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Nanocomposite Materials for Neurotransmitters and Nucleotide Bases Sensors

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Abstract :

Conductive composite films with the combination of nanomaterials such as multi-walled carbon nanotubes (MWCNTs), nano platinum (Pt), nano gold (Au), nafion (NF) and hydroxypropyl- β -cyclodextrin (HP β CD) as catalysts have been synthesized on glassy carbon, gold and indium tin oxide electrodes (ITO) by potentiostatic methods. Both, the cyclic voltammetry and differential pulse voltammetry have been used for the measurement of electroanalytical properties of neurotransmitters and nucleotide bases by means of nanocomposite film modified electrodes. The presence of MWCNTs in the composite films enhances the active surface coverage concentration of catalysts. The presence of nanoparticles in the films enhances the functional properties and produces an overall increase in the sensitivity of the modified electrodes. One such composite film (MWCNTs-NF-PtAu) exhibit promising catalytic activity towards the oxidation of mixture of biochemical compounds such as ascorbate anion, epinephrine and urate anion in aqueous buffer solution (pH 6.75). Well separated voltammetric peaks have been obtained for ascorbate, epinephrine and urate anions with the peak separations of 0.222 and 0.131 V. Another type of composite film (MWCNTs-Au-HP β CD) exhibits promising electrocatalytic activity towards the oxidation of tyrosine (TYR), guanine (GU), adenine (AD) and thymine (THY) present in pH 7.4 aqueous solutions. Well separated voltammetric peaks have been obtained between TYR and GU (80 mV), GU and AD (290 mV), AD and THY (185 mV). Electrochemical quartz crystal microbalance, scanning electrochemical microscope, atomic force microscope and scanning electron microscope techniques have been used for the electrochemical characterizations and surface morphology studies.

Keywords : Multiwall carbon nanotubes; composite film; modified electrodes; electrocatalysis; neurotransmitters; nucleotide bases.

1. Introduction :

Ascorbic acid (AA), being a constituent of the cell, has momentous biological functions and is an analyte of great importance because it is vital to immune response and wound healing [1]. It is found in fruits, vegetables and in beverages. Epinephrine (EP) is one of the

important catecholamine neurotransmitter in the mammalian central nervous system, which plays a very important role in the function of central nervous, renal, hormonal and cardiovascular systems. In mammalian central nervous systems, tyrosine (TYR) plays as a precursor of hormones or neurotransmitter. The absence of tyrosine could cause albinism and alkaptonuria, while a high tyrosine concentration results in increased sister chromatid exchange [2]. Uric acid (UA) and other oxypurines are the principle final products of purine metabolism in the human body [3]. Abnormal levels of UA are symptoms of several diseases, including gout, hyperuricemia, etc [4]. Similarly, guanine (GU), adenine (AD) and thymine (THY) are the important components found in deoxyribonucleic acid. Determination of individual concentrations of guanine, adenine and thymine or their ratio in DNA is important for the measurement of the nucleic acid concentration itself [5]. The oxidation of these compounds is interesting as this process occurs in the human body. Due to their crucial role in neurochemistry and industrial applications, several traditional methods have been used for its determination. Among these, the electrochemical methods have more advantage over the other methods in sensing the neurotransmitters in living organisms [6]. But, the electrochemical analysis on the unmodified electrodes, for example GCE has limitations because of the overlapping of oxidation potentials of the analytes which, often cause a pronounced fouling effect that results in rather a poor selectivity and reproducibility [7,8]. In this article we report novel nanocomposite films (MWCNTs-NF-PtAu and MWCNTs-Au-HP β CD) made of functionalized multiwall carbon nanotubes (MWCNTs) with NF, nano Pt and nano Au, along with its characterization and its enhanced catalytic activity towards biochemical compounds even at very low concentrations. The film formation processing involves the modification of glassy carbon electrode (GCE) with uniformly well dispersed MWCNTs aqueous solution and then coated with a uniform thin layer of NF or bimetallic Pt and Au or HP β CD which were electrochemically deposited from an aqueous solution mixture.

