Graphite Electrodes Modified With Polysaccharides-Osmium (VI) Tmen Layer Catalyzing the Electrochemical Oxidation of Dopamine, Ascorbate, and Urate, Forming a Multi-Analyte Sensor

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Abstract

Mannan was modified with Os(VI)N,N, N',N' tetramethylethylenediamine complex to form a compound denoted Man-Os(VI)tmen and was cast on graphite electrodes to make a modified electrode (PGE/Man-Os(VI)tmen) and used as mediator to catalyze the electrochemical oxidation of compounds such as: dopamine (DA), uric acid (UA) and ascorbic acid (AA). A catalytic current was exhibited for the PGE/Man-Os(VI)tmen for oxidation of DA, UA and AA when studied with differential pulse voltammetry and cyclic voltammetry and compared with the response at bare graphite electrodes. For all the three analytes a higher current response was obtained and at lower over-potentials, however, the PGE/Man-Os (VI)tmen shows a distinct catalytic for oxidation of DA, while this response was lowest than that for UA or AA. The performance of PGE/Man-Os(VI)tmen showed an excellent sensitivity towards DA 110.500 ± 0.017 μA.μM^{-1} in comparison with that for UA and AA, which were 39.793 ± 0.042 and 20.616 ± 0.395 μA.μM^{-1} respectively, with lower limit of detection (3 SD/slope) for DA, UA, and AA of 0.003, 4.0 and 9.0 μM respectively. Additionally, a hybrid film consisting of a mixture of DA and Man-Os (VI)tmen was applied on the modified PGE, producing a modified electrode (PGE/DA-Man-Os(VI)tmen) showed long-term stability. Moreover, scanning electron microscopy of both the PGE/Man-Os (VI)tmen and PGE/DA-Man-Os(VI)tmen modified electrodes pointed out a morphological structure, where both electrodes offered a porous nature..

Keywords: ascorbate, dopamine, Man-Os (VI)tmen film-coated electrodes, urate,

1. Introduction

Substantial efforts have been dedicated to develop electrochemical sensors based on chemically modified electrodes, CMEs [1-4]. Such investigations have established that coating of electrode surfaces with polymer films is an attractive means of increasing the sensitivity and selectivity of electrodes suitable for voltammetric analysis [5, 6]. Various approaches have been proposed, involving electrostatic accumulation [7] complexation [8], adsorption-extraction [9], bioaccumulation [10] and covalent reaction [11]. CMEs have also been improved as convenient electrochemical sensors for determination of neurotransmitters both *in vivo* and *in vitro* [12-14]. Electrochemical sensors based on graphite electrodes have demonstrated to be simple, rapid, sensitive, and with easily renewed surfaces.

However, there is an observed overlap in the voltammetric response for dopamine (DA), uric acid (UA), and ascorbic acid (AA) at bare graphite electrodes [15, 16]. Thus sensors based on optimal mediators have to display higher catalytic currents and lower operating potentials, minimizing interfering reactions at the electrode surface. As an attempt to propose such a mediating layer, it is important to refer to the initial work reported from this laboratory, where it was proven that pyrimidine bases in DNA and RNA can be modified by osmium tetroxide (Os(VIII)L) complexes (where L stands for a nitrogenous ligand) forming covalently bound electro active labels [17, 18] . It was also shown that such complexes represent chemical probes of the DNA structure at single nucleotide resolution in vitro [18] and in cells [19]. Os(VI)L binding to diols of carbohydrates [20] was used for modification of electro inactive carbohydrates, easily transforming them into electro active species producing reduction and oxidation signals at mercury, carbon, or gold electrodes [21, 22].

