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Electrochemical determination of diazepam in real samples based on fullerene-functionalized carbon nanotubes/ionic liquid nanocomposite

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ABSTRACT

A modified electrode using fullerene-functionalized carbon nanotubes and ionic liquid (IL, 1-butyl-3-methylimidazolium tetrafluoroborate) has been applied for the determination of diazepam in real samples including serum, urine, and tablets. The properties of fullerene-functionalized carbon nanotubes and ionic liquid were characterized by transmission electron microscopy, scanning electron microscope, electrochemical impedance spectroscopy, and voltammetry. The experimental results confirmed that modified electrode with fullerene-functionalized carbon nanotubes and ionic liquid has good electrocatalytic activity toward the reduction of diazepam. The electrocatalytic current increases linearly with the diazepam concentration in the ranges of 0.3–700.0 μM, and the detection limit is 87 ± 2 nM. The proposed electrode displayed excellent repeatability and long-term stability and it was satisfactorily used for determination of diazepam in real samples (commercially tablet, urine, and serum samples) with high recovery.

1. Introduction

Benzodiazepines are the chemicals having the important medicinal applications, thanks to their sedative, anticonvulsant and hypnotic properties [1]. Benzodiazepines are prescribed for the therapy of anxiety, anti-depressive, sleep disorders, and convulsive attacks. Diazepam is the most commonly benzodiazepine drug used as a popular drug. A short term use of this medication is generally safe and effective. Although, the long term use of diazepam is very controversial, because of the potential of tolerance, dependence, withdrawal, and other adverse effects. However, abuse of diazepam can have serious consequences, even causing death when taken in overdose. Therefore, from the health viewpoints, development of a sensitive analytical method for the determination of diazepam in biological fluids is very important. In recent years a variety of analytical and pharmacological studies on diazepam have been described. The dominant assay methods include gas chromatography–mass spectrometry [2,3], radioimmunoassay [4], spectrophotometry [5,6], enzymatic and immunoassay methods [7,8], capillary electrophoresis [9], and electrochemical techniques [10–13]. Among them, electrochemical methods are practical and attractive because electrochemical instrumentation is usually compact, relatively inexpensive, reliable, and sensitive [14–18]. Moreover, the development of a rapid, simple, and sensitive electrochemical method for diazepam determination that does not require sample pretreatment is possible.

Carbon based nanotubes composites, especially carbon nanotubes (CNTs), have attracted much attention due to their novel structural, mechanical, electronic, and chemical properties [19,20]. Modification of CNTs with various functions paves the way for their future applications in different fields [21–25]. Recently, we have introduced benzofuran derivative onto CNTs and used it for the electrochemical detection of isoproterenol and serotonin with high specificity and sensitivity [26]. Fullerene has attracted much attention of researchers owing to its remarkable electrochemical properties and electrocatalysis [27,28]. One possible field of its application is the modification of electrodes for the enhancement of peak currents in electro-analysis, because it is chemically stable, metallic impurity free and relative simple to implement, and gives rise to reproducible electrocatalytic responses [29,30]. Various

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electrochemical sensors based on fullerene have been developed to detect biologically active analytes such as dopamine [31], NADH [32], iodine [33], bisphenol-A [34], carbamazepine [35] and atenolol [36]. However, electrocatalytic properties of C_{60} modified electrodes have not been widely accepted [37]. The group of Compton has extensively explored the electrochemistry of fullerene and has shown that the observed electrocatalysis in some cases is caused by either the small amount of graphite impurity, or introduction of surface oxygenated species with electrode pretreatment, while electrocatalysis mediated by C_{60} is only likely "where C_{60} itself becomes oxidized or reduced" [37–39].

It is well-known that the composite of fullerene with other nanomaterials, such as carbon nanotubes, plays an important role in the dispersion and catalytic activity of the fullerene [40]. Recent investigations revealed that C_{60}-functionalized CNTs films were found to be electrocatalytic for a range of analytical targets [41–43]. We have recently reported the electroanalytical applications of C_{60}-functionalized CNT composite for the electrocatalytic determination of catecholamines [44]. In fabricating electrochemical sensing layers, the CNT/IL-based composite materials are advantageous due to the following reasons: (i) retain inherent mechanical, electrical, and thermal properties of CNT, (ii) better solvent and conductivity property of ILs [26], and (iii) proper interaction between CNT and ILs [27], therefore, combination of the IL and CNT-based materials can create unique materials for fabrication of electrochemical sensors and biosensors.

