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Square Wave Voltammetric Determination of Dexamethasone on a Multiwalled Carbon Nanotube Modified Pencil Electrode

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O comportamento eletroquímico de dexametasona, um hormônio esteroidal, foi estudado em um eletrodo de grafite modificado por nanotubos de carbono de camadas múltiplas, por voltametria de onda quadrada e espectroscopia de impedância eletroquímica. Dexametasona mostrou um pico de oxidação com potencial em torno de + 0,80 V na superfície do eletrodo modificado. Sob condições ótimas, a corrente de pico de oxidação depende da concentração da dexametasona e foi linear no intervalo de 0,15-100 μmol L^{-1} de dexametasona com um limite de detecção de 0,09 μmol L^{-1} de dexametasona. Os efeitos de substâncias interferentes potenciais na determinação de dexametasona foram investigados e encontrou-se que o sensor proposto é sensível e rápido para a determinação de dexametasona em produtos farmacêuticos e em amostras de urina humana sem qualquer tratamento prévio.

The electrochemical behavior of dexamethasone, a steroid hormone, was studied on a multiwalled carbon nanotube (MWCNTs) modified pencil electrode (PE) by square wave voltammety and electrochemical impedance spectroscopy (EIS). Dexamethasone showed one oxidation peak potential around +0.80 V on the surface of the modified electrode. Under optimized conditions, the oxidation peak current depended on the dexamethasone concentration and was linear in the range of 0.15-100 μmol L^{-1} dexamethasone with a detection limit of 0.09 μmol L^{-1} dexamethasone. The effects of potential interfering substances on the determination of dexamethasone were investigated to find that the proposed sensor is a sensitive and fast tool for the determination of dexamethasone in both pharmaceutical and human urine samples without any pretreatment.

Keywords: multiwalled carbon nanotubes, dexamethasone, square wave voltammetry, impedance

Introduction

Dexamethasone (DXA) is a potent synthetic member of the glucocorticoid class of steroid hormones (Scheme 1). It acts as an anti-inflammatory and immunosuppressant agent. Dexamethasone acetate (DXA), dexamethasone sodium phosphate (DSP), and dexamethasone are derivatives of the adrenal corticosteroid group. They are broadly used to treat many inflammatory and autoimmune conditions (*e.g.*, rheumatoid arthritis) and are useful to counteract the allergic anaphylactic shock. They are often administered before antibiotics in cases of bacterial meningitis to reduce the inflammatory response of the body to the bacteria killed by the antibiotics. Application of dexamethasone with alcohol, mineralocorticoids, oral antidiabetic drugs, insulin, certain antibiotics, estrogens, ephedrine, and digoxin increases of side effect risks. Recently, abuse of adrenal corticosteroid in cosmetics has caused skin addiction and dermatitis has also been reported.[1] The importance of controlling their therapeutic use and supervising its addition in animal food and cosmetics has warranted simple, sensitive, rapid, efficient, and practical methods to determine DXA, DSP, and dexamethasone in various samples. In addition, the therapeutic importance of dexamethasone required the development of sensitive and rapid methods for industrial quality control and clinical monitoring.

Various methods have been proposed for the analysis of DXA and other adrenal corticosteroid derivatives in different matrices. These methods are essentially based on separation methods such as liquid chromatography and HPLC-UV,[2,3] thin-layer chromatography,[4] LC-MS,[5-7] LC-MS-MS,[8] GC-MS with negative chemical ionization

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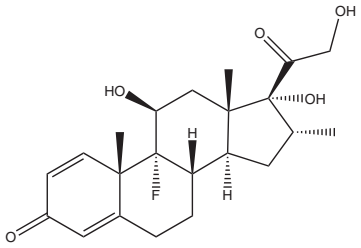

Scheme 1. Structure of dexamethasone.

