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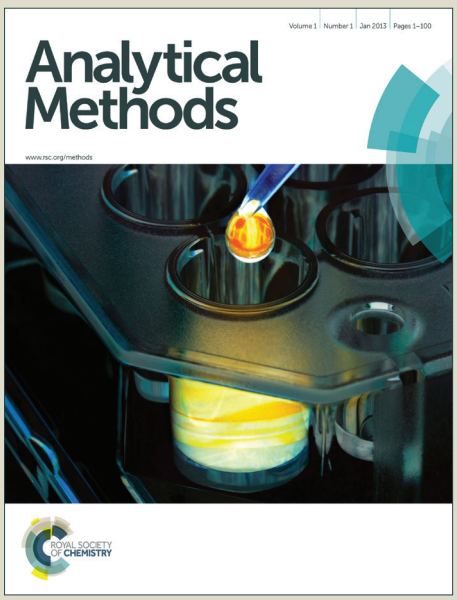

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ARTICLE

Selective determination of mefenamic acid in the presence of 1000-fold excess paracetamol and caffeine using multiwalled carbon nanotubes-polymer composite electrode

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This article describes the selective and sensitive determination of Mefenamic acid (MA) using carboxylated multiwalled carbon nanotubes (FMWCNTs) -nanostructured conducting polymer (p-ATT) composite modified glassy carbon (GC) electrode in 0.2 M phosphate buffer solution (PBS, pH 7.2). The bare GC electrode failed to show a stable response for MA oxidation due to the surface fouling caused by the oxidized product of MA. However, the FMWCNTs/p-ATT composite electrode showed 2.4-fold higher oxidation current with 70 mV less positive potential for MA when compared to bare GC electrode. The higher electrocatalytic activity of MA at composite modified electrode may be due to the hydrogen bonding and electrostatic interactions between p-ATT and MA besides the π-π interaction between the FMWCNTs and p-ATT. Further, the FMWCNTs/p-ATT composite electrode showed extreme selectivity towards MA in the presence of 1000-fold excess of paracetamol (PA) and caffeine (CAF) and 3330-fold excess of other common interferences. The amperometric current response was increased linearly with increasing MA concentration in the range of 40-5000 nM with a correlation coefficient of 0.9980 and the limit of detection was found to be 90 pM (S/N=3). The practical application of the present modified electrode was successfully demonstrated by determining MA in commercial drug samples.

Introduction

Mefenamic acid (MA, 2-[(2,3-dimethylphenyl)amino]benzoic acid) (Chart 1) is a non-steroidal anti-inflammatory drug with analgesic and antipyretic properties. [1] It is widely used in the treatment of diseases like osteoarthritis, non-articular rheumatism, sports injuries and rheumatoid arthritis.[1] It is also used to treat autoimmune haemolytic anaemia.[2] The degree of menstrual blood loss was reduced with the treatment of MA.[3] MA inhibits prostaglandin action, prophylaxis and it is used as a first line treatment in contraceptives. [4] However, the excessive intake of MA leads to diarrhoea, vomiting, lactic acidosis, purpura, hepatic necrosis, liver injury, morbidity and mortality in humans. [5-7] Hall et al. reported that the treatment with MA leads to acute colitis in patients. [8] The lower tolerance value of MA concentration in serum is 25 μg L^{-1}. [9] Hence, development of a suitable method for the sensitive determination of MA in biological samples is very essential.

Paracetamol (PA (N-acetyl p-aminophenol)) (Chart 1) is an effective analgesic for fever, moderate pain, lumbar pain and migraine. [10] It is an effective drug, alternative to aspirin, while using PA the secondary effects of salicylates on the gastric mucosa are absent.[11] An overdose of PA can lead to the accumulation of toxic metabolites, which may cause fatal hepatotoxicity, nephrotoxicity, liver disorders, skin rashes and inflammation of the pancreas. [12] On the other hand, caffeine (CAF (1,3,7-trimethylxanthine) (Chart 1) is a N-methyl derivative of xanthine that is widely present in coca nuts, coca-cola, coffee, coffee bean and tea leaves.[13] A dose of 200-500 mg of CAF is sufficient for a mild stimulation by increasing the release of adrenaline. [14] However, use of CAF for a prolonged time can lead to hypertension, nervousness, vomiting, irritability, anxiety, cardiac arrest and tremorsa. [15] When consumed excessively, it leads to inhibition of DNA repair and cyclic AMP phophodiesterase activity. [16]

