammograms were recorded at various scan rates and the potential and current were measured so as to compare the results with stored calibrated data. All measurements were performed at room temperature (~25 °C).

2.3. Preparation of bare and the modified electrode

To begin with, the ratio of graphite powder to binder (silicone oil) was optimized; CPE was prepared by thoroughly hand mixing the graphite powder and silicone oil in the ratio 70:30 (w/w) and was homogenized in an agate mortar using a pestle to obtain a paste. This paste was packed tightly into the cave of the Teflon tube. A copper wire fitted to a graphite rod was inserted into the Teflon tube in order to establish electrical contact with the external circuit. Similarly, iron nanoparticle decorated MWCNTs modified CPE was prepared by homogeneous mixing of graphite powder, iron nanoparticle decorated MWCNTs and silicone oil in the ratio 20:1:9 (w/w), respectively. The modified electrode was designated as Fe-MWCNTs/MCPE. For every reading, the surface was renewed by removing some amount of paste and polishing with tissue paper following which fresh paste was filled and the surface was smoothened.
3. Results and discussion

3.1. Characterization of electrodes by FE-SEM and EDX

Comparison of morphological characteristics of the surfaces of CPE and Fe-MWCNTs/MCPE was done by FE-SEM. Fig. 1(a–b) illustrates the FE-SEM images of CPE and Fe-MWCNTs/MCPE, respectively. In Fig. 1(a) flakes of graphite are seen on the surface of CPE which appears smooth. It is obvious from Fig. 1(b) that iron nanoparticle decorated MWCNTs are homogeneously mixed with CPE matrix and the iron nanoparticle decorated multi-wall carbon nanotube is exposed to the surface. Fig. S1(a) and (b) (supplementary information) show the EDX analysis of CPE and Fe-MWCNTs/MCPE, respectively. The EDX analysis of electrodes shows the presence of C, Si and O. Fig. S1(b) reveals the presence of Fe in Fe-MWCNTs/MCPE.

3.2. Characterization of the electrodes by electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) is an efficient tool to study the properties of electrode/solution interface. Fig. S2 (supplementary information) illustrates the Nyquist plot of CPE and Fe-MWCNTs/MCPE. The EIS data of the electrodes were obtained in ac frequency range varying from 0.1 Hz to 100 kHz at the oxidation peak potential (E_p) of UA in 0.1 M phosphate buffer solution of pH 3.0. The charge transfer resistance (R_{ct}) value was obtained from the equivalent electrical circuit, well matched with impedance spectra (inset of Fig. S2). R_s represents uncompensated resistance due to the electrolyte, R_{ct} is charge transfer resistance, C_{dl} is double layer capacitance, W is Warburg impedance which represents the diffusion process. The term R_0 is the resistance due to double layer capacitance. The R_{ct} values of 5.0 × 10^{-4} M UA at CPE and Fe-MWCNTs/MCPE were 4.918 × 10^5 Ω and 7.809 × 10^4 Ω, respectively. The lower charge transfer resistance at Fe-MWCNTs/MCPE implies that it facilitates electron transfer at Fe-MWCNTs/MCPE as compared to bare CPE.

3.3. Electrochemical behavior of UA

The electrochemical behavior of UA was studied at different electrodes using the CV technique. Fig. 2 shows the cyclic voltammograms of 5.0 × 10^{-4} M UA in 0.1 M phosphate buffer solution of pH 3.0 at CPE and Fe-MWCNTs/MCPE. UA exhibits electrochemically irreversible behavior at both the electrodes. The anodic peak potentials (E_{pa}) of UA were 577.3 ± 2.5 mV and 576.6 ± 2.2 mV at CPE and Fe-MWCNTs/MCPE, respectively. The corresponding anodic peak current (I_{pa}) of UA were ~ 41.8 ± 4.6 μA and ~ 51.7 ± 2.2 μA at CPE and Fe-MWCNTs/MCPE, respectively. It is clear from the cyclic voltammogram that the presence of iron nanoparticle decorated MWCNTs increases the current sensitivity of electrode by 1.2 times. Also, a slight negative shift in E_{pa} was observed. This enhancement in current can be attributed to good electrical conductivity, large surface area and more electroactive interaction sites of Fe-MWCNTs/MCPE, all of which combine to offer increased mass transport and easier accessibility to the active sites.