Moreover, Os(VI)L complexes bind specifically to the ribose at the 3'-ends of RNAs (but not to DNA) making thus possible easy determination of RNAs and particularly of micro RNAs [23]. Recently, it was shown that using Os(VI)tmen, glycans can be detected directly in glycoproteins at carbon electrodes [24]. Osmium-based redox polymers have been used for decades e.g. [25-27] to "wire" various substances (including enzymes) to various electrodes. Here, we introduce a new type of such a polymer, which is based on a simple reaction between a polysaccharide (PS) and an Os(VI)complex with N,N,N',N'- tetramethylethylenediamine (tmen) forming [PSs-Os(VI)tmen] [28]. Properties of the new osmium redox films can be adapted for a given purpose by changing the ligands in the Os(VI)complexes (reviewed in [29, 30]) and/or polysaccharide lengths and compositions. In this approach, we just used (tmen), as a ligand and mannan (Man) as a PS to produce Man-Os (VI)tmen.

A number of different ligands have been shown to form Os (VI) L complexes binding to PSs. Such nitrogenous ligands can be composed of aromatic rings and/or carry an electric charge. Thus Os (VI)-modified PSs can be prepared possessing different properties and offering a new version of adducts-polymer, which could be adopted as mediator in the electrochemical analysis of biologically important compounds, such as DA. To the best of our knowledge, no information is available about using such a polymer as a mediator in this field. The major goal of this work was to investigate whether Man-Os(VI)tmen modified graphite electrode can electrocatalyze the oxidation of DA, UA, and AA successfully. The Man-Os(VI)tmen modified electrode shows a decrease in the operating potential and an increase in the catalytic current, with a distinct affinity for DA oxidation. The observed stability of the obtained PGE/Man-Os(VI)tmen was restricted to 3-4 h. Accordingly, it was improved after covering the surface of the PGE with both DA and Man-Os(VI)tmen to form a hybrid of DA-Man-Os(VI)tmen-film.

2. Experimental

2.1. Chemicals and equipment

Lyophilized yeast mannan (Man) from *Saccharomyces cerevisiae* (Sigma cat. no. M7504, MW in range from 34,000 to 62,500 Da), consists of a backbone of α-1,6-linked mannose units with short 1 to 3 mannose units branches attached by α-1,2- and α-1,3-linkages [31, 32], and potassium osmate dihydrate were obtained from Sigma-Aldrich (St-Louis, MO, USA), N,N,N´,N´-tetramethylethylenediamine (tmen) from (Merck Darmstadt, Germany), dopamine hydrochloride from (Steinheim, Germany), ascorbic acid from ICN Biomedical Inc. (Malmö-Stockholm, Sweden), uric acid from Sigma-Aldrich, 0.1 M phosphate buffer solution (PBS, pH7), was prepared from sodium dihydrogen phosphate, purchased from BDH Analar, VWR International Ltd. (Poole, UK), and disodium hydrogen phosphate dehydrate from Sigma-Aldrich. The redox polymer, Man-Os (VI)tmen was synthesized as reported previously [28]. All aqueous solutions were prepared with purified water in Milli-Q water purification system (Millipore. Bedford, MA, USA).

Cyclic voltammetry (CV) experiments were performed with an Autolab PGSTAT30 (Utrecht, The Netherlands) equipped with GPES 4.9 software using an Ag|AgCl|(KClsat.) as reference electrode, a platinum foil counter electrode and a modified pyrolytic graphite electrode PGE [33] (Ringsdorff Werke, GmbH Bonn, Germany) as working electrode. Scanning electron microscopy (SEM) measurements were done using a DEM-instrument, Hitachi SU3500, at secondary electron detector (SE-detector), 5kV in a high vacuum, a pH meter 827 (Metrohm, Switzerland). Nitrogen gas was purged through the solution cell for at least 20 min prior to experiments. For all results given four equivalent electrodes were used.

2.2. Electrode modification process:

PGEs were polished with emery SiC paper (Tufback Durite, P1200), and then rinsed thoroughly with Milli-Q-water. After being dried, 2 µl of a freshly prepared 0.02 mg.µl^{-1} Man-Os(VI)tmen solution was drop cast onto the polished end of the PGE to prepare the modified electrode (PGE/Man-Os(VI)tmen). The second type of modified electrode was prepared by thorough mixing of 4 µl of a 100 µM DA solution with 2 µl of a 0.02 mg.µl^{-1} Man-Os(VI)tmen solution and then evenly spread on the PGE surface. The modified electrodes were left at room temperature for 15 min until complete dryness.

