

*Full Paper*

## **Simultaneous Determination of Ascorbic Acid, Uric Acid and Acetaminophen on a Glassy Carbon Electrode Coated with a Novel Single Walled Carbon Nanotubes/ Chitosan/ MCM-41 Composite**

**Mohammad Afrasiabi\* and Shokat Kianipour**

*Young Researchers Club, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran*

\* Corresponding Author, Tel.: +98-2177722469; Fax: +98-2177722469

E-Mail: [Mohammad\\_Afrasiabi07@yahoo.com](mailto:Mohammad_Afrasiabi07@yahoo.com)

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**Abstract-** The present work demonstrates that simultaneous determination of ascorbic acid (AA), uric acid (UA) and acetaminophen (ACT) can be performed on a novel single walled carbon nanotubes (SWCNTs), chitosan (CHIT) and MCM-41 composite modified glassy carbon electrode (SWCNTs-CHIT-MCM-41/GCE). The electro-oxidations of AA, UA and ACT were investigated by using differential pulse voltammetry (DPV), chronoamperometry (CA) and electrochemical impedance spectroscopy (EIS) methods. Under optimum conditions application of DPV method showed that the linear relationship between oxidation peak current and concentration of AA, UA and ACT were 1-160  $\mu\text{M}$ , 0.1-24  $\mu\text{M}$  and 0.1-21  $\mu\text{M}$  with detection limits of 0.41, 0.04 and 0.03  $\mu\text{M}$ , respectively

**Keywords-** Ascorbic acid, Uric acid, Acetaminophen, Sensor, Carbon nanotubes

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### **1. INTRODUCTION**

The unique properties of carbon nanotubes (Multi Walled Carbon Nanotubes and Single Walled Carbon Nanotubes) such as high electrical conductivity, great chemical stability and extreme mechanical strength lead to interest in use of them as a modifier in various sensors [1-5].

Chitosan (CHIT), a natural-polymer, is attractive one in that it is biocompatible, biodegradable, nontoxic, and has high mechanical strength and hydrophilicity. In addition, it is of low cost, is chemically inert, good adhesion and exhibits excellent membrane-forming ability. Because CHIT-CNTs can form a stable complex through noncovalent binding, the stability of CNTs in aqueous CHIT solution is greatly improved [6]. As a result it has been applied as a modifier in electrochemical sensors [7-9].

MCM-41 was the first synthetic mesoporous material and one member of the mesoporous molecular sieves M41S family, with regularly ordered pore arrangement and a very narrow pore distribution, which was disclosed by the Mobil scientists in 1992 [10]. MCM-41 can act as a catalyst or as a catalyst support [11], as a host for host-guest nanocomposite materials [12], it has also been applied as an adsorbent material [13], as a material for electrochemiluminescence [14] and electrochemical sensors [15,16]. This is mainly because MCM-41 can offer a large surface area, controllable pore size, high porosity, ordered uniform pore structure and high loading capacity. It is a strong Lewis acid, and therefore has been used for catalysis purposes in organic synthesis reactions. It means that substances with strong base properties have a strong interaction with MCM-41.

Ascorbic acid (AA) is a naturally occurring organic compound with antioxidant properties. It is a white solid, but impure samples can appear yellowish. It dissolves well in water to give mildly acidic solutions. AA is one form of vitamin C. Because it is derived from glucose, many animals are able to produce it, but humans require it as part of their nutrition. Reviewing the literature revealed that several methods have been used for the determination of AA, these methods include high performance liquid chromatography (HPLC) [17], spectrophotometry [18] and electrochemical methods [19,20].

Uric acid (UA) is the primary end product of purine metabolism, and considered as a species of great importance in human diagnosis. An abnormal concentration of UA can indicate the presence of one of numerous diseases and/or physiological disorders. An elevated concentration of UA is observed in patients suffering from diseases such as gout and hyperuricemia [21]. Because of its clinical relevance, it is crucial to develop simple and rapid methods for UA determination in routine analysis. The most common analytical techniques currently used to determine UA concentrations are HPLC [22], capillary electrophoresis [23], chemiluminescence [24] and electrochemical methods [25,26].

Acetaminophen (ACT) is classified as a mild analgesic. It is commonly used for the relief of headaches and other minor aches and pains and it is a major ingredient in numerous cold and flu remedies. However, overdose consumption of ACT can cause fatal liver damage. In fact, ACT toxicity is the main cause of acute liver failure and accounts for most drug overdoses in the world. So, there has been a great deal of effort to determine concentration of ACT in biological samples. A variety of analytical methods have been developed in order to

measure ACT levels in different sample matrices, such as spectrophotometry [27], HPLC [28] and electrochemical methods [29,30].