Combinations of MA and PA are frequently prescribed for analgesic and anti-inflammatory use in rheumatoid arthritis [17] and combination of MA and CAF also widely used for migraine attacks.[18] Creapigny et al. reported that CAF potentiates the

nephrotoxicity of the MA on rat renal papilla.[19] Bach et al. reported that the analgesic efficacy was increased while using analgesics like PA and MA with coformulation of CAF. [20] Normally, in this combination, low concentration of MA present along with high concentrations of PA. [17] Thus, it is essential to determine trace level of MA in the presence of high concentration of PA and CAF.

Several methods are available to determine MA which include UV-Vis spectrophotometry [17], HPLC [21] and potentiometry [22]. However, spectrophotometry method failed to determine MA in the presence of PA because the absorption bands of these compounds overlapped with each other. [23] The other techniques have disadvantages of high cost, time consuming, low sensitivity and selectivity and involving tedious method. On the other hand, electrochemical method has advantages of less time consuming, high sensitivity and selectivity, easy to handle and low cost. MA is an electrochemically active compound. The electrochemical determination of MA has been already reported.[23-27] In the reported papers, MA or PA or CAF was either individually determined or PA and MA or PA and CAF were simultaneously determined.[23-27] To the best of our knowledge, no report is published in the literature for either the determination of low concentration of MA in the presence of high concentrations of PA and CAF or simultaneous determination of all the three compounds.

Carbon based materials and their composites have received enormous interest because of their interesting electrochemical sensing applications.[28,29] Recently, we have successfully prepared a composite film containing multiwalled carbon nanotubes (FMWCNTs)-nanostructured film of 5-amino-2-mercapto-1,3,4-thiadiazole (p-ATT) film on GC electrode.[30] The objective of the present study is to utilize the FMWCNTs/p-ATT composite film for the selective determination of MA in the presence of large excess of PA and CAF and also simultaneous determination of these analytes using the FMWCNTs/p-ATT composite modified electrode. It was found that the composite modified electrode dramatically enhanced the MA oxidation current and also shifted the oxidation potential towards less positive potential when compared to bare GC electrode. Further, it shows high selectivity towards MA even in the presence of 1000-fold excess of both PA and CAF and also 3330-fold excess of other common interferences. The amperometric current response was increased linearly with a correlation coefficient of 0.9980 and the limit of detection was found to be 90 pM (S/N = 3).

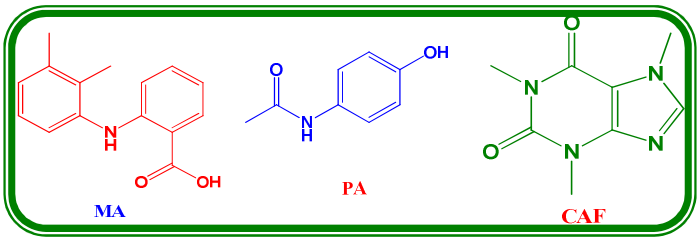


Chart 1 Structures of mefenamic acid, paracetamol and caffeine.

Experimental

Chemicals

5-amino-1,3,4-thiadiazole-2-thiol (ATT), multiwalled carbon nanotubes (FMWCNTs), dicyclohexylcarbodiimide (DCC), 1,8-octanediamine (OD), mefenamic acid (MA), paracetamol (PA) and caffeine (CAF) were purchased from Aldrich, India and were used as received. Na_2HPO_4 and NaH_2PO_4 were used to prepare the phosphate buffer solution (PBS, pH 7.2) with double distilled water and pH of the PBS was varied by adjusting with ortho-phosphoric acid and sodium hydroxide. All other chemicals used in this investigation were of analytical grade.

Instrumentation

Electrochemical measurements were performed in a conventional two-compartment three electrode cell with a mirror polished 3 mm GC electrode as a working electrode, Pt wire as a counter electrode and NaCl saturated Ag/AgCl as a reference electrode. The electrochemical measurements were carried out with a CHI model 634B electrochemical workstation (CH Instruments, Austin, TX, USA). For differential pulse voltammetry (DPV) measurements, a pulse width of 0.06 s, amplitude of 0.05 V, a sample period of 0.02 s and a pulse period of 0.20 s were used. All the electrochemical measurements were carried out under a nitrogen atmosphere at 27°C.

