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A sensitive voltammetric sensor for determination of synthetic corticosteroid triamcinolone, abused for doping

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ABSTRACT

Edge plane pyrolytic graphite electrode (EPPGE) modified with single-wall carbon nanotubes (SWNTs) has been used as a sensor to determine triamcinolone, abused by athletes for doping. A comparison of the voltammetric behavior between SWNTs modified EPPGE and fullerene – C_{60}-modified EPPGE indicated that SWNTs modified EPPGE is more sensitive. The electrode exhibited an effective catalytic response with good reproducibility and stability. The effect of several parameters such as pH, square wave frequency and steroid concentration were studied. The square wave voltammetric response of the electrode to triamcinolone is linear in the range 0.1–25 nM with a detection limit and sensitivity of 8.9×10^{-10} M and 2.06 μA nM^{-1}, respectively. The method was applied for the determination of triamcinolone in several commercially available pharmaceuticals and real urine samples obtained from patients undergoing pharmacological treatment with triamcinolone. A comparison of the observed results with HPLC analysis indicated a good agreement. The product obtained after reduction of triamcinolone was also characterized using ^{1}H NMR and GC–MS and the site of reduction is found to be carbonyl group at position 20. The method described is rapid, simple and accurate and can be easily applied for detecting cases of doping.

1. Introduction

Corticosteroids are high potency drugs which decrease inflammation by acting within cells to prevent the release of certain chemicals that are important in the immune system. The use of corticosteroids in combination with other hormonal substances has been known to result in increased mass gain with bovines (Vedhi et al., 2008). For both the potential positive effects on sport performance and the associated toxicological risks, the systemic administration of glucocorticoids has been forbidden by the International Olympic Committee (IOC) and World Anti-Doping Agency (WADA) so that synthetic glucocorticoids including triamcinolone **(I)** are presently included in the list of doping agents (Fluri et al., 2001; Amendola et al., 2003; Spyridaki et al., 2006). Doping is a hindrance to sports ethics and a threat to the health of athletes. On July 3, 2008, Olympic team member Morgan Hamm of Columbus, an athlete in the sport of gymnastics received an official warning from the United States Anti Doping Agency after testing positive for triamcinolone acetonide after the second day of the US Nationals on May 24, 2008.

Triamcinolone chemically known as 9α-fluoro-11β, 16α, 17α, 21-tetrahydroxy-l, 4-pregnadiene-3, 20-dione is a synthetic steroid of the glucocorticoid family which is a mimic of natural hydrocortisone. It is one of the several derivatives of hydrocortisone widely used in medical practice. It is an anti-inflammatory drug which is applied to the skin to relieve irritation, rashes and infection (Li et al., 2007). It is used in the treatment of allergic disorders, ulcerative colitis, arthritis, lupus, psoriasis, skin infections and respiratory disorders (Smith, 1959; Malik et al., 2007; Simon, 2008; Gunasekera et al., 2008; Wang, 2005; Mosbaugh and Carlson, 2008; Pellico and Bosco, 2007). Triamcinolone is used in the postoperative period of certain cosmetic surgery procedures, notably rhinoplasty (Hanasono et al., 2002). It is occasionally used to treat severe cases of hayfever and diabetic retinopathy (Mcmillin, 1972; Chang and Sarraf, 2008) and has also been studied for the prevention of nonmelanoma skin cancer (Ross and Bradlow, 1988). It is a nasal steroid that works directly on nasal tissue and is also used to treat swelling or discomfort of the mouth and gums (Potter et al., 2003; Thongprasom et al., 2007). Triamcinolone acetonide is a more potent type of triamcinolone, being about eight times as effective as prednisone. It is a long-acting corticosteroid with reported efficacy when given by intravitreal or subtenon injection as a treatment for diabetic macular edema, uveitis, retinal vein occlusion and age-related macular degeneration (Nemutlu et al., 2005). It is for this reason that the analysis of triamcinolone is of great significance in pharmaceutical research and clinical chemistry.

The review of literature revealed that few methods have been reported for the determination of triamcinolone in human

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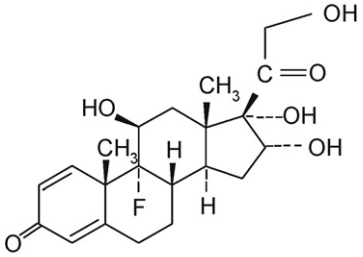
urine and pharmaceutical formulations with most of them relying on the use of chromatographic techniques. Liquid chromatography–electrospray ionisation mass spectrometry (Fluri et al., 2001), gas chromatography–mass spectrometry (Amendola et al., 2003), high performance liquid chromatography (Nemutlu et al., 2005), adsorptive cathodic stripping voltammetry (Hammam, 2007) and liquid chromatography–atmospheric pressure ionization mass spectrometry (Koupai-Abyazani et al., 1995) have been used for the determination of triamcinolone in the past years. Although these techniques are sensitive, they include the need for derivatisation, time-consuming extraction procedures, expensive instrumentation and running costs. Therefore, the purpose of this work is to investigate triamcinolone using an electrochemical method like Osteryoung square wave voltammetry (SWV) which has proved to be highly sensitive for the analysis of organic molecules including drugs owing to its simplicity, low cost and relatively short analysis time as compared to the other routine analytical techniques including chromatography. Edge plane pyrolytic graphite (EPPG) has been taken as the substrate for modification as it has been found better in comparison to other conventional electrodes like glassy carbon electrode (Goyal et al., 2009) due to its strong adsorption, easy maintenance, low background current and wide potential window. A comparison between single-wall carbon nanotubes (SWNTs) and fullerene as modifiers have been made which clearly indicates that SWNTs because of its remarkable elctrocatalytic properties serves as a better modifier. Hence, the studies have been carried out at SWNTs modified edge plane pyrolytic graphite electrode (EPPGE). The developed protocol has been applied with emphasis on quantification of triamcinolone in biological and pharmaceutical samples. HPLC method is used to compare the results obtained for the quantitative estimation of the drug in the biological fluids.