In our previous work we introduce two electrodes for simultaneous determination of AA, UA and ACT [31,32]. However these methods suffer from the high detection limit, low sensitivity and low stability. Therefore, development of a simple, sensitive and accurate analytical method for simultaneous determination of AA, UA and ACT would be of great importance. In this study, we report the preparation and application of SWCNT-CHIT-MCM-41/GCE as a novel sensor for nanomolar simultaneous determination of AA, UA and ACT. The modified electrode showed high sensitivity, lower detection limit with a good linear dynamic range.

## 2. EXPERIMENTAL

### 2.1. Reagents and solutions

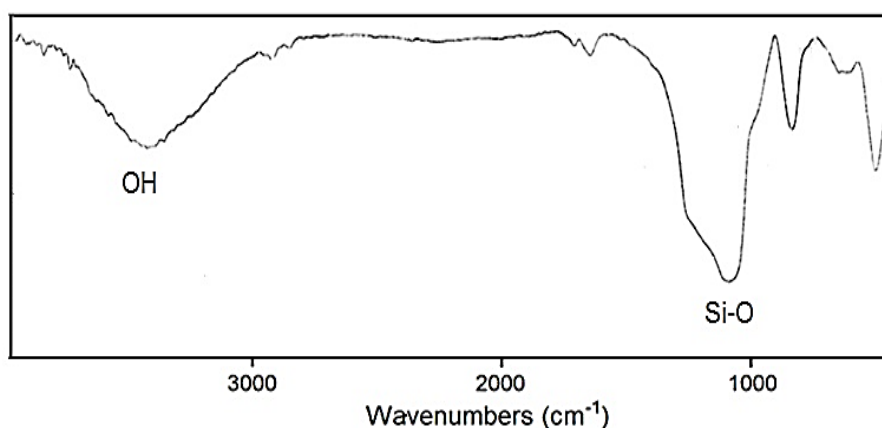
All chemicals were analytical grade and used without further purification. AA, UA and ACT were obtained from Merck chemical company. CHIT (MW  $1.0\text{--}3.0 \times 10^5$ ) and SWCNTs were purchased from Acros and Sigma chemical companies, respectively. The purity of SWCNTs was 90% with a surface specific area of  $480 \text{ m}^2 \text{ g}^{-1}$  and length of  $0.5\text{--}2 \mu\text{m}$ . Stock standard solutions of 10 mM AA, 10 mM UA and 10 mM ACT were freshly prepared in 0.1 M phosphate buffer solutions (PB solutions) of pH=7. All AA, UA and ACT solutions were prepared by diluting the stock standard solutions using 0.1 M PB solution (pH=7). Electrochemical experiments on AA, UA and ACT were carried out in 0.1 M PB solution at pH=7.

### 2.2. Instrumentation

All voltammetric measurements were carried out using SWCNTs-CHIT-MCM-41/GCE as the working electrode, an Ag/AgCl/KCl (3 M) electrode as the reference electrode and a platinum wire as an auxiliary electrode. Differential pulse voltammetry (DPV), chronoamperometry (CA) and electrochemical impedance spectroscopy (EIS) experiments were carried out using an Autolab PGSTAT 30 Potentiostat Galvanostat (EcoChemie, The Netherlands) coupled with a 663 VA stand (Metrohm Switzerland). All potentials given are with respect to the potential of the reference electrode. The pH measurements were performed with a Metrohm 744 pH meter using a combination glass electrode. The morphological analyses were carried out by using MIRA3 TESCAN scanning electron microscope (SEM). FTIR spectra were recorded as a KBr disc on a galaxy series fourier transform infrared 5000 spectrometer.

### 2.3. Synthesis of MCM-41

MCM-41 was synthesized through the self-assembly of inorganic silica precursor in our laboratory. A typical MCM-41 preparation procedure is described as follows: 13.0 g of hexa decyl trimethyl ammonium bromide (CTAB) was dissolved in 170 mL of deionized water; then, 163.7 g of the aqueous ammonia and 131.5 g of the ethanol was added to the surfactant solution. The solution was stirred for 15 min, and afterward 24.8 g of tetraethyl orthosilicate (TEOS) was added. After 2 h of the vigorous stirring with the mechanical stirrer at 1500 rpm and filtration, the white precipitate was successfully washed with plenty of deionized water and dried at 60 °C for 24 h. Before using MCM-41, a part of MCM-41 sample was calcined under oxygen atmosphere by applying a heating ramp of 1 °C per min up to 600 °C and then kept at this same temperature for 6 h [33]. The FTIR spectrum was assigned for MCM-41 (Fig. 1). The band at 3434  $\text{cm}^{-1}$  is assigned to the silanol groups linked to the molecular water via hydrogen bonds with internal Si-OH groups. The band at 1631  $\text{cm}^{-1}$  is contributed to the absorbed molecular water. Other bands are as follow: 1083  $\text{cm}^{-1}$  for Si-O-Si asymmetry stretching vibration, 812  $\text{cm}^{-1}$  for Si-O-Si symmetry stretching vibration and 464  $\text{cm}^{-1}$  for Si-O-Si bending vibration.



