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SENSITIVE ELECTROCHEMICAL DETERMINATION OF BETAMETHASONE IN DAIVOBET AND IN WATER SAMPLES

Bianca-Maria TUCHIU[1]*, Raluca-Ioana STEFAN-VAN STADEN[2]*, Jacobus (Koos) Frederick VAN STADEN[3]

An electrochemical sensor designed using ZnO and carbon nanopowder is proposed for determining betamethasone in gel formulation as well as in water samples, using differential pulse voltammetry. The betamethasone can be determined within 0.1 and 100.0 nmol L^{-1}. The validation of the proposed procedure was done using a betamethasone-based gel as well as surface water samples. BIAS were calculated and reported.

Keywords: Betamethasone, electrochemical determination, gel, surface water.

1. Introduction

Part of the corticosteroid class with anti-inflammatory and immunosuppressive actions that can be administered orally, by injection, or topically, betamethasone (Figure 1) is commonly used in antenatal treatment to reduce neonatal respiratory complications, subsequently decreasing the risks of neonatal mortality and morbidity [1]. Betamethasone can also be used as monotherapy but also in combination with other drugs in inflammatory diseases such as rheumatoid arthritis and asthma and in immune-mediated diseases such as psoriasis, lupus, and lichen planus [2–6].

Like other corticosteroids, betamethasone has a number of side effects, especially when administered over long periods. These include endocrine, hematological, bone, gastric, cardiovascular, ocular, skin, and mental disorders [7]. Latest utilization of betamethasone includes on-site treatment of ulcer [8],

[1] PhD student, National University of Science and Technology Politehnica Bucharest, Faculty of Chemical Engineering and Biotechnologies, Bucharest, Romania, e-mail: bianca.tuchiu@gmail.com.

[2] CS I, Prof. Dr. habil, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, Splaiul Independentei No. 202, Bucharest, Romania, National University of Science and Technology Politehnica Bucharest, Faculty of Chemical Engineering and Biotechnologies, e-mail: ralucavanstaden@gmail.com.

[3] CS I, Prof. Dr, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, Splaiul Independentei No. 202, Bucharest, Romania, e-mail: koosvanstaden2012@gmail.com; koosvanstaden2012@yahoo.com.

epilepsy treatment [9], and the treatment of Stevens-Johnson syndrome [10], and cushing syndrome [11].

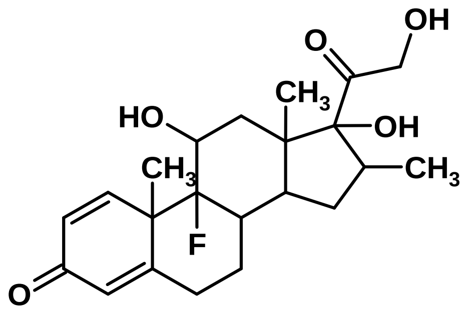

Fig. 1. Chemical structure of betamethasone

Both UV-Vis spectroscopy and liquid chromatography (LC) are recommended in the European Pharmacopoeia as compendial methods for determining betamethasone and its esters [12]. Numerous chromatographic techniques were used for the assay of betamethasone [13-17]. These methods are reproducible, sensitive, accurate, and robust; however, they have a number of drawbacks, like the need for large quantities of expensive solvents, expensive and complex equipment that require the supervision of trained personnel, long run times, and laborious sample processing. On the other hand, electrochemical methods do not suffer from these limitations as they are based on simple principles, are cost-effective, and require minimal to no sample processing. Furthermore, portable instruments are available and the sensors used can be miniaturized to facilitate on-site determination.

This paper proposed the utilization of a carbon paste electrode designed using ZnO nanopowder and carbon nanopowder (ZnO/CnpPE) for the sensitive quantitative investigation of betamethasone in DAIVOBET and in water stream samples. Nanostructured ZnO is one of the most frequently used metal oxide nanoparticles owing to its superior electrochemical activity due to its considerable sized active area and high electrical conductivity, stability, and low-cost synthesis [18,19]. Carbon nanopowder is a nanostructured material; its electroactive surface is bigger than of a carbon powder paste, and this size facilitates electron transfer, superior electrical and thermal conductivity, and low production costs. These characteristics have made this material a promising option in the manufacture of electrochemical sensors [20].