3. Results and Discussion

3.1. Electrochemical characterization of PGE/Man-Os (VI) tmen

Fig.1. A shows the cyclic voltammograms of PGE/Man-Os (VI)tmen in 0.1 M PBS at pH7. The CV shows two anodic current peaks Ia_1 and IIa_2 at -500 and 125 mV respectively, and two cathodic peaks current, I_{c1} and $II_{c2,}$ at -625 and -450 mV respectively, which is in agreement with previous studies [28, 34]. The nature of the redox reactions giving rise to the two redox waves might be attributed to the oxidation state of $Os^{+4}|Os^{+6}$, however, no direct evidence has been reported up to now [34] proving the adsorption of the modified layer on the electrode surface [34]. The more positive redox wave of the CVs is broad with its anodic peak current located between 0-500 and its cathodic peak around -450 mV. The formal potential, $E^{0'}$ equal to the mean value of anodic and cathodic peak potentials [$E^{0'} = ½ (E_{pa} + E_{pc})$], was -220 mV, this wave covers the range of the oxidation peak currents of DA, AA and UA see Figs. 1B-D which might be a reasonable explanation for the reactivity of this polymer towards these donor compounds, see below.

3.2. Electrocatalytic oxidation of DA, UA and AA at PGE|Man-Os (VI)tmen

Differential pulse voltammetry (DPV) was used the difference to compare bare PGE and PGE|Man-Os(VI)tmen in plain 0.1 M PBS buffer at pH7 and in buffer containing 50 µM DA, 0.5 mM UA, or 2 mM AA. Figs. 1B-D illustrate the DPVs for bare PGE in plain buffer where virtually no oxidation peaks are seen and bare PGE in solution containing the analytes 50 µM DA, 0.5 mM UA, or 2 mM AA yielding peak currents of 10, 15, and 18 µA for DA, UA, and AA respectively. For the PGE|Man-Os (VI)tmen in plain buffer there is somewhat higher background current and some electrochemical activity of the Man-Os(VI)tmen can be traced around -100 mV. In the presence of the analytes, sharp well-defined anodic peaks appeared between 0 and 500 mV for DA and UA with peak current of 27.16 and 22.50 µA respectively Figs. 1B, C and a broader peak current of 22.5 µA between -200 and 200 mV for AA Fig. 1D.

Thus, Man-Os(VI)tmen gives clear evidence for its ability to catalyze the electrooxidation of DA distinctly, among UA and AA, whereas less for AA. The results in Fig. 2 were calculated to show two linear ranges for DA oxidation starting between 0.15 and 2.4 nM followed by one between 0.0024 and 20 µM, covering a wider range than the linear dynamic concentration in biological systems [35] and also showing the lowest LOD (3SD/slope) of 3.9 nM and the highest sensitivity 110.5 ± 0.007 ± µA.µM^{-1} Fig. 2A. Whereas adequate analytical characteristics were obtained as 0.002 - 17.8 µM , 39.793 ± 0.042 µA.µM^{-1} and 4 µM linear range, the sensitivity, and LOD respectively for UA see Fig. 2B and 0.5 – 60 µM, 20.1616 ± 0.395 µA.µM^{-1}, and 9 µM linear range, the sensitivity, and LOD for AA respectively, as in Fig. 2C. Hence, it can be estimated that the Man-Os(VI)tmen based modified electrode has a higher performance towards the catalytic oxidation of DA versus the other analytes species, UA and AA.