2. Experimental :

2.1. Materials, apparatus and composite modified electrode preparation :

Electrochemical experiments were done using CHI-400 and CHI-900 potentiostats. A conventional three-electrode cell assembly consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode were used for the electrochemical measurements. The working electrode was either an unmodified GCE or a GCE modified with the composite films; all the potentials were reported versus the Ag/AgCl reference electrode. The working electrode for electrochemical quartz crystal microbalance (EQCM) measurements was an 8 MHz AT-cut quartz crystal coated with a gold electrode. The diameter of the quartz crystal was 13.7 mm; the gold electrode diameter was 5 mm. The morphological characterization of composite films was examined by means of SEM (Hitachi S-3000H) and AFM (Being Nano-Instruments CSPM4000). A Pt ultramicroelectrode (UME) of 7 μ m diameter was employed as a scanning electrochemical microscopy (SECM) tip. The dispersion of MWCNTs have been done using previous studies [9]. Before starting each experiment, the GCEs were polished by a BAS polishing kit with 0.05 μ m alumina slurry, rinsed and then ultrasonicated in double distilled deionized water. The GCEs studied were uniformly coated with 6.4 μ g cm⁻² of MWCNTs, dried and then 2 μ l of NF or HP β CD, dried at about 40° C. The concentrations of homogeneously dispersed MWCNTs and NF or HP β CD were exactly measured using micro-syringe. The electrochemical deposition of nano Pt and nano Au were done using electrochemical reduction of the K₂PtCl₆ and KAuCl₄.3H₂O (1 mM and 2 mM respectively) on the MWCNTs modified GCE in 0.5 M H₂SO₄ aqueous solution. It was performed by consecutive CV over a suitable potential range of 1.5 to -0.15 V.

3. Results and discussions :

3.1. Electrochemical and topographic characterizations of MWCNTs-NF-PtAu film :

For a detailed comparison, various films PtAu, NF-PtAu, MWCNTs-PtAu and MWCNTs-NF-PtAu as shown in Fig. 1 were prepared using 0.5 M H₂SO₄ aqueous electrolyte and were studied using cyclic voltammograms method in pH 6.75 of 0.1 M phosphate buffer solution. The CV Fig. 1(a) of NF-PtAu film with two peak currents $I_{p_c} = 38.70$ and $39.78 \mu\text{A}$ for Pt and Au reduction respectively, has lower peak current magnitude comparing to that of MWCNTs-NF-PtAu composite film. Fig. 1(b) shows the CV for nano PtAu film only, where $I_{p_c} = 75.0$ and $24.63 \mu\text{A}$ for Pt and Au respectively. By comparing (a) and (b) it was clear that the NF plays an important role in reducing the peak current magnitude of Pt, increasing the reduction peak current of Au and balancing the reduction peak currents of both Pt and Au. Similar cases were found in (c) MWCNTs-NF-PtAu and (d) MWCNTs-PtAu too. Among these, for (c) the $I_{p_c} = 98.30$ and $77.18 \mu\text{A}$ for Pt and Au respectively and for (d) the $I_{p_c} = 133.50$ and $63.07 \mu\text{A}$ for Pt and Au respectively. Further, in (c) and (d) the magnitudes of the currents were higher comparing to that of (a) and (b).

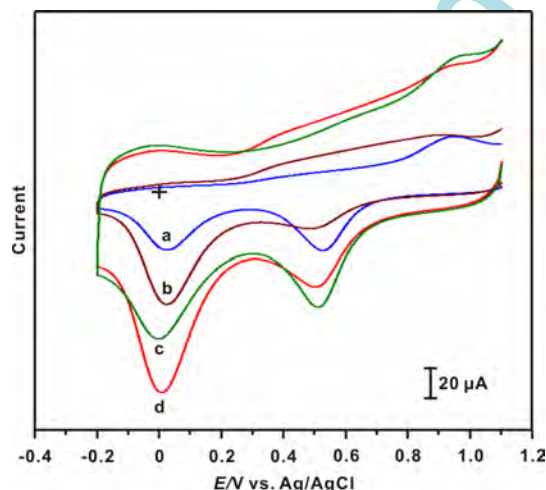


Fig. 1. CVs of the (a) NF-PtAu, (b) PtAu, (c) *f*-MWCNT-NF-PtAu composite film and (d) *f*-MWCNT-PtAu in 0.1 M phosphate buffer pH 6.75 aqueous solution : scan rate 100 mVs^{-1} . (Reproduced with permission from Yogeswaran et al. Anal. Biochem. 365 (2007) 122-131).

These results show that due to their large surface area, the MWCNTs play an important role in increasing the current magnitude and activity of the composite film. The oxidation peak current $I_{p_a} = -91.30 \mu\text{A}$ of Au was also higher in (c) when compared with all other films. These studies prove that the MWCNTs and NF enhance the activity and the current magnitude of the MWCNTs-NF-PtAu composite film. From the EQCM results, the increase and decrease in frequency of the gold electrode during reduction and oxidation of Pt and Au are confirmed. The mass change during Pt and Au incorporation on the MWCNTs-NF modified gold electrode for total cycles is $20.51 \mu\text{g cm}^{-2}$. The topographic characterization (figure not shown) indicates the particle size distribution of Pt and Au, which varies from 200-300 nm and 50-80 nm respectively. From the horizontal cross section of the topographic images we could clearly notice the approximate thickness of NF-PtAu was 110 nm and for MWCNTs-NF-PtAu it was about 210 nm. Results are similar in SEM, AFM and SECM for both the films.

