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Fabrication and characterisation of high performance polypyrrole modified microarray sensor for ascorbic acid determination

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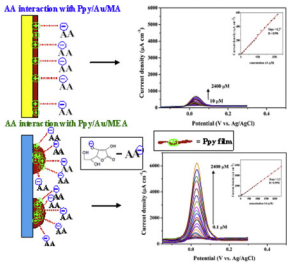
HIGHLIGHTS

- Gold microelectrode array (Au/MEA) with electrode of 12 μm diameter was fabricated by photolithography technique.
- Subsequently, polypyrrole (Ppy) modified gold microarrays sensor (Ppy/Au/MEA) was prepared.
- Ppy/Au/MEA used for ascorbic acid determination in the presence of different neurotransmitters.
- The micro array exhibited wide linear range, very high sensitivity and very low LOD than the earlier reports.
- It was used successfully to test ascorbic acid in different types real samples.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, gold microelectrode array (Au/MEA) with electrode of 12 μm diameter was fabricated by photolithography technique. Subsequently, polypyrrole (Ppy) modified gold microarrays sensor (Ppy/Au/MEA) was prepared by cyclic voltammetry technique. The deposition potential range and number of cycles were optimised in order to get optimum thickness of Ppy film. Scanning Electron Microscope and Atomic Force Microscope investigations reveal that Ppy coating formed at 3 cycles is porous with thickness of 1.5 μm which exhibiting high catalytic current for ascorbic acid (AA) in square wave technique (SWV). In contrast to earlier sensors designs, these Ppy/Au/MEA sensors exhibits lower detection limit (LOD) of 10 nm towards AA at physiological conditions. It also exhibits enhanced sensitivity (2.5 mA cm^{-2} mM^{-1}) and long range of linear detection limit from 10 nm to 2.8 mM. In the same way, polypyrrole modified macro Au (Ppy/Au/MA) biosensor was also fabricated and its electro catalytic property towards AA was compared with that of Ppy/Au/MEA. The Ppy/Au/MA exhibits sensitivity of only 0.27 mA cm^{-2} mM^{-1}, LOD of 5 μM and linear range of 10 μM to 2.2 mM. Hence, our investigations indicate that the Ppy/Au/MEA could serve as highly sensitive sensor for AA than any of the earlier designs. So, the Ppy/Au/MEA electrode was utilised for determination AA in a wide variety of real samples.

1. Introduction

Currently, improvements in electrochemical sensors include the development of new electrode material, size, and geometry of electrodes. The development of lithography and plasma etching

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permits the fabrication of surfaces with a precisely defined geometry at the micro- or nano-scale that allows the fabrication of MEA [1,2]. The small size of microelectrodes opens up other interesting research opportunities, since they can be used for the study of concentrations in confined volumes or spatial regions of otherwise difficult to access. In vivo studies are the perfect examples of MEA sensor. Hence, the unconventional properties of micro electrodes make them attractive as sensitive electrochemical sensors in a variety of media [3–6].

Ascorbic acid (vitamine C or AA) is usually used in large scale as an antioxidant in food, animal feed, beverages, pharmaceutical formulations and cosmetic applications [7]. Nowadays, considerable attention is being paid on monitoring of the extracellular AA during the early period of cerebral ischaemia because AA has been known to be involved in many neurochemical processes [8], such as energy failure, anoxic depolarization, glutamate excitotoxicity, peri-infarct depolarization, oxidative stress and necrosis [9].

Conducting polymers show very interesting chemical and physical properties owing to their unique conjugated π-electron system. Polypyrrole shows its electric conductivity and electrochemical redox activity even in neutral solutions, which allows the entrapment of a wide range of biocatalysts [10]. Hence, conducting polymers modified electrodes have been used as biosensors for simultaneous determination of AA, dopamine and uric acid as they exhibit enhanced and well separated responses for AA, catecholamines and NADH, and at lower over potentials than bare glassy carbon and gold electrodes [11,12]. Cun Wang et al. demonstrated that chloro [3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropanoato (2⁻)]iron(III)/multi-walled carbon nanotubes (Fe(III)P/MWCNTs) composites electrode was fabricated and used successfully for the simultaneous determination of AA, DA and UA [13]. Likewise, Au-nanoclusters incorporated 3-amino-5-mercapto-1,2,4-triazole film modified electrode, [14], 3D nanoporous gold thin film modified electrode [15], poly(N-methylpyrrole)/Pd-nanoclusters electrode [16] were used for AA determination.

Similarly some research groups also reported modified macro and micro electrodes for selective detection and determination of catecholamine neurotransmitters in the presence of AA. They are nanostructured Pt–Au hybrid film for simultaneous determination of catecholamines in the presence of AA [17], boron-doped diamond electrode modified with gold nanoparticles/polyelectrolyte-coated polystyrene colloids [18], nano-Au self-assembly on GCE [19], Palladium nanoparticles modified electrode [20], graphene/poly(p-aminobenzoic acid) composite film [21], and fictionalized-graphene modified graphite electrode [22].

