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FULL PAPER

Voltammetric Determination of Diethylstilbestrol in Pharmaceutical Formulation

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Abstract:

A simple, inexpensive and highly sensitive new electrochemical method was developed to quantify Diethylstilbestrol (DES) in medication samples using carbon paste electrode (CPE). The electrode was electrochemically characterized using cyclic voltammetry (CV) and square wave voltammetry (SWV). From the cyclic voltammograms obtained in the presence of diethylstilbestrol could be observed two peaks, one of oxidation at ≈ 590 mV and other of reduction at 200 mV. The development of method for estrogen quantification under study involved optimization of instrumental parameters (frequency, amplitude and potential step) and the experimental parameter pH. The electrochemical procedure applied to diethylstilbestrol was developed using CPE under optimal conditions. Diethylstilbestrol oxidation currents exhibited linear concentration in the 0.017 - 2.08 μmol L^{-1} range, with a limit of detection of 14.15 nmol L^{-1}. The amount of DES found in the analyzed samples was 0.98 mg, with a relative standard deviation of less than 2%, indicating high agreement with the amount described in the label of the drug (1 mg). The results indicate that the method is applicable for quantifying diethylstilbestrol in pharmaceutical formulation.

Keywords: hormone; estrogen; voltammetry; carbon paste electrode

1. Introduction

The Diethylstilbestrol (4-[(E)-4-(4-hydroxyphenyl)hex-3-en-3-yl] phenol, DES), is a synthetic estrogen developed in 1938 used in the treatment of estrogen deficiency in women and in treatment of advanced breast and prostate cancer [1, 2, 3]. Years after its development and commercialization, it was verified that the exaggerated use of DES caused side effects such as vaginal and breast cancer as well as poor reproductive organ formation in children [4, 5]. Its use was banned in the USA and others Europe countries [6]. In Brazil, its use has been banned in animal production since 2001, according to the Normative Instruction of the Ministry of Agriculture, Livestock and Supply [7]. However, it is still used as a medicine in the treatment of advanced breast and prostate cancer.

Recent studies show that the use of this drug in the proportion of 1 mg per day has a lower rate of side effects with high efficacy in the treatment of prostate cancer and low cost [8]. Therefore, in order to follow with the official regulations and to assure the quality of medicines containing DES, sensitive analytical methods are required for this purposes.

Chromatographic methods are used in the determination of DES, such as gas chromatography with mass spectrometry (GC-MS) was used in its determination in cattle urine samples [9], high performance liquid chromatography (HPLC) in water samples of the sea [10], with ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-QTOF-MS) in milk samples [11]. These methods can be sensitive and accurate, however, requiring long period of analysis and pre-treatment of the sample [12]. The electrochemical method has been highlighted

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due to its high sensitivity, simplicity and lower cost when compared with chromatographic methods [12].

The molecular structure of DES contains two hydroxyl groups attached to the phenolic ring, which makes possible the electrochemical study of this compound, since these compounds can oxidize on the surface of the working electrode. The literature describes some electrochemical methods to determine DES in several types of samples using the most varied types of materials including glassy carbon electrode modified with cobalt-functionalized multilayer carbon nanotube (CoPc/MWCNTs/AuNPTs/GCE) [7], a glassy carbon electrode modified with cyclodextrins deposited on the reduced graphene oxide surface (βCD/RGO/GCE) [13], electrochemically modified glassy carbon electrode with reduced graphene oxide and multiple carbon nanotubes (ERGO/MWCNTs/GCE) [14] and with platinum nanoparticles functionalized carbon nanotubes (SWNT/Pt/GCE) [15]. The carbon paste electrode (CPE) is simple to prepare, inexpensive and sensitive. In the development of sensors, graphite has several advantages: high surface area, high sensitivity and electrocatalytical activity [16].