Fabrication of FMWCNTs and FMWCNTs/p-ATT modified GC electrode

The FMWCNTs and FMWCNTs/p-ATT modified GC electrodes were prepared according to our recent report.[30] Briefly, the GC electrode was polished with 0.05 µm alumina slurry and rinsed thoroughly with water. Then, the electrode was sonicated in water for 5 min to remove the adsorbed alumina particles. The cleaned GC electrode was immersed in an ethanolic solution of 1 mM OD for 8 h. The electrode was then washed with ethanol and subsequently with water. The OD modified GC electrode was immersed into a solution containing 1:1 volume ratio of 0.2 mg/ml FMWCNTs and 2 mM DCC in ethanol for 4 h. The FMWCNTs were attached on the GC electrode through amide bond by the condensation reaction between the amine groups at the terminal end of the SAM and acid groups of the FMWCNTs. This electrode is termed as GC/OD/FMWCNTs. The FMWCNTs-polymer composite electrode was prepared by electropolymerizing 1 mM ATT on GC/OD/FMWCNTs by 15 successive potential cycles between -0.2 V and +1.7 V at a scan rate of 50 mV s^{-1} in 0.1 M H_2SO_4.[30] For control experiments, the GC/p-ATT electrode was prepared under identical conditions on bare GC electrode. After the deposition of the p-ATT film, the electrode was washed with water and kept in PBS before used for electroanalysis.

Results and discussion

Electrochemical oxidation of MA at different modified electrodes

Fig. 1 displays the cyclic voltammograms (CVs) obtained for 0.5 mM MA at bare GC, GC/p-ATT, GC/OD/FMWCNTs and GC/OD/FMWCNTs/p-ATT modified electrodes in 0.2 M PBS (pH 7.2). The bare GC electrode shows a broad irreversible oxidation wave at 0.69 V for MA in the first cycle (curve a). The oxidation of MA involves one electron and one proton. This is evidenced its oxidation at different pH. The plot of potential vs. pH gives the slope value of 60 mV/pH. It indicates that MA oxidation involves equal number of proton and electron. [31,32] The reaction mechanism is shown in Scheme 1.

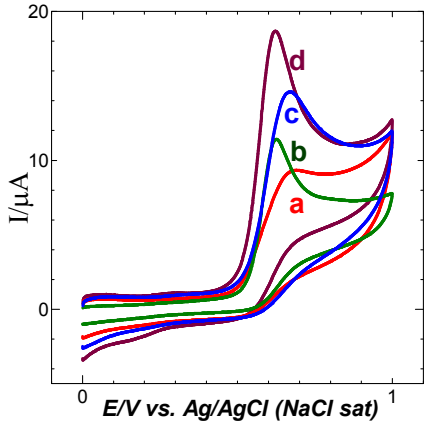
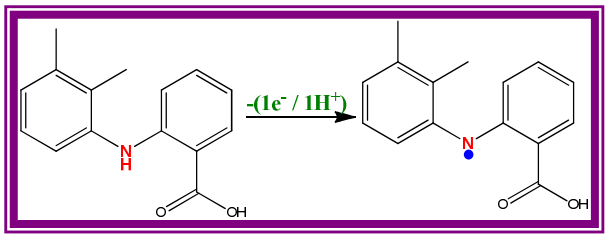
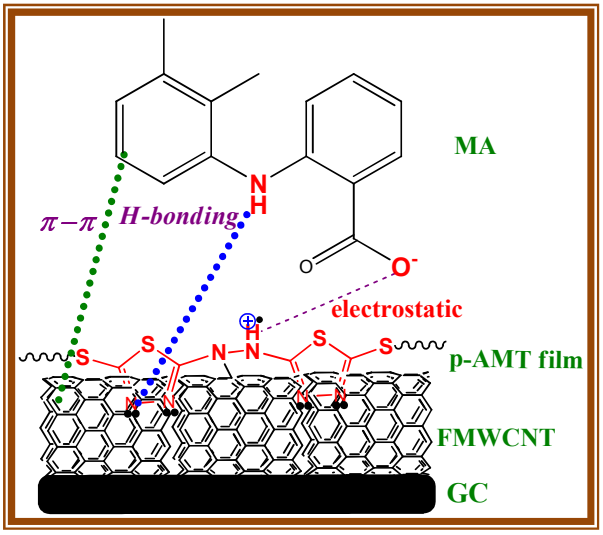


Fig. 1 CVs obtained for 0.5 mM of MA at (a) bare GC, (b) GC/p-ATT, (c) GC/OD/FMWCNTs and (d) GC/OD/FMWCNTs/p-ATT electrodes in PBS (pH 7.2) at a scan rate of 50 mV s^{-1}.



