A Sensitive Simultaneous Determination of Acetaminophen and Indomethacin at Multi-walled Carbon Nanotubes Modified Glassy Carbon Electrode

Ali Babaei¹,²*, Mojtaba Farshbaf¹, Mohammad Afrasiabi³ and Aliyeh Dehdashti¹

¹Department of Chemistry, Arak University, Arak, P.O. Box 38156-8-8349, Iran
²Research Center for Nanotechnology, Arak University, Arak, P.O. Box 38156-8-8349, Iran
³Young Researchers Club, Islamic Azad University, Shoushtar Branch, Shoushtar, Iran

*Corresponding Author, Tel.: +98 861 4173401; Fax: +98 861 4173406
E-Mail: a-babaei@araku.ac.ir

Received: 4 November 2012 / Accepted in revised form: 28 November 2012 / Published online: 30 December 2012

Abstract- A chemically modified electrode is constructed based on multi-walled carbon nanotube modified glassy carbon electrode (MWCNTs/GCE) as a sensor for simultaneous determination of acetaminophen (ACT) and indomethacin (INDO) in phosphate buffer (pH 7) solution. The measurements were carried out using differential pulse voltammetry (DPV), cyclic voltammetry (CV) and chronoamperometry (CA) methods. DPV measurements showed that the linear relationship between oxidation peak current and concentration of ACT and INDO were in the range of 3 µM to 400 µM, and 10 µM to 330 µM, respectively. Under optimal conditions the modified electrode exhibited high sensitivity and stability for both ACT and INDO determination, making it a suitable sensor for the simultaneous submicromolar detection of ACT and INDO in solutions. The analytical performance of this sensor has been evaluated for detection of ACT and INDO in human serum, human urine and a pharmaceutical preparation with satisfactory results.

Keywords- Acetaminophen, Indomethacin, Carbon Nanotube, Modified Glassy Carbon Electrode
1. INTRODUCTION

Carbon nanotubes (CNTs) are an important group of nanomaterials. They have attracted much attention because of their high surface area, high chemical stability, and high electrical conductivity, unique geometrical and mechanical strength [1]. Therefore CNTs have received enormous attention for the development of electrochemical sensors. Actually, sensors based on CNTs have largely improved the voltammetric responses (lower over voltages and higher peak currents) for determinations a variety of biological, clinical and environmental compounds [2]. Indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1-H-indole-3-acetic acid) (Scheme 1) is a non-steroidal anti-inflammatory drug exhibiting analgesic, anti-inflammatory and antipyretic effects. It is used in the treatment of rheumatoid arthritis and is a potent inhibitor of cyclo-oxygenase, reducing prostaglandin synthesis, relieving pain and reducing temperature in febrile patients and often also used topically in the eye to reduce local inflammation. Several methods for the determination of indomethacin in pharmaceuticals and biological fluids have been reported. They include chromatography [3-7], fluorimetry [8-10], spectrophotometry [11] and other methods. An electrochemical PVC-based sensor for the determination of indomethacin was also reported [12], it is based on an ionic associate bis(triphenyl-phosporanylidene) ammoniumindomethacin. However, it is characterized by low selectivity which significantly limits its practical application.

Scheme 1. Chemical structures of INDO and ACT

Acetaminophen (ACT) (Scheme 1) is an important medicine, extensively used both in pure form and in pharmaceutical preparations. It is mainly used as an alternative to aspirin as an analgesic and antipyretic agent that lacks the disadvantageous secondary effects of the salicylates on the gastric mucosa [13]. It is often self-prescribed, without medical control, for relief of moderate pain, fever, lumbar pain, migraine or even non-specific indications [14]. It
has been reported to be a useful drug in osteoarthritis therapy [15]. However an overdose of ACT can result in the accumulation of toxic metabolites, which may cause severe and sometimes fatal hepatotoxicity [16-18]. It can also cause liver disorders, skin rashes and inflammation of the pancreas [19]. A number of analytical techniques such as titrimetry [15], spectrophotometry [20], spectrofluorometry [21], voltammetry [22], HPLC [23], TLC [24], colorimetry [25] and Fourier transform infrared spectrometry [26], have been proposed for the determination of ACT in pharmaceutical formulations and biological samples.