**Fig. 1.** FTIR spectrum of MCM-41

### 2.4. Preparation of the modified electrode

Prior to modification, the GCE (3 mm diameter, Metrohm) was first polished with 0.3 and 0.05  $\mu\text{m}$  aluminum slurry and rinsed thoroughly with triply distilled water. The electrode was then cleaned by sonication for 5 min, first in ethanol and then in distilled water, and then it dried under a nitrogen gas flow. To obtain a good quality SWCNTs-CHIT-MCM-41/GCE, the mass ratios of CHIT, SWCNTs and MCM-41 in the mixture was optimized in control experiments. A stock solution of 0.4 wt % CHIT was prepared by dissolving 4 mg of CHIT in 1 mL of 1% acetic acid solution and the pH of the solution was adjusted to 5.0 with

concentrated NaOH. With the aid of ultrasonic agitation for 30 min, 1.5 mg SWCNTs were dissolved in 1 mL of 0.4 wt % CHIT and resulted in a homogeneous black SWCNTs-CHIT solution. Typically, a homogeneous solution containing 0.4 wt.% CHIT, 1.5 mg mL<sup>-1</sup> SWCNTs and 0.3 mg mL<sup>-1</sup> was obtained. Then 20  $\mu$ L of prepared suspension was casted on the electrode with a micro syringe and dried at room temperature to obtain the modified electrode. Finally the fabricated SWCNTs-CHIT-MCM-41/GCE was placed in the electrochemical cell containing 0.1 M PB solution. Then it subjected to 20 cycles in the potential windows of -0.2 to 0.6 V using the CV method to obtain stable responses.

## 2.5. General procedure

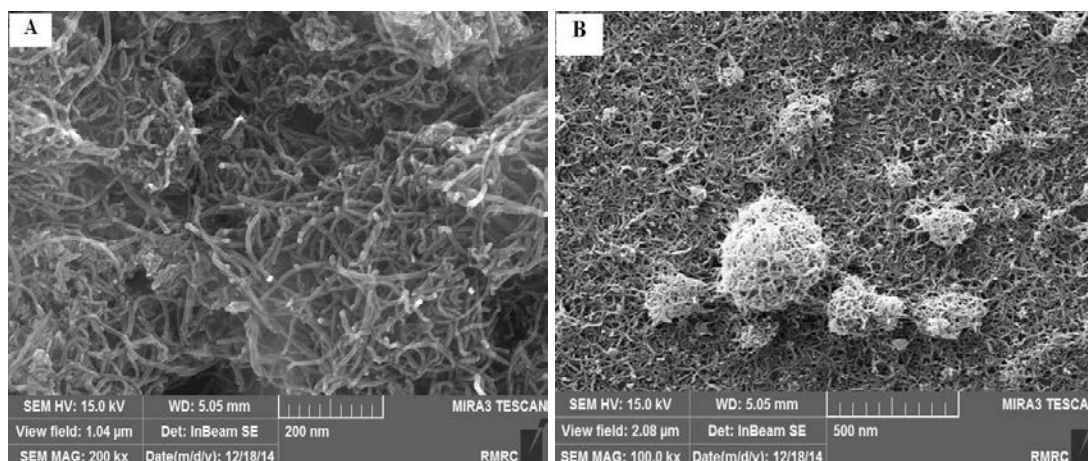
Each sample solution (10 mL) containing appropriate amounts of AA, UA and ACT in 0.1 M PB solution at pH of 7 were transferred into the voltammetric cell. The differential pulse voltammograms were recorded by applying positive-going potential from - 0.1 to 0.4 V. The voltammograms showed anodic peaks around 0.05, 0.22 and 0.32 V corresponding to the AA, UA and ACT compounds, respectively. Calibration curves were obtained by plotting the anodic peak currents of AA, UA and ACT against the corresponding concentrations. After each measurement, the SWCNTs-CHIT-MCM-41/GCE was regenerated by thoroughly washing the electrode with triply distilled water and then 5% sodium hydroxide solution to remove all adsorbate from electrode surface. The electrode was finally rinsed carefully with distilled water to provide a clean surface for next experiments.

