Application of Multi-Walled Carbon Nanotubes Modified Glassy Carbon Electrode for Determination of Mefenamic Acid in Pharmaceutical Preparations and Biological Fluids

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Summary: A chemically modified electrode is constructed based on multi-walled carbon nanotube modified glassy carbon electrode (MWCNTs/GCE). It is demonstrated that this sensor could be used for determination of Pharmaceutically important compound mefenamic acid (MEF). Differential pulse voltammetry (DPV) experiments of various concentration of MEF showed two linear dynamic ranges. The first linear dynamic range was from 2 μM to 40 μM, and the second linear dynamic range was between 50 μM to 360 μM. A detection limit of 0.21 μM (S/N = 3) was obtained. Under optimal conditions the modified electrode exhibited high sensitivity and stability for determination of MEF, making it a suitable sensor for the submicromolar detection of MEF in solutions. The analytical performance of this sensor has been evaluated for the detection of MEF in human serum, human urine and a pharmaceutical preparation with satisfactory results.

Introduction

Mefenamic acid (MEF) is a non-steroidal drug which has analgesic, anti-inflammatory and antipyretic actions and it is used specially in the treatment of rheumatoid arthritis and osteoarthritis and other muscular-skeletal diseases [1] and to relieve short-term moderate pain lasting less than one week, such as muscular aches and pains, menstrual cramps, headaches, and dental pain [2-8]. Mefenamic acid works by reducing hormones that cause inflammation and pain in the body. This medicine can increase risk of life-threatening heart or circulation problems, including heart attack or stroke and this medicine should not be used just before or after having heart bypass surgery (also called coronary artery bypass graft, or CABG). Mefenamic Acid can also increase risk of serious effects on the stomach or intestines, including bleeding or perforation (forming of a hole). These conditions can be fatal and gastrointestinal effects can occur without warning at any time while everyone is taking mefenamic acid. Older adults may have an even greater risk of these serious gastrointestinal side effects. Overdose can lead to a range of symptoms including convulsions, nausea, vomiting, vomiting blood, shallow breathing, coma. Therapeutic mefenamic acid level is 10 μg/mL [9].

Various analytical methods have been reported for the determination of mefenamic acid as pure and in dosages forms and biological fluids. These methods include titrimetric [10], luminescence [11], flow injection [12, 13], spectroflouro-metric [14,15], fluorimetry [3, 4], chemiluminescence (CL) [5], high performance liquid chromatography [16-19], high performance liquid chromatography /mass spectrometry (HPLC/MS) [20], liquid chromatography/mass spectrometry (LC/MS) [21], gas chromatography (GC) [22], gas chromatography/high performance liquid chromatography (GC/HPLC) [23], gas chromatography/mass spectrometry (GC/MS) [24-27], high performance thin layer chromatography (HPTLC) [28], ion pair partition chromatography [29], capillary electrophoresis (CE) [30], capillary electrophoresis/mass spectrometry (CE/MS) [31, 32], capillary isotachophoresis [33] and other spectrophotometric methods [34-43]. However, some of these procedures suffer from disadvantage such as extraction into organic solvent [36], requiring non-aqueous medium [40] and others need control of temperature [39, 41], time consuming or require expensive and sophisticated instruments. In addition, in some cases, low sensitivity and selectivity makes them unsuitable for routine analysis. Consequently, the development of a simple, inexpensive, sensitive and accurate analytical method for determination of MEF is of considerable importance.

MEF is an electroactive compound which can be oxidized electrochemically. The development and application of electrochemical sensors for MEF analysis, with respect to its sensitivity, accuracy, and simplicity, has been of greater interest in recent years [44-50]. Success of a sensor for routine analysis in biological media requires its applicability in biological pH. Carbon nanotubes (CNTs) consisting of cylindrical graphite sheets with nanometer

diameter, combine in a unique way with large surface area, high electrical conductivity and remarkable mechanical properties. CNTs are widely used to prepare modified electrodes and construct sensing films as they can enhance electron transfer rate and sensitivity [51-55].