Electrochemical sensors based on single-walled carbon nanotubes [21, 22], and on an amalgam film [23] were proposed to date for the quantitative investigation of betamethasone.

2. Experimental

2.1. Materials and reagents

Betamethasone, ZnO nanopowder, carbon nanopowder (<50 nm), phosphate buffer solution, pH=5.00, and dimethyl sulfoxide were bought from Sigma Aldrich, while the paraffin oil (d_4^{20}, 0.86 g × cm^{-1}) was acquired from Fluka. The stock solution of betamethasone (1.0 × 10^{-2} mol L^{-1}) was obtained in dimethyl sulfoxide. Serial dilution method was applied to prepare solutions of different concentrations of betamethasone, in phosphate buffer solution (PBS) pH 5.00. Daivobet, gel (Leo Pharmaceutical, Denmark) was bought from a local pharmacy.

2.2. Equipment

An EmStat Pico mini potentiostat (PalmSens BV, Houten, The Netherlands) was employed for electrochemical measurements. The device was accessible by a computer through the PSTrace software 5.8 version for data acquisition. The experimental setup involved an electrochemical cell with three electrodes: ZnO/CnpPE as the indicator electrode, an Ag/AgCl wire (1.0 mol L^{-1} KCl) as the reference electrode, and a Pt wire as the auxiliary electrode.

2.3. Preparation of the working electrode ZnO/CNP

5.0 mg of ZnO nanopowder was added to 50.0 mg of carbon nanopowder followed by adding 20.0 µl paraffin oil. The paste was pushed into a plastic tube; an Ag wire was utilised as electrical contact. The sensor surface was polished on aluminum foil before its first utilization. The sensor has been kept at room temperature, away from light and moisture.

2.4. Procedure

A potential range of -1.4–0 V, with the step potential of 25.0 mV, and a scan rate of 50.0 mV s^{-1} were used for all differential pulse voltammetry (DPV) measurements. All measurements were conducted at 25°C. Calibration equation of the sensor was calculated applying the linear regression analysis. To determine the unknown concentrations of betamethasone in Daivobet and water samples, the current intensity was measured and used in the equation of calibration.

2.5. Samples

Daivobet, pharmaceutical gels containing different concentrations of betamethasone was acquired from a pharmacy. To dissolve the betamethasone, approximately 0.5 g of the gel was treated with a 1:10 dimethyl sulfoxide and deionized water mixture and then filtered. The obtained solution was buffered with PBS pH 5.0. The surface water sampling was done from a local stream. All water samples were buffered with PBS pH 5.0 (1:1, v/v) before analysis.

3. Results and discussion

3.1. Response characteristics of the ZnO/CNP electrode

The response characteristics of the ZnO/CNP electrode were obtained using the procedure mentioned above. Table 1 shows all the calibration curve parameters.

Table 1

Calibration curve parameters values of ZnO/CNP electrode for the assay of betamethasone.

Linear concentration range (nmol L^{-1})	0.1 – 100.0
Calibration equation	$1.24 \times 10^{-3} + 6.47 \times 10^{-5} \times C_{betamethasone}$
Correlation coefficient	0.9946
Sensitivity (μA nmol L^{-1})	6.47×10^{-5}
Quantitation limit (nmol L^{-1})	0.1
Determination limit (nmol L^{-1})	0.03
Number of data points	6

The large linear concentration range is covering concentrations found in pharmaceutical formulations of betamethasone, as well as the betamethasone concentrations in surface water. Very low values of the limits of quantification and determination were obtained. Fig. 2 displays the obtained voltammograms and the calibration graph obtained for the calibration of betamethasone using the proposed electrode. Comparing with the results obtained earlier using electrochemical sensors [21-23], the proposed electrode provides wider linear concentration range, and lower limits of determination and detection, making from it an ideal tool especially for surface water monitoring for the presence of betamethasone.