## 3. RESULTS AND DISCUSSION

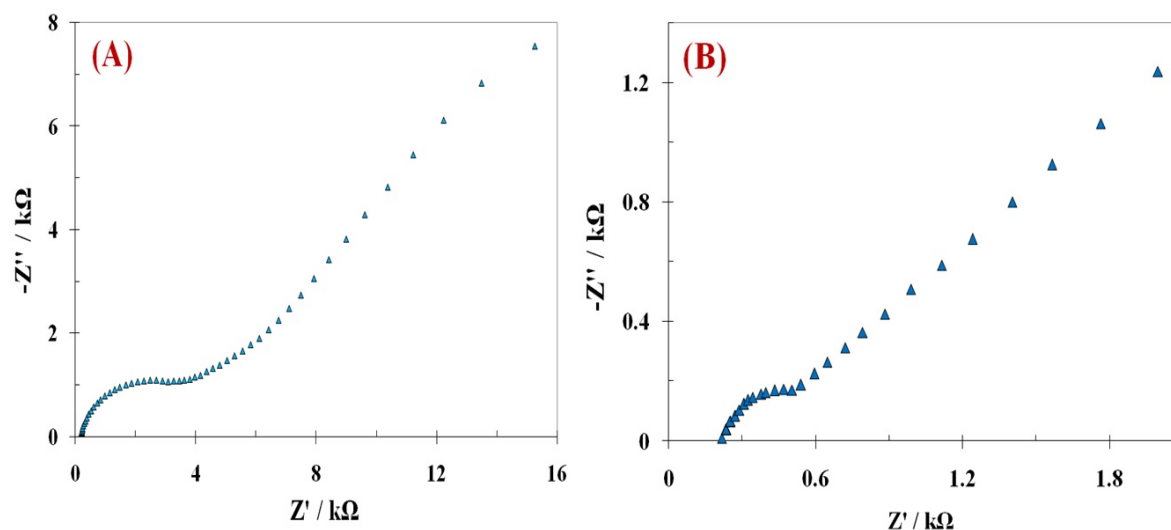
### 3.1. Characterization of the SWCNTs-CHIT-MCM-41 composite

Fig. 2a is shown the SEM image of the SWCNTs-CHIT composite. It can be seen that the SWCNTs is well covered with CHIT film. Fig. 2b shows a typical image of the SWCNTs-CHIT-MCM-41 composite. The particles sizes of MCM-41 are between 100 to 300 nm. It can be seen that the MCM-41 is well covered with SWCNTs-CHIT that this leads to increase of the surface area of the composite, therefore higher electrical conductivity is expected to be obtained on the modified electrode with this composite.

The Nyquist plots ( $-Z''$  vs.  $Z'$ ) for SWCNTs-CHIT-MCM-41/GCE and GCE electrodes obtained when the electrodes were immersed in a 0.1 M KCl solution containing 5 mM in both  $K_3[Fe(CN)_6]$  and  $K_4[Fe(CN)_6]$  (Fig. 3). In the Nyquist diagram, the semicircle diameter of electrochemical impedance spectroscopy (EIS) is equal to  $R_{ct}$ . The results showed the diameter of the semicircle for the SWCNTs-CHIT-MCM-41/GCE is 1.42 k $\Omega$  which are smaller than that of the unmodified GCE (3.014 k $\Omega$ ). The results suggest the SWCNTs-CHIT-MCM-41 composite modification of the electrode provides significant acceleration for  $[Fe(CN)_6]^{3-/4-}$  redox reaction on the electrode surface.



**Fig. 2.** SEM micrographs of the samples: (A) SWCNTs-CHIT and (b) SWCNTs-CHIT-MCM-41



**Fig. 3.** Nyquist plots for (A) GC and (B) SWCNTs-CHIT-MCM-41/GC electrodes obtained when the electrodes immersed into solutions of 5 mM  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  and 0.1 M KCl solution

The effect of modification of the electrode on active surface area was investigated by cyclic voltammetry of 2 mM potassium ferricyanide in PB solution (pH 7.0) at GCE and SWCNTs-CHIT-MCM-41/GCE.  $K_3Fe(CN)_6$  exhibited a pair of reversible redox peaks at a bare and modified GC electrode. For the modified electrodes the redox peak currents are larger than for the GCE. In addition the  $K_3Fe(CN)_6$  redox reaction behave like quasi reversible system at unmodified GCE by shifting potential at higher scan rates. However at SWCNTs-CHIT-MCM-41/GCE the corresponding redox peaks of  $K_3Fe(CN)_6$  did not shift in potentials by increasing scan rates, which confirm higher electron transfer rate on the modified electrode. On the other hand, under the same conditions, the cathodic peak currents

were linear with the square root of scan rate (in the range of 10-140 mVs<sup>-1</sup>) on the GCE and SWCNTs-CHIT-MCM-41/GCE. The obtained regression equations for the tow electrodes are as follows:

$$I_{pa} (\mu\text{A}) = 43.75 v^{1/2} (\text{V s}^{-1})^{1/2} + 3.28 \quad (R^2 = 0.994) \quad \text{GCE}$$

$$I_{pa} (\mu\text{A}) = 622.87 v^{1/2} (\text{V s}^{-1})^{1/2} + 8.98 \quad (R^2 = 0.992) \quad \text{SWCNTs-CHIT-MCM-41/GCE}$$

A reversible system should satisfy the Randles-Sevcik equation [34]:

$$I_p = (2.69 \times 10^5) n^{3/2} A C_0 D^{1/2} v^{1/2}$$

By application of the above equation and comparison of the slope of the equations respect to GCE, the apparent area of the SWCNTs-CHIT-MCM-41/GCE was estimated about 14.2 times as large as that of the GCE. It can be concluded that the application of a SWCNTs-CHIT-MCM-41 composite leads to higher electrochemically active surface area than each composite.