3.3. Cyclic voltammetric of DA, UA and AA at PGE|Man-Os (VI) tmen and PGE|DA-Man-Os (VI) tmen

The electro catalytic behavior of the PGE|Man-Os(VI)tmen was evaluated for the oxidation of the analytes species by cyclic voltammetry. Fig. 3 shows the CVs for bare PGE in the absence and presence of 4 mM of DA, UA, AA in 0.1M PBS, pH7 at a scan rate of 50 mV Figs. 3A-C respectively. No redox peaks appeared in the absence of analytes, while clear anodic peaks were observed with peak currents of about 150, 90, 85 µA, respectively in the presence of these analytes, whereas the PGE/Man-Os (VI)tmen in the absence/presence of analytes, showed anodic peaks current at 300, 150, 100 µA respectively, all with a 50 mV negative shift in the peak potential. The behavior of the CVs confirms again the distinct catalysis of Man-Os (VI)tmen film for the oxidation of DA, and the reason for that may be related to appropriate diffusion of DA into the film of the PGE|Man-Os(VI)tmen.

DA has an aromatic ring structure with resonance hybrid that can be easily oxidized [36] therefore the intermediate state could be easily stabilized by an electrostatic interaction with the Man-Os(VI)tmen redox center, while UA and AA are non- aromatic compounds, for which the intermediate state has a higher energy. These rules might give us sufficient evidence to explain the excellent electro catalytic effect of the PGE|Man-Os(VI)tmen for the oxidation of DA among the other two analytes, producing a desirable sensor that could be successfully adopted as an alternative tool in neurotransmitter measurements. However, the stability of this sensor was finite with 3-4 h. Concerning that, the lifetime of this sensor was improved by using a hybrid film of DA and Man-Os(VI)tmen resulting in a PGE/DA-Man-Os(VI)tmen-film.

This surface was evaluated using CV in the absence and presence of DA, UA, and AA. Anodic peak currents appear at 550, 230, 200 µA with a noticeable negative shift yielding peaks at 70, 100, and 60 mV, respectively. Then further studies were achieved on both types of modified electrodes; PGE/Man-Os (VI)tmen and PGE/DA-Man-Os(VI)tmen to evaluate the morphologic structure, by using scanning electron micrographs (SEM) Figs. 4A and B. As is shown both surfaces show a porous nature, thus there are no negative effects on the morphologic structure resulting from the incorporation of DA within the Man-Os (VI)tmen polymer. Hence, we can state that such a surface would facilitate diffusion of analytes within the PGE|Man-Os (VI)tmen film modified electrode surface in a neutral electrolyte. On the other hand the structure of the DA-Man-Os(VI)tmen film might offer a synergistic effect between DA and the redox center of Man-Os(VI)tmen at the electrode producing a stable surface. Fig. 4C summarizes our speculation about this surface, on the basis of the following principles; AA and UA exist in their anionic form at pH7, since the pK_a values for AA is 4.1 [37] and that of UA is 5.4 [38] whereas the pK_a value for DA is 8.87 [36]. Thus DA exists in a cationic form and such a surface character may provide with an effective platform for nucleophiles attack.

3.4. Stability

The activity of the PGE|DA-Man-Os(VI)tmen was measured via a constant flow of a 0.1 µM DA solution through the flow system for 15 h see Fig. 4D which displays a relative current response versus time. This plot shows that a 50 % of the initial response was retained after 15 h of continuous operation, in comparison with a 100 % during the first 3 h of operation. It was shown a slow decrease in the current response for the PGE|DA-Man-Os(VI)tmen during 15 h. It can be concluded that simple adsorption, in combination with a sufficient electrostatic binding between the redox centers of a Man-Os(VI)tmen and DA, at the PGE surface was the reason of this improvement in stability, compared with the PGE|Man-Os(VI)tmen, which shows no guarantee to be active longer than 3 h under the same operational conditions.

4. Conclusion

This work demonstrated that Man-Os(VI)tmen modified graphite electrodes, have shown through DPV and CV measurements a higher current and a lower operating potential compared with bare PGE in the presence of the analyte species; DA, UA and AA, offering prominent catalytic oxidation signal towards DA compared with the other two analytes, UA and AA. Moreover, the stability of the proposed multi-analyte sensor was improved when a hybrid PGE/DA-Man-Os (VI)tmen-film was adopted. This new insight was shown to be a suitable surface for nucleophilic attack at pH7. Furthermore SEM images proved that the Man-Os (VI)tmen polymer retained a porous nature after combination with DA. Thus this multi-analyte sensor might be offering a feasible alternative in detection of each of the three analytes at a distinct sensitivity.