source,[9] HPLC-chemiluminescence,[10-13] and finally flow-injection chemiluminescence method coupled with solvent extraction.[14] Separation-detection methods such as GC-MS and HPLC-MS methods are expensive and time-consuming procedures, whereas the other separation methods suffer from such disadvantages as large amounts of high purity organic solvents required, long equilibration, and derivatization treatment. A few electrochemical papers have been published on the determination of dexamethasone based on reduction current, such as differential-pulse polarography,[15] and voltammetric methods using fullerene-C_{60}-modified pyrolytic graphite or modified carbon paste electrodes.[16,17] Study of reduction current behavior of dexamethasone is difficult and limited to the use of mercury or specially modified electrodes. Many cations and organic substances could interfere with the reduction peak potential, especially at negative potentials. Also the mercury is very toxic and difficult to work so its application is limited. In this work, we used multiwalled carbon nanotubes to modify a pencil electrode to develop a sensitive, selective, and useful method for the determination of dexamethasone. This method is based on the unusual properties of carbon nanotubes such as strong adsorptive ability, huge specific area, subtle electronic properties, and excellent electrocatalytic activity. This work is the first reported on the electrochemical oxidation behavior of dexamethasone that is based on specific behavior of activated carbon nanotubes in electrocatalysis of dexamethasone oxidation. In addition, impedance spectroscopy was used to determine the oxidation mechanism of dexamethasone at the surface of the modified electrode.

Experimental

Apparatus

Voltammetric measurements were carried out using a Metrohm potentiostat/galvanostat (Model 797 VA), connected to a computer (Pentium IV, 1200 MHz) and with 797-VA computrace 1.2 Metrodata software. A conventional three-electrode system comprising a modified pencil electrode as a working electrode, a Pt-wire as an auxiliary electrode, and an Ag/AgCl (saturated KCl) reference electrode were used for the measurements.

Impedance measurements were performed with a conventional three-electrode cell, powered by an electrochemical system comprising the Autolab system (PGSTAT 12 and FRA2 boards, Eco Chemie B.V., Utrecht, and The Netherlands). The system was run on a PC using GPES and FRA 4.9 software. For impedance measurements, a frequency range of 100 kHz to 10 Hz was employed. The AC voltage amplitude used was 5 mV, and the equilibrium time was 15 s. The MWCNTs modified pencil electrode, a graphite electrode and a saturated Ag/AgCl reference electrode were employed as a working, auxiliary and reference electrode, respectively.

Chemicals

All chemicals were of analytical reagent grade purchased from Merck (Darmstadt, Germany) unless otherwise stated. Deionized water of resistivity not less than 18.0 MΩ at 25 ºC was used throughout.

Dexamethasone was purchased from Aldrich. Dexamethasone ampoule (2 mg mL^{-1}) was prepared from Amin Company (Tehran, Iran). Dexamethasone solution was prepared from 0.010 mol L^{-1} stock solution in deionized water.

Universal, acetate, phosphate and tris buffer (all 0.10 mol L^{-1}) solutions with different pH values were used for the study of the pH influence.

MWCNTs was prepared from Irans Research Institute of Petroleum Industry with a diameter of 8-15 nm, a length of 50 μm and the purity of 95%. The modified electrodes with carbon nanotube layers were characterized by scanning electron microscopy (SEM) and 1578 B. energy dispersive X-ray (EDX) analysis.

Pencil core graphite (diameter equal to 0.5 mm) from Micro was used for the preparation of the electrode.

Preparation of the electrode

To activate MWCNTs and to remove metal residuals in the nano-structure, 3.0 g of MWCNTs plus 20 mL of 3.0 mol L^{-1} HNO_3 were placed in a 25 mL flux and then refluxed for 15 h. Then, the MWCNTs were washed with water and dried at room temperature. The stable suspension of activated MWCNTs was obtained by ultrasonicating of MWCNTs in dimethyl formaldehyde (0.10 mg MWCNTs *per* 10 mL). The surface of the pencil electrode (PE) was carefully polished onto a filter paper until it had a glossy appearance and then cleaned in an ethanol/water solution (50% v/v) in the ultrasonic bath. The cleaned PE was

immerged in a stable MWCNT suspension for 1 min and dried in a hot air flow at 50 °C. The two last steps were repeated for 20 times.

Recommended procedure

Ten mL of the buffer solution (pH 5.0) were transferred into an electrochemical cell using a three-electrode system containing the modified electrode as a working electrode. Then, square wave voltammogram (SWV) was recorded in the potential range of +0.40 to +1.20 V with a frequency of 50 Hz and a potential step amplitude of 25 mV. The peak current was measured and recorded as a blank signal (I_b). Once the background voltammogram was obtained, aliquots of the sample solution containing 5 mL of the buffer solution, pH 5.0, plus a sufficient amount of dexamethasone solution was introduced into the cell. The SWV was recorded again in the potential range of +0.40 to +1.20 V as described above to give the sample peak current. The peak current was measured and recorded as a sample signal (I_s). The difference in current ($Ip_s - Ip_b$) was considered as a net signal (ΔI_p) for each of the species. Calibration graph was prepared by plotting the net peak currents *vs.* dexamethasone concentrations in the solutions.