### 3.2. Electrochemical behavior of AA, UA and ACT on SWCNTs-CHIT-MCM-41/GCE

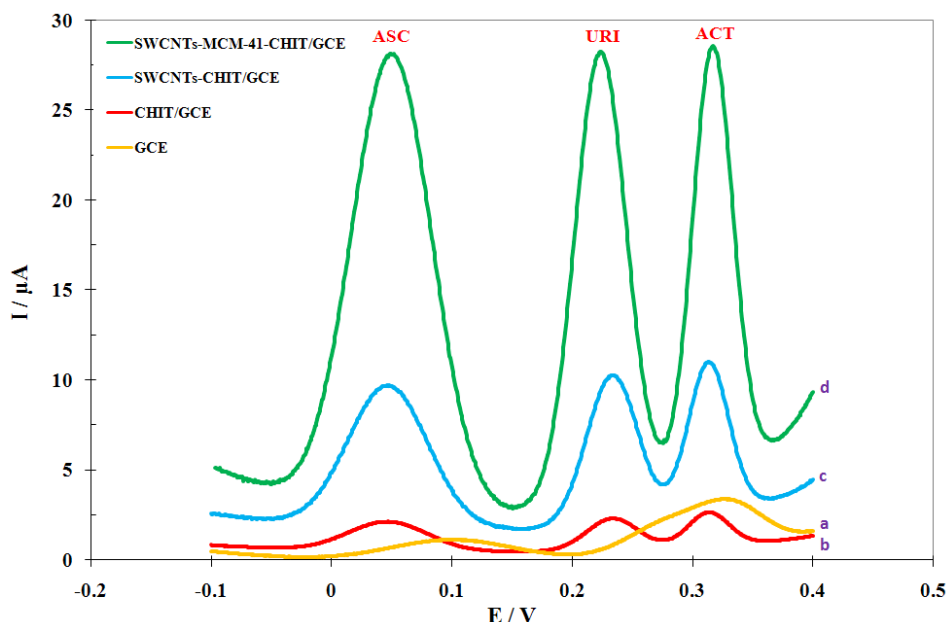
Fig. 4 shows the differential pulse voltammograms of a solution of 150 μM of AA, 20 μM of UA and 15 μM of ACT of in PB solution (pH=7) at bare and modified GC electrodes. The voltammograms were recorded for AA, UA and ACT at (a) GCE, (b) CHIT/GCE, (c) SWCNTs-CHIT/GCE and (d) SWCNTs-CHIT-MCM-41/GCE. As can be seen (curve b in Fig. 4) the presence of CHIT lead to the oxidation peak of URI shifts to less positive potentials and leads to more current peak separation between URI and ACT. The presence of SWCNTs (curve c in Fig. 4) lead to significantly enhance the corresponding peak currents compared with the bare GCE which can be related to electro-catalytic behavior of SWCNTs. The oxidation peak currents of AA, UA and ACT significantly increase at the SWCNTs-CHIT-MCM-41/GCE as a result of large surface area of MCM-41 and electro-catalytic behavior of SWCNTs (curve d in Fig. 4).

### 3.3. Effects of solution pH

The effect of solution pH on the electrochemical response of the SWCNTs-CHIT-MCM-41/GCE towards AA, UA and ACT in the simultaneous determination of 100 μM AA, 5 μM UA and 8 μM ACT was investigated using DPV method (Fig. 5A). The results showed higher oxidation peak currents for AA, UA and ACT at pH of 7. Therefore the pH value of 7 was chosen as an optimum solution pH for further experiments.

In addition the relationship between oxidation peak potential of AA, UA and ACT with pH was investigated (Fig. 5B). The slopes of the variations of  $E_p$  as a function of solution pH

are close to the Nernstian slope 59 mV/pH which suggests an equal number of electrons and protons involved in the electrochemical oxidations of AA, UA and ACT.