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Figure list of Manuscript and supplementary results within captions:

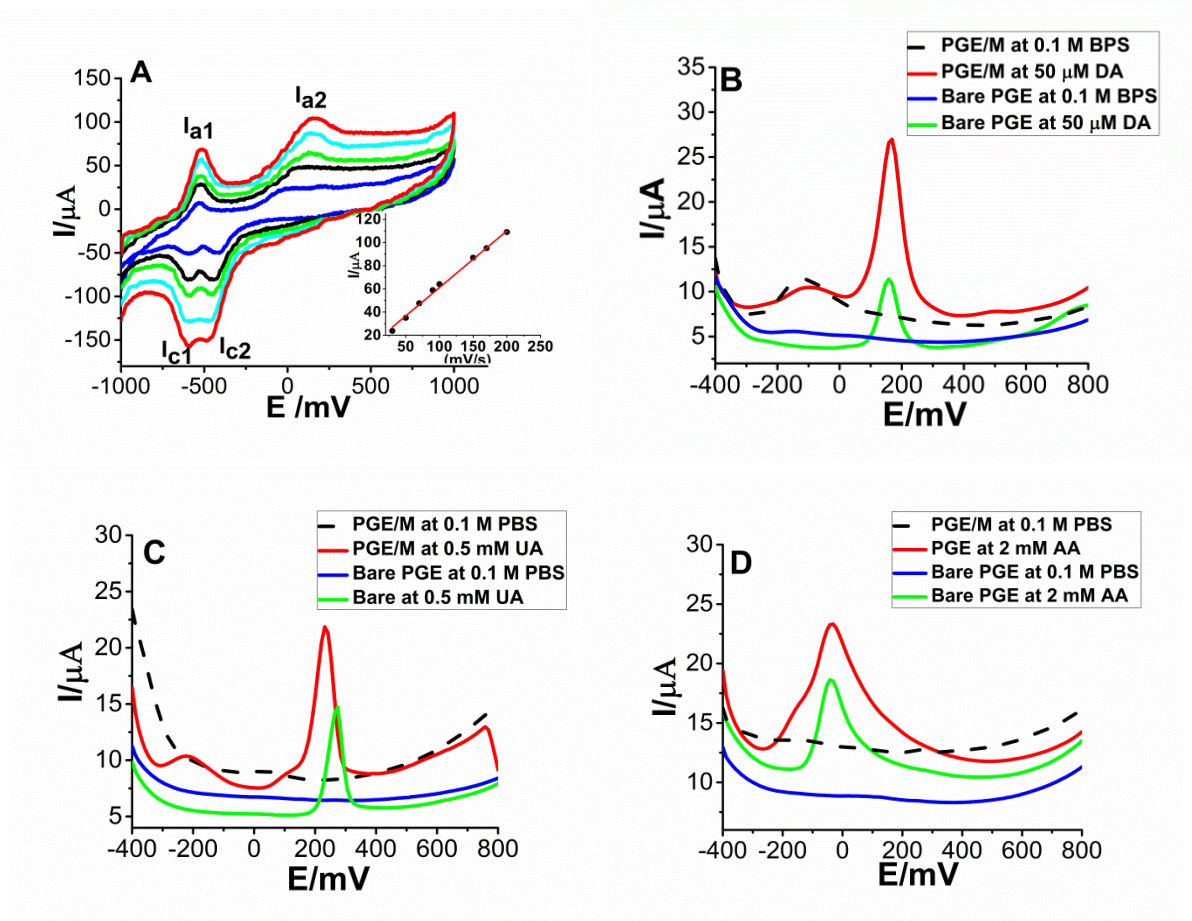
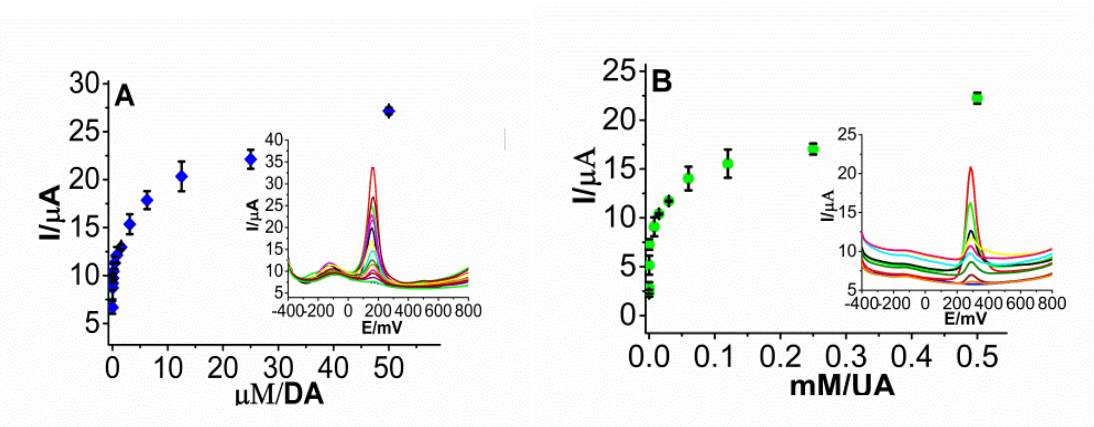