Further, some other reports discuss sensors for selective determination of AA, for example: Pt and Ni foil electrodes [23], a movable assembly of a pencil rod working electrode [24], flexible poly(dimethylsiloxane) (PDMS)-based gold electrode [25], DNA-decorated nanoparticles electrode as nanosensors [26] and an ionic liquid carbon nanotube composite electrode [27].

Recently, the ability of easy fabrication of gold MEA has made it a prime candidate for wide range of electrochemical studies, including multi-electrode arrays with the ability to detect and differentiate multiple neurotransmitters [28–30].

To the best of our knowledge, there is no earlier report on the application of Ppy/Au/MEA for AA determination. We have utilized Ppy/Au/MEA for the determination AA in wide variety of samples like fresh fruit, blood serum and celin tablet. The Ppy/Au/MEA showed higher sensitivity and lowest detection limit, i.e. nobody has reported so far, for the detection of AA with excellent selectivity and sensitivity.

2. Experimental

2.1. Materials

Ascorbic acid, dopamine hydrochloride, uric acid, tyramine, histamine dihydro chloride and pyrrole monomer were purchased from Sigma–Aldrich. All other chemicals (Merck) used were analytical grades and aqueous solutions were prepared with millipore water. A phosphate buffer solution (PBS) of pH 7.0 was prepared from Na_2HPO_4 (0.05 mol L^{-1}) and NaH_2PO_4 (0.05 mol L^{-1}).

2.2. Apparatus

Scanning Electron Microscopy (SEM) was performed with a VEGA3 TESCAN under 15 kV accelerating voltage (TESCAN, Japan). Atomic Force Microscope images were recorded with atomic scanning probe microscope, Agilent Technology 5500, UK. All electro chemical measurements were performed using Gamry 300 electrochemical Workstation. A three-electrode system, consisting of Ppy-film modified and unmodified gold micro electrode array (Au/MEA) (area 0.02 cm^2) or gold macro electrode (Au/MA) (area 0.02 cm^2) as working electrode, Ag/AgCl as reference electrode and a platinum wire as counter electrode were used for electrochemical studies. Electro chemical cleaning of both electrodes was carried out in 0.5 M H_2SO_4 in the potential range of −0.5 V to +1.0 V vs Hg/Hg_2SO_4 at 100 mV s^{-1}.

2.3. Fabricated MEA

Au/MEA with electrode of 12 μm diameter was fabricated by photolithography technique. Rectangular array device is having over-all diameter of 8 mm × 12 mm & electrode active area is 0.02 cm^2 containing approximately 20,000 electrodes of 12 μm size with inter-electrode spacing of 60 micron. Fig. S1 shows the SEM images of bare Au/MEA with different magnification and Ppy/Au/MEA (Supporting Information).

2.4. Preparation of Ppy/Au/MEA and Ppy/Au/MA

The electrochemical polymerisation of the pyrrole was carried out by cyclic voltammtric (CV) technique in aqueous solution containing 0.1 M pyrrole and 0.1 M KCl (Fig. S2A). The polymerisation was carried out between −0.2 and 0.8 V at a scan rate of 50 mV s^{-1} for different cycles, for example 1, 3 and 6 cycles, to control thickness of the film. After polymerisation, the electrode was washed in distilled water and electro chemically treated using CV in 0.1 M phosphate buffer solution until a stable background current was obtained. So the working electrode is either an unmodified Au/MA or Au/MEA or Ppy film modified Au/MA or Au/MEA.

3. Results and discussion

3.1. Characterisation of Ppy/Au/MEA

3.1.1. Measurement of thickness of Ppy-film by AFM

The thickness of the Ppy-film coated on MEA was determined by using AFM three dimensional imaging techniques. The images in Fig. 1A and B depict three dimensional versions of topography and amplitude of AFM images of Au/MEA surface with the Ppy film obtained for 3 and 6 cycles, respectively. From the 3D analyses, the thickness of the Ppy film on the individual micro electrode was estimated to be 1.5 μm for three cycles and 3 μm for 6 cycles of polymerisation. The above results hence indicate that the thickness of the Ppy film increases with number of cycle of polymerisation.