In the present investigation, the electrochemical process that happens on the surface of the working electrode was performed using cyclic voltammetry (CV), and the chemical reaction that occurs on the surface of the working electrode was proposed. After optimization of the experimental and instrumental parameters, a novel electrochemical method to quantify DES in a drug sample was developed and applied using carbon paste electrode and square wave voltammetry (SWV). The analytical performance, precision, repeatability and stability of the sensor were also evaluated.

2. Results and Discussion

2.1. Electrochemical characterization of DES

The cyclic voltammograms obtained in the presence and absence of 8.53 x 10^{-6} mol L^{-1} of DES using CPE are shown in Figure 1. In the buffer solution PB (voltammogram 1, Figure 1), can be observed the absence of peaks which indicates that in this potential range there is no interference of the buffer, and it can be used in the study of the redox process of DES. In the presence of DES in the electrochemical cell (voltammogram 2, Figure 1) can be observed an oxidation peak at approximately 595 mV and a reduction peak at approximately 195 mV.

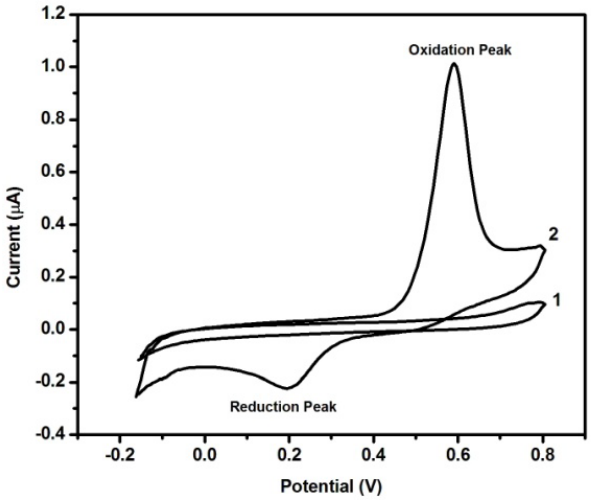

Figure 1. CVs. Experimental conditions: [DES] = 8.53 μmol L^{-1} (voltammogram 2) in Buffer [PB] = 0.1 mol L^{-1} pH 6.5 (voltammogram 1). Instrumental parameters: ν = 0.1 V s^{-1}.

2.2. Influence of pH

The influence of pH on electrochemical behavior of DES was investigated using CV in buffer PB 0.1 mol L^{-1} in the pH 5.0 - 8.0 range for a solution of DES 4.3 μmol L^{-1} (Figure 2A and 2B). The peak of potential (E_p) obtained showed a linear shift to more negative values of the potential with increasing pH (the right side of Figure 2B), after adjustment the following equation was obtained: Ep (mV) = 890.64 - 51.99 pH, r = 0.996. The value of the obtained coefficient (slope) was 51.99 mV/pH, indicating the participation of protons ($2H^+$) in the oxidation reaction of DES on the surface of CPE, and the same number of protons and electrons are involved in the electrochemical reaction [17]. The variation in the peak current (I_p) in relation to pH values (the left side of Figure 2) showed maximum value of oxidation current at pH 6.0 and based on this, pH 6.0 was chosen for the electrochemical study to quantify DES in a pharmaceutical formulation.

2.3. Scan rates effect

The influence of the scan rate (ν, mVS^{-1}) on Ip and Ep was evaluated in a range of ν = 1 - 500

mV s^{-1} in cyclic voltammetry. The obtained voltammograms showed a peak of oxidation, direct scan, a peak of reduction, and reverse scan (Figure 3A). The relation Ip *vs.* ν (Figure 3B) shows a linear behavior, according to equation Ip (μA) = 0.02 + 1.13 ν, r = 0.99, for oxidation peak and show a linear behavior according to the equation Ip (μA) = -0.01 - 0.22 V s^{-1}, r = 0.99, for reduction, which suggests an adsorption-controlled process on the surface of working electrode [17]. The relation log Ip vs log ν (Figure 3C) was also linear, according to log equations log Ip = 0.02 + 0.84 log ν, r = 0.99, with a slope of 0.84, for oxidation, and log Ip = -0.07 + 0.89 log ν, r = 0.99, with a slope 0.89, for reduction peak, suggesting a process controlled by adsorption [17]. The electrons released in the oxidation process on the surface of the CPE are adsorbed electrostatically on their surface, characterizing a chemical reaction controlled by adsorption. In the relation $Ip/\nu^{1/2}$ *vs.* ν (Figure 3D), a peak current increase is observed in a non-linear way for both oxidation and reduction, and these results indicate that the electrochemical oxidation of DES involves a coupled chemical reaction, suggesting an electrochemical- electrochemical mechanism [17]. Considering that the number of electrons and protons involved in the DES oxidation process is the same (see Section 2.2), the electrochemical oxidation of DES in CPE is a process of two electrons and two protons, which can be described in Figure 4 [13].