Combination of ACT with INDO is occasionally prescribed as an analgesic and anti-inflammatory agent in rheumatoid arthritis [27]. The most of the methods for simultaneous determination of ACT and INDO were based on solid phase extraction [28], liquid chromatography [29], and electrophoresis [30]. The most of the proposed analytical methods suffer from some disadvantages such as high cost, long analysis times and requirement for sample pretreatment, and in some cases low sensitivity and selectivity that makes them unsuitable for routine analysis. Therefore, development of a simple, inexpensive, sensitive and accurate analytical method for simultaneous determination of ACT and INDO is of great importance. Both ACT and INDO are electroactive compounds and can be oxidized electrochemically. To the best of our knowledge there has not been a report of the use of an electrochemical sensor for the simultaneous determination of ACT and INDO compounds. In this work we outline for the first time the use of a multi-walled carbon nanotubes modified glassy carbon electrode (MWCNTs/GCE) as a new sensor for this purpose. In addition, the analytical performance of this sensor for determination of ACT and INDO in human serum, human urine and in actual pharmaceutical preparation samples is evaluated.

2. EXPERIMENTAL

2.1. Reagents and solutions

All chemicals were analytical grade and used without further purification. ACT and INDO were obtained from Merck and Sigma chemical companies, respectively. Multi-walled carbon nanotubes (MWCNTs) (>95 wt%, 5-20 nm) were purchased from PlasmaChem GmbH companies, respectively. The 0.1 M phosphate buffer solutions (PBS) (pH 7.0) used was prepared by dissolving appropriate amounts of sodium hydrogen phosphate and sodium dihydrogen phosphate in triple distilled water. Stock standard solutions of 10 mM ACT and 10 mM INDO were freshly prepared in PBS at pH 9.0. Stock standard solutions of 10 mM ACT and 10 mM INDO were freshly prepared in PBS at pH 7.0. All subsequent ACT and INDO solutions used were prepared by diluting these standard solutions with PBS (pH 7.0).

Fresh human serum samples were obtained from the Razi Institute of Vaccine and Serum Company (Tehran, Iran). The serum and urine samples were filtered and diluted 100 times
with PBS (pH 7.0) before spiking with ACT and INDO. Ten tablets of Tylenol (McNeil-PPC Inc., USA) (each tablet labeled as 500 mg of ACT content) or INDO (Zahravi company, Iran) (each tablet labeled as containing 25 mg of INDO) were accurately weighed and powdered in a mortar. A weight equivalent to one in tablet content was dissolved in 70 mL of 0.1 M PBS (pH 7). After a 10 min sonication, the solutions were filtered and the residual was washed three times with 10 mL of PBS, then the volume was adjusted to 100 mL with the same solvent. These solutions were diluted either 1000 times (ACT) or 100 times (INDO) with 0.1 M PBS of pH 7.0. These solutions were used for the determination and spiking with ACT and INDO compounds.

2.2. Instrumentation

All voltammetric measurements were carried out using a multi-walled carbon nanotube modified glassy carbon electrode (MWCNTs/GCE) as a working electrode, an Ag/AgCl/(3 M KCl) as a reference electrode and platinum wire as an auxiliary electrode. DPV, CV and CA experiments were carried out using an Autolab PGSTAT 30 Potentiostat Galvanostat (EcoChemie, The Netherlands) coupled with a 663 VA stand (Metrohm Switzerland). All potentials cited in this work are expressed with respect to the potential of the reference electrode. The pH measurements were made with a Metrohm 744 pH meter using a combination glass electrode.

2.3. Modification of the electrodes

A glassy carbon electrode (GCE, 2-mm diameter, Metrohm) was polished using 0.3 and 0.05 µm aluminum slurries and rinsed thoroughly with triple distilled water. The GCE was cleaned by ultrasonic agitation for 5 min in ethanol and then in the distilled water, individually.

A stock solution of 1 mg mL$^{-1}$ MWCNTs–DMF was prepared by dispersing 1 mg of MWNTs in 1 mL DMF. 20 µL of the MWCNTs–DMF solution was coated on GC electrode surface. To obtain MWCNTs/GCE, the electrode was dried at room temperature overnight. The fabricated MWCNTs/GCE was placed in the electrochemical cell containing 0.1 M PBS. In order to obtain stable responses, several cycles in the potential window of -0.1 to 1.0 V were applied using CV method.

2.4. General procedure

A 10 mL solution containing the appropriate amounts of ACT and INDO in 0.1 M PBS at pH 7.0 was transferred into the voltammetric cell. The voltammograms were recorded by applying positive-going potential from 0.2 to 1 V. The DPV results showed anodic peaks around 0.32 and 0.7 V corresponding to ACT and INDO, respectively, with heights proportional to their concentrations in solutions. The calibration curves were obtained by
plotting anodic peak currents of ACT and INDO vs. the corresponding concentrations. All experiments were carried out under open circuit conditions.