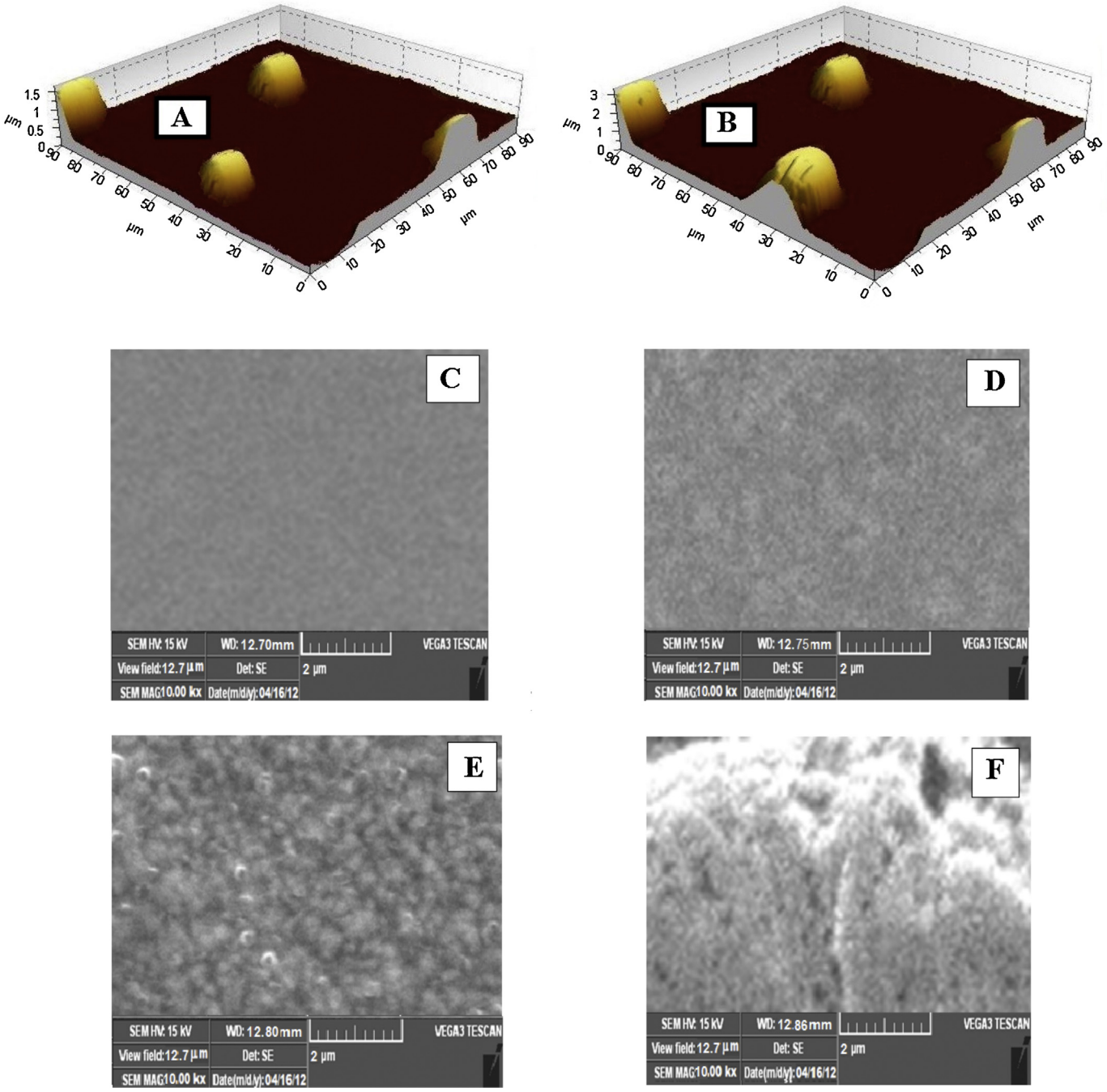

Fig. 1. AFM three dimensional images of the Ppy/Au/MEA: (A) 3 cycles (B) 6 cycles; SEM image of the one Au/MEA of different thickness: (C) bare Au/MEA, (D) Ppy/Au/MEA one cycle, (E) Ppy/Au/MEA 3 cycles and (F) Ppy/Au/MEA 6 cycles.

3.1.2. Imaging of surface morphology of Ppy-film by SEM

To examine the influence of thickness of Ppy-film on its surface morphology and its catalytic property towards AA, SEM images were captured for different thickness of the Ppy-film. Fig. 1(C–F), display the SEM images of the Ppy film with different thickness coated on single Au-micro electrode. As images reveal that the surface of bare Au-micro electrode is looking smooth but after one cycle of polymerisation, there is an appearance of surface roughness which indicates that the surface is partially covered with polymer coating Fig. 1C and D. However, after 3 and 6 cycles of deposition, it is clearly observed that the electrode is fully covered with polymer film. Further, the film obtained at three cycles displays topography with hierarchical roughness and high porosity whereas the film prepared at 6 cycles exhibits the surface topography of the highly dense spongy film (Fig. 1E and F).

