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Short communication

Voltammetric investigation of diethylstilbestrol

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Abstract

In this work electrooxidation of diethylstilbestrol (DES) was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) using a glassy carbon (GC) electrode. It was statistically shown that both methods could be used for the determination of DES in the concentration range of 2×10^{-5}–6×10^{-4} M by CV and 1×10^{-5}–1×10^{-3} M in methanol (MeOH) and 4×10^{-5}–6×10^{-4} M in acetonitrile (ACN) by DPV and both of the methods could be applied to human serum. A mechanism was proposed about the electrooxidation of this substance.

Keywords: Diethylstilbestrol; Electrooxidation; Cyclic voltammetry; Differential pulse voltammetry; Determination

1. Introduction

Estrogenic activity is a property shared by a great number of steroidal and non-steroidal compounds. Diethylstilbestrol [(*E*)-4,4′-(1,2-diethyl-1,2-ethenediyl) bis phenol] (DES) is among the first non-steroidal estrogens to be encountered and it is the most potent. In contrast to the natural estrogens, it is highly active when given by mouth and the duration of action of a single dose is longer because of its slower rate of degradation in the body. Its oxidative mechanism affects its hormonal activity [1,2].

Anabolic compounds stimulate synthesis and thus increase the muscle size and strength in both humans and animals. Although the fact that it is a known carcinogen, DES is used for both human and animal application because of its estrogenic activity and it is the one of the cheapest synthetic estrogens to produce. It has been banned from use in animal feeds in the USA and in many European countries [3] but it is used illegally. Therefore, to comply with official regulations and to guarantee the absence of xenobiotic anabolic residues in meat and meat products and biological fluids, practical and reliable analytical methods are needed. The most common methods found in literature are different types of chromatography such as thin layer (TLC) [4–6], high performance liquid chromatography (HPLC) [7–10], gas and mass spectrometry [11–14], immuno affinity chromatography

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(IAC) [15], radioreceptor assays [16] and immuno assays [17]. Although most of these methods are sensitive and specific, they are also expensive and take a long time. But electrochemical methods are rapid and inexpensive and generally directly applicable to many of the pharmaceutical preparations.

As far as we know polarographic and voltammetric studies on DES are very limited e.g. a paper related to the direct current (DC) and alternating current polarography (AC) of some pharmaceuticals including DES was published, but in this paper it was reported that no response could be obtained for DES by both of the methods [18]. Séquaris et al. [19] investigated the anodic oxidation mechanisms, peroxidase-mediated oxidation of DES has been proposed as a pathway in DES-induced carcinogenity.

In the present study electrooxidation of DES was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) mainly for analytical purposes and the evaluation of the data revealed that DES could be determined by CV and DPV and also it was shown that CV and DPV techniques could be applied for the determination of DES in human serum. Recently a paper [20] was published related to the determination of DES at carbon paste electrode using cetylpyridine bromide as medium. Although the determination limit of this absorptive method was found very low there is no knowledge about the application to serum.

2. Experimental

2.1. Apparatus

The measurements were taken and curves were obtained using a BAS 100 W/B electrochemical analyser and a HP SL printer. Working and counter electrodes were a BAS MF 2012 glassy carbon (GC) disc and a BAS MV 1032 platinum, respectively. A BAS MF 1063 type silver/silver chloride electrode was used as reference. The potentials in the text were given versus silver/silver chloride electrode.