3.2. Simultaneous determination of AA, EP and UA at MWCNTs-NF-PtAu film :

The CV and DPV were obtained for ascorbate, epinephrine and urate anions coexisting (AAEPUA mixture) at the bare and composite film modified GCEs. The electrolyte used for electrocatalysis was pH 6.75 of 0.1 M phosphate buffer aqueous solution, where the biochemical compounds are highly stable. The CVs exhibited two reduction peaks and one oxidation peak for MWCNTs-NF-PtAu composite film in the absence of AAEPUA mixture. Upon the addition of AAEPUA mixture, three new growth in the oxidation peak for AA, EP and UA were appeared at $E_{p_a} = 0.07, 0.29$ and 0.42 V respectively. The peak separation between ascorbate anion and epinephrine is 0.222 V and the peak separation between epinephrine and urate anion is 0.131 V. An increase in the concentration of AAEPUA mixture simultaneously produced an increase in the oxidation peak current of AA, EP and UA with good film stability. Similar results were obtained in DPV. The sensitivity of MWCNTs-NF-PtAu film towards the analytes in different techniques is given in Table.1.

Table. 1. Sensitivities and correlation co-efficient of MWCNT-NF-PtAu film for AA, EP and UA using different techniques. (Reproduced with permission from Yogeswaran et al. Anal. Biochem. 365 (2007) 122-131).

Type of technique	Sensitivity ($\mu\text{A mM}^{-1}\text{cm}^{-2}$) [Correlation co-efficient]		
	AA in mixture	EP in mixture	UA in mixture
CV	36.24 [0.983]	587.9 [0.940]	500.4 [0.972]
DPV	8.48 [0.904]	761.6 [0.903]	9.03 [0.942]

3.3. Electrochemical and topographic characterizations of MWCNTs-Au-HP β CD film :

Initially, there were six different film modified GCEs which have been studied as shown in Fig. 2. The six different types are the different combinations of MWCNTs, Au and HP β CD. They are (a) only Au, (b) Au with HP β CD, (c) MWCNTs with HP β CD (d) only MWCNTs, (e) MWCNTs with Au and (f) MWCNTs with Au and HP β CD. In all these studies, care has been taken to remove H_2SO_4 present on the film before transferring it in to pH 7.4 PBS. The corresponding CVs have been measured at 100 mVs^{-1} scan rate in the potential range of 1.0 to -0.4 V. The broad redox peak with very low peak current at $E^{0'} = -0.19$ V represents the redox peak for MWCNTs. From the same figure, it is found that the presence of MWCNTs and HP β CD shows the catalytic effect on Au redox peak currents. Further, it has been observed that the presence of MWCNTs and HP β CD in the composite film increases the overall back ground current. This could be explained in three step comparisons. The first explanation is by comparing (a), (b), (e) and (f) reveals that the HP β CD has increased the Au redox peak current. The second comparison is (a), (b) with (e), (f), which reveals that the presence of MWCNTs has enhanced the peak currents with overall background current. The final comparison is the (c) with (d) reveals that the HP β CD has decreased the overall current of MWCNTs. However, this decrease is very low, which will not affect the enhanced peak current obtained in (f).

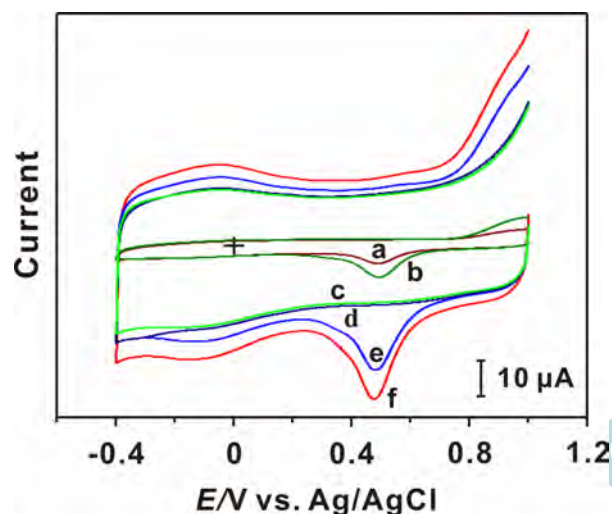


Fig. 2. CVs of (a) only Au, (b) Au-HP β CD, (c) MWCNTs-HP β CD (d) only MWCNTs, (e) MWCNTs-Au and (f) MWCNTs-Au-HP β CD composite films using 0.1 M pH 7.4 PBS, scan rate at 100 mV s⁻¹. (Reproduced with permission from Yogeswaran et al. Carbon 45 (2007) 2783–2796).

All these results are evident with the active surface coverage concentration of Au in the MWCNTs-Au-HP β CD increased. From the EQCM results, the increase and decrease in frequency of the gold electrode during reduction and oxidation of Au are confirmed. The mass change during Au incorporation on the MWCNTs modified gold electrode for total cycles is 2.9 $\mu\text{g cm}^{-2}$. From topographic studies (figures not shown), it is significant that there are morphological differences between all the four films.