In this work we present an application of low cost multi-walled carbon nanotube modified glassy carbon electrode (MWCNTs/GCE) as a sensor for determination of MEF in biological pH. This study has led to the development of a voltammetric method with good characteristics, such as simplicity of electrode preparation with low cost material, good selectivity, wide linear dynamic range. Finally the analytical performance of this sensor for determination of MEF in human serum, human urine and a pharmaceutical preparation samples is evaluated.

Results and Discussion

Scanning Electron Microscopy (SEM) Analysis of MWCNTs/GCE

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.

Electrochemical Behavior of MEF on MWCNTs/GCE

In Fig. 2 the differential pulse voltammograms recorded for MEF at bare GCE and MWCNTs/GCE are shown. Voltammogram (A) is that for 60 μM of MEF in PBS (pH 7) at GCE. Voltammogram (B) is that of MEF at MWCNTs/GCE at the same conditions. As can be seen, for the GCE the oxidation peak for MEF is very small. However the DPV for the MEF at the MWCNTs/GCE (voltammogram B) shows considerable increase in its oxidation peak current. The presence of MWNTs could both increase the electrode surface area and facilitate the electron transfer between electrode and the analyte, therefore the enhancement in the corresponding electrochemical oxidation peak current was observed.

The effect of potential scan rate on peak current of 100 μM MEF in PBS (pH 7.0) was investigated. Fig. 3 shows the cyclic voltammograms of the MEF using the modified electrode at different scan rate in the potential range of 0.35 to 0.95 V. The results showed that the anodic peaks current of MEF were proportional to the scan rate over the range 10–100 mV s^{-1} (Figure 3, Inset A) indicating adsorptive properties of the electrochemical process. At higher sweep rates, up to 350 mV s^{-1}, the plot of peak currents versus scan rate plot deviates from linearity and the peak current becomes proportional to the square root of the scan rate (Fig. 3), indicating a diffusion controlled process.

Effect of Operational Parameters

The effect of pH of solutions on the electrochemical response of the MWCNTs /GCE towards the determination of 100 μM MEF was investigated using CV method in 0.1M phosphate buffer solutions. Variations of the observed peak current with electrolyte pH of the electrolyte in the pH range from 5 to 10 are shown in Fig. 4. It can be seen that the anodic peak currents of MEF increases with raising the solution pH until it reaches 7 (Inset A). However at higher pH the MEF oxidation peak current starts to diminish. Therefore the pH value of 7 was chosen as an optimum solution pH for further experiments. Variation of MEF oxidation peak potential with pH is in accordance with equation of Ep = -0.0526 pH + 1.0261 (Inset B). For a Nernstian process which numbers of transferred electrons are equal to number of transferred proton, the slope would be expected to be -59 mV pH^{-1} unit. The slope of -52.6 mV pH^{-1} suggests that the numbers of electrons and protons transferred in the oxidation reaction of MEF are equal.

The plot of the cyclic voltammogram anodic peak current versus accumulation time for 100 μM MEF solution was obtained. Initially, peak currents for this compound increase with accumulation time up to 60 s. However after 60 s of accumulation time, the peak currents reach a slight increasing and then plateau. As a consequence, the accumulation time of 60 s was chosen as an optimum time for further experiments.

Linear Dynamic Range and Detection Limit of the Method

The electrochemical response for additions of MEF in a 0.1M PBS of pH 7 using MWCNTs/GCE are depicted in Fig. 5 and 6. Fig. 5 shows differential pulse voltammograms together with the corresponding calibration curves obtained using a MWCNTs/GCE in various concentrations of MEF. By application of DPV method, two linear

ranges were obtained. The first linear dynamic range was from 2 μM to 40 μM, with a calibration equation of Ip(μA) = 0.3371c (μM) + 0.931 (R^2=0.9963) and the second linear dynamic range was between 50 μM to 360 μM with a calibration equation of Ip(μA) = 0.1127c (μM) + 8.6069 (R^2=0.9977). A detection limit of 0.21 μM (S/N = 3) was obtained.