3.1.3. SWV of AA on unmodified and modified Au/MEA and Au/MA

As per the earlier reports [31], it is well known that the Ppy film has high catalytic effect for AA. With this idea, we have selected the Ppy film as catalyst on Au/MEA mainly to the catalytic oxidation of AA using Ppy film coated Au/MEA. Fig. S2B show SWVs obtained for bare Au/MA and Au/MEA and Ppy modified Au/MA (25 µA cm^{-2} at 25 mV) and Au/MEA (325 µA cm^{-2} at 25 mV) electrodes for the 100 µM AA. The electrochemical parameters calculated from above SWVs are summarised in the Table 1. From the Table 1, it could be observed that anodic peak current of AA was observed three times

Table 1
Electrochemical parameters obtained for 100 μM AA on modified electrodes.

S. No.	Type of electrode	Peak potential (mV)	Peak current (μA cm^{-2})
1	Bare Au/MA	330	15
2	Bare Au/MEA	330	50
3	Ppy/Au/MA	25	25
4	Ppy/Au/MEA	25	325

higher at unmodified Au/MEA than that of unmodified Au/MA (Fig. S2B) and at the same time there is no change in peak potential. The phenomenon observed at peak current is due to dominated radial diffusion in the mass transport of the reactant which leads to larger mass transport in the electrode/electrolyte interface compared to planar diffusion occurring on macro electrode [32]. More importantly, the shape and magnitude of the voltammetric response are highly dependent on two factors: the size of the individual microelectrodes vs. the size of the diffusion zones, δ; and the size of the diffusion zones vs. the size of the insulating material which separates neighbouring microdiscs (the centre-to-centre separation, d, of the microdiscs). In order to get the highest faradaic current/background current ratio, the diffusion layer thickness, δ, must be larger than the microelectrodes but not so large as to cause adjacent diffusion layers to overlap. Indeed, design of our microelectrode arrays must be obeyed this principle. So highest faradaic current is obtained on our Au/MAE electrode for AA than that on Au/MA.

When both working electrodes are modified with Ppy film of same thickness, the oxidation peak current of AA increases drastically along with a oxidation peak potential of AA shift about 300 mV to the negative side than on bare Au. The results obtained can be attributed to the electrostatic attraction between the oxidised Ppy-film and AA (exists as ascorbate anions at pH = 7) that resulted in preconcentration of ascorbate anions at the electrode surface/solution interface. Because generally, polypyrrole and polythiophene exhibit two distinct regions that are conducting (oxidised form) and non-conducting (reduced form) at neutral pH. Whereas at Ppy/Au/MEA, the peak current increases one more order higher than that of Ppy/Au/MA due to synergistic effect of the above said radial diffusion as well as electrostatic interaction of AA with Ppy/Au/MEA.

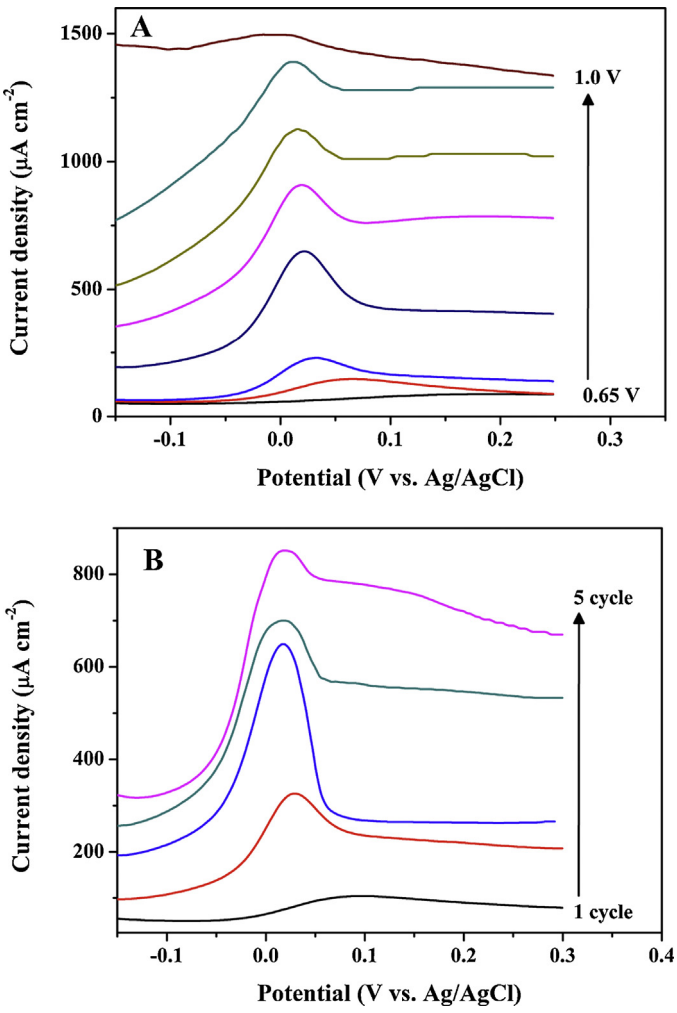

Fig. 2. (A) SWVs of 100 μM AA on Ppy/Au/MEA prepared at different anodic switching potentials range between −0.2 (from inner to outer) and 0.65, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95 and 1.00 V; (B) SWVs of 100 μM AA on Ppy/Au/MEA prepared at different cycle number between 1 cycle, 2 cycles, 3 cycles, 4 cycles and 5 cycles.