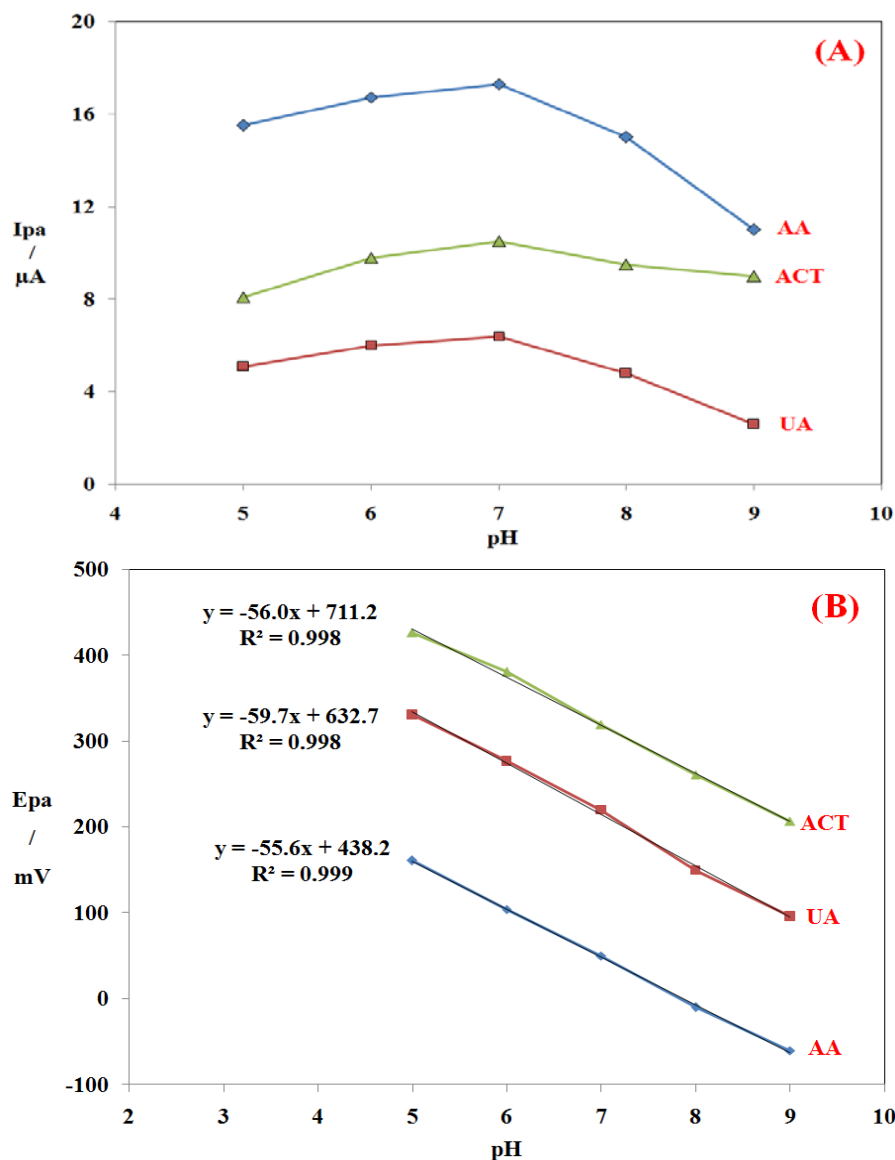


**Fig. 4.** Differential pulse voltammograms of 150  $\mu\text{M}$  AA, 20  $\mu\text{M}$  UA and 15  $\mu\text{M}$  ACT at (a) GCE, (b) CHIT/GCE, (c) SWCNTs-CHIT/GCE and (d) SWCNTs-CHIT-MCM-41/GCE in 0.1 M PB solution (pH=7). Other conditions:  $t_{\text{acc}}=60$  s, pulse amplitude=50 mV, scan rate=10  $\text{mV s}^{-1}$ , interval time 0.5 s, modulation time=0.2 s and step potential=5 mV