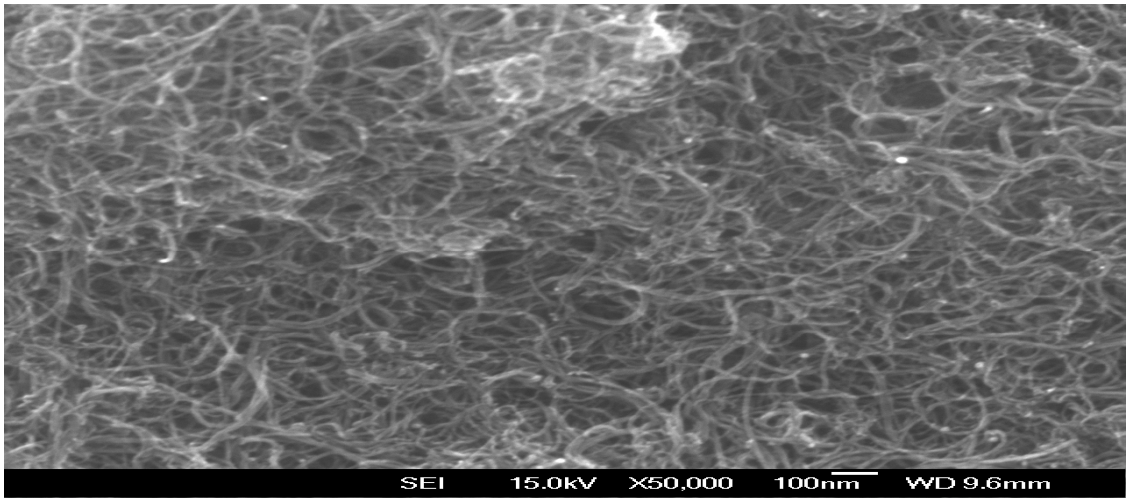

Fig. 1: SEM image of MWCNTs film on a GCE.

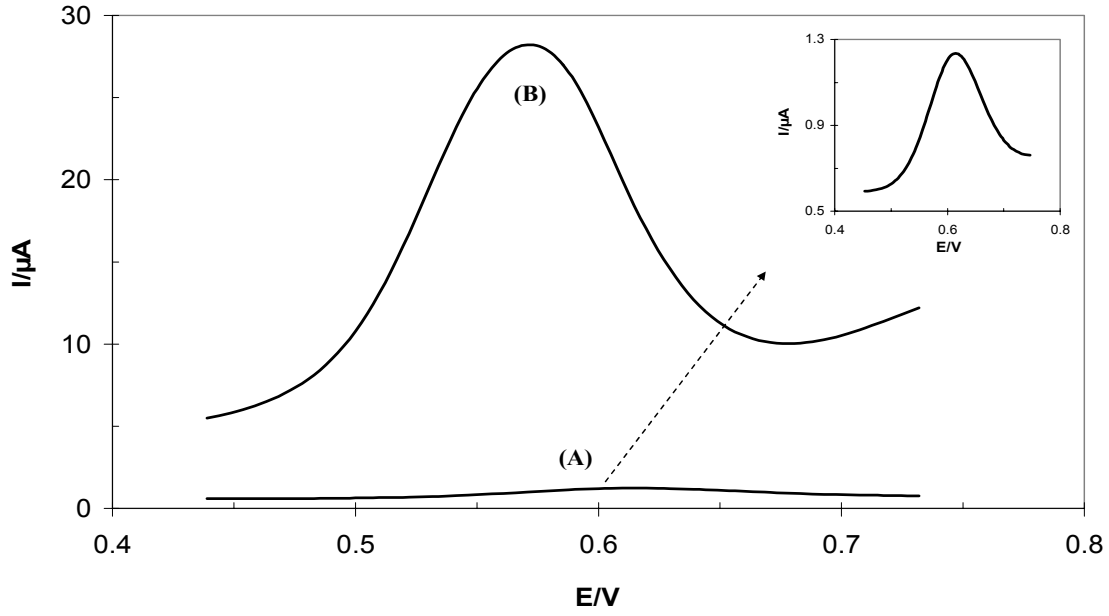

Fig. 2: Differential pulse voltammograms of 60 μM MEF at (a) GC and (b) MWCNTs/GCE in 0.1M phosphate buffer solution (pH 7). Other conditions: Open circuit, t_{acc}= 60s, pulse amplitude = 50 mV and scan rate = 10 mV s^{-1}, interval time = 0.5 s modulation time = 0.2 s and step potential = 5 mv.