3.2. Optimisation of experiment conditions

In order to achieve good catalytic activity, two parameters like anodic switching potential of polymerisation and thickness of the polymer were optimised.

3.2.1. Optimisation of anodic switching potential

Fig. 2A, demonstrates the effect of anodic switching potentials of electro polymerisation towards oxidation of AA. The Ppy-film was prepared at different polymerisation potential ranges in cyclic voltammetry between −0.2 and 0.65, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95 and 1.00 V. It could be noticed that when the anodic switching potential of polymerisation was increased from 0.65 to 8.00 V, the oxidation current of AA increased considerably. On further increase of anodic switching potential decreased oxidation current of AA. The increase in peak current of AA up to 0.8 V of switching potential must be due to increase in active sites of conducting Ppy-film on Au/MEA. However, beyond 0.8 V, the reverse trend in peak current is observed which may be due to the passivation effect or the irreversible damage of polymer film at higher switching potentials. Moreover, by increasing the oxidation potential of the monomer, the thickness of the polymer is being increased and so the capacitive background current is also increased, which causes lowering of signal to noise ratio in the detection limit of the voltammetric measurements.

3.2.2. Optimisation of thickness of Ppy-film for effective catalysis of AA

To find out the suitable thickness of Ppy-film for effective catalysis to AA, we controlled the polymer thickness in terms of cycle number. As shown in Fig. 2A, the oxidation peak potential of AA shifts maximum 300 mV towards positive side for first two cycles and remained constant on further increase of polymerisation cycles. But in the case peak current of AA, it increases upto from 3 cycles of pyrrole polymerisation. However, the peak current of AA decrease beyond the third cycle of polymerisation. This catalytic property of Ppy-film up to 3 cycles can be explained by two factors: firstly, increasing the polymer film thickness improves the active surface area of the Ppy/Au/MEA and secondly due to the thin-layer effect of polymer film. At 3 cycles, Ppy film could have contained more pinholes and the electro active species that trapped inside the pores are equivalent to the small volume of solution in a thin layer cell model, which allows us to assume negligible mass transfer and shorter diffusion times [33]. In consequence, sensitivity of the sensor is improved. But, at higher film thickness, while the electrostatic interaction is enhanced by increasing the thickness of Ppy film, the diffusion barrier for AA also increases simultaneously,

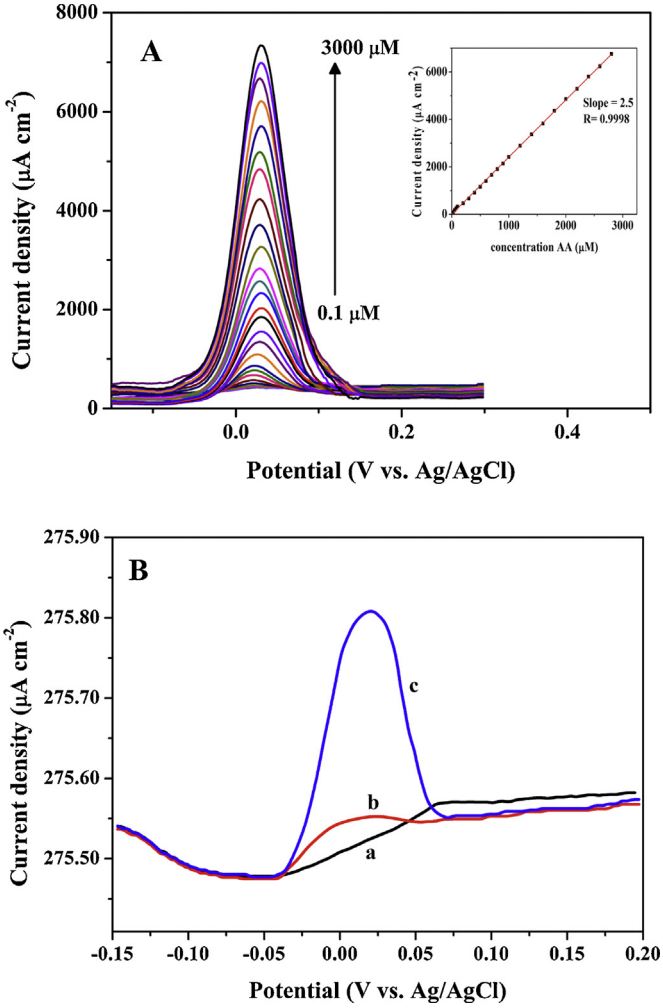

Fig. 3. SWV profiles at Ppy/Au/MEA in 0.1 M PBS for (A) different concentrations of AA from 0.1 μM to 3000 μM. Inset: The linear relationship between SWV peak current and AA concentration. (B) Containing 0 μM AA (a), 0.01 μM AA (b) and 0.1 μM AA (c).