Scheme 1 Electrochemical oxidation mechanism of MA.

After four cycles, the oxidation current was decreased markedly (S1: curve a'). This may be due to the surface fouling caused by the adsorption of the radical species of MA. The GC/p-ATT electrode shows a sharp oxidation peak at 0.63 V with an enhanced oxidation current for MA when compared to bare GC electrode (curve b). The obtained 60 mV less positive potential and 1.4-fold higher oxidation current at GC/p-ATT electrode were attributed to the hydrogen bonding interaction between the -NH- group of MA and the heterocyclic nitrogen atom present in p-ATT. At physiological pH (7.2), MA exists as an anionic form. Hence, the electrostatic interaction between $-COO^-$ of MA and $-N^+H-$ of the p-ATT is also possible [33] (Scheme 2). After four cycles, the oxidation current of MA was slightly decreased (S1: curve b'). The GC/OD/FMWCNTs modified electrode shows an oxidation peak for MA at 0.66 V with 1.7-fold higher oxidation current when compared bare GC electrode (curve c). This may be due to the π-π attraction between MA and the FMWCNTs. After four cycles, the oxidation peak was appeared at 0.69 V with decreased peak current (S1: curve c'). The obtained results revealed that the oxidation of MA is sluggish at the above electrodes and not suitable for the stable determination of MA. On the other hand, the GC/OD/FMWCNTs/p-ATT electrode shows a sharp oxidation peak at 0.62 V with an enhanced current for MA (curve d). When compared to bare GC electrode, the oxidation peak of MA remains stable even after 4 cycles (S1: curve d'). The obtained 2.4-fold higher oxidation current with 70 mV less positive potential for MA at FMWCNTs/p-ATT electrode are attributed to the hydrogen bonding interaction between the -NH- group of MA and the heterocyclic nitrogen atom present in p-ATT and the electrostatic interaction between the $-COO^-$ of MA and $-N^+H-$ of the p-ATT besides of π-π interaction between FMWCNTs and MA (Scheme 2). These effects enhanced the oxidation current of MA with 70 mV less positive potential shift than bare GC. The obtained higher oxidation current at FMWCNTs/p-ATT in contrast to p-ATT electrode clearly indicates that even after the deposition of p-ATT, π-π interaction between MA and FMWCNTs is not completely vanished.



Scheme 2 Possible interactions between MA and the composite electrode.

Simultaneous determination of PA, MA and CAF

One of the objectives of the present study is to simultaneously determine PA, MA and CAF using FMWCNTs/p-ATT electrode. Fig. 2 shows the DPVs obtained for 3 μM PA, 5 μM MA and 40 μM CAF at GC/OD/FMWCNTs/p-ATT electrode in 0.2 M PBS (pH 7.2). It shows well separated voltammetric signals for 3 μM PA, 5 μM MA and 40 μM CAF (curve a) at 0.32, 0.54 and 1.36 V, respectively. When the concentration of PA was increased from 3 μM to 27 μM, MA was increased from 5 μM to 45 μM and CAF was increased from 40 μM to 360 μM (curve a-i), the corresponding peak currents were

increased linearly with correlation coefficients of 0.9971 (inset A), 0.9957 (inset B) and 0.9974 (inset C), respectively.