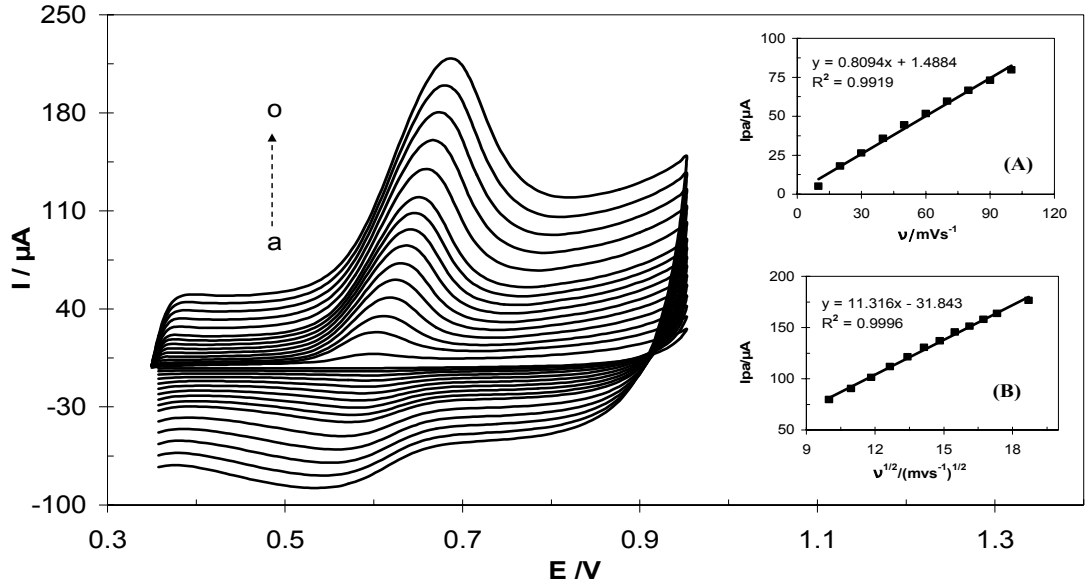

Fig. 3: Effect of scan rate of potential on the cyclic voltammograms peak currents of 100 µM MEF in phosphate buffer solution for different scan rate as (a) 10, (b) 20, (c) 30, (d) 40, (e) 50, (f) 60, (g) 70, (h) 80, (i) 90, (j) 100, (k) 120, (l) 140, (m) 160, (n) 180 and (o) 200 mVS^{-1}. Insets: (A) Plot of peak currents as a function of scan rate of potential; (B) Plot of peak currents as a function of square root of the scan rate of potential.