3.4. Effect of variation of Fe-MWCNTs loading in the carbon paste matrix

The amount of modifier is also one of the parameters which affect the current sensitivity. Fig. S3 (supplementary information) shows the effect of loading different weight percentages of Fe-MWCNTs on anodic peak current. Weight percentage (wt.%) of Fe-MWCNTs with respect to weight of graphite powder varies from 1.64 to 9.09%. It is evident from Fig. S3 that with increase in wt.% of Fe-MWCNTs, the anodic peak current increased and attained a maximum value at 4.76 wt.%. This may be ascribed to an increase both in the surface area as well as in the number of catalytic sites with increase in wt.% of Fe-MWCNTs. Beyond 4.76 wt.%, I_{pa} was found to decrease and finally I_{pa} remained constant, which could possibly be due to the saturation reached at the surface. So an optimum of 4.76 wt.% Fe-MWCNTs was used for further experiments since it gave the maximum response.

3.5. Effect of solution pH

Since pH is one of the important factors which play a crucial role in the electrochemical reaction, the effect of variation of pH on the electrochemical parameters was studied with the help of CV technique using 5.0 × 10^{-4} M UA in 0.1 M phosphate buffer solution of pH in the range of 3.0–8.0. Fig. S4 (supplementary information) depicts the cyclic
voltammograms of $5.0 \times 10^{-4}$ M UA in 0.1 M phosphate buffer solution of various pH. Fig. 3(a) illustrates the variation of $E_{pa}$ with pH. As depicted Fig. S4, oxidation potential of UA decreased with the increase of pH, suggesting an easier oxidation at higher pH. The linear regression equation of $E_{pa}$ versus pH plot is $E_{pa}$ (mV) = 785.4 − 64.9 pH with $R^2 = 0.9962$. Eq. (1) denotes the Nernst equation, which describes the relationship between pH and $E_{pa}$, where $n$ and $m$ denote the number of electrons and protons involved in the reaction, a and b are coefficients of oxidant and reductant in the reaction [70].

$$E_{pa} = E^0 + \frac{0.0591}{n} \log \left( \frac{[OX]^a}{[R]^b} \right) - \frac{0.0591}{n} pH$$

The slope of $−64.9$ mV/pH obtained, closely matches with the Nernstian slope for the electrode reaction which involves an equal number of electrons and protons ($m = n$). As reported in the literature [71], the UA oxidation involved two electrons and hence the number of protons taking part in the reaction is also suggested to be two. Scheme 1 represents the electrochemical oxidation mechanism of UA at FeMWCNTs/MCPE.

The anodic peak current exhibits non-linear behavior with variation in pH as shown by Fig. 3(b). A dip in peak current for oxidation of UA was observed with a rise of pH between the pH range 3 and 5. A slight increase in current was observed in the range of pH 5−6 and then beyond 6 the current remained constant. The pKa of UA is approximately 5.4. Hence UA exists as neutral molecule in the pH range 1.0−4.0 [72]. At pH 5.0, some of the protons of UA will dissociate and in the pH range 6.0 to 8.0, all protons of UA get dissociated i.e. it gets deprotonated. As the mechanism of UA oxidation involves transfer of two protons, they are no more available to take part in UA oxidation. Hence $I_{pa}$ is almost constant in the pH range 6.0−8.0, and with decrease in the pH, the number of protons available for UA will increase, resulting in increase in $I_{pa}$. The observed voltammogram at pH 5.0 had recorded wider peaks with decreased current sensitivity. Maximum $I_{pa}$ was observed at pH 3.0 and hence pH 3.0 was chosen for further experiments.