2.4. Linear range, limit of detection and quantification

To develop the methodology using with CPE, the following instrumental and experimental parameters were optimized in relation to the peak oxidation current of DES (frequency, amplitude and potential step) (Table 1). Due to its high sensitivity and low detection limit (LD), SWV electrochemical technique was used to determine DES under optimized conditions. To develop a new electrochemical method for the quantification of DES pharmaceutical formulation, the previously optimized parameters and working conditions were used (Table 1). The SWVs and the curve of the variation of Ip with the concentration (Figure 5) were obtained in the range of 17.20 nmol L^{-1} to 2.08 μmol L^{-1}. A linear increase of Ip with concentration is observed, and the equation that represents the analytical curve was calculated using the least squares method as Ip (μA) = 0.006 + 2.119 [DES] μmol L^{-1}, r = 0.999. The LD and LQ were determined using the ratio LD = 3SD/s LQ = 10SD/s [18], where SD is the standard deviation of the intercept, and *s* is the angular coefficient of analytic curve. The values found for the analytical curve of DES are shown in Table 2. The values were compared to those already reported by other authors [12-15] (Table 3) it can be observed that the limit of detection obtained in this work is close to that reported in the reference [15] and lower than that reported in the reference [7] showing the viability of the electrode used.

Table 1. Optimized parameters in SWV. Buffer PB 0.1 mol L^{-1}. [DES] = 4.3 μmol L^{-1}.

Parameters	Range of values	Optimized value
pH	5.0 - 8.0	6.0
Amplitude (mV)	5 - 140	40
Step(mV)	1 - 35	10
Frequency (Hz)	1 – 70	40

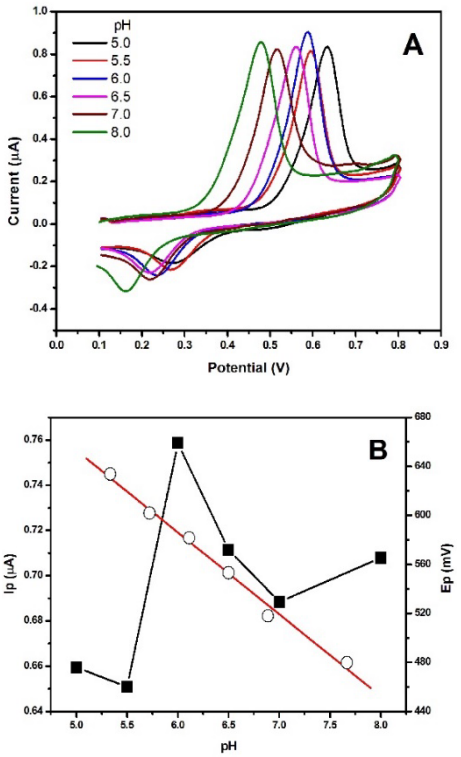

Figure 2. A) CVs and B) Ip x pH and Ep x pH. Experimental conditions: [DES] = 4.3 μmol L^{-1} in buffer. [PB] = 0.1 mol L^{-1}. Instrumental parameters: ν = 0.1 V s^{-1}. Working electrode: CPE.