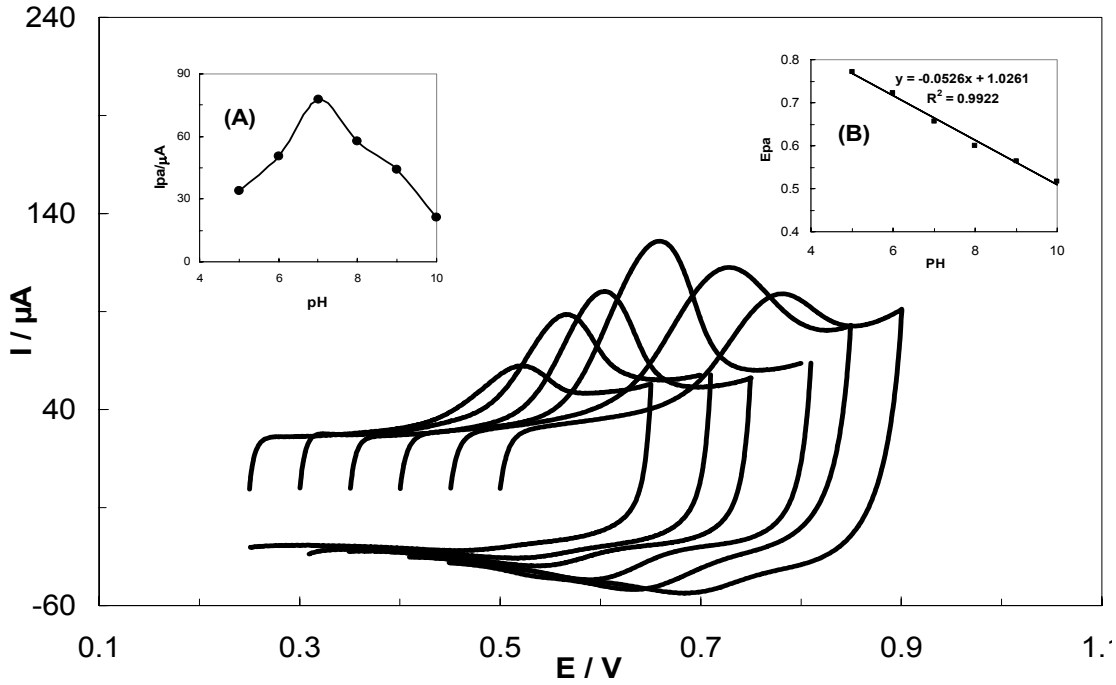

Fig. 4: Effect of pH on the cyclic voltammogram peak currents of oxidations of MEF compound at MWCNTs/GCE in phosphate buffer solutions. Concentrations: MEF: 100 µM at scan rate of 100 mVs^{-1}. Inset Insets: (A) Plot of peak currents as a function of pH buffer. (B) Plot of potential of peaks (Ep) as a function of pH buffer.

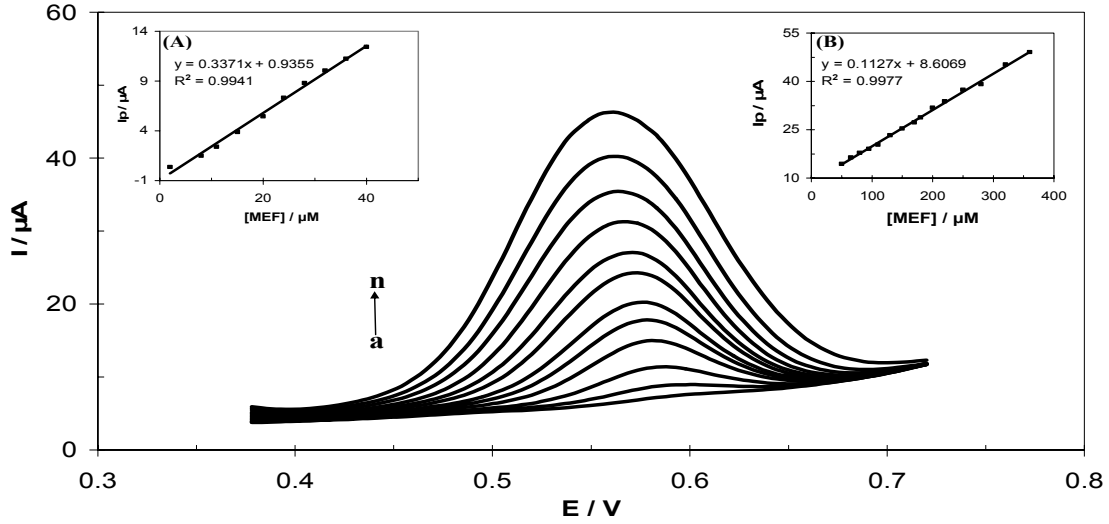

Fig. 5: Differential pulse voltammograms for different concentrations of MEF as (a) 2, (b) 8, (c) 15, (d) 24, (e) 32, (f) 40, (g) 65, (h) 95, (i) 130, (j) 170, (k) 200, (l) 250, (m) 280 and (n) 360. Insets: (A) Plot of peak currents as a function of MEF concentration. (B) Plot of peak currents as a function of MEF concentration.