### 3.4. Linear dynamic range and detection limit of the method

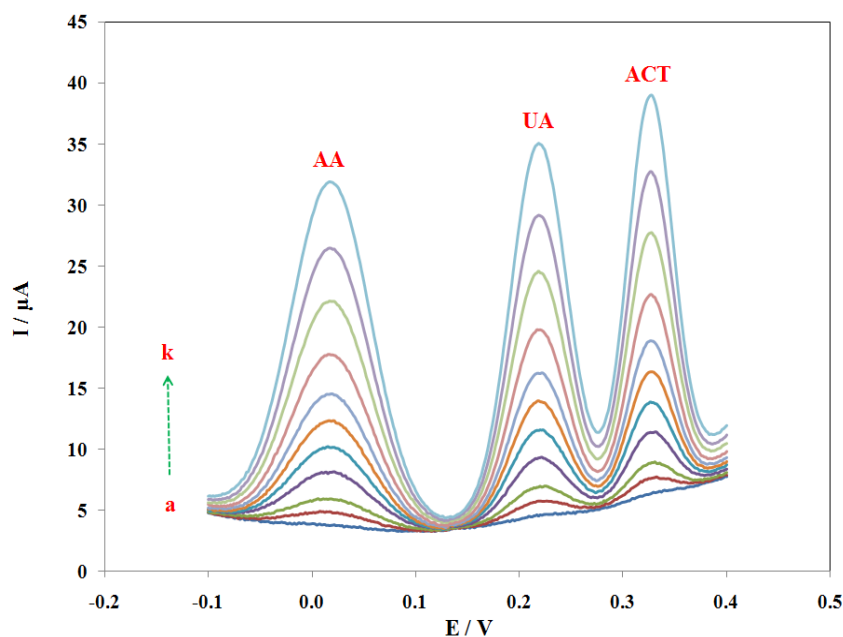
The electrochemical responses of simultaneous determinations of AA, UA and ACT in a 0.1 M PB solution pH 7 using SWCNTs-CHIT-MCM-41/GCE are depicted in Fig. 6. Fig. 6 show differential pulse voltammograms and the corresponding calibration curves obtained for various concentrations of AA, UA and ACT at SWCNTs-CHIT-MC-41/GCE. For AA a linear dynamic range from 1  $\mu\text{M}$  to 160  $\mu\text{M}$  with a calibration equation of  $I_p(\mu\text{A})=0.164C(\mu\text{M})+0.263$  ( $R^2=0.999$ ), and a detection limit of 0.41  $\mu\text{M}$  ( $S/N=3$ ) were obtained. For UA a linear dynamic range from 0.1  $\mu\text{M}$  to 24  $\mu\text{M}$  with a calibration equation of  $I_p(\mu\text{A})=1.079C(\mu\text{M})+0.689$  ( $R^2=0.998$ ), and a detection limit of 0.04  $\mu\text{M}$  ( $S/N=3$ ) were obtained. For ACT a linear dynamic range from 0.1  $\mu\text{M}$  to 21  $\mu\text{M}$  with a calibration equation of  $I_p(\mu\text{A})=1.274C(\mu\text{M})+0.703$  ( $R^2=0.997$ ), and a detection limit of 0.03  $\mu\text{M}$  ( $S/N=3$ ) were obtained. The investigations showed that these linear ranges were kept in mixture solutions of AA, UA and ACT, revealing high efficiency of the fabricated modified electrode for simultaneous determinations of these compounds in mixed pharmaceutical samples.





**Fig. 5.** (A) Plots of peak currents of URI, PAR and MEF as a function of pH and (B) Plots of peak potentials of URI, PAR and MEF as a function of pH for 100  $\mu\text{M}$  AA, 5  $\mu\text{M}$  UA and 8  $\mu\text{M}$  ACT at SWCNTs-CHIT-MCM-41/GCE in 0.1M PB solution (pH=7)

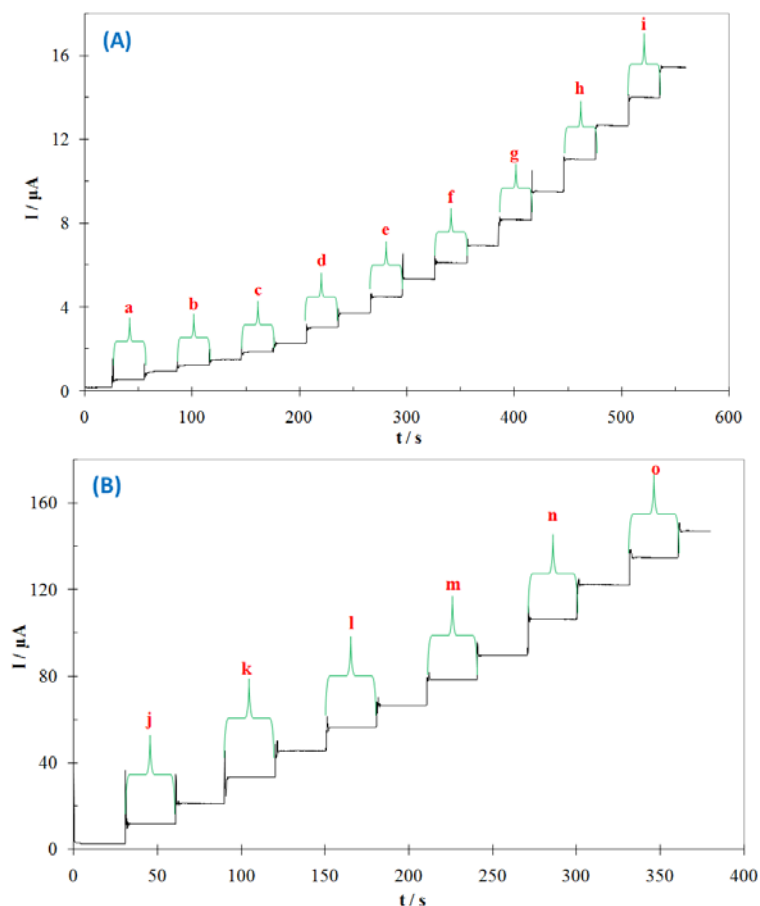
Fig. 7 displays a chronoamperogram of the response of a rotated modified electrode (3000 rpm) following the successive injection of AA, UA and ACT at an applied potential of 0.45 V in PB solution (pH=5). For AA the linear dynamic range was from 1  $\mu\text{M}$  to 156  $\mu\text{M}$ , with a calibration equation of  $I_p(\mu\text{A})=0.332c(\mu\text{M})+0.281$  ( $R^2=0.999$ ) and a detection limit of 0.17  $\mu\text{M}$  ( $S/N=3$ ) was obtained. For UA the linear dynamic range was from 0.1  $\mu\text{M}$  to 48  $\mu\text{M}$ , with a calibration equation of  $I_p(\mu\text{A})=1.282c(\mu\text{M}) + 0.179$  ( $R^2=0.998$ ) and a detection limit of 0.04  $\mu\text{M}$  ( $S/N=3$ ) was obtained. For ACT the linear relationship was in the range of 0.2 to 40  $\mu\text{M}$  with a calibration equation of  $I_p(\mu\text{A})=1.170c(\mu\text{M})+0.186$  ( $R^2=0.999$ ) and a detection limit of 0.05  $\mu\text{M}$  ( $S/N=3$ ) was obtained.