Selective determination of MA in presence of PA and CAF

The main intention of the present study is to determine low concentration of MA in the presence of high concentrations of PA and CAF. Since high dose of MA leads to several diseases and MA mainly co-formulated with PA and CAF an accurate determination of MA is very important in the presence of PA and CAF. Fig. 3 shows the DPVs obtained for the increment of 0.5 µM of MA to a solution of 0.5 mM PA and 0.5 mM CAF at FMWCNTs/p-ATT in 0.2 M PBS (pH 7.2). A well-defined peak at 0.55 V was observed for 0.5 µM MA even in the presence of 1000-fold excess of PA and CAF. This reveals that the detection of low concentration of MA is possible even in the presence of high concentration of PA and CAF. The oxidation current for each addition of 0.5 µM MA to a solution of 1000-fold excess of PA and CAF increases linearly with a correlation coefficient of 0.9960 (inset A) while the peak currents of both PA and CAF were unchanged. These results show that the FMWCNTs/p-ATT electrode was more selective towards the oxidation of MA even in the presence of 1000-fold higher concentrations of PA and CAF

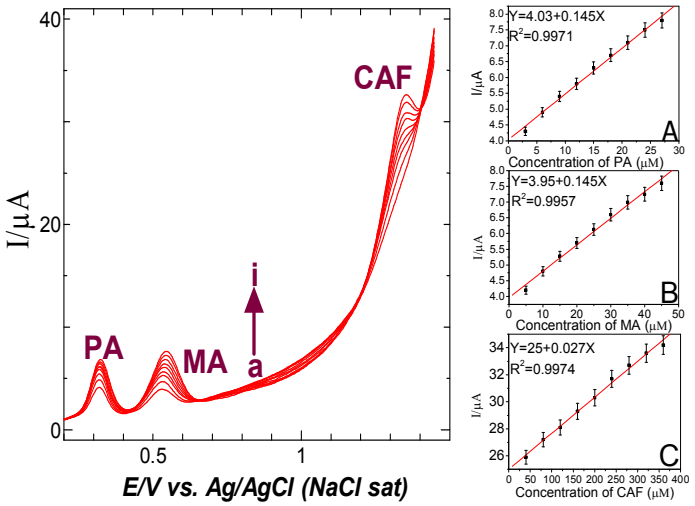


Fig. 2 DPVs obtained for each increment of 3 µM PA, 5 µM MA and 40 µM CAF (curves a-i) at GC/OD/FMWCNTs/p-ATT electrode in 0.2 M PBS (pH 7.2). **A, B** and **C** are the plots obtained for current vs. concentration of PA, MA and CAF, respectively.

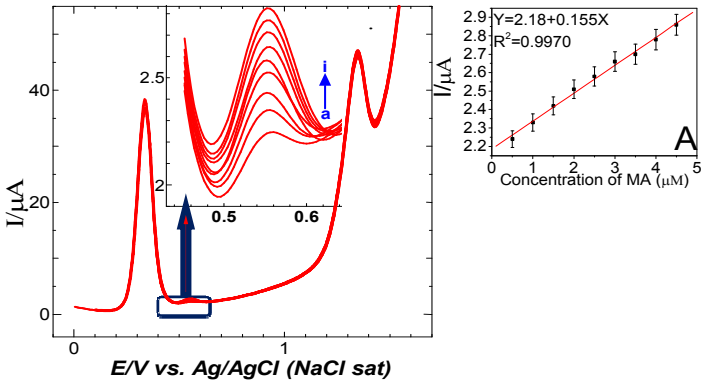


Fig. 3 DPVs obtained for each increment of 500 nM MA (curves a-i) at GC/OD/FMWCNTs/p-ATT electrode in the presence of 0.5 mM PA and 0.5 mM CAF in 0.2 M PBS (pH 7.2). **Insets:** Expanded view of 500 nM MA addition and (A) corresponding calibration plot.

Amperometric determination of MA

Amperometric method was used to examine the sensitivity of GC/OD/FMWCNTs/p-ATT electrode towards the detection of MA. Fig. 4A and Fig. 4B show the amperometric i-t curves for MA at FMWCNTs/p-ATT composite electrode in a homogeneously stirred 0.2 M PBS (pH 7.2) by applying a constant potential of 0.70 V. The modified electrode shows the initial current response due to 5 nM MA (Fig. 4A) and further addition of 5 nM MA in each step with a sample interval of 50s increases the current response. The dependence of response current with respect to concentration of MA was linear from 5 to 50 nM with a correlation coefficient of 0.9997 (inset Fig. 4A).