Fig. 6 displays hydrodynamic chronoamperogram response of the rotated modified electrode (3000 rpm) with successive injection of MEF at an applied potential of 0.65 V in PBS (pH 7). For MEF, the linear dynamic range was from 10 µM to 310 µM. A calibration equation of Ip(µA) = 0.0932c (µM) + 0.5006 (R^2=0.9982) (Inset of Fig. 6) and a detection limit of 0.314 µM (S/N = 3) were obtained.

Repeatability and Long-Term Stability of the Electrode

The repeatability of the analytical method for determination of MEF has been studied. Indeed, the relative standard deviation (RSD) of 1.83 and 1.04 % for 10 and 50 µM MEF in ten consecutive determinations has been obtained, respectively.

Another attraction of the proposed modified electrode is that the resulting modified electrode is of a good long-term stability. Stability of the proposed electrode was tested by measuring the decrease in voltammetric current during repetitive DPV measurements of MEF with MWCNTs/GCE stored in solution or air for certain period of time. For example, in the determination of 60 µM MEF in 0.1 M PBS (pH 7), subjecting the modified electrode to an experiment every 30 min, led to a less than 7 % decrease in the voltammetric currents after 24 h. When the electrode was stored in the atmosphere for 7 days, the corresponding current response fell less than 10% in a solution containing 60 µM MEF.

Interference Studies

The effects of common interfering species in solution of 100 µM MEF under the optimum conditions were investigated. The results are summarized in Table-1 and show that they do not significantly affect the height of the peak currents for MEF. The tolerance limit listed is the concentrations of interfering species that still gives an error of ≤ 10% in the determination of MEF compound. The data confirm that the proposed method is free from interferences of the most common interferants.

Table-1: Maximum tolerable concentration of interfering species.

Interfering species	C_{int}/µM
L-dopa	**600**
dopamine	**550**
L-alanin	**1300**
L-glutamic acid	**1500**
uric acid	**450**
ascorbic acid	**800**
Aspartic acid	**2500**