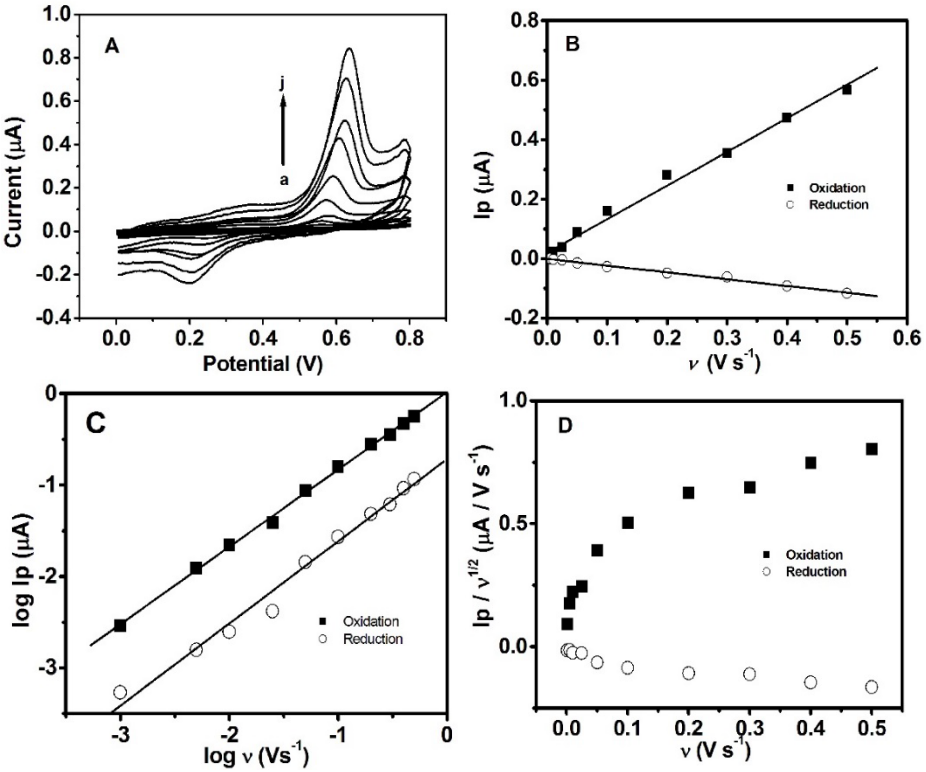

Figure 3. A) CVs. B) Ip *vs.* ν. C) log Ip *vs.* log ν. D) $Ip/\nu^{0.5}$ *vs.* ν. Experimental conditions: [DES] = 2.15 μmol L^{-1} in Buffer [PB] = 0.1 mol L^{-1}, pH = 6.0. Scan rate: a) 0.001; b) 0.005; c) 0.010; d) 0.025; e) 0.050; f) 0.100; g) 0.200; h) 0.300; i) 0.400 and j) 0.500 V s^{-1}.

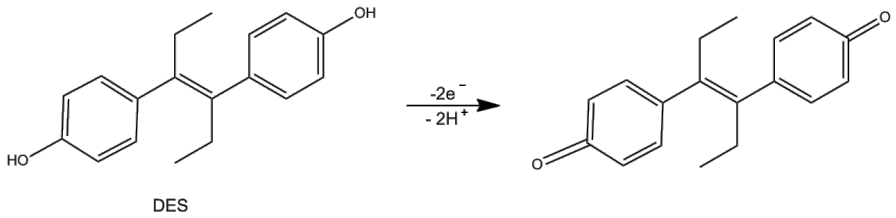

Figure 4. Proposed reaction mechanism for the electrochemical oxidation of DES [17].