3.4. Simultaneous determination of AD, GU, THY and TYR at MWCNTs-Au-HP β CD film

The electrochemical oxidation CVs and DPVs have been obtained for TYR, GU, AD and THY coexisting (analyte mixture) at MWCNTs-Au-HP β CD film using pH 7.4 PBS at 100 mV s⁻¹ in the potential range of -0.2 to 1.3 V. The CVs for MWCNTs-Au-HP β CD exhibits two redox couples in the absence of the analytes, upon the addition of analyte mixture, a new growth in the oxidation peaks of respective analytes has appeared. An increase in concentration of analyte mixture simultaneously produces a linear increase in the oxidation peak currents of all the four analytes with good film stability. The peak separation values between analytes are TYR and GU (80 mV), GU and AD (290 mV), AD and THY (185 mV). Similar results have been obtained in DPV also. From the slopes of the linear calibration curves the sensitivity of MWCNTs-Au-HP β CD modified GCEs and their correlation coefficients have been calculated and where given in Table. 2.

Table. 2. Sensitivities and correlation co-efficient of MWCNTs-Au-HP β CD film for AD, GU, THY and TYR using different techniques. (Reproduced with permission from Yogeswaran et al. Carbon 45 (2007) 2783–2796).

Type of technique	Sensitivity ($\mu\text{A mM}^{-1}\text{cm}^{-2}$) [Correlation co-efficient]			
	AD in mixture	GU in mixture	THY in mixture	TYR in mixture
CV	58.13 [0.988]	06.08 [0.989]	25.67 [0.863]	35.32 [0.990]
DPV	127.1 [0.946]	013.2 [0.962]	032.8 [0.988]	070.3 [0.987]

4. Conclusions :

We developed combination of nanocomposite materials with MWCNTs, NF, HP β CD, nano Pt and nano Au (MWCNTs-NF-PtAu and MWCNTs-Au-HP β CD) at the GCE, gold and ITO surfaces in aqueous solution containing 0.5M H₂SO₄ solution, which was also highly stable in other pH solutions. The developed composite films for the simultaneous determination combine the advantages of ease of fabrication, high reproducibility and sufficient long-term stability. The experimental method of CV and DPV with composite film biosensors integrated into a GCE presented in this paper provides an opportunity for qualitative, quantitative characterization and simultaneous determination of AA, EP and UA at MWCNTs-NF-PtAu; and AD, GU, THY and TYR at MWCNTs-Au-HP β CD in physiologically relevant conditions. This work establishes and illustrates, in principle and potential, a simple and novel approach for the development of simultaneous neurotransmitters and nucleotide bases voltammetric sensors which are based on the modified GCE.

5. Acknowledgement :

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6. References :

- [1] G.F. Combs, 1992. The Vitamins: Fundamental Aspects in Nutrition and Health, second ed., Academic Press, San Diego, CA.
- [2] Q. Xu, S.F. Wang, Electrocatalytic oxidation and direct determination of L-Tyrosine by square wave voltammetry at multi-wall carbon nanotubes modified glassy carbon electrodes, *Microchim Acta* 151 (2005) 47-52.
- [3] G. Dryhurst, 1977. *Electrochemistry of Biological Molecules*, Academic Press, New York.
- [4] J.M. Zen, J.J. Jou, G. Ilangovan, Selective voltammetric method for uric acid detection using pre-anodized Nafion-coated glassy carbon electrodes, *Analyst* 123 (1998) 1345-1350.
- [5] E.E. Ferapontova. Electrochemistry of guanine and 8-oxoguanine at gold electrodes, *Electrochim Acta* 49 (2004) 1751-1759.

- [6] B. Jil venton, R.M. Wightman, Psychoanalytical electrochemistry: Dopamine and behavior, *Anal. Chem.* 1 (2003) 414A-421A.
- [7] J. Chen, C.S. Cha, Detection of dopamine in the presence of a large excess of ascorbic acid by using the powder microelectrode technique, *J. Electroanal. Chem.* 463 (1999) 93-99.
- [8] M.A. Dayton, A.G. Ewing, R.M. Wightman, Response of microvoltammetric electrodes to homogeneous catalytic and slow heterogeneous charge-transfer reactions, *Anal. Chem.* 52 (1980) 2392-2396.
- [9] Y. Yan, M. Zhang, K. Gong, L. Su, Z. Guo, L. Mao, Adsorption of Methylene Blue Dye onto Carbon Nanotubes: A Route to an Electrochemically Functional Nanostructure and Its Layer-by-Layer Assembled Nanocomposite, *Chem. Mater.* 17 (2005) 3457-3463.