3.6 Spectroelectrochemical study of $2.5 \times 10^{-4}$ M UA at Fe-MWCNTs/MCPE

The spectroelectrochemical experimental set up consisted of a cell with three electrodes, namely, platinum mesh as a support for the modified working electrode, saturated calomel electrode as a reference electrode and platinum wire as a counter electrode. These three electrodes were connected to a potentiostat while a UV/Vis probe was connected to a spectrophotometer. The probe was used to conduct the light beam from the deuterium-halogen light source to the spectroelectrochemical cell and from the spectroelectrochemical cell to the spectrometer, a CCD detector. The probe was set approximately to the maximum closest distance from the surface of the working electrode which was supported on the Pt mesh.

Absorbance of $2.5 \times 10^{-4}$ M UA in 0.1 M KPBS of pH 3.0 was measured without the application of the potential. UA showed two absorbance peaks at 235 and 288 nm. Further, oxidation of UA was carried out by chronoamperometry at a potential of 600 mV. Absorbance was recorded continuously; however, only a few of the spectra are shown in Fig. 4. The first absorption peak observed at 235 nm was assigned to the n to π* transition due to the presence of conjugated double bond in UA. The other absorption peak, which appeared at 288 nm, was assigned to the n to π* transition, which was due to the keto group present in the molecule. As depicted in Fig. 4, the absorbance corresponding to both the
transitions were found to decrease with duration of electrolysis. No new absorption peaks were observed, suggesting that the extinction coefficient of the oxidation product of UA might be much lower compared to UA. Since the absorbance decreased only due to the electrochemical oxidation of UA, the Cottrell treatment was applied to correlate between the decrease in the absorbance and the duration of the process. The enhanced sensitivity and resolution of UA signal at Fe-MWCNTs/MCPE, clearly indicates that the latter can be effectively used for the simultaneous trace level determination of UA in the presence of AA, DA and Tyr.

3.10. Analytical characterization

Trace level detection of UA was carried out using differential pulse technique because of its high sensitivity and better resolution as compared to CV. Prior to the analysis the parameters such as pulse interval, pulse width and pulse amplitude were optimized. The peak to peak separation of AA, DA, UA and Tyr was optimized. The peak to peak separation of AA, DA, UA and Tyr at Fe-MWCNTs/MCPE is an adsorption controlled process [73]. The anodic peak potential shifts to more positive potential with increasing scan rate.

3.7. Effect of scan rate

The influence of variation of scan rate on \( I_{pa} \) of AA and Tyr at Fe-MWCNTs/MCPE. Fig. S5 (supplementary information) illustrates the cyclic voltammograms of various concentrations of AA, DA, UA and Tyr at Fe-MWCNTs/MCPE. \( I_{pa} \) showed a linear correlation with scan rate in the range 10–125 mV s\(^{-1}\), as shown in inset of Fig. S5. The linear regression equation for this range of scan rate is \( I_{pa} (\mu A) = -19.9–0.5 \nu (mV s^{-1}) \) with \( R^2 = 0.9929 \). This implies that the electrochemical oxidation of UA at Fe-MWCNTs/MCPE is an adsorption controlled process [73]. The anodic peak potential shifts to more positive potential with increasing scan rate.

3.8. Effect of variation of concentration of UA on the electrochemical parameter

Effect of variation of concentration of UA was studied in 0.1 M phosphate buffer solution of pH 3.0 at a scan rate of 50 mV s\(^{-1}\) using CV. The concentration of UA was varied from 1 × 10\(^{-4}\) M to 1 × 10\(^{-3}\) M. Fig. S6 (supplementary information) shows the voltammograms recorded at various concentrations of UA. Fig. S6 (inset) clearly shows a linear dependency of concentration with \( I_{pa} \). A linear correlation with scan rate in the range 10–125 mV s\(^{-1}\) with \( R^2 = 0.9943 \). The good linearity suggests that quantitative estimation of AA can be carried out at Fe-MWCNTs/MCPE.