After each measurement, the MWCNTs/GCE was regenerated by washing the electrode successively with triple distilled water and 5% sodium hydroxide solution consecutively. Finally the electrode was rinsed carefully with distilled water, 5% sodium hydroxide solution and finally with the water to remove any adsorbate from the electrode surface.

3. RESULT AND DISCUSSION

3.1. Characterizing of the MWCNTs/GCE

Scanning electron microscopy (SEM) was used to observe directly the morphology of MWCNTs/GCE. The SEM images of the MWCNTs/GCE (Fig. 1) showed that the GCE surface was mostly covered with homogenous MWCNTs, which were in the form of small bundles or single tubes.

The relative electrochemical surface areas of the modified MWCNTs/GCE and bare GCE were determined by CVs measured between -0.1 and 0.6 V in 4 mM ferricyanide solution (PBS) at several scan rates. The modified MWCNTs/GCE showed a surface area 9.8 times that of GCE.

Fig. 1. SEM image of MWCNTs film on glassy carbon
3.2. Electrochemical behavior of ACT and INDO at the MWCNT/GCE

The cyclic voltammograms recorded for 300 µM ACT and 200 µM INDO using MWCNTs/GCE is shown in Fig. 2. The ACT, unlike the INDO, showed a reversible oxidation that is related to the electrocatalytic behavior of the MWCNTs [31].

![Cyclic voltammograms](image)

**Fig. 2.** Cyclic voltammograms of 300 µM ACT and 200 µM INDO at (a) GCE and (b) MWCNTs/GCE in 0.1 M phosphate buffer solution (pH 7.0) at scan rate of 50 mV s⁻¹

The differential pulse voltammograms (DPVs) recorded for ACT and INDO for bare GCE, and modified MWCNTs/GCE are shown in Fig. 3. Voltammograms is that for 250 µM of ACT, and 200 µM of INDO in PBS (pH of 7.0) at GCE. Voltammogram b is that of ACT and INDO at MWCNTs/GCE under the same conditions. As can be seen at GCE surface the oxidation peaks for ACT and INDO are very small. However at MWCNTs/GCE surface (voltammogram b), both ACT and INDO showed considerable increases in their oxidation peak currents. These phenomena could be attributed to the larger active surface area of the modified electrode and its catalytic effects.

The effect of potential scan rate on the oxidation responses of ACT and INDO were investigated in the 10-100 mV s⁻¹ scan rate range (not shown). A linear relationship between the anodic peak current and scan rate were found for ACT and INDO as follows:

\[
\begin{align*}
I_{pa}(\mu A) &= 0.557v \text{ (mV s}^{-1}\text{)} + 3.74 \quad (R^2=0.993) \quad \text{ACT} \\
I_{pa}(\mu A) &= 0.178v \text{ (mV s}^{-1}\text{)} + 2.50 \quad (R^2=0.997) \quad \text{INDO}
\end{align*}
\]
The linear relationship between peak currents and scan rates, suggests that the redox reactions of ACT and INDO compounds at MWCNTs/GCE, are adsorption-controlled processes.

3.3. Effects of operational parameters

3.3.1. Effects of supporting electrolyte and solution pH

The oxidation peak current of ACT and INDO at MWCNTs/GCE in 0.1 M PBS was found to be higher than that in other supporting electrolytes, such as acetate, citrate and ammonia buffer solutions. Therefore a 0.1 M PBS was adopted as the electrolyte for experiments. The effect of solution pH on the electrochemical response of the MWCNTs/GCE towards the simultaneous determination of 150 µM ACT and 250 µM INDO was investigated using the DPV method. Variations of the observed peak current with electrolyte pH over the range 5 to 10 are shown in Fig. 4. It can be seen that the anodic peak currents of ACT increase with increasing solution pH until it reaches 7.0. However at higher pH the oxidation peak current starts to diminish. In addition the ACT is not stable above pH of 7. These factors would favor a decrease in the oxidation peak height of ACT. The oxidation peak current for INDO also increases with pH but starts to fall away from pH 7.0. The reasons are probably the same as for ACT with the earlier maximum being reached because the INDO, being the more acidic of the two being more readily deprotonated. A pH value of 7.0, which is close to biological pH value, was selected as the optimum pH for further experiments.