Urine and blood plasma samples were centrifuged (10000 rpm) to remove solid particles. Then, 1.0 mL of the sample solution plus 9.0 mL of 0.10 mol L^{-1} buffer (pH 5.0) were transferred into the cell to measure the dexamethasone contents using the standard addition method.

Results and Discussion

Study of electrochemical behavior of dexamethasone

The characterization of the MWCNTs film on the PE was investigated using the SEM method.[18] It is obvious that the MWCNTs were distributed uniformly on the surface of the pencil electrode. The spaghetti-like MWCNTs formed a porous structure. The entangled cross-linked fibrils offered a highly accessible surface area. Similar observations have been reported in the literature.[19]

The voltammetric response of 2.5 μmol L^{-1} dexamethasone at the surface of bare and MWCNTs-modified electrodes at pH 4.0 was recorded (Figure 1). The results confirm that the oxidation of dexamethasone on the surface of PE has a high overpotential. On the other hand, with the MWCNTs-modified electrode, the overpotential decreased sharply because of the activation of the surface of the modified electrode which proved the catalytic role of MWCNTs in dexamethasone oxidation. This clearly shows that dexamethasone does not have any oxidation peak on the bare PE that confirms previous reports about only reduction of dexamethasone on surface of carbon paste and glassy carbon electrode. On the other hand, the oxidation of dexamethasone occurred on the surface of MWCNTs-modified electrode at a potential near 0.90 V by elecrocatalytic effect of activated multiwalled carbon nanotubes.

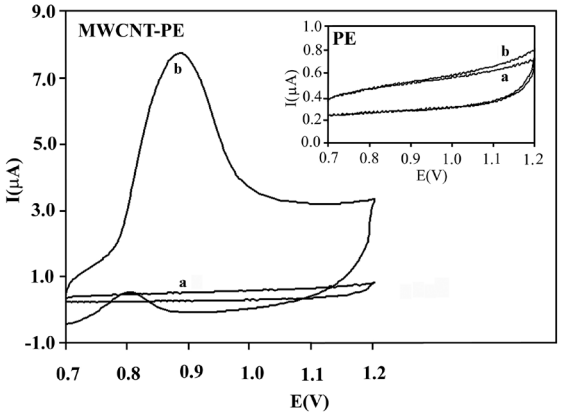

Figure 1. Voltammetric response of a) electrolyte and b) 1.0 μmol L^{-1} dexamethasone on the bare (insert voltammogram) and MWCNTs modified electrode in pH 4.0 acetate buffer.

Influence of variables

Buffer composition and pH are important parameters for the oxidation behavior of dexamethasone at the MWCNTs modified electrode. We, therefore, investigated the influence of buffer composition with identical pH values including acetate, universal, and phosphate solutions on the peak potential and current of dexamethasone. The results showed that the oxidation peak current of dexamethasone was maximized in the universal buffer as compared with the phosphate and acetate buffer solutions. Therefore, the universal buffer (containing H_3PO_4, HBO_4, and acetic acid, 0.10 mol L^{-1}) was selected for further study.

To find the optimum solution pH, the influence of pH (between 2.0 to 8.0) on the peak current of 1.0 μmol L^{-1} dexamethasone was studied. Figure 2 shows that the peak potential and peak current of dexamethasone strongly depend on the pH of the solution. Our study showed that the oxidation peak of dexamethasone in an alkaline solution at 0.90 V fundamentally decomposed into three ill-defined oxidation peaks. When the solution pH was increased, it was observed that the peak potential shifted to less positive values and showed that the oxidation of dexamethasone is dependent on proton transfer. Based on this observation, dexamethasone can be said to be a multiproton donor and acceptor, which is in agreement with chemical structure of

DXA.[20] By treating MWCNTs in nitric acid, a carboxylic group is created on the surface of nanotubes. Therefore, the modified surface of MWCNTs will catalyze the proton transfer of dexamethasone oxidation and will decrease its oxidation potential. Under alkaline conditions, other sites of dexamethasone are activated so that new oxidation peaks appear. The relation of E_P *versus* pH (in the pH range of 2.0-6.0) has a slope of 55.3 mV *versus* pH. This slope suggests that the number of electrons transferred is approximately equal to that of the hydrogen ions taking part in the electrode reaction. Finally, the alkaline solution was found not suitable for dexamethasone measurements; hence, a pH value of 5.0 was chosen as the optimum pH value with a high oxidation peak current.