(I)

2. Materials and methods

2.1. Chemicals and reagents

Triamcinolone was obtained from Sigma–Aldrich Inc., USA and was used as received. SWNTs of purity >95% and fullerene of purity >99.5% were purchased from Bucky USA, Houston, TX, USA. Triamcinolone containing tablets and injection marketed by different medical companies were purchased from the local market. Phosphate buffer solutions (1 M) were prepared according to the method of Christian and Purdy (Christian and Purdy, 1962). All other solvents and reagents used were of analytical grade. Double distilled water was used to prepare the solutions.

2.2. Instrumentation

Square wave voltammetric experiments were performed using Bioanalytical System (BAS, West Lafayette, USA) CV-50 W voltammetric analyzer which was equipped with a three electrode system incorporating a bare or modified EPPGE (~6 mm^2) as the working electrode, an Ag/AgCl (3 M NaCl) as reference electrode (BAS Model MF-2052 RB-5B) and a platinum wire as counter electrode. The edge plane pyrolytic graphite piece was obtained from Pfizer Inc., New York, USA and the electrode was prepared as reported in literature (Goyal et al., 2009). All potentials are reported with respect to Ag/AgCl electrode at an ambient temperature of 25 ± 2 °C. The surface morphology of electrode was characterized by recording FE-SEM using Quanta 200 FE-SEM instrument. HPLC studies were performed on Agilent 1100 series system with RP-18e (5 μm) column. The mobile phase used for HPLC experiments was a mixture of water:methanol (40:60) at a flow rate of 1 mL min^{-1} and detection was carried out at 250 nm.

Controlled potential electrolysis was carried out in a conventional cell equipped with a three electrode system having a pyrolytic graphite plate (6 cm × 1 cm) as working electrode, cylindrical platinum gauze as auxillary electrode and silver–silver chloride as a reference electrode at an ambient temperature of 25 ± 2 °C. UV–vis spectral studies were carried out using a Perkin–Elmer–Lambda 35 UV–vis Spectrophotometer. Gas chromatography–mass spectrometric analysis (GC–MS) of the sample was performed with Perkin Elmer Clares 500 Spectrometer in EI mode at 70 eV using HP–17 column. The ^1H NMR spectrum of the product was recorded in DMSO-d_6 with TMS as an internal standard using Avance 500 digital NMR spectrometer from Brucker (500 MHz). Chemical shifts (δ) are reported in parts per million (ppm) of the applied field and coupling constants (J) are expressed in Hertz (Hz).

2.3. Procedure

A stock solution of triamcinolone (1 μM) was prepared by dissolving the required amount of the compound in double distilled water. For recording voltammograms, aliquots of the stock solution of triamcinolone were diluted with appropriate amount of phosphate buffer of desired pH. Before recording each voltammogram, high-purity nitrogen was bubbled for 12–15 min to deoxygenate the solutions as oxygen might be a major interference at such a negative measurement potential and a complete exclusion of oxygen is important. Optimized square wave voltammetry parameters used were: Initial E: −800 mV, Final E: −1600 mV, Square wave amplitude (E_{sw}): 25 mV, Potential step (E): 4 mV, Square wave frequency (f): 15 Hz.

Urine sample of normal person received from the laboratory personnel was used as control. The human urine samples of patients undergoing treatment with triamcinolone were obtained from the Indian Institute of Technology hospital. The samples were obtained after 10 h of administration of 4 mg tablet of Tricort. The anthropometric data of the patients were, Sample 1: female, age 25 yrs, height 152 cm, weight 42 kg; Sample 2: female, age 42 yrs, height 167 cm, weight 58 kg and Sample 3: male, age 45 yrs, height 175 cm, weight 75 kg. The biological samples did not show any reduction peak on scanning from −0.8 to −1.6 V indicating thereby the absence of other reducible compounds in this potential window. The samples were therefore used for analysis without any dilution. The analysis of urine is carried out at pH 7.2 as the pH of urine of normal person is close to neutral.

2.4. Product characterization

For product identification, 15–20 mg of the compound **(I)** was exhaustively electrolyzed at pH 7.2 by applying a potential ~50 mV more negative than the reduction peak potential. The progress of the electrolysis was monitored by recording cyclic voltammograms and UV spectra at different time intervals. With progress of electrolysis colourless solution of triamcinolone changed to light yellow