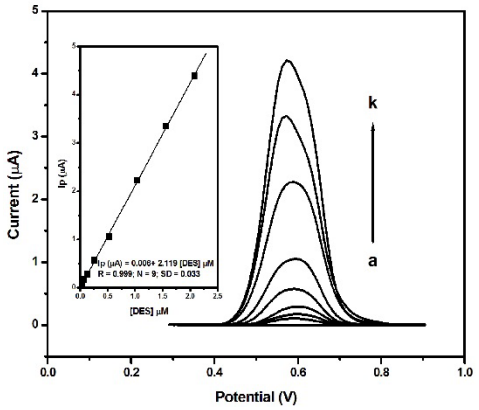

Figure 5. SWVs and analytical curve for DES, using CPE as the working electrode. Experimental conditions: Buffer [PB] = 0.1 mol L^{-1} (pH 6.0). Variable concentration of DES a: Buffer [PB] alone; b: 0.017 c: 0,034; d: 0.068; e: 0.130; f:0.260; g: 0.520; h:1.040; i: 1.560; j: 2.080 μmol L^{-1}. Inserted analytical curve.

2.5. Precision, repeatability and stability

Precision, repeatability and stability (Figure 6) are three important aspects of any method of determination. Precision was determined in three experiments performed on intraday measurements with a 5 hour interval. The results showed a relative standard deviation (RSD) of 6.0%, indicating good precision. Repeatability was determined in experiments performed with 30 consecutive measurements. The results presented RSD lower than 2.0%. In addition, stability of electrode was also investigated for three electrodes with same composition, RSD lower than 6.0%. These low RSD values demonstrate the high quality of CPE electrode in terms of precision, repeatability and stability.

Table 2. Parameters obtained from the analytical Figure 5.

Parameters	Values
Concentration range (mol L^{-1})	17.00 x 10^{-9} - 2.08 x 10^{-6}
Correlation coefficient (r)	0.999
Intercept (±sd) (μA)	0.006 ± 0.010
Slope (±sd) (μA/μmol L^{-1})	2.119 ± 0.010
Detection limit (nmol L^{-1})	14.15
Limit of quantification (nmol L^{-1})	47.19

Table 3. Comparison of the proposed sensor for determination of DES with others.

Electrode	Range (μmol L^{-1})	Detection limit (μmol L^{-1})	References
CoPc/MWCNTs/AuNPTs/GCE	0.7 – 5.66	0.199	7
βCD/RGO/GCE	0.01 – 13	0.004	13
ERGO/MWCNTs/GCE	0.01 – 40	0.003	14
SWNT/Pt/GCE	0.109 - 20.9	0.015	15
CPE	0.017 - 2.08	0.014	This work

MWCNT: multi-walled nanotube. CoPc: cobalt phthalocyanine. AuNPTs: gold nanoparticules GCE: Glassy carbon electrode. βCD: Cyclodextrins. GRO: reduced graphene oxide. ERGO: reduced oxidized graphene.

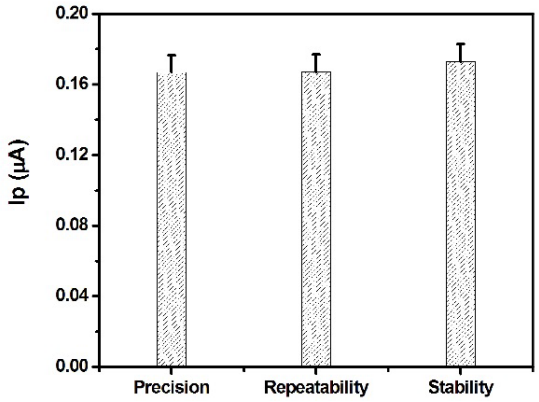

Figure 6. Precision, repeatability and stability. Experimental conditions: [DES] = 0.13 μmol L^{-1} in Buffer [PB] = 0.1 mol L^{-1} (pH 6.0).

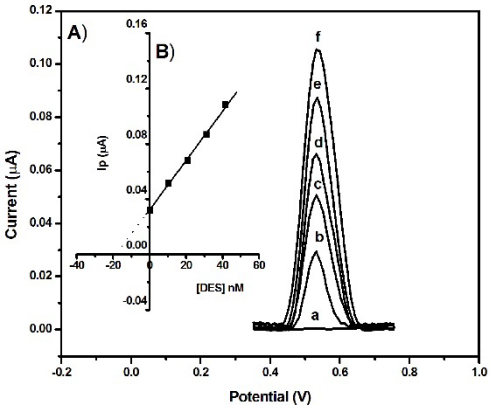

Figure 7. SWVs determination for DES, using CPE as the working electrode. Experimental conditions: Buffer [PB] = 0.1 mol L^{-1} (pH 6.0). A) [DES]: a: Buffer; b: Sample; c: 10.40; d: 20.80 e: 31.20 f: 41.60 nmol L^{-1}. B) Standard addition curve.