The effects of frequency (affecting scan rate) on the SWV peak current and peak potential at MWCNTs modified electrode were investigated in the presence of 1.0 μmol L^{-1} dexamethasone at pH 5.0 (Figure 2). The results showed that the anodic peak current increased linearly with increasing frequency according to the equation $I(\mu A) = 0.560f(Hz) + 3.993$, $R^2 = 0.986$. This result indicates an adsorption controlled oxidation process occurring at the surface of the modified PE.[21] Based on the results, a frequency of 50 Hz was selected as the optimum value for dexamethasone determination. The peak potential of dexamethasone was also found to shift to more positive potential values with increasing frequency. The plot of E_P *versus* log*f* was linear with a correlation coefficient of 0.934. Such a behavior indicates the reversible nature of the redox reaction.[21] The variation of E_P with log*f* can be expressed by the equation $E_p(mV) = 51.40 \log f(Hz) + 881.1$.

The effect of amplitude voltage on peak current was studied in the range of 1-45 mV. It was found that the peak current increased sharply up to voltage amplitude of 30 mV, which tended to level off afterwards. So an amplitude voltage of 30 mV was chosen as the best value for further study.

As already described, the influence of pH on peak potential showed that the current is dependent on the analyte adsorption at the surface of PE. Thus, the influence of accumulation potential (from 0.40 to +0.90 V) on the peak current of 1.0 μmol L^{-1} dexamethasone was studied at the MWCNTs modified electrode using SWV with an accumulation time of 30 s at pH 5.0 (Figure 3a). The results showed that the accumulation time increases the oxidation peak current of dexamethasone but it is nearly independent