3.9. Electrochemical behavior of a mixture of AA, DA, UA, and Tyr at Fe-MWCNTs/MCPE

Since the main objective of our present work was to determine UA in the presence of interfering molecules such as AA and DA and also coexisting biologically important electroactive molecule Tyr, we studied the cyclic voltammetric response of a mixture containing 5.0 × 10\(^{-4}\) M AA, 5.0 × 10\(^{-3}\) M DA, 5.0 × 10\(^{-4}\) M UA and 5.0 × 10\(^{-4}\) M Tyr in 0.1 M phosphate buffer solution of pH 3.0 at Fe-MWCNTs/MCPE. The resulting cyclic voltammogram is illustrated in Fig. 6.

The cyclic voltammograms of quaternary mixture at Fe-MWCNTs/MCPE as depicted in Fig. 6(a) showed four well defined anodic peak potentials at 277, 420, 576 and 830 mV corresponding to the oxidation of AA, DA, UA and Tyr, respectively. The peak potential of these molecules in the quaternary mixture coincided with the peak potential of AA, DA, UA and Tyr, respectively. The peak potential of these molecules in the quaternary mixture coincided with the peak potential when they were being studied individually as shown in Fig. 6(b). The peak to peak separation of AA–DA (\( \Delta E_{AA-DA} \)), UA–DA (\( \Delta E_{UA-DA} \)), UA–AA (\( \Delta E_{UA-AA} \)) and UA–Tyr (\( \Delta E_{UA-Tyr} \)) is 143, 156, 299 and 254 mV, respectively. The enhanced sensitivity and resolution of UA signal at Fe-MWCNTs/MCPE, clearly indicates that the latter can be effectively used for the simultaneous trace level determination of UA in the presence of AA, DA and Tyr.
The calibration plot of Ipa versus concentration of UA offered two linear dynamic ranges for UA in the concentration range $7.0 \times 10^{-8}$ M–$1.0 \times 10^{-6}$ M and $2.0 \times 10^{-6}$ M–$1.0 \times 10^{-5}$ M. The linear regression equations for the calibration plots were $I_{pa} (\mu A) = -0.007 - 0.134 C_{UA} (\mu M)$ with $R^2 = 0.9967$ and $I_{pa} (\mu A) = -0.213 + 0.338 C_{UA} (\mu M)$ with $R^2 = 0.9978$ respectively. The detection limit of UA was found to be $(4.80 \pm 0.35) \times 10^{-8}$ M. Inset shows the calibration plot of $I_{pa}$ versus concentration of UA from 1.0 μM to 0.07 μM.

3.11. Interference studies

AA, DA and Tyr coexist with UA in biological samples and are electroactive. AA and DA have oxidation potentials close to UA. Therefore, it is essential to investigate the mutual interferences of these biomolecules in the sensitive and selective detection of UA at Fe-MWCNTs/MCPE. Fig. 8 depicts the differential pulse voltammograms of quaternary mixture containing UA, AA, DA and Tyr at Fe-MWCNTs/MCPE. We had carried out two sets of experiments to ensure that the quantification of UA at Fe-MWCNTs/MCPE was free from interferences. In the first set of experiments, we had varied the concentration of UA while keeping the concentration of AA (30.0 μM), DA (10.0 μM), and Tyr (50.0 μM) constant as depicted in Fig. 8(a). The linear regression equation for the variation of concentration of UA in this case was $I_{pa} (\mu A) = 0.18 - 0.34 C_{UA} (\mu M)$ with $R^2 = 0.9963$. This implies the selectivity of Fe-MWCNTs/MCPE towards UA in the presence of AA, DA and Tyr. In the second set of experiments, concentrations of all analytes were varied as shown in Fig. 8(b). The peaks were well separated and there was no indication of any mutual interference in their detection. This suggests applicability of this method for real sample analysis in the simultaneous quantification of UA in the presence of AA, DA and Tyr at Fe-MWCNTs/MCPE. In both cases, the current was directly proportional to the concentration of the analyte.