**Fig. 6.** Differential pulse voltammograms for different concentrations of AA, UA and ACT mixture as (a) 1+0.1+0.1, (b) 5+0.5+0.5, (c) 12+1.5+1.5, (d) 20+3+3, (e) 35+5+4.5, (f) 45+7+6, (g) 60+9+8, (h) 80+11+10, (i) 100+15+14, (j) 120+19+17 and (k) 160+24+21, respectively, in which the first, second and third value is the concentration of AA, UA and ACT in  $\mu\text{mol L}^{-1}$

### 3.5. Repeatability and long-term stability of the electrode

The repeatability of the proposed SWCNTs-CHIT-MCM-41/GCE under optimum condition was studied. The relative standard deviations (RSD) of 1.12% and 0.94% for ten consecutive determinations of 50  $\mu\text{M}$  AA, 10  $\mu\text{M}$  UA and 10  $\mu\text{M}$  ACT were obtained, respectively. The proposed modified electrode has a further attraction of good long-term stability. It was tested by measuring the decrease in voltammetric current during the repetitive DPV measurements of solutions of AA, UA and ACT with SWCNTs-CHIT-MCM-41/GCE which was stored in solution (wet condition) or atmosphere air (dry condition) for certain period of time. For example at wet condition, in the determination of 50  $\mu\text{M}$  AA, 10  $\mu\text{M}$  UA and 10  $\mu\text{M}$  ACT in 0.1 M PB solution (pH=7), when the modified electrode was subjected to experiment several times, after 36 h it gave less than 1.1, 1.2 and 1.2% decrease in the voltammetric oxidation peak current of AA, UA and ACT, respectively. When the electrode was stored in the atmosphere air for 7 days, the oxidation peak current of AA, UA and ACT in the solution was reduced less than 1.0, 0.8 and 0.9%, respectively. The excellent stability of the electrode could be related to present of the SWCNTs that are coated with CHIT and MCM-41, which make it suitable for long-term electrochemical sensing applications.



**Fig. 7.** Amperometric response at rotating SWCNTs-CHIT-RTIL/GCE (rotating speed 2000 rpm) held at 0.45 V in PB solution (pH=7) for determination of ACT, UA and AA by successive additions of (a) 0.2, (d) 0.6, (g) 1.2, (j) 8 and (m) 10  $\mu\text{mol L}^{-1}$  ACT, (b) 0.1, (e) 0.4, (h) 1.5, (k) 10 and (n) 12  $\mu\text{mol L}^{-1}$  UA and 50  $\mu\text{mol L}^{-1}$  ACT, (c) 1, (f) 2, (i) 5, (l) 30 and (o) 40  $\mu\text{mol L}^{-1}$

#### 4. CONCLUSIONS

In this report the new application of the novel sensor based on SWCNTs, CHIT and MCM-41 composite modified glassy carbon electrode (SWCNTs-CHIT-MCM-41/GCE) is introduced. The combination of SWCNTs, CHIT and MCM-41 as a composite in the modified electrode leads to very high sensitivity for the simultaneous determination of AA, UA and ACT due to excellent electro-catalytic performance and high surface area of the composite and on the other hands the anodic peak currents for ACT and UA are well separated in comparison with those from the GCE. The electrode also shows high stability in repetitive experiments. The simple fabrication procedure, high speed, reproducibility, high stability, wide linear dynamic range, low detection limit and high sensitivity, suggest that the proposed sensor is an attractive candidate for practical applications.

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