and therefore the advantageous effect of electrostatic interaction is greatly reduced. Therefore, the thickness obtained at 3 cycles is fixed as optimal thickness. Again, this behaviour is complemented by the SEM morphology images of Ppy film obtained at various cycles.The Ppy-film prepared at 3 cycles reveals a lots of pine holes in SEM image. This result is also in agreement with previous reports [34].

3.3. Quantitative analysis of AA at Ppy/Au/MEA and Ppy/Au/MA

The analytical performances of Ppy/Au/MEA and Ppy/Au/MA electrodes obtained at optimised conditions of polymerisation (in the potential range from −0.2 V to 0.8 V for 3 cycles) were tested using SWV. In this section, the peak current of AA at Ppy/Au/MEA and Ppy/Au/MA are quantitatively analysed so that the sensitivity of different sensors could be evaluated. Figs. 3A and S3A present SWV recorded for AA on Ppy/Au/MEA and Ppy/Au/MA at varying concentrations, respectively. When the different concentrations of AA are added, peak current increase linearly upto 2.8 mM at Ppy/Au/MEA and 2.2 mM at Ppy/Au/MA, respectively. Conversely, when AA is added beyond these concentrations, the peak current decreases. It implies that the oxidation of AA is kinetically controlled above this limit. The phenomenon may also be due to electrode fouling because of accumulation of oxidised products of AA inside the Ppy-film at higher concentrations.

3.4. Comparison of the sensitivities and LODs on Ppy/Au/MEA and Ppy/Au/MA for AA

The peak current density of SWV were measured for different concentrations of AA and plotted against the concentration (Figs. 3A and S3). From the calibration curve, the linear range and sensitivity of Ppy/Au/MEA and Ppy/Au/MA were calculated as 10 nM to 2.8 mM and 2.5 mA cm^{-2} mM^{-1} and 10 μM to 2.2 mM and 0.27 mA cm^{-2} mM^{-1}, respectively, with high correlation coefficient of 0.9998. But in lower range of concentration (10 nM to 100 μM), the sensitivity was found to be little bit higher (2.8 mA cm^{-2} mM^{-1}) at Ppy/Au/MEA (Fig. S4). The sensitivity and LOD of Ppy/Au/MEA for AA were compared with performance of MEA modified with poly(ethylene dioxythiophine modified) (50 μm dia.) [35]. The LOD is three fold lower and sensitivity is three times higher than that obtained at poly(ethylene dioxythiophine modified)/MEA.

In order to compare the low detection limit (LOD) on Ppy/Au/MEA and Ppy/Au/MA for AA, the SWVs were recorded under the same experimental conditions in the presence of very low concentrations of AA (Figs. 3B and S3B). The LOD for AA on Ppy/Au/MEA is 10 nM and on Ppy/Au/MA is 5 μM.

These analytical performances were compared to those obtained on different modified electrodes reported recently in the literature (Table 2), [36–43]. To the best of our knowledge, the Ppy/Au/MEA the lowest LOD for AA that never reported earlier using pulse and amperometric techniques and also exhibited higher sensitivity which is comparable with the values reported earlier.

3.5. Simultaneous determination of AA, DA, UA and HA and TA on Ppy/Au/MEA

To test the practical application of Ppy/Au/MEA, the SWV was recorded in the electrolyte containing AA along with neurotransmitters like Dopamine (DA), Histamine (HA), Tyramine (TY) and Uric acid (UA) at physiological pH. Five well resolved independent anodic peaks are observed for AA, DA, UA, TY and HA at −0.15 mV, 190 mV, 350 mV, 610 mV and 770 mV, respectively on Ppy/Au/MEA whereas at bare Au/MEA only one single broad peak with a hump at positive potential is obtained at 210 mV. The anodic peak separation between AA and DA is 205 mV, between AA and UA is 365 mV, between AA and TY is 625 mV and between AA and HA is 785 mV. In this experiment, it is important to note that, the oxidation peak potential of AA appears at 50 mV less positive potential (−0.025 mV) in the presence of neurotransmitters.