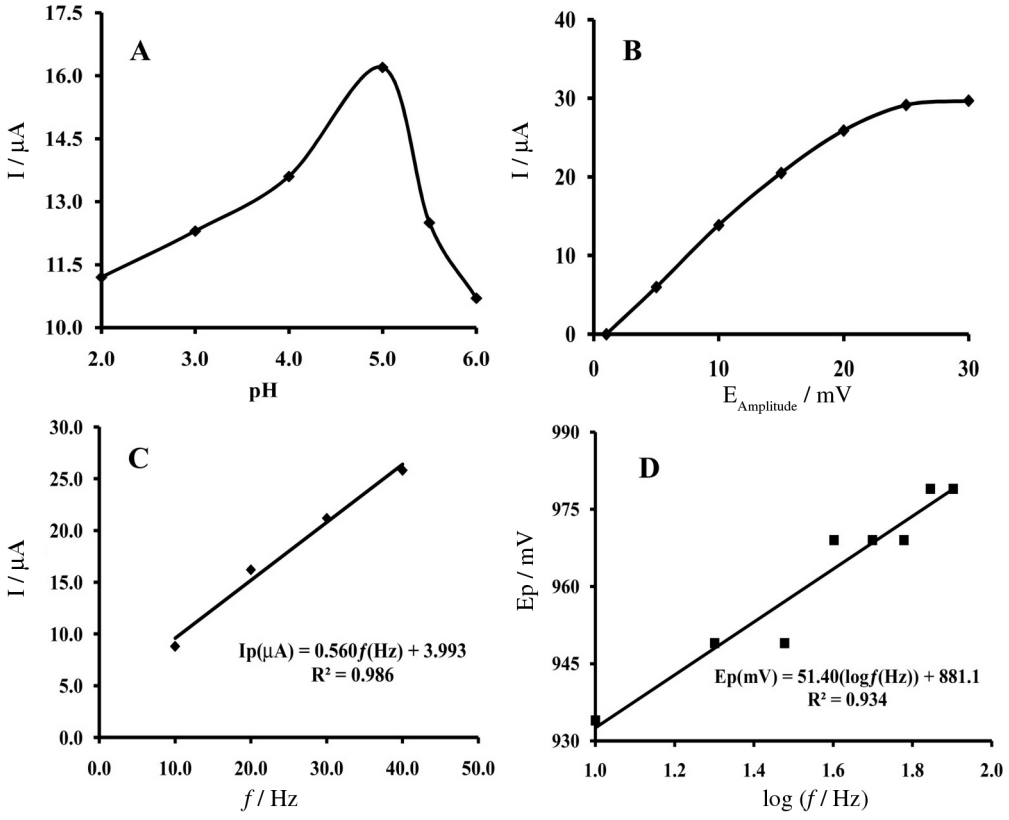

Figure 2. A) Current of 1.0 μmol L^{-1} dexamethasone in 0.1 mol L^{-1} universal buffer solution at different pHs from 2.0 to 6.0 on the surface of the PE; B) peak current *versus* potential amplitude; C) peak current *versus* frequency; D) peak potential *versus* log*f* for 1.0 μmol L^{-1} dexamethasone in 0.1 mol L^{-1} universal buffer solution at pH 5.0.

of increasing accumulation potential up to +0.80 V, which subsequently decreased. This is due to the fact that beyond +0.80 V, the oxidation of dexamethasone occurred during accumulation. Hence, +0.50 V was selected as the optimum accumulation potential.

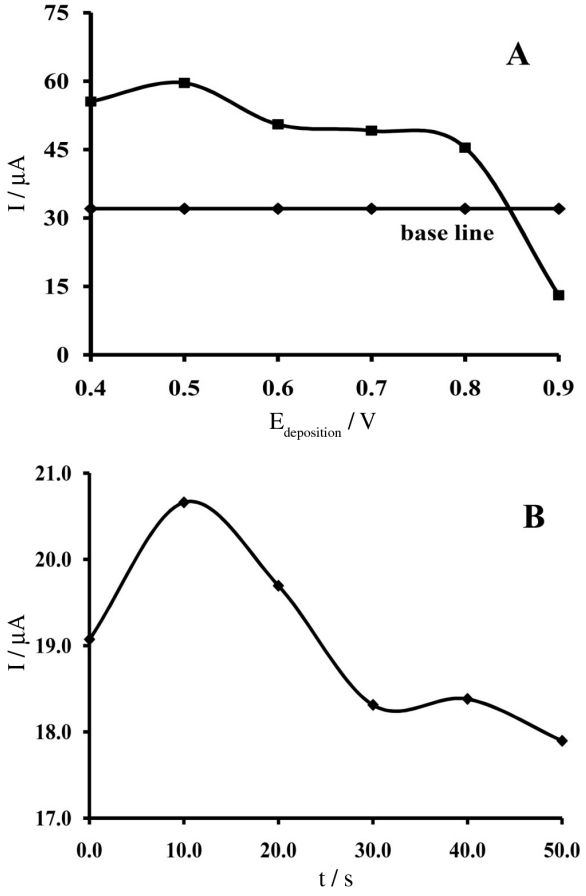

Figure 3. A) The oxidation peak currents of 1.0 µmol L^{-1} dexamethasone after 30 s of accumulation at different potential from 0.40 to 0.90 V at pH 5.0; B) effect of accumulation time in accumulation potential on 0.50 V.

The effect of accumulation time on peak currents with an accumulation potential of +0.50 V for the oxidation of 1.0 µmol L^{-1} dexamethasone at pH 5.0 was studied (Figure 3b). The results showed that increasing the accumulation time caused the peak current to increase. After 60 s, it was leveled off due to adsorption saturation. Thus, 10 s was selected as the accumulation time.

Response time of electrode was examined by following chronoamperometry of 1.0 µmol L^{-1} dexamethasone at pH 5.0, oxidation potential of +0.80 V and duration time 5, 10, 15 and 20 s. The results show that the change in current after 10 s is less than 1%. Also, it was mentioned that, the adsorption process of dexamethasone on surface of MWCNTs modified electrode after 60 s are saturated and current is leveled off.

Electrochemical impedance spectroscopy

Oxidation of dexamethasone on both PE and MWCNTs electrodes was investigated using the impedance spectroscopic method. Figure 4 shows the bode plot and the Nyquist plot of the impedance surface densities (Ω cm^{-2}) and admittance surface densities (S cm^{-2}) on the PE (A) and modified PE (B) electrodes recorded at +0.60 V as DC offset for 1.0 µmol L^{-1} DXA at pH 5.0. Impedance and admittance quantities depend on the microscopic areas of the electrodes. The surface area of the bare PE and modified electrodes were different; therefore, the impedance elements were normalized after the experiments with respect to the electrode surface area during data analysis.