$C_{int.}$ refers to interfering compound concentration

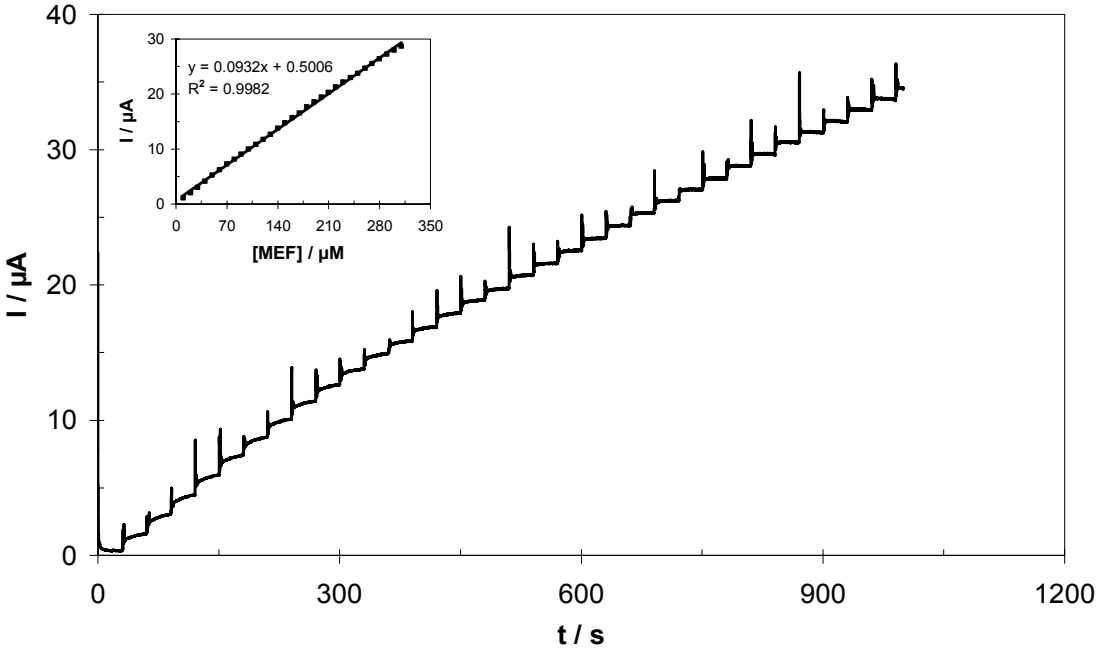

Fig. 6: Amperometric response at rotating MWCNTs/GCE (rotating speed 3000 rpm) held at 0.65 V in PBS (pH 7) for determination of MEF by successive additions of 10 µM MEF. Inset: corresponding calibration curve.

Analytical Applications

The applicability of the MWCNTs/GCE was examined for the determination of MEF in human serum and human urine (Table-2) and drug samples (Table-3). The differential pulse voltammograms were obtained by spiking known amounts of MEF in the prepared real solutions using MWCNTs/GCE at optimum conditions as described earlier. The concentrations were obtained by using the calibration plots. The recoveries were acceptable and they confirm that the proposed methods could be efficiently used for the determination of trace amounts of MEF in biological systems and pharmaceutical preparations.

Table-2: Determination of MEF in human serum and urine with MWCNTs/GCE .

Sample	*Added (µM)*	*Found[a] (µM)*	*R.S.D. (%)*	*Recovery (%)*
	10.00	**9.89**	**2.6**	**98.9**
serum	**20.00**	**19.42**	**1.7**	**97.1**
	30.00	**30.54**	**1.1**	**101.8**
	10.00	**9.67**	**2.3**	**96.7**
urine	**20.00**	**19.50**	**1.8**	**97.5**
	30.00	**29.46**	**1.4**	**98.2**

a. Average of five determinations at optimum conditions.

Table-3: Determination of MEF in mefenamic acid capsule with MWCNTs /GCE

Analyte	*Added (µM)*	*Found[a] (µM)*	*R.S.D. (%)*	*Recovery (%)*
MEF	**0.00**	**24.33[b]**	**2.4**	**102.8**
	10.00	**34.26**	**2.0**	**99.6**
	20.00	**44.43**	**1.7**	**101.7**

a. Average of five determinations at optimum conditions.
b. This amount is equal to 257.1 mg per tablet.

Experimental

Materials and Methods

All chemicals were analytical grade and used without further purification. MEF were obtained from Sigma chemical company. Multi-walled carbon nanotubes (MWCNTs) (>95 wt%, 5-20nm) were purchased from PlasmaChem GmbH company. 0.1 M Phosphate buffer solution (PBS) was prepared by dissolving appropriate amounts of sodium hydrogen phosphate and sodium dihyrogen phosphate in 250 mL volumetric flask. Electrochemical experiments on the MEF were carried out in 0.1 M PBS at pH 7.

Fresh human serum samples were

available from Razi Institute of Vaccine and Serum Company (Tehran, Iran). serum and urine samples were filtered and diluted 25 times with 0.1 M PBS of pH 7 and checked for the determination of the recovery by spiking with MEF. Ten capsules of MEF (Alhavi company, Iran) (each labeled as containing 250 mg of MEF) were accurately weighed and powdered in a mortar. An amount equivalent to one in capsule content was dissolved in 70 mL of 0.1M PBS (pH 7). After sonication for 10 minutes, the solution was filtered, the residue was washed three times with 10 mL appropriate solvent, and the filterate volume was adjusted to 100 mL also using the same solvent. This solution was diluted 500 times with 0.1 M PBS of pH 7. The solution was used for the determination of the recovery in spiking of MEF compound.

Instrumentation

All the voltammetric measurements were carried out using nanotube modified glassy carbon electrode (MWCNTs/GCE) as a working electrode, an Ag/AgCl/3M 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 given are with respect to the potential of the reference electrode. pH measurements were performed with a Metrohm 744 pH meter using a combination glass electrode.

