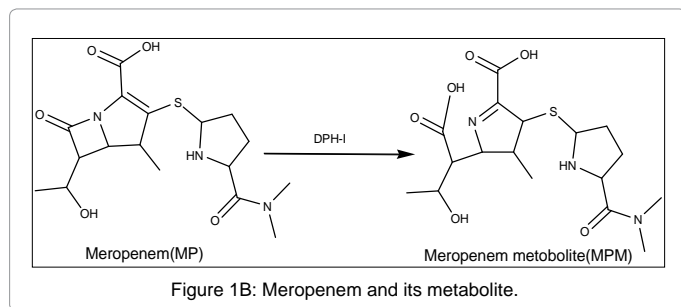


Keywords: Doripenem; Meropenem metabolites; Polyaniline; Graphene; Cyclic voltammetry; Differential pulse voltammetry

Introduction

Doripenem (DP) and meropenem (MP) are new parenteral carbapenems (Figures 1a and 1b). Both are similarly to ertapenem



detected only one metabolite in the urine, the ring-open lactam. Iolanda Cirillo et al. [23] reported the metabolism of doripenem in urine. In this work, the electrochemical behaviour of the metabolites DPM and MPM has been studied, the reduction mechanism has been suggested on the modified electrode and differential pulse voltammetry method have been offered and applied to the determination of DPM and MPM in human urine and serum samples [24].

In the present study, graphene (Gr) was firstly fabricated on a glassy carbon electrode (GCE), then polyaniline by in situ electrochemical polymerized on the graphene modified electrode. Nevertheless, to the best of our knowledge, there is no electroanalytical report concerning the DP and MP metabolites at the graphene (Gr) and polyaniline (PAN) modified electrode.

Materials and Methods

Chemicals and reagents

Doripenem, meropenem metabolites obtained from Aurobindo pharmaceuticals, Hyderabad, AP. N,N c-dimethylformamide (DMF), polyaniline and grapheme (Gr) were obtained commercially from sigma Aldrich (Mumbai, India). Double distilled water was used to prepare all the experimental solutions. Phosphate buffer was prepared using potassium dihydrogen phosphate. All reagents were used as analytical reagent grade.

Apparatus

Electrochemical studies were carried out by Autolab PG STAT101 supplied by Metrohm Autolab B.V. Netherlands. A three electrode system comprising of a glassy carbon electrode modified with polyaniline (PAN) and graphene (Gr) composites as a working electrode. Graphene (Gr) and polyaniline (PAN) obtained from Aldrich. Saturated Ag/AgCl/KCl as a reference electrode and Pt wire as a counter electrode obtained from local scientific labs. Electrode surface morphology study was carried out by SEM instrument model OXFORD INCA PENTA FETX3 CARL ZEISS from Japan. An Elico LI-120 pH meter supplied by Elico LTD, Hyderabad, India was used to determine the pH of the buffer solution.

Preparation of samples

An aliquot containing 2.5×10^{-6} M of DPM and MPM was placed into a 25 ml calibrated flask and 5 ml of buffer solution, pH 6.0 was added. The solution was diluted to the mark with water and mixed well. The solution was transferred into electrolytic cell. A pre-deoxygenating for 10 min with a stream of pure nitrogen up to 2 ml of untreated urine and serum samples containing 2.5×10^{-6} M of DPM and MPM was placed each separately into a 25 ml volumetric flask and diluted with water to the mark. To this 0.5 ml of the solution with 5 ml of pH 6.0 phosphate buffer solution was diluted with water to 25 ml into a

volumetric flask. The voltammograms were recorded according to the above recommended procedure.

Preparation of PAN/Gr/GCE

Before modification, a bare glassy carbon electrode (GCE) with 3 mm in diameter was polished with abrasive paper and rinsed with ethanol and redistilled water to remove the trace remainder. Then, the electrode was cycled in 1 mM $K_3Fe(CN)_6$ solution between 0.3 and 0.5 V (vs SCE) at a scan rate of 100 mV/s until a pair reversible CV peaks were obtained, indicating that the surface of glassy carbon electrode was cleaned. The electrode was again rinsed with redistilled water and cleaned in an ultrasonic bath. A given amount of graphene powder was dispersed in double distilled water with the help of ultra-sonication for one hour, to obtain a homogeneous, well-distributed black solution. A 5 μ L graphene solution was dropped onto the cleaned glassy carbon electrode and dried under air, to obtain a graphene modified glassy carbon electrode, denoted as Gr/GCE. The polyaniline (PAN) film was in situ polymerized on the Gr/GCE by cyclic voltammetry between 0.20 V to 1.50 V at 50 mV/s for 5 cycles in the solution of 0.25 mol/L H_2SO_4 and 0.1 mol/L aniline. The modified Gr/GCE was obtained and it was denoted as PAN/Gr/GCE. Figures 2 and 3 have shown the polymerization and schematic diagram of electrode modification with PAN and Gr composites. Figure 4 shows the typical cyclic voltammogram for 50 cycles of polymerisation of graphene and polyaniline composite doping on glassy carbon electrode.