3.12. Comparison of analytical performance of present sensor with other sensors reported in the literature

The analytical performance of Fe-MWCNTs/MCPE has been compared in terms of detection limit and the linear dynamic range with other reported electrodes and the results are displayed in Table 1. Apart from superior lower detection limit at Fe-MWCNTs/MCPE, the other disadvantages of the sensors reported in Table 1 are as follows: in the case of CTAB-GO/MWNT/GCE [74], the preparation of the modified electrode was a tedious process and it required pretreatment of GCE. At dsDNA-coated CNTPE [75], a positive shift in anodic peak potential was observed for UA as compared to the bare electrode, which makes the electrochemical process at the modified electrode more energy demanding; also it demands 5 min of accumulation time for UA. There are no reports on qualitative analysis of UA at bienzymatic/GCE [76] and the deposition and drying of the enzymatic

Fig. 7. (a) DPVs of various concentrations of UA (μM): 10.0, 9.0, 8.0, 6.0, 4.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.09, 0.08, 0.07 and blank (a→n) at Fe-MWCNTs/MCPE in 0.1 M phosphate buffer of pH 3.0. Scan rate: 5 mV s$^{-1}$ and pulse amplitude 75 mV. (b) Calibration plot of $I_{pa}$ versus concentrations of UA (μM): 30.0, 26.0, 21.0, 14.0 and 10.0 μM. Inset is enlarged view of calibration plot at lower concentration of UA (μM): 1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.09, 0.08 and 0.07.

Fig. 8. (a) DPVs of solutions containing 30 μM AA, 10 μM DA, 50 μM Tyr and various concentrations of UA (μM): 10.0, 6.5, 5.5, 4.5, 3.5, 2.5, 1.0 and 0 at Fe-MWCNTs/MCPE in 0.1 M phosphate buffer of pH 3.0. Scan rate: 5 mV s$^{-1}$ and pulse amplitude 75 mV. Inset is calibration plot of $I_{pa}$ versus concentration of UA. (b) DPVs of varying concentration of AA (90.0, 75.0, 50.0, 40.0 and 30.0 μM), DA (30.0, 26.0, 21.0, 14.0 and 10.0 μM), UA (30.0, 24.0, 16.0, 13.0 and 10.0 μM) and Tyr (75.0, 65.0, 50.0, 32.0 and 25.0 μM) at Fe-MWCNTs/MCPE in 0.1 M phosphate buffer of pH 3.0. Scan rate: 5 mV s$^{-1}$ and pulse amplitude 75 mV.
Table 1
Comparison of analytical performance of present sensor with other sensor reported in literature.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>pH</th>
<th>Linear dynamic range (M)</th>
<th>Detection limit (M)</th>
<th>Technique used</th>
<th>Reference</th>
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<tr>
<td>CTAB-GO/MWNT/GCE</td>
<td>7.0</td>
<td>3.0 × 10^{-6}–6.0 × 10^{-5}</td>
<td>1.0 × 10^{-5}</td>
<td>DPV</td>
<td>[74]</td>
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<tr>
<td>dsDNA-coated CNPTE</td>
<td>3.0</td>
<td>7.0 × 10^{-7}–1.1 × 10^{-4}</td>
<td>1.8 × 10^{-7}</td>
<td>DPV</td>
<td>[75]</td>
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<tr>
<td>bioenzymatic/GCE</td>
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<td>2.0 × 10^{-6}–1.0 × 10^{-5}</td>
<td>3.3 × 10^{-7}</td>
<td>Amperometry</td>
<td>[76]</td>
</tr>
<tr>
<td>Plmox-GO/GCE</td>
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<td>3.6 × 10^{-6}–2.4 × 10^{-4}</td>
<td>5.9 × 10^{-7}</td>
<td>DPV</td>
<td>[77]</td>
</tr>
<tr>
<td>UOx/Fe3O4NPs/CHIT-g-PANU/PTi</td>
<td>7.5</td>
<td>1.0 × 10^{-7}–8.0 × 10^{-4}</td>
<td>1.0 × 10^{-7}</td>
<td>Amperometry</td>
<td>[78]</td>
</tr>
<tr>
<td>Fe3O4PSbO3/MWNTs/CPE</td>
<td>6.0</td>
<td>6.0 × 10^{-7}–1.0 × 10^{-4}</td>
<td>1.3 × 10^{-7}</td>
<td>SWV</td>
<td>[79]</td>
</tr>
<tr>
<td>Trp-GR/GCE</td>
<td>7.0</td>
<td>1.0 × 10^{-5}–1.0 × 10^{-3}</td>
<td>1.2 × 10^{-5}</td>
<td>DPV</td>
<td>[80]</td>
</tr>
<tr>
<td>PD-Cu(II)/GCE</td>
<td>7.5</td>
<td>6.0 × 10^{-5}–1.6 × 10^{-3}</td>
<td>2.4 × 10^{-5}</td>
<td>DPV</td>
<td>[81]</td>
</tr>
<tr>
<td>GEF/CPE</td>
<td>7.0</td>
<td>3.7 × 10^{-5}–1.8 × 10^{-4}</td>
<td>2.0 × 10^{-5}</td>
<td>DPV</td>
<td>[82]</td>
</tr>
<tr>
<td>Fe-MWCNTs/MCPE</td>
<td>3.0</td>
<td>7.0 × 10^{-5}–1.0 × 10^{-4} &amp; 2.0 × 10^{-5}–1.0 × 10^{-5} (4.80 ± 0.35)</td>
<td>10^{-4}</td>
<td>DPV</td>
<td>This work</td>
</tr>
</tbody>
</table>