Figure 1): (A) cyclic voltammograms of the modified PGE|Man-Os(VI)tmen in 0.1M PBS (pH7), Inset plot, anodic peak current (Ia2) at about 125 mV vs. different scan rates (30, 70, 100, 150 , and 200) mV.s-1, from blue to red color respectively, (B) DPV of PGE|Man-Os(VI)tmen at: 0.1 M PBS, pH7 (black dashed line), then in a 50 µM DA (red line), and bare PGE at 0.1 M PBS, pH7 (blue line) and then in a 50 µM DA(green line) respectively, same measurement where repeated for (C) and (D) in the presence of 0.5 mM UA and 2 mM of AA respectively, with same condition that described in (B).



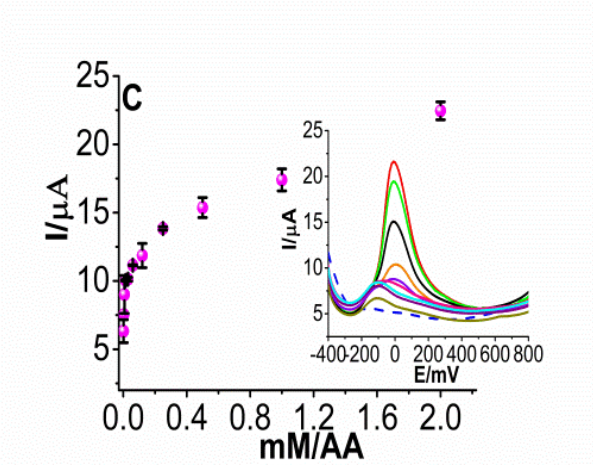


Figure 1):Calibration curve of the modified PGE|Man-Os(VI)tmen based on DPV measurements inset plot shown for the three analytes in PBS, pH7, at which (A) in the concentration range of DA (0.1 nM – 50 µM), (B) UA at the concentration range of a (0.15 nM – 0.5 mM), and (C) AA at the concentration range of a (0.004 µM – 2 mM). Scan rate: 50 mV s^{-1} vs Ag|AgCl|(KCl sat.) reference electrode.