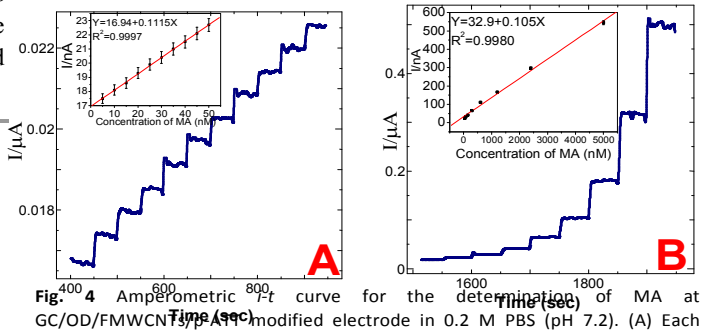


Fig. 4 Amperometric *i-t* curve for the determination of MA at GC/OD/FMWCNTs/p-ATT modified electrode in 0.2 M PBS (pH 7.2). (A) Each addition increases the concentration of MA by 5 nM at a regular interval of 50 s. (B) Each addition increases the concentration of 40, 80, 150, 300, 600, 1200, 2400 and 5000 nM MA at a regular interval of 50 s. E_{app} = +0.7 V (Insets: corresponding linearity plots).

The amperometric current response was also increased linearly with increasing MA concentration in the dynamic range of 40 to 5000 nM (Fig. 4B). The amperometric current response was increased linearly from 40-5000 nM with a correlation coefficient of 0.9980 and the limit of detection was found to be 90 pM (S/N = 3). Further, the dynamic range and the limit of detection of MA obtained in the present study was compared with the reported papers and are given in Table 1. It can be seen from Table 1, the present method showed the lowest limit of detection for MA when compared to the reported papers. [23-27] For example, the limit of detection of 40 nM was reported at copper (II) doped zeolite modified carbon paste electrode[23], while 10 nM was reported at MWCNTs-AuNPs-DHP/GC electrode .[24] At RTIL-MWCNTs-CHIT/GC modified electrode, limit of detection of 1.2 µM was reported .[26] Besides high sensitivity of the present method, electrode modification procedure is also easy when compared to the reported papers .[24,26]

Effect of interferences

Fig. 5 shows amperometric response of 30 nM MA in 0.2 M PBS (pH 7.2) in the presence of various physiological interferences at GC/OD/FMWCNTs/p-ATT modified electrode. It shows no change in the amperometric current response for 30 nM MA (a) in the presence of 100 µM of each proline, methionine, alanine, asparagine, aspartic acid, glucose and arginine. This indicates that the present composite electrode is highly selective towards the determination of MA in the presence of 3330-fold excess of these interferences.

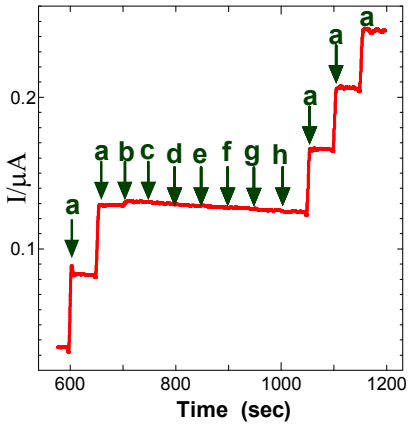


Fig. 5 Amperometric *i-t* curve for the determination of (a) 30 nM MA in the presence of 100 µM each (b) proline, (c) methionine, (d) alanine, (e) asparagine, (f) aspartic acid, (g) glucose and (h) arginine at GC/OD/FMWCNTs/p-ATT electrode in 0.2 M PBS (pH 7.2) at a regular interval of 50 s. E_{app} = +0.7 V.

Table 1 Comparison of present method of limit of detection for MA with the previously reported papers.