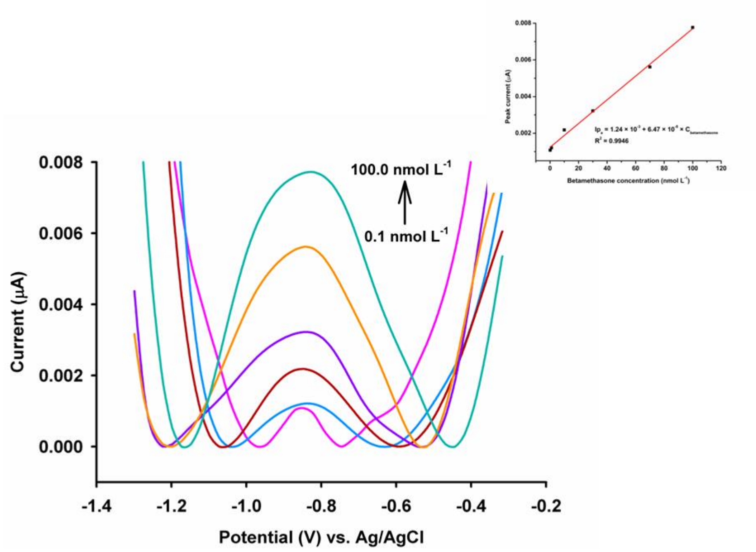

Fig. 2. Calibration of the ZnO/CNP electrode using differential pulse voltammetry ($C_{betamethasone}$ ranging from 0.1 to 100.0 nmol L^{-1}).

3.2. Electroanalysis of betamethasone in Daivobet and water samples

The concentrations of betamethasone in the samples were calculated using the calibration curve (Table 1), and the procedure described above.

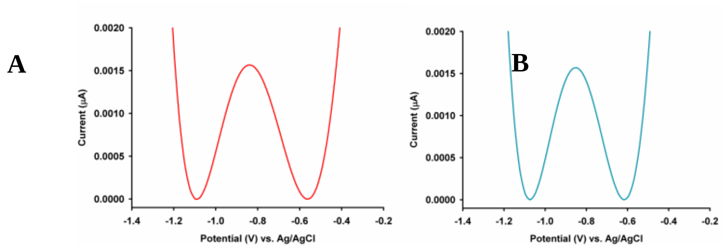

Fig. 3. Voltammograms recorded for the quantitative analysis of betamethasone in **A** DAIVOBET gel and **B** stream water sample.

Figure 3 shows specific diagrams obtained for the quantification of betamethasone in the DAIVOBET gel, and stream water samples. Table 2 displays the results of the betamethasone assay in gel formulation and river network water samples.

Table 2

Quantitative analysis of betamethasone in DAIVOBET and stream water samples using ZnO/CNP electrode.

Sample type and number	Betamethasone declared amount (nmol L^{-1})	Betamethasone found amount (nmol L^{-1})	RSD (%)	Recovery (%)	Bias (%)
DAIVOBET 1	0.1	0.11	0.33	108.43	-7.77
DAIVOBET 2	5.0	5.03	2.87	100.61	-0.61
DAIVOBET 3	100.0	99.64	1.94	99.64	0.36
Surface water 1	0.1	0.1	0.18	97.65	2.41
Surface water 2	5.0	5.04	0.43	100.87	-0.86
Surface water 3	100.0	98.27	2.85	98.27	1.76

Following the evaluation of the applicability of ZnO/CNP electrode for the quantification of betamethasone in real samples, good values of recovery (ranging from 97.65 to 108.43%), RSD (≤2.87%), and bias (ranging from -7.77 to 2.41%) were obtained. These values proved that the proposed electrode can be reliably used for the uniformity content tests of DAIVOBET as well as for the quantitative analysis of the quality of stream water, by determining small amount of betamethasone in surface water samples.

4. Conclusions

This study presents the application of a voltammetric sensor based on ZnO and carbon nanopowder for the sensitive quantification of betamethasone in gel formulation (DAIVOBET) and stream water samples. The electrode exhibited a broad linear concentration range, good sensitivity, and very low limits of detection and quantitation. The proposed electrode was validated in real samples, obtaining satisfactory results, thus indicating that this method could be used on a large scale in the quality check of active substances in pharmaceuticals. Moreover, it may be employed for the on-site surveillance of chemical water status.

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