This behaviour may be due to the interactions between ascorbate anions and accumulated protonated neurotransmitters within the Ppy film. It is well known that the pK_a values of dopamine are 8.9 (pK_{a1}) and 10.6 (pK_{a2}) [44] and uric acid is (pK_a = 5.4). The pK_a value of the aliphatic amino group of histamine is 9.4 [45]. So in the physiological pH, dopamine and histamine must be existing in fully protonated form whereas uric acid and TY may be existing in partially protonated form. Though conducting polymer and above neurotransmitters are existing in positively charged form in pH = 7, the entrapment of above amino molecules can be occurred through hydrophobic interactions between of the reduced form of conducting polymer (the reduced Ppy-film is hydrophobic) [32] and the above neurotransmitter, since they are aromatic in nature [46].

As the peak separation between AA and TY and between AA and HA are very large, we just ignore interferences of these compounds during the determination of AA (Fig. 4A). So the inference test was carried out only for DA and UA. Fig. S5. shows the SWVs obtained during the simultaneous change of concentrations of the AA, DA and UA at the Ppy/Au/MEA. The calibration curves for AA, DA and

Table 2
Comparison of the analytical data's obtained by modified electrodes proposed for the determination of AA.

Electrode materials	pH	Detection limit (μM)	Linear range (μM)	Sensitivity (μA/μM cm^{-2})	Reference
Modified glassy carbon (GCE) electrode in basic media	7.0	23.38	25–300	0.3255	[36]
Nitrogen doped grapheme modified GCE	7.0	0.92	7.5–180	4.3878	[37]
Nitrogen doped grapheme modified GCE	6.0	2.2	5–1300	0.081	[38]
Electrodeposition of Au-nanoclusters (nano-Au) on poly(3-amino-5-mercapto-1,2,4-triazole) (p-TA) film modified GCE	4.0	1.1	2.1–50.1	3.069	[14]
Hollow nitrogen-doped carbon microspheres modified GCE	7.0	0.91	100–1000	0.4246	[39]
Poly(3-methylthiophene)/Pd, Pt nanoparticle modified platinum electrode	7.4	7.0	10–160	0.75	[40]
Poly(3,4 ethylenedioxythiophene) modified gold electrode	7.0	2.5	5–300	0.87	[35]
Poly (3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxynaphthalene-2,7-disulfonic acid) film modified GCE	4.0	1.43	5–240	0.4331	[41]
Poly(p-toluene sulfonic acid)-modified GCE	7.0	50	100–2000	0.2831	[42]
Poly (vinyl alcohol) covalently modified GCE	7.0	7.6	10–250	7.64	[43]
Polypyrrole modified Au/MEA	7.0	0.01	0.01–2800	2.5	Our report

UA are drawn from the above SWVs data. They show linearity for a wide range of concentrations from 1 μM to 1000 μM, from 0.1 μM to 50 μM and from 0.1 μM to 50 μM, AA, DA and UA respectively. These analytical performances are well suited to the assay of AA in biological fluids like blood serum, as well as in fruits, because, the physiological concentration in blood serum are between 34 and 79 μM for AA along with the coexistence of DA in nanomolar and UA in micromolar [47].

To find out independency in catalytic activities of these analytes, three different experiments were carried out at same experimental conditions. In each experiment, concentration of one of the three analytes was varied and keeping the concentrations of other two constant. The SWVs was recorded in the electrolyte in which the concentration of AA was varied while keeping the concentrations of DA and UA constant as shown in Fig. 4(B–D). The peak current of AA increases linearly up to 2000 μM while the peak currents of the other two remained almost constant. The same experiment was repeated for the other analytes also. So, the peak current of DA and UA increase linearly up to 100 μM each in presence of other two analytes. From the above results it is predicted that peak potential