S. No	Electrodes	Limit of detection (nM)	References
1	MWCNTs-Graphite/Ag electrode	16	25
2	RTIL-MWCNTs-CHIT/GC electrode	1235	26
3	MWCNTs-CHT/GC electrode	660	27
4	GC/OD/FMWCNTs/p-AMT electrode	0.09	This work

Determination of MA in commercial drugs

The practical application of FMWCNTs/p-ATT modified electrode was tested by measuring the concentration of MA in commercially available tablets. The MA containing three different commercial tablets were employed. The labeled concentrations of MA in tablets is 25, 250 and 500 mg, respectively. The modified electrode showed the concentration of 24.9 for the addition of 25 mg MA with a recovery of 99.6 (Fig.S2A, Table 2). Similarly, for the addition of 250 and 500 mg MA showed the concentration of 249.5 and 500 with recoveries of 99.8 and 100%, respectively (Table 2). The obtained good recovery results reveal that the present method could be applied to determine MA in real samples. The estimated RSD values for five times parallel detection is 1.12 and 1.16%. These results indicated that the present method could be efficiently used for the determination of MA in pharmaceutical formulations. We have compared the present method of determination of MA with HPLC method and obtained 1.2% variation between the two methods. We also carried out linear addition of MA contained commercial drug in 0.2 M PBS and shown in Fig.S2B. It shows good linearity with the oxidation current and concentration of MA with a correlation coefficient of 0.9938. This indicates that the present method is highly reliable for the determination of MA in real samples.

Table 2 Determination of MA in commercial drugs using GC/OD/FMWCNTs/p-ATT electrode.

Commercial drugs	Labelled (mg)	Found (mg)	Recovery (%)
Tablet 1	25	24.9	99.6
Tablet 2	250	249.5	99.8
Tablet 3	500	500	100

Stability of the GC/OD/FMWCNTs/p-ATT electrode

In order to investigate the stability of the GC/OD/FMWCNTs/p-ATT electrode, the CVs for 5 µM MA in 0.2 M PBS (pH 7.2) were recorded at every 5 min interval. It was found that the peak currents remained same with a relative standard deviation of 1.1 % for 15 repetitive measurements indicating that the modified electrode has a good reproducibility and does not experience surface fouling during the voltammetric measurements. The current response decreased about 1.4 % in 2 weeks when the electrode was kept in PBS (pH 7.2). To ascertain the reproducibility of the results further, three different GC/OD/FMWCNTs/p-ATT modified electrodes and their response towards the oxidation of 5 µM MA was recorded by 15 repeated measurements. The peak current showed a relative standard deviation of 1.9 %, confirming that the results are reproducible. The above results showed that the present modified electrode was very stable and reproducible towards MA.

Conclusions

The present work demonstrates that the FMWCNTs/p-ATT composite modified electrode offers a simple and reliable method for the sensitive and selective determination of MA. The bare GC, GC/p-ATT, GC/OD/FMWCNTs electrodes failed to show a stable electrochemical response towards MA oxidation. On the other hand, the GC/OD/FMWCNTs/p-ATT modified electrode not only shifted the oxidation potential towards less positive potential but also enhanced the oxidation current with stable voltammetric signal for MA when compared to bare GC electrode. The enhanced oxidation current with 70 mV less positive shift of MA at FMWCNTs/p-ATT composite modified electrode was due to the hydrogen bonding interaction

between the -NH- group of MA and the heterocyclic nitrogen atom present in p-ATT and the electrostatic interaction between the $–COO^-$ of MA and $-N^+H-$ of the p-ATT besides facilitation of π-π interaction between FMWCNTs and MA. The DPV current increases linearly while increasing the concentration of MA from 0. 5 to 4.5 µM even in the presence of 1000-fold excess of PA and CAF. The detection of 5 nM of MA was achieved at the FMWCNTs/p-ATT composite modified electrode using the amperometry method. The amperometric current response was increased linearly while increasing MA concentration in the dynamic range of 40 to 5000 nM with a correlation coefficient of 0.9980 and the limit of detection was found to be 90 pM (S/N=3). The practical application of the present modified electrode was successfully demonstrated by determining the concentration of MA in commercial drug samples.

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Electronic Supplementary Information (ESI) Available:

CVs obtained for 0.5 mM MA (a) bare GC, (b) GC/p-ATT, (c) GC/OD/FMWCNTs and (d) GC/OD/FMWCNTs/p-ATT composite modified electrodes in PBS (pH 7.2) at a scan rate of 50 mV s^{-1} (dashed lines are the respective fourth cycles) and (A) DPVs obtained for (a) PBS only (b) after spiked with MA drug sample, (B) DPVs obtained for the linear addition of commercial drug sample of MA at GC/OD/FMWCNT/p-ATT modified electrode in 0.2 M PBS (pH 7.2). See DOI: 10.1039/b0000.

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