3.13. Analytical application

The practical analytical application of the sensor was demonstrated by good percentage recovery in the determination of UA in human blood serum and urine sample by standard addition method [83] without it being subjected to any preliminary treatment. 0.5 ml of blood serum and 9.5 ml of 0.1 M phosphate buffer solution of pH 3.0 were added to it. This solution was spiked with standard solution of UA. The resultant solution was placed in the cell and differential pulse voltammograms of the same were recorded at Fe-MWCNTs/MCPE.

In the case of urine sample, 0.1 ml of urine was made up to 25 ml using 0.1 M phosphate buffer solution of pH 3.0. This solution was then spiked with standard solution of UA and the corresponding analytical response was recorded. The percentage recoveries of UA from blood serum and urine were satisfactory and are displayed in Tables 2 and 3 respectively. Three replicate measurements (n = 3) were performed for each of the concentrations at Fe-MWCNTs/MCPE.

3.14. Reproducibility and stability of the electrode

To find out the reproducibility of UA response at Fe-MWCNTs/MCPE, a series of repetitive measurements were carried out using CV for 5.0 × 10^{-4} M UA in 0.1 M phosphate buffer solution of pH 3.0. The relative standard deviation (RSD) of responses for 5 measurements was 4.6%, which implies a satisfactory reproducibility. The electrode was prepared and after it was stored in a dry place for over a week, the voltammetric response of 5 × 10^{-4} M UA was determined. The sensor registered an almost constant voltammetric response, which suggests its long term stability. Fig. S7 (supplementary information) illustrates the amperometric response of 5.0 × 10^{-4} M UA at Fe-MWCNTs/MCPE over a period of 30 min. The amperometric response was constant throughout the experiment, which confirms the stability of Fe-MWCNTs/MCPE.

4. Conclusions

A simple, rapid, sensitive, voltammetric method was developed by the modification of CPE with Fe-MWCNTs for the simultaneous determination of UA in the presence of interfering molecules such as AA, DA and coexisting biologically important molecule Tyr. The qualities of the electrode that makes it an attractive and suitable candidate for the function of an electrochemical sensor are simple methods of preparation, reproducibility, long term stability, very minimal use of modifier, reduction in cost and increased efficiency. The Fe-MWCNTs/MCPE was successfully employed for the spectroelectrochemical investigation of UA. The modified electrode can be successfully applied for the determination of UA in clinical samples such as human blood serum and human urine samples. The sensor may be employed without subjecting it to any preliminary treatment. Hence, this electrode has all the qualities needed for it to be used commercially as an electrochemical sensor for the determination of UA in various real samples.
References


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