Fig. 3. Differential pulse voltammograms of 250 µM of ACT and 200 µM INDO at (a) GC and (b) MWCNTs/GCE in 0.1 M phosphate buffer solution (pH 7.0). Other conditions: Open circuit, \( t_{\text{acc}} = 60 \) s, pulse amplitude=50 mV and scan rate=10 mV s\(^{-1}\), interval time 0.5 s modulation time=0.2 s and step potential=5 mV
3.3.2. Effects of accumulation time

Fig. 5 shows variations of obtained DPV anodic peak currents with accumulation time for 200 µM ACT and 150 µM INDO. Initially, peak currents for these compounds increase with accumulation time up to 60 s. However after 60 s the rate of increase falls away and they eventually plateau. Therefore an accumulation time of 60 s was chosen for further experiments.

3.4. Linear dynamic range and detection limit of the method

The electrochemical responses at MWCNTs/GCE for simultaneous additions of ACT and INDO to a 0.1 M PBS of pH 7.0 are depicted in Fig. 6 and 7. Fig. 6 shows differential pulse voltammograms and corresponding calibration curves obtained at MWCNTs/GCE in various concentrations of ACT and INDO. For ACT, a linear dynamic range from 3 µM to 400 µM, with a calibration equation of $I_p(\mu A)=0.2569c(\mu M)+9.394$ ($R^2=0.9968$) (Fig. 6, Inset A) and a detection limit of 0.11 µM (S/N=3) were obtained. A linear relationship was obtained for INDO over a range of 10 to 330 µM with a calibration equation of $I_p(\mu A)=0.3161c(\mu M)-6.718$ ($R^2=0.9965$) (Fig. 6, Inset B) and the detection limit of 0.12 µM.

![Fig. 4. Effect of pH on the differential pulse voltammogram peak currents of oxidations of 150 µM ACT and 250 µM INDO compounds at MWCNTs/GCE in phosphate buffer solutions. Insets: Plots of anodic peaks currents ($I_{pa}$) as a function of pH of buffer solutions](image-url)
**Fig. 5.** Effect of accumulation time on the differential pulse voltammogram peak currents of 200 µM ACT and 150 µM INDO in phosphate buffer (pH 7.0) solution. Other conditions as shown in Fig. 3

**Fig. 6.** Differential pulse voltammograms for different concentrations of ACT and INDO mixture as (a) 3+10, (b) 50+35, (c) 100+65, (d) 140+100, (e) 180+140, (f) 220+180, (g) 300+240, (h) 400+330, respectively, in which the first value is the concentration of ACT in µM and the second value is the concentration of INDO in µM. Insets: (A) Plot of peak currents as a function of ACT concentration. (B) Plot of the peak currents as a function of INDO concentration.
Fig. 7 displays hydrodynamic chronoamperogram response of the rotated modified electrode (3000 rpm) with successive injection of ACT and INDO at an applied potential of 0.8 V in PBS (pH 7). For ACT, a linear dynamic range was from 5 µM to 400 µM. A calibration equation of $I_p(\mu A)=0.3554c(\mu M)+1.4383$ ($R^2=0.9981$) (Inset of Fig. 7A) and a detection limit of 0.46 µM (S/N=3) were obtained. For INDO, a linear relationship was in the range of 4 to 350 µM. A calibration equation of $I_p(\mu A)=0.3113c(\mu M)+2.2327$ ($R^2=0.994$) (Inset of Fig. 7B) and a detection limit of 0.69 µM were obtained.

3.5. Interference studies

The effect of common interfering species in solutions of 100 µM ACT and 100 µM INDO under our optimum conditions were investigated. The results are summarized in Table 1. The tolerance limit listed is the concentration of the interfering species that still gives an error of $\leq 10\%$ in the determination of ACT and INDO. The data show that interferences are only significant at relatively high concentrations, confirming that the proposed method is likely to be free from interferences from common components of biological samples.

<table>
<thead>
<tr>
<th>Interfering species</th>
<th>ACT $C_{int}^a$/µM</th>
<th>INDO $C_{int}^a$/µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-dopa</td>
<td>600</td>
<td>850</td>
</tr>
<tr>
<td>Dopamine</td>
<td>650</td>
<td>900</td>
</tr>
<tr>
<td>L-alanin</td>
<td>1400</td>
<td>1800</td>
</tr>
<tr>
<td>L-glutamic acid</td>
<td>1100</td>
<td>1700</td>
</tr>
<tr>
<td>uric acid</td>
<td>300</td>
<td>700</td>
</tr>
<tr>
<td>ascorbic acid</td>
<td>500</td>
<td>750</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>3000</td>
<td>2500</td>
</tr>
</tbody>
</table>