Analytical performance

Using square wave voltammetry and under the optimum conditions (selected as: pH 5.0 (universal buffer) with a

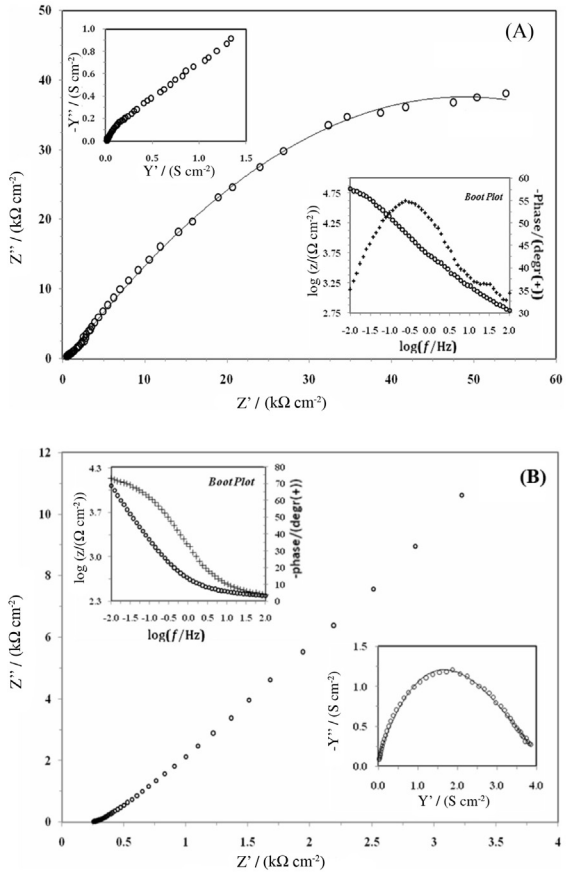

Figure 4. The Nyquist plots of the impedance surface densities (Z'' cm^{-2} *vs.* Z' cm^{-2}) acquired for 1.0 µmol L^{-1} dexamethasone in pH 5.0 on the PE electrode (A) and MWCNTs modified electrode (B). Bias in both diagrams was 0.60 V with 5 mV ac voltage amplitude and frequency range of 0.01 Hz to 100 kHz. Insets show their related a) bode plots and b) the admittance surface densities (Y'' cm^{-2} *vs.* Y'cm^{-2}) plots. Points show the experimental data and the full line is calculated from the fitted parameters.

frequency of 50 Hz and a potential amplitude of 30 mV), the calibration plot of dexamethasone determination was linear over a concentration range of 0.15-100 μmol L^{-1} with a regression equation of $I(\mu A) = 0.122C_{DXA} + 1.470$ ($R^2 = 0.9991$) (Figure 5).

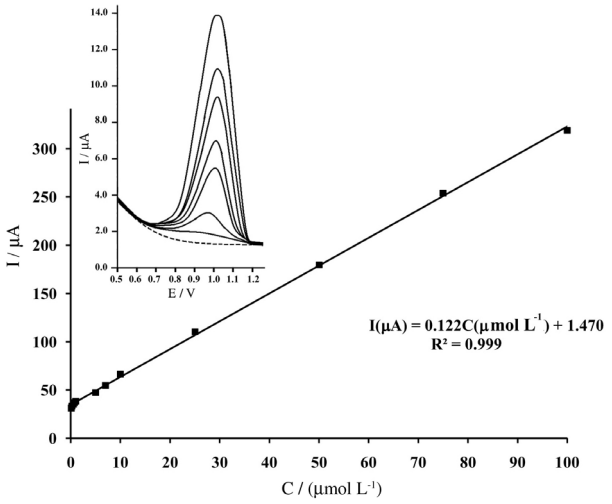

Figure 5. Calibration curve of dexamethasone at pH 5.0 with frequency of 50 Hz, pulse amplitude of 25 mV and with 10 s accumulation potential at 0.50 V.

The detection limit, defined as the blank signal plus three times its standard deviation, was 0.090 μmol L^{-1} dexamethasone. The relative standard deviation (RSD%) is less than 1% for 2.5 μmol L^{-1} dexamethasone (for five analysis), which shows excellent reproducibility.