Modification of the Electrodes

A glassy carbon electrode (GCE, 2-mm diameter, Metrohm) was polished with 0.3 and 0.05 μm aluminum slurries and rinsed thoroughly with triply distilled water. The GC electrode was individually cleaned by ultrasonic agitation for 5 min in ethanol and then distilled water. The electrode was then dried under nitrogen gas flow. Variation of concentration of MWCNTs in DMF solution and volume of the suspension of MWCNTs/DMF for drop coating of the GCE, showed that the best sensitivity for the modified electrode could be obtained when concentration of 1mg/mL and volume of 20 μL of MWCNTs/DMF were used. A solution of 1 $mgml^{-1}$ MWCNTs–DMF was prepared by dispersing 1.0 mg MWCNTs in 1mL of DMF. Then the solution was sonicated by ultrasonic agitation for 30 minute. 20 μl of MWNTs–DMF solution was placed on the GC electrode surface. The electrode was then dried at room temperature to obtain MWCNTs /GCE. The fabricated MWCNTs /GCE was placed in the electrochemical cell containing 0.1M PBS and several cycle in the potential windows of 0.1 to 0.9 V were applied using CV method to obtain stable responses.

The MWCNTs/GC modified electrode was characterized by electrochemical methods. $K_3Fe(CN)_6$ exhibited a pair of quite reversible redox peaks at a bare GC electrode. At the modified electrode, a pair of higher and reversible redox peaks could still be observed. On the other hand, under the same conditions, the anodic peak of $K_3Fe(CN)_6$ at both the GC and MWCNTs/GC electrodes increased in proportion to the square root of the scan rate. It was found that in both cases the electrode process was diffusion controlled. The regression equations for the 4 mmol L^{-1} $K_3Fe(CN)_6$ were:

$Ipa(\mu A) = 92.57\nu^{1/2}$ $(V\ s^{-1})^{1/2} + 8.440$ $(R^2 = 0.995)$ **GC**

$Ipa(\mu A) = 904.53\nu^{1/2}$ $(V\ s^{-1})^{1/2} + 7.267$ $(R^2 = 0.999)$ **MWCNTs/GC**

A reversible system should satisfy the Randles-Sevcik equation:

$$I_P = 2.99\times10^5 \alpha^{1/2} n^{3/2} A C_0 D_R^{1/2} \nu^{1/2}$$

According to the ratio of the slopes of the two lines, the apparent area of the MWNTs/GC modified electrode was about 9.8 times greater than that of the GC electrode.

General Procedure

Solutions (10 mL) containing appropriate amounts of MEF in 0.1 M PBS at pH 7 were transferred into the voltammetric cell. The stirrer was switched on and 60 s accumulation time under open circuit condition was applied. Following the accumulation period, the stirrer was stopped, and after 5 s quiescence time, the voltammogram was recorded by applying a positive going potential from 0.3 to 0.9 V. The voltammogram showed anodic peak around 0.58 V corresponding to MEF compound with height proportional to MEF concentration in solution. All experiments were carried out under open circuit condition. After each measurement, the MWCNTs/GCE was regenerated by thoroughly washing the electrode successively with triply distilled water and then 5% sodium hydroxide

solution consecutively. Finally the electrode rinsed carefully with distilled water to remove any adsorbate from electrode surface and provide a fresh one for subsequent experiments.

Conclusions

In this paper we have shown that the application of MWCNTs on GCE can increase anodic peak current for MEF on the electrode surface. The results indicated that the use of a MWCNTs/GCE allows the determination of MEF at biological pH with high sensitivity and wide linear dynamic range. The electrode showed high stability in repetitive experiments due to high water stability and high mechanical strength of MWCNTs. The effects of potential interfering compounds were studied, and it was found that the proposed procedure is free from interferences of most common interfering compounds. The proposed sensor was used in determination of MEF in some real samples like human serum, urine and some drugs, without the necessity of sample pretreatments with short analysis time using economic electrochemical instrument, with satisfactory results. The simple and low cost the electrode fabrication procedure, high speed, reproducibility, high stability, wide linear dynamic range and high sensitivity, suggest that the proposed sensor is an attractive candidate for practical applications.

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