$^a$ Cint refers to interfering compound concentration
Fig. 7. Hydrodynamic Amperometric response at rotating MWCNTs/GCE (rotating speed 3000 rpm) held at 0.8 V in PBS (pH 7.0) for simultaneous determination of ACT and INDO by successive additions of (A) (a) 5 µM ACT and (b) 4 µM INDO (Inset: corresponding calibration curve for ACT), and (B) (c) 50 µM ACT (d) 50 µM INDO (Inset: corresponding calibration curve for INDO)
3.6. Repeatability and long-term stability of the electrode

The repeatability of the DPV responses were examined giving a relative standard deviations (RSD) of 1.29% (n=10) and 1.44% (n=10) for consecutive determinations of 20 µM ACT and 20 µM INDO, respectively.

The Stability of the proposed electrode was tested by measuring the decrease in voltammetric current during repetitive DPV measurements of ACT and INDO solutions with MWCNTs/GCE stored in solution or air for certain period of time. For instance, in the determination of 20 µM ACT and 20 µM INDO in 0.1 M PBS (pH 7.0), subjecting the modified electrode to an experiment for ten measurements over 24 h, led to a less than 9.5 and 11% decrease in the voltammetric peak currents of ACT and INDO, respectively. When the electrode was stored in the atmosphere for 10 days, the corresponding current responses fell less than 12% and 14% in a solution containing 30 µM ACT and 30 µM INDO, respectively. These results suggest that the modified electrode has a relatively good long-term stability.

Table 2. Determination of ACT and INDO in human serum and human urine with MWCNTs/GCE

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Added (µM)</th>
<th>Found&lt;sup&gt;a&lt;/sup&gt; (µM)</th>
<th>R.S.D. (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACT</td>
<td>INDO</td>
<td>ACT</td>
<td>INDO</td>
</tr>
<tr>
<td>Human serum</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>9.81</td>
<td>9.74</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>20.0</td>
<td>19.35</td>
<td>19.62</td>
</tr>
<tr>
<td>Human urine</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>9.81</td>
<td>9.86</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>20.0</td>
<td>19.54</td>
<td>19.78</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average of five determinations at optimum conditions

3.7. Analytical applications

The applicability of the method used for the determination of ACT and INDO in human serum, human urine and drug samples was studied by spiking diluted samples with known amounts of ACT and INDO simultaneously. DPVs of unspiked and spiked samples were
obtained and the concentrations of ACT and INDO were determined using standard addition method and corresponding calibration plots. The results are summarized in Tables 2 and 3. The obtained good recoveries confirm that the proposed methods could be efficiently used for simultaneous determination of ACT and INDO in typical biological systems and pharmaceutical preparations.

Table 3. Determination of ACT and INDO in mixture of Tylenol tablet and indomethacin capsule sample with MWCNTs/GCE

<table>
<thead>
<tr>
<th>Added (µM)</th>
<th>ACT</th>
<th>IND</th>
<th>ACT</th>
<th>IND</th>
<th>ACT</th>
<th>IND</th>
<th>ACT</th>
<th>IND</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32.7b</td>
<td>27.5c</td>
<td>2.1</td>
<td>2.2</td>
<td>98.9</td>
<td>98.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>42.22</td>
<td>37.1</td>
<td>1.5</td>
<td>1.6</td>
<td>98.0</td>
<td>97.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>54.1</td>
<td>48.3</td>
<td>1.1</td>
<td>1.3</td>
<td>101.9</td>
<td>100.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Average of five determinations at optimum conditions
b This amount is equal to 494.5 mg per tablet
c This amount is equal to 24.52 mg per tablet

4. CONCLUSION

In this paper we introduced a new application of multi-walled carbon nanotube modified glassy carbon electrode for simultaneous determination of ACT and INDO. The results showed MWCNTs can increase anodic peak currents for ACT and INDO compounds on the electrode surface. The electrode showed high stability in repetitive experiments due to high water stability and high mechanical strength of MWNT. The effects of potential interfering compounds were studied, and it was found that the proposed procedure is free from interferences of the most common interfering compounds. When the procedure was used for the determination of ACT and INDO in real samples like human serum, urine and some drugs, satisfactory results were obtained without the necessity of sample pretreatments, time-consuming extractions, or requiring overlapped data analysis. The simple fabrication procedure, high speed, reproducibility, high stability, wide linear dynamic range, low detection limit and high sensitivity, all suggest that the proposed sensor is an attractive candidate for practical applications.
Acknowledgments

The authors gratefully acknowledge the research council of Arak University for providing financial support for this work.

REFERENCES