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Electro-oxidation and voltammetric determination of oxymetholone in the presence of mestanolone using glassy carbon electrode modified with carbon nanotubes

Abbas Afkhami *, Hamed Ghaedi, Tayyebeh Madrakian, Davood Nematollahi, Banafsheh Mokhtari

Faculty of Chemistry, Bu-Ali Sina University, Hamedan, Iran

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ABSTRACT

A new chemically modified electrode was constructed and applied to the electro-oxidation of the oxymetholone. Also, the electrode was applied to the simple, rapid, highly selective and sensitive determination of oxymetholone (OXM) in pharmaceutical and plasma samples using square wave voltammetry (SWV). The multi-walled carbon nanotubes modified glassy carbon electrode (MWCNT/GCE) were prepared by casting of the multi-walled carbon nanotubes (MWCNT) suspension on the glassy carbon electrode surface. The limit of detection and the linear range were found to be 1.36 and 2.00–90.00 ng mL^{-1} of OXM, respectively. The effects of potentially interfering substances on the determination of this compound were investigated and found that the electrode is highly selective. The proposed modified electrode was used for the determination of OXM in human plasma and pharmaceutical samples. This reveals that MWCNT/GCE shows excellent analytical performance for the determination of OXM in terms of very low detection limit, high sensitivity, very good repeatability and reproducibility over other methods reported in literature.

1. Introduction

Oxymetholone (OXM), 17-hydroxy-2-[hydroxymethylenel-17-methyl-5a-androstim-3-one, is an orally active 17a-alkylated anabolic–androgenic steroid first described by Ringold et a1. [1]. It has a fully saturated A-ring structure, which may reduce the risk of hepatotoxicity [2]. It is used in treating anemia. Clinical assessments of OXM have been made to establish the degree of anabolism and androgenicity of this steroid [3] and information on its side effects have been documented [3–5]. Despite the wide use of this steroid in pharmaceutical preparations and significant studies on side effects [4], relatively little is known about the solution chemistry of this compound. OXM [5] is a l,3-dicarbonyl compound, which can exist in three tautomeric forms I–III (Fig. 1) [6]. There is no quantitative information available regarding the positions of these equilibria and on solvent effects on them. Nevertheless, the structure of OXM is usually presented as I, which in nonhydroxylic media is stabilized by formation of an intramolecular hydrogen bond (IV). In hydroxylic solvents it is able to form intermolecular hydrogen bonds, the role of intramolecular hydrogen bonds is usually negligible whereas covalent solvation, resulting in formation of hydrates and hemiacetals, may occur in nonhydroxylic solvents. As aldehydes form hydrates and hemiacetals more readily than ketones [7], nucleophilic addition of the solvent will favor the formyl groups in structures II and III. As conjugation deactivates the carbonyl function toward hydrate and hemiacetal formation [7], stronger hydration and reaction with alcohols is predicted to involve structure II rather than III. In this structure, the presence of the electron-withdrawing carbonyl groups in the α-position can be expected to enhance the reactivity of the formyl group toward nucleophilic addition [6,7].

OXM is also used as a doping agent [8,9]. Intramuscular or deep subcutaneous injection is the principal route of administration of all anabolic steroids except the 17-alpha-substituted steroids as OXM, which are active, orally [10]. This is feasible because substitution at the 17-carbon protects the compound from the hepatic metabolism [11]. Many side-effects have reportedly been associated with chronic use of high doses of all oral anabolic–androgenic hormones including OXM. These include high blood pressure, water retention, prostate gland enlargement, gynecomastia (abnormal breast tissue growth in males) and liver damage. OXM is the anabolic steroid most associated with premature hair loss [8–11]. Consequently, the development of rapid, simple and accurate method with high sensitivity for the determination of

* Corresponding author. Tel./fax: +98 811 8272404.
E-mail address: afkhami@basu.ac.ir (A. Afkhami).

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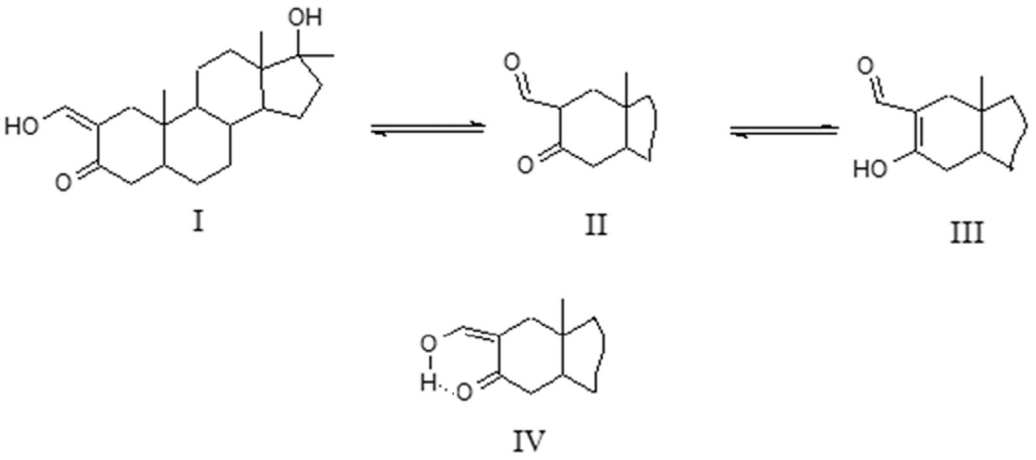