2.6. Application of the proposed method

The determination of the amount of DES in destilbenol tablet was made under optimized conditions by SWV with working electrode CPE. Using the standard curve and addition methods, the Figure 7 shows the SWVs and the addition curve. The found concentration of DES for the indicated on label (1 mg/tablet), with relative errors of less than 1%, demonstrating the viability of the proposed method (Table 4 and Figure 7).

Table 4. Found amounts of DES sample (n = 3).

Sample	Indicated (mg)	Found (mg ± SD)	RSD %
Destilbenol	1	0.98 ± 0.01	1.01

3. Material and Methods

3.1. Equipment

The measurements of CV (pH) and SWV (frequency, amplitude and potential step) were performed in a Potentiostat/Galvanostat AUTOLAB PGSTAT 128N (Ecochemie, Utrecht, The Netherlands) interfaced to a computer and managed by NOVA 1.10 software for data acquisition. The experiments were performed in a cell of three electrodes at room temperature (25±1 °C) using a platinum wire as the counter electrode, and Ag / AgCl in KCl (3 mol L^{-1}) as reference electrode. The cell was placed in a

Faraday cage to minimize background noise.

3.2. Reagents and solutions

The phosphate buffer [PB] was prepared by mixing solutions of disodium hydrogen phosphate (Na_2HPO_4) (Merck, 99.5%) and sodium dihydrogen phosphate (NaH_2PO_4) (Vetec, 99%) at concentrations of 0.1 mol L^{-1}, pH adjusted in 5.0 - 8.0 range. The stock solution of DES ($C_{18}H_{20}O_2$) (Sigma-Aldrich, 99.9%, CAS number 56-53-1) was prepared in ethanol (Dinâmica, 99.5%). From the stock solution, dilutions were made in buffer itself to obtain the concentrations used in this study.

3.3. Preparation of working electrode

The carbon paste working electrode (CPE) was prepared using: 0.750 g of graphite and 0.250 g of mineral oil (Fluka). The obtained mixture was homogenized by hand in glass mortar for 40 min, and placed into a plastic syringe with a geometric area of 0.03 cm^2. Electrical contact was established via copper wire.

3.4. Precision, repeatability and stability

The study of precision, repeatability and stability for CPE was investigated with 0.13 µmol L^{-1} of DES. Precision was mande with 3 measurements performed at 5 hours intervals. Repeatability was made with 30 consecutive measurements, and stability was drawn 3 electrodes with same composition to obtain 10 consecutive scans.

3.5. Preparation of the pharmaceutical formulation

To determine DES, a pharmaceutical formulation (Destilbenol) purchased from the local pharmacy. The amount described on the label was 1 mg/tablet. A stock solution of the formulation was prepared in ethanol (1.57 mg L^{-1}). From the stock solution working solutions were prepared on own buffer used in this study at pH = 6.0. Using the standard addition method, the concentration of drug was evaluated against value given on the label.

4. Conclusions

In this work, a new electrochemical method based on the simplicity of the working electrode (CPE) was developed and applied to determine DES. The electrode prepared, although simple, has a lower limit of detection, less than that already reported in the literature, besides, it exhibited good precision, repeatability and stability. In the application of the Destilbenol sample, the electrode manufactured obtained a relative standard deviation of less than 3% and the determination revealed a similar amount as indicated on the label, showing that it could be applied to determine DES in pharmaceutical formulation samples. Together, these data indicate the high analytical efficiency of the new method, suggesting its potential usefulness in routine laboratory analysis.

Acknowledgments

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