In order to explore the electrochemistry of C_{60}-functionalized CNTs and ionic liquid (IL, 1-butyl-3-methylimidazolium tetrafluoroborate) composites as well as their potential application in diazepam reduction, in this work, a modified electrode by C_{60}-functionalized CNTs/IL nanocomposite was used for determination of diazepam in real samples. The main object of this work was to develop an electrochemical method for determination of diazepam in real samples. To the best of our knowledge, a few articles have introduced different modified electrodes that can be used for reduction of diazepam [45]. This demonstrates that modified electrode by this nanocomposite facilitates electron transfer due to unique electrochemical properties of C_{60}-functionalized CNTs and IL has exhibited some advantages including high ionic conductivity, wide electrochemical windows, and good solubility, which results in the enhanced sensitivity and stability of modified electrode.

2. Experimental

2.1. Apparatus and chemicals

All measurements were conducted using a three electrode system, where a GCE or a modified GCE served as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl/KCl (3.0 M) electrode as the reference electrode. The electrochemical experiments were performed by an Autolab potentiostat/galvanostat (PGSTAT–302 N, Eco Chemie, Netherlands). A Metrohm 691 pH/Ion Meter was used for pH measurements. The morphology of nanomaterial was characterized using transmission electron microscopy (TEM) (Philips EM208) and scanning electron microscopy (SEM, Hitachi S-4160).

All solutions were freshly prepared with double distilled water. Diazepam, sodium hydroxide, phosphate salt, fullerene, 1-butyl-3-methylimidazolium tetrafluoroborate, carbon nanotube, solvents and reagents were of pro-analysis grade from Merck (Darmstadt, Germany). These chemicals were used without further purification.

2.2. Preparation of the electrode

Preparation of the modified electrode includes the pretreatment of GCE and the immobilization of nanocomposite. GCE was successively polished with alumina powder, and washed with double distilled water for 10 min. C_{60}–CNT/IL nanocomposite was prepared by simple solution mixing method that was similar to our previous paper [44]. Briefly, purified MWCNTs and C_{60} (MWCNTs/C_{60} = 2:1) with a total amount of about 5 mg were dispersed in 10 mL toluene solution using ultrasonic bath and then filtered and thoroughly rinsed with acetonitrile to remove the excipients and then dried at 40 °C under vacuum overnight to obtain the C_{60}-CNT composites. For the preparation of C_{60}-CNT-IL nanocomposite, 2.0 mg C_{60}-CNT was uniformly dispersed in 4 mL ethanol and then 30 μL of IL were added and with the aid of ultrasonic agitation, a uniform solution was prepared.

2.3. Preparation of real samples

Ampoule was prepared (10 mg mL^{-1}, Caspian Tamin Co., from Iran) and then suitable amount of the solution plus 10 mL of 0.1 M phosphate buffer solution (pH 7.0) were used for the analysis.

The tablet sample (diazepam tablet 5 mg from Sobhan Darou Co., Iran) was purchased from the local drug store. Seven tablets were weighed and finely powdered. Then, 10 mg of each tablet powder was accurately weighed and dissolved in 100 mL water by ultrasonication. Then the solution was filtered and diluted quantitatively to an appropriate concentration for assay with phosphate buffer solution (pH 7.0). Standard addition method was used for the determination of diazepam in the samples.

Urine samples were stored in refrigerator immediately after collection. Ten milliliters of the sample were centrifuged for 40 min at 3000 rpm. The supernatant was filtered out using a filter and then diluted 3-times with the PBS (pH 7.0). The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. Also, serum samples were directly subjected to the voltammetric measurement after filtration using a 0.45 μm filter.

3. Results and discussion

3.1. Characterization of the C_{60}-CNT/IL composites

The electrochemical response of a nanocomposite is related to its physical morphology of its surface. TEM images of C_{60}-CNT composites reveal the C_{60} decorated on the CNT surface and it was indicated that C_{60}-CNT composites were obtained under our experimental conditions (Fig. 1A). Typical SEM images of C_{60}-CNT/IL are shown in Fig. 1B. In the composite without IL the CNTs could be clearly distinguished, when IL is introduced in this composite, the film becomes more uniform and even, this is related to the binding and blanketing effect of IL (Fig. 1B).

Furthermore, the C_{60}-CNT were added into toluene and sonicated for 60 min, but the toluene solution did not turn purple, a characteristic color of C_{60} in toluene. These results indicate that C_{60} has been strongly attached on the CNT surface.

In order to understand the electrochemical performance of the modified electrode by nanocomposite, we investigated various modified electrodes by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV).

Fig. 2C showed the impedance spectra represented as Nyquist plots for bare GCE (a), CNT/GCE (b), C_{60}-CNTs/GCE (c), and C_{60}-CNTs/IL/GCE (d) in 1.0 mM $K_4Fe(CN)_6$ and 1.0 mM $K_3Fe(CN)_6$.

The charge transfer resistance (R_{ct}) reflects the electron transfer kinetic processes of the redox probe at the electrode interface, and the linear segment of the EIS at lower frequencies signifies