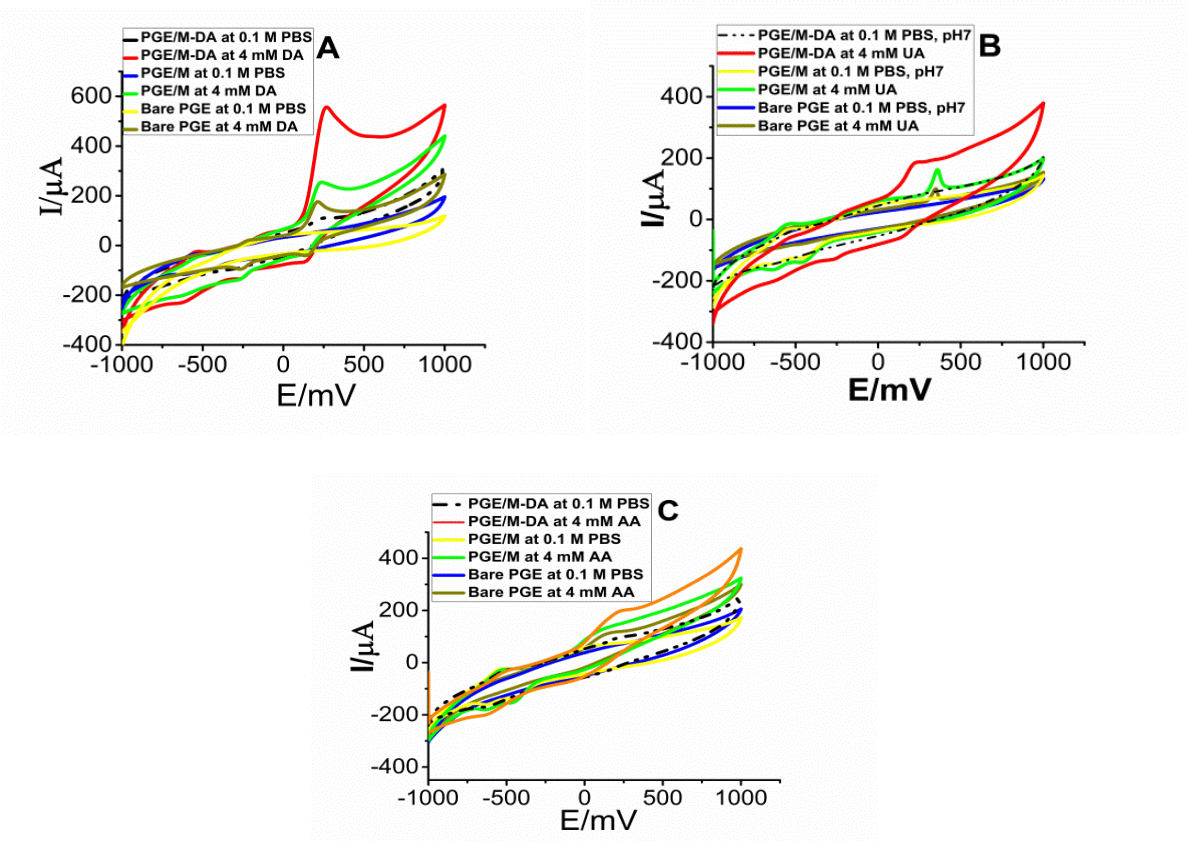


Figure.2): (A, B, C) cyclic voltammograms of bare PGE at 0.1M PBS pH7 (yellow lines), then in the analytes of 4 mM DA, UA, AA (brown lines) respectively, PGE/Man-Os(VI)tmen modified electrode in the absence of analytes at 0.1M PBS (blue lines), and then the presence of analytes of 4 mM DA,UA and AA in 0.1M PBS (green lines), and the PGE/DA-MPO-Os(VI)tmen modified electrode in a 0.1M PBS (black dashed lines) then in the analytes of 4 mM DA ,UA and AA (red lines) respectively, vs. AglAgCl| (KCl sat.) reference electrode, and 50 mV/s scan rate.