The stability of the modified electrode was good. No obvious changes were observed in the peak currents for the same sample concentration after several runs of the SWV when the modified electrode was kept at a room temperature of about 25 °C for one month. In fact, the performance of the modified electrode completely depends on the yield of surface modification with MWCNTs. Furthermore, electrode preparation is a critical measurement step. But the preparation procedure is repeatable which is confirmed by using of F-test to comparison of standard deviation of determination of DXA in 5 days electrode preparation. The results show that there is no significant difference between different days preparation electrode. So the repeatability of electrode like its reproducibility is good. The figures of merit of dexamethasone determination with the sensor were compared with other electrochemical methods reported as given in Table 1. In this table it is obvious that MWCNTs modified PE based on its specific design is the only one to show an oxidation behavior for dexamethasone in lower potential in relation to other electrodes (hanging mercury electrode and modified graphite electrode). Also this new electrode linear dynamic range is three orders of magnitude better with regard to other electrodes with one,[15] or two,[16,22] orders of magnitude. As well, proposed electrode without any preliminary stripping step has good detection limit in comparison to other electrodes, only long time stripping on hanging mercury electrode has shown lower detection limit.[22] In addition, MWCNTs modified pencil electrode in comparison to mercury electrode is safe and easy to work and can be used for analysis of different pharmaceutical samples.

Interference study

Under the optimized experimental conditions described above, the effects of some ordinary compounds in biological media and drugs typically used with dexamethasone were investigated using 1.0 μmol L^{-1} dexamethasone. The tolerance limit was defined as the maximum concentration of the substance that caused an error of less than 3% in dexamethasone determination. The results in Table 2 show that common cations and anions do not affect dexamethasone determination, but some related pharmaceutical compounds have similar responses and, hence, interfere with the dexamethasone signal.

Analytical application

To evaluate the applicability of the proposed method, the recovery of dexamethasone was determined in common

Table 1. Analytical parameters of several electroanalytical methods used to dexamethasone determination

Method	E_p / V	Electrode	Limit of detection / (μmol L^{-1})	Linear dynamic range / (μmol L^{-1})	Reference
DPV	−1.14	HMDE	7.6	25.5-122.3	15
DPV	−1.04	Graphite	0.36	0.41-20	16
SWV	−1.20	Graphite	0.06	0.07-100	17
SWP	−1.20	HMDE	0.003	0.009-0.4	22
SWV	+0.90	MWNT	0.09	0.15-100	This work

DPV: differential pulse voltammetry; SWV: square wave voltammetry; SWP: square wave polarography; HMDE: hanging mercury drop electrode; CPE: carbon paste electrode; LOD: limit of detection; LDR: linear dynamic range.

Table 2. Interference study under the optimum conditions

Species	Tolerance limit[a]
Na^+, K^+, NO_3^-, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Cl^-, CO_3^{2-}, Cortion, Amoxicilin, Methyprednisolone, Phenazopyridine, Isobuthyl phenyl propionicacid	1000*
DNA, Aminoantipyrene, Chloroamine-T hydrate, Arginine, Valine, Histidine, Tryptophan, Glycine, Cysteine, Glucose, Starch, Cysteic acid, Citric acid, Urea, Uric acid, Tartaric acid, Ascorbic acid, Sacarose	100
Betametasone, Dibucaine hydrochloride, 5-chlorosalycilic acid, Dimenhydrinate	10

[a]Interference limit concentration of species times than dexamethasone concentration. *Maximum concentration of tested species.

Table 3. Determination of dexamethasone in real sample at pH 5.0 (3 measurements on each sample)

No.	DXA added / (μmol L^{-1})	DXA found / (μmol L^{-1})	Recovery / (%)
Ampoule			
1	1.000[a]	1.015 ± 0.010	101.5
2	1.200[a]	1.218 ± 0.012	101.5
Urine			
1	1.000	1.027 ± 0.010	102.7
2	1.200	1.235 ± 0.012	103.0
Plasma			
1	1.000	0.999 ± 0.010	99.9
2	1.200	1.191 ± 0.011	99.3

[a]ampoule content of 1mg $mL^{-1}$of dexamethasone diluted to sufficient concentration.

injection ampoule which are commercial available for dexamethasone, plasma, and urine samples by spiking the samples with standard values of dexamethasone. The standard addition method was used for the analysis. The results are given in Table 3, which indicate that the results are satisfactory for analytical determination of dexamethasone in real samples.