Results and Discussions

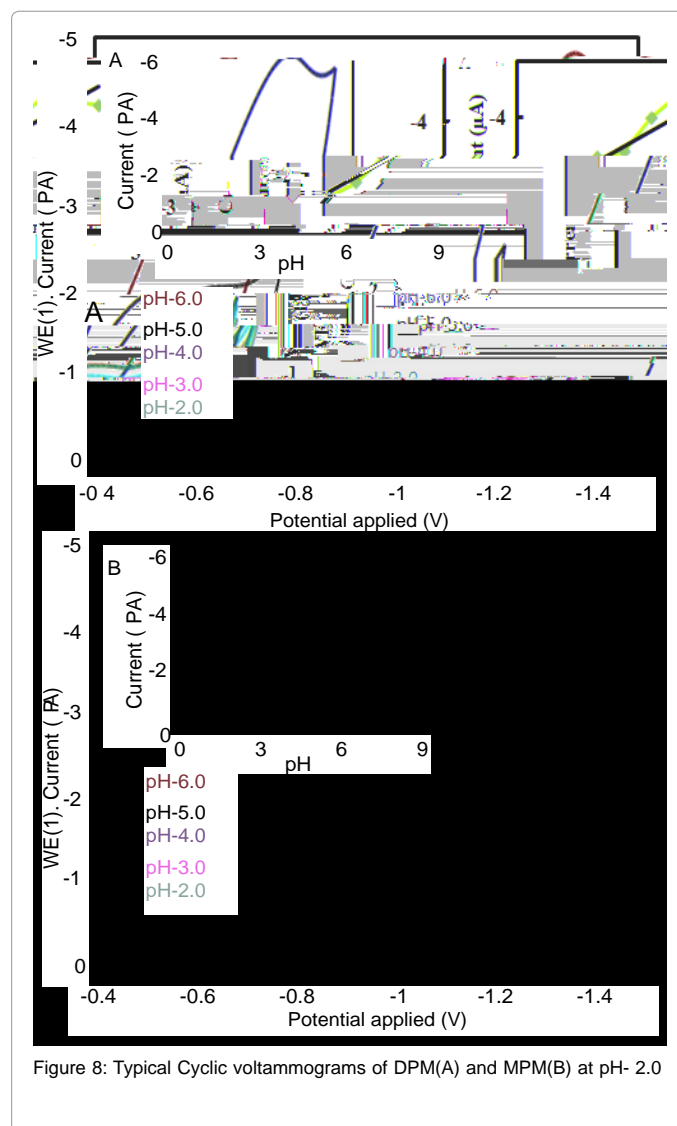
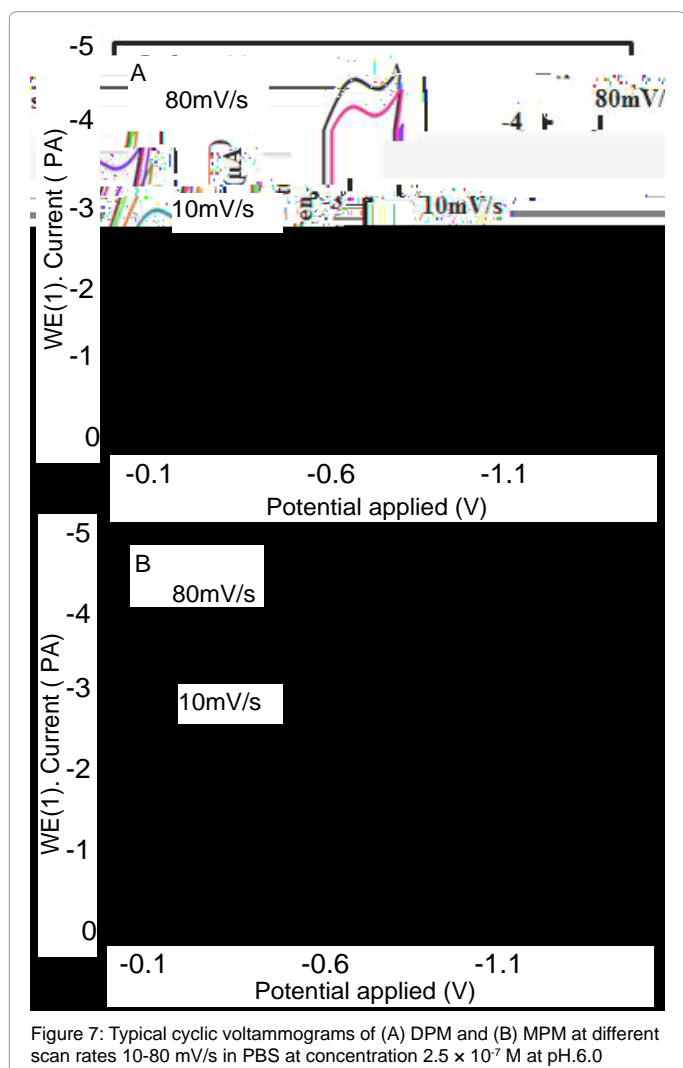
Characterization of PAN/Gr/GCE

The thinning out and bonding of the PAN and Gr composites are most important subject in producing the PAN/Gr composite