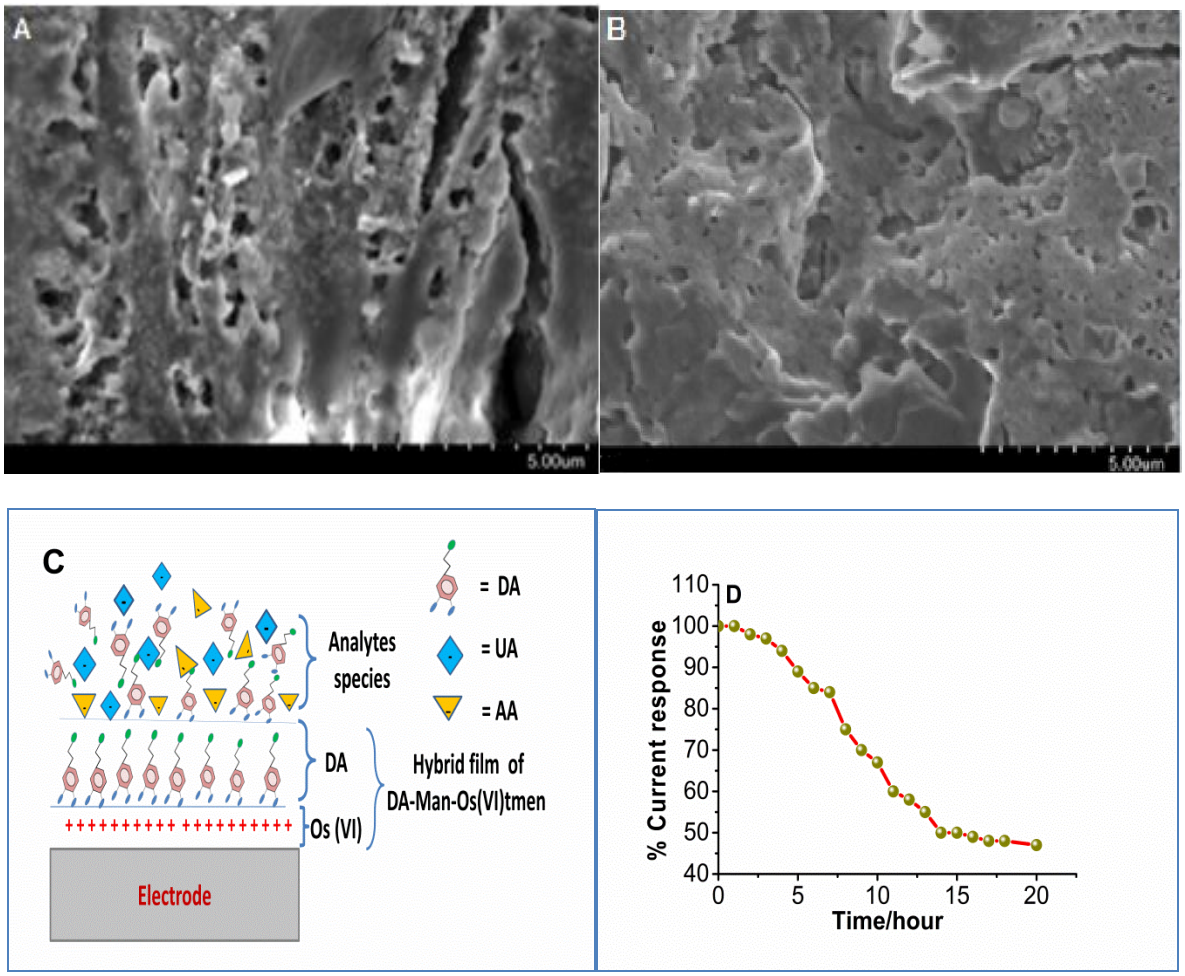


Figure 3): (A) SEM for PGE|Man-Os(VI)tmen modified electrode. (B) PGE|DA-Man-Os(VI)tmen electrode, magnification; $8000_{X,}$ accelerating voltage; 5 Kv: (C) scheme diagram represent the structure of DA-Man-Os(VI)tmen hybrid film based modified PGE surface within analytes species; DA, AA and UA; (D) stability of modified PGE|DA-Man-Os(VI)tmen electrode that measured by flow system in a 0.1 µM DA solution at 200 mV vs Ag|AgCl (0.1M KCl), for 20 hours.