Fig. 1. Different tautomeric forms of OXM [7].

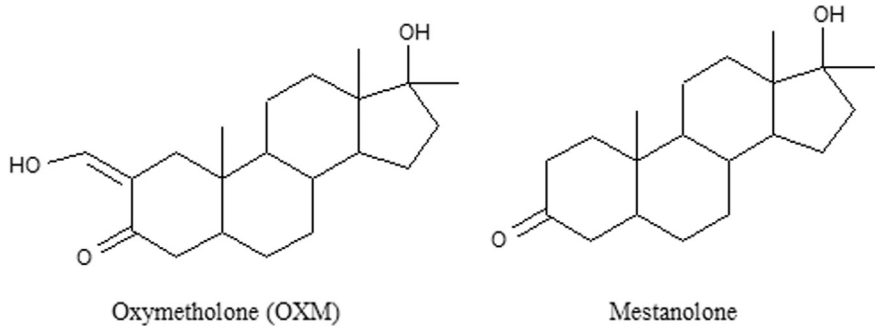

Fig. 2. Structures of OXM and mestanolone.

OXM at (ultra)trace levels in pharmaceutical and biological samples is of particular significance. To the best of our knowledge, there is no report on the determination of OXM by electrochemical methods. This shows the importance of the development of rapid, simple, sensitive and accurate electrochemical methods for measuring OXM.

The development of electrochemical sensors has been widely researched as an inexpensive method to sensitively detect a variety of biological analytes. Carbon based electrodes have been commonly used because of their low cost, good electron transfer kinetics and biocompatibility. Recently, carbon nanotubes (CNTs) have also been incorporated into electrochemical sensors. While they have many of the same properties as other types of carbon, CNTs offer unique advantages including enhanced electronic properties, a large edge plane/basal plane ratio, and rapid electrode kinetics [12].

To find new electrocatalytic surfaces, a suitable electrode substrate, such as glassy carbon (GC) or gold, is modified with a film or layer of CNTs. Several methods have achieved the electroanalysis of different analytes by using CNT modified electrodes. Benefits of low detection limits, increased sensitivity, decreased overpotentials and resistance to surface fouling are found by these CNTs-based electrodes [13]. Electrochemistry implies the transfer of charge from one electrode to another. Due to the curvature of the carbon graphene sheet in nanotubes, the electron clouds change from a uniform distribution around the C−C backbone in graphite to an asymmetric distribution inside and outside the cylindrical sheets of the nanotube [14]. Because the electron clouds are distorted, a rich π-electron conjugation forms outside the tube, therefore, making CNTs electrochemically active [14].

There are numerous reports of CNT-modified electrodes for the detection of different analytes with low detection limits, decreased overpotentials, and resistance to surface fouling [15–18].Therefore, CNT-based sensors generally have higher sensitivities, lower limits of detection, and faster electron transfer kinetics than traditional carbon electrodes. Many variables need to be tested and then optimized to create a CNT-based sensor. Electrode performance can depend on the synthesis method of the nanotube, CNT surface modification, the method of electrode attachment, and the addition of electron mediators [12].

The physical and catalytic properties make CNTs ideal for use in sensors. Most notably, CNTs display high electrical conductivity, chemical stability, and mechanical strength. The two main types of CNTs are single-walled CNTs (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). SWCNTs are sp^2 hybridized carbon in a hexagonal honeycomb structure that is rolled into hollow tube morphology [19]. MWCNTs are multiple concentric tubes encircling one another [20].

In this work, a modified glassy carbon electrode was used as the working electrode for investigation of electro-oxidation behavior of OXM and determination of this anabolic steroid in the presence of its main metabolite mestanolone (Fig. 2) that its chemical structure is very similar to OXM, in the biological and pharmaceutical samples. To the best of our knowledge, this is the first report on the investigation of the oxidation and determination of OXM with the carbon electrode.

2. Experimental

2.1. Apparatus and chemicals

All electrochemical experiments including cyclic voltammetry (CV), square wave voltammetry (SWV) and impedance spectroscopy