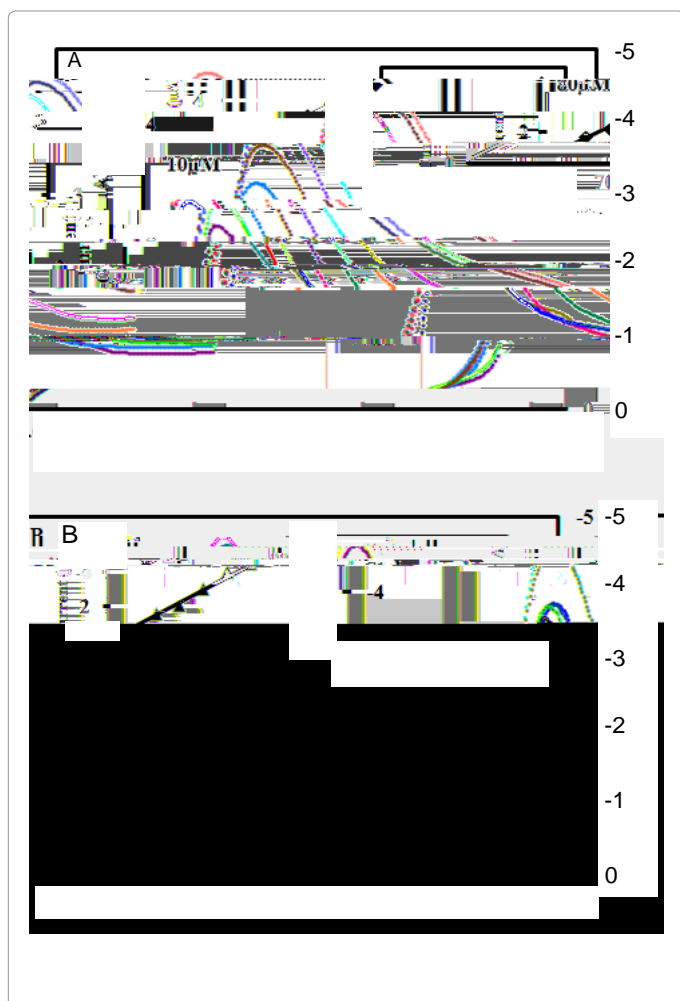


materials. SEM microscopy was used to gain insight into the surface characteristics of the PAN/Gr/GCE composite. Figure 5 shows the SEM image of the PAN/Gr/GCE composite electrode and it can be seen that PAN and Gr was dispersed and distributed surrounded the glassy carbon electrode. Cyclic voltammetry (CV) is one of the most versatile electrochemical techniques used in the study of electroactive behaviour and the characterization of sensors. In order to determine the electroactive surface area of the PAN/Gr/GCE composite electrode, the electrochemical behaviour of potassium ferrocyanide ($[Fe(CN)_6]^{4-}$) in 1 M KNO_3 supporting electrolyte was studied using cyclic voltammetry recorded at different scan rates. According to the Randles-Sevcik Equation:

the accumulation of the metabolites. The CV curve of PAN/Gr/GCE shifted to more negative current at pH 6.0. There is no corresponding oxidation peaks observed at the reverse scan, indicating that the electrochemical reduction of DPM and MPM were totally irreversible reaction under the above experimental conditions. The suggested reduction mechanisms of the metabolites are shown in scheme 1 and scheme 2.



This occurrence should be attributed to the reduction medium of deposition and the surface area was significantly increased in the resulting nanocomposite, suggesting the effect of PAN/Gr/GCE composite provides efficient results for the electrochemical reaction of DPM and MPM with enhanced voltammetric response. In sequence about the mechanism of electrochemical reactions can be determined from the relationship between scan rate and peak current. Therefore,



standard deviation of residuals and 'a' is the slope of the calibration plot). The LOQ was found to be 4.2×10^{-10} M and 2.4×10^{-10} M for DPM and MPM respectively. The relative standard deviation (RSD) of DPM and MPM were 2.45% and 3.52% in human urine samples and the RSD of DPM and MPM in human serum samples were 2.52% and 4.25%. The peak potentials and peak heights of the given compounds at concentration 2.5×10^{-10} M was compared at GCE modified electrode shown in Figure 9.

Conclusion

The studies have shown that the peak potentials and detection limits at modified glassy carbon electrode (PAN/Gr/GCE) for the determination of DPM and MPM are -1.25 mV and -1.10 mV, and 3.5×10^{-10} and 1.75×10^{-10} M respectively and the peak potentials, detection limits at bare glassy carbon electrode are -1.12 mV and -1.0 mV and 5.0×10^{-10} and 6.2×10^{-10} M respectively. The lower value shows that the modified glassy carbon electrode is superior to bare glassy carbon electrode. Further, it is believed that this alternative approach of determining DPM and MPM by differential pulse voltammetry is convenient, faster and accurate. It is thus better than on hand spectrophotometric and chromatographic methods which are pricey and time consuming. Thus it can be said that this sensor (PAN/Gr/GCE) is a useful addition in the field of analytical chemistry for the determination of drugs and their metabolites.

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