Conclusions

The electrochemical results discussed above demonstrate the electrochemical response of dexamethasone on the modified pencil electrode. An adsorption process was found to occur during the redox process of dexamethasone on the electrode surface as revealed by impedance measurements. Electrochemical studies for identifying the dexamethasone oxidation mechanism confirmed the contribution of one electron and one proton in the process. It was also shown that the proposed method outperforms the other reported electrochemical methods in analyzing dexamethasone with a satisfactory sensitivity and determination range and with a low experimental detection limit of 0.09 μmol L^{-1}. Based on our findings, this modified electrode can be properly used for the determination of dexamethasone in pharmaceutical and urine samples with satisfactory results.

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References

1. Van de Beek, D.; de Gans, J.; McIntyre, P.; Prasad, K.; *Cochrane Database Syst. Rev.* **2007**, *1*, CD004405.
2. Gallego, J. M .L.; Arroyo, J. P.; *J. Pharm. Biomed. Anal*. **2002**, *30*, 1255.
3. Grippaa, E.; Santinia, L.; Castellanob, G.; Gattoa, M. T.; Leonea, M. G.; Sasoa, L.; *J. Chromatogr., B* **2000**, *738*, 17.
4. Huetos, O.; Ramos, M.; Pozuelo, M. M.; Andr, M. S.; Reuvers, T. B. A.; *Analyst* **1999**, *124*, 1583.
5. Wasch, K. D.; Brabander, H. D.; Courtheyn, D. C.; Peteghem, V.; *Analyst* **1998**, *123*, 2415.
6. Shibasaki, H.; Furuta, T.; Kasuya, Y; *J. Chromatogr., B* **1997**, *692*, 7.
7. Creaser, C. S.; Feely, S. J.; Houghton, E.; Seymour, M.; *J. Chromatogr., A* **1998**, *794*, 37.
8. Cherlet, M.; Baere, S. D.; Backer, P. D; *J. Chromatogr., B* **2004**, *805*, 57.

9. Hidalgo, O. H.; Lopez, M. J.; Carazo, E. A.; Larrea, M. S. A.; Reuvers, T. B. A.; *J. Chromatogr., B* **2003**, *788*, 137.
10. Iglesias, Y.; Fente, C.; Mayo, S.; Vazquez, B.; Franco, C.; Cepeda, A.; *Analyst* **2000**, *125*, 2071.
11. Iglesias, Y.; Fente, C.; Vazquez, B. I.; Franco, C.; Cepeda, A.; Mayo, S.; *Anal. Chim. Acta* **2002**, *468*, 43.
12. Toriba, A.; Kubo, H.; *J. Liq. Chromatogr. Relat. Technol.* **1997**, *20*, 2965.
13. Niwa, Y.; Somiya, K.; Miyachi, Y.; Kanoh, T.; Sakane, T.; *Inflammation* **1987**, *11*, 163.
14. Wu, F.; Lv, J.; *Talanta* **2007**, *72*, 1811.
15. Jeyaseelan, C.; Joshi, A. P.; *Anal. Bioanal. Chem.* **2002**, *373*, 772.
16. Balaji, K.; Raghunadha R. G. V.; Madhusudana R. T.; Jayarama R. S.; *Afr. J. Pharm. Pharmacol.* **2008**, *2*, 157.
17. Goyal, R. N.; Gupta, V. K.; Chatterjee, S.; *Biosens. Bioelectron.* **2009**, *24*, 1649.
18. Rezaei, B.; Mirahmadi Z., S. Z.; *Anal. Lett*. **2008**, *41*, 2267.
19. Rezaei, B.; Mirahamdi Z., S. Z.; *Sens. Actuators, B* **2008**, *134*, 292.
20. Nth, M.; Chau, T.; Thwaites, G.; *N. Engl. J. Med.* **2007**, *357*, 2431.
21. Quentel, F.; Elleouet, C.; *Electroanalysis* **2001**, *13*, 1030.
22. Ghoneim, E. M.; El-Attra, M. A.; Ghoneim, M. M.; *J. AOAC Int.* **2009**, *92*, 597.

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