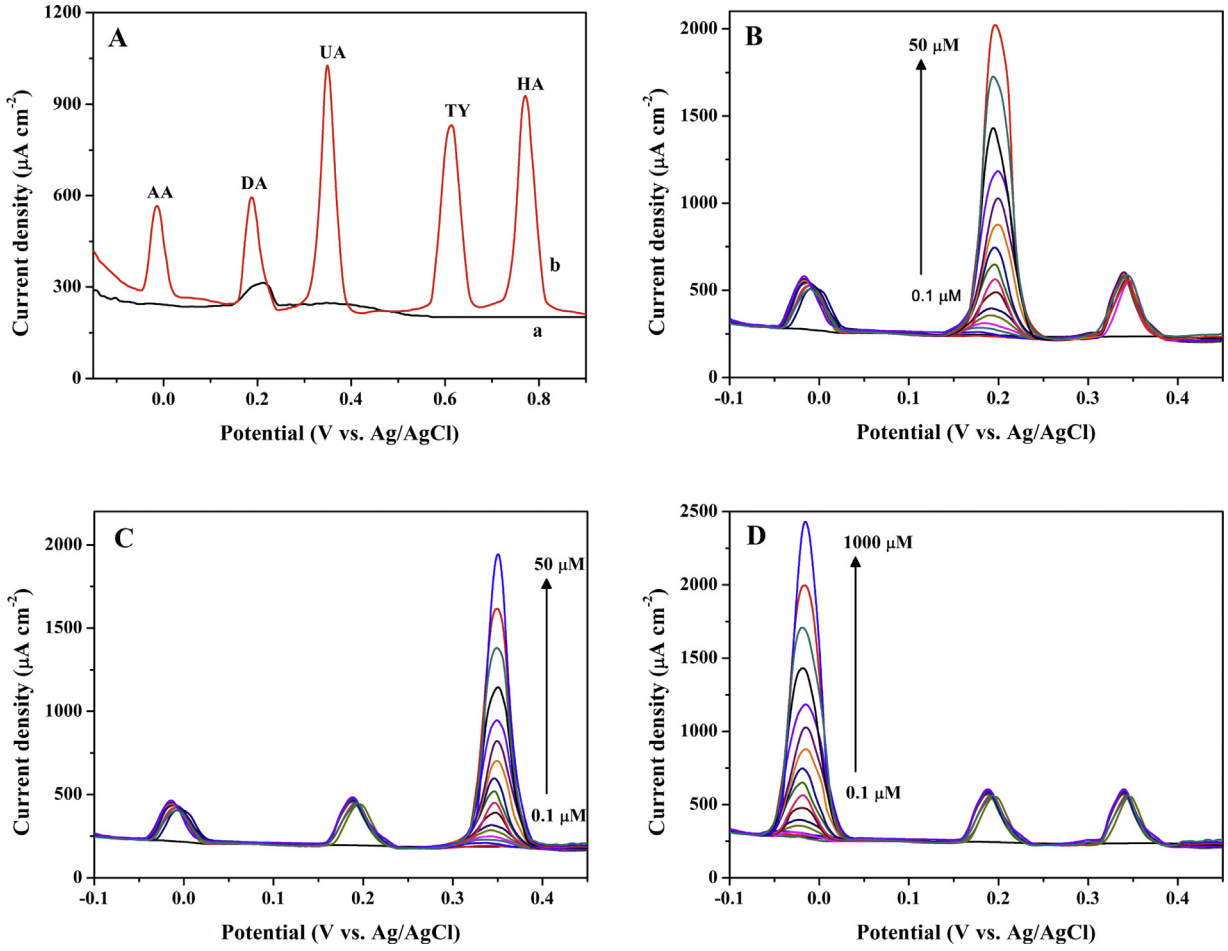

Fig. 4. SWV profiles at Ppy/Au/MEA in 0.1 M PBS for (A) Containing 100 μM AA, 10 μM DA, 100 μM UA, 50 μM TY and 50 μM HA at bare Au MEA (a) and Ppy/Au/MEA (b), respectively. (B) Containing 100 μM AA, 10 μM UA and different concentrations of DA from 0.1 μM to 50 μM, (C) Containing 100 μM AA, 10 μM DA and different concentrations of UA from 0.1 μM to 50 μM, (D) containing 10 μM UA, 10 μM DA and different concentrations of AA from 0.1 μM to 1000 μM.

Table 3
Determination of AA in lemon, human serum and celin tablet.

Sample	Dilution factor	Detected (μM)	Added (μM)	Found (μM)	Recovery (%)
Lemon	500	5	5	9.9	99
Serum	50	4	6	9.9	99
Celin tablet	5000	10	10	10	100

and peak current of AA are found to remain unaffected in presence of DA and UA. Further, the oxidation of AA occurred totally independent and free from interferences of DA and UA at above modified array. Finally, the long term stability of Ppy/Au/MEA electrode was also tested for one month of storage time. It retains its 95% sensitivity after one month period of storage.

The Ppy/Au/MEA has been applied to detection of AA in real samples like serum, lemon juice and celin tablet chewable (300 mg) by SWV method (Figs. S6–S8). Prior to measurements, samples were diluted many times with PBS and then used without any pre-treatment process. The concentration of AA in the real samples and the recovery rates of the spiked AA samples are given in the Tables 3 and S1. The recovery rate is varying between 99% and 102%. The content of AA sensed in the real samples are found to be comparable and better with those repotted in the literatures for lemon [48], serum [49,50] and celin tablet [51].

4. Conclusion

It is concluded that Ppy/Au/MEA and Ppy/Au/MA were fabricated and used for sensing AA. The Ppy/Au/MEA exhibited high sensitivity and very low LOD when compared with earlier report. It also exhibits very high selectivity toward AA with excellent storage and operational stability. Therefore, it is used to fabricate a facile, selective, and rapid biosensor for the precise determination of AA in a large number of real samples. The amount of AA detected in real samples using the above Ppy/Au/MEA is approximately equal to those given in the literature. This modified sensor is also very much suitable for simultaneous determination of DA, HA, TY, UA and AA.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2013.06.049.

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