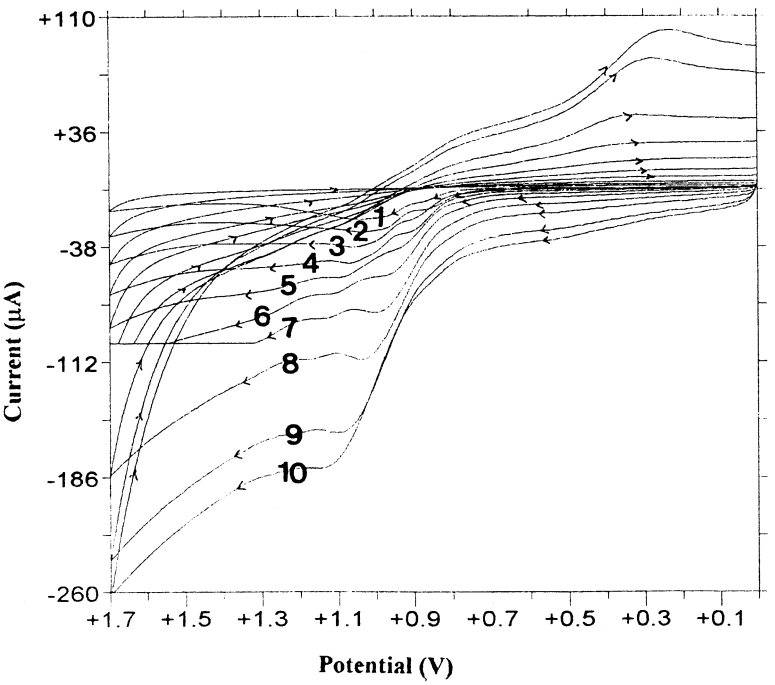


Fig. 1. Cyclic voltamograms of 6×10^{-4} M DES in 0.05 M H_2SO_4 ACN:water (9:1 v/v) obtained with various scan rates. (1, 10 mV/s; 2, 25 mV/s; 3, 50 mV/s; 4, 100 mV/s; 5, 200 mV/s; 6, 400 mV/s; 7, 800 mV/s; 8, 1505 mV/s; 9, 3011 mV/s; 10, 4015 mV/s).

Table 1
Statistical analysis of calibration plots of DES obtained with CV and DPV under different conditions

Technique	Medium	Linearity range (mol/l)	Slope (A/M)	S.E. of slope (A/M)	Intercept (A)	S.E. of intercept (A)	Correlation coefficient	Limit of detection (M)	Limit of quantitation (M)
CV (scan rate 25 mV/s)	0.05 M H_2SO_4 ACN/water (9/1 v/v)	2×10^{-5}–6×10^{-4}	4.48×10^{-2}	1.55×10^{-4}	6.03×10^{-7}	4.00×10^{-8}	0.99	1.12×10^{-5}	1.72×10^{-5}
DPV	0.05 M H_2SO_4 MeOH/water (45/55 v/v)	1×10^{-5}–1×10^{-3}	1.00×10^{-2}	3.40×10^{-4}	8.20×10^{-7}	1.60×10^{-7}	0.99	8.85×10^{-6}	1.35×10^{-5}
DPV	0.05 M H_2SO_4 ACN/water (9/1 v/v)	4×10^{-5}–6×10^{-4}	3.07×10^{-2}	1.08×10^{-3}	1.00×10^{-7}	3.10×10^{-7}	0.99	1.14×10^{-5}	1.74×10^{-5}

2.2. Reagents

Standard DES, was supplied by Sigma. All other reagents were of analytical grade. All the solutions were prepared using doubly distilled water. Two stock solutions of 10^{-3} M DES were prepared in two different solvent systems namely 45/55 (v/v) MeOH/water and 9/1 (v/v) acetonitrile (ACN)/water. Standard solutions were prepared using these stock solutions and contained MeOH/water and ACN/water as the same ratio in the stock. Tests were performed in 0.05 M H_2SO_4 and Britton–Robinson (BR) buffers. BR buffers were prepared using 0.04 M phosphoric, acetic and boric acids. pH was adjusted by the addition of 6 M NaOH solution.

2.3. Pretreatment of the working electrode

Before each experiment surface of the GC electrode was polished using alumina ($\phi = 0.01$ μm) on a polishing pad and then carefully washed with bidistilled water and dried on a filter paper.

2.4. Analysis of DES in serum

Trichlor acetic acid, sulphuric acid and ACN were tested in order to precipitate serum proteins. ACN was found as the proper precipitant because small volumes of ACN were enough for complete precipitation. DES was analysed in serum using ACN/water and MeOH/water solvent systems. Serum samples (0.5 ml) were added to 10 ml screw-capped centrifugation tubes and DES was added from a stock solution followed by 0.5 ml ACN addition and the volume was completed to 10 ml with 45/55 (v/v) MeOH/water and 9/1 (v/v) ACN/water mixture containing 0.05 M H_2SO_4. After centrifugation (10 min) the upper solutions filtrated and filtrates were transferred to voltammetric cell.

3. Results and discussion

Tests were performed using a GC electrode in 0.05 M H_2SO_4 and BR buffers in the pH range covering 2.88–8.66. ACN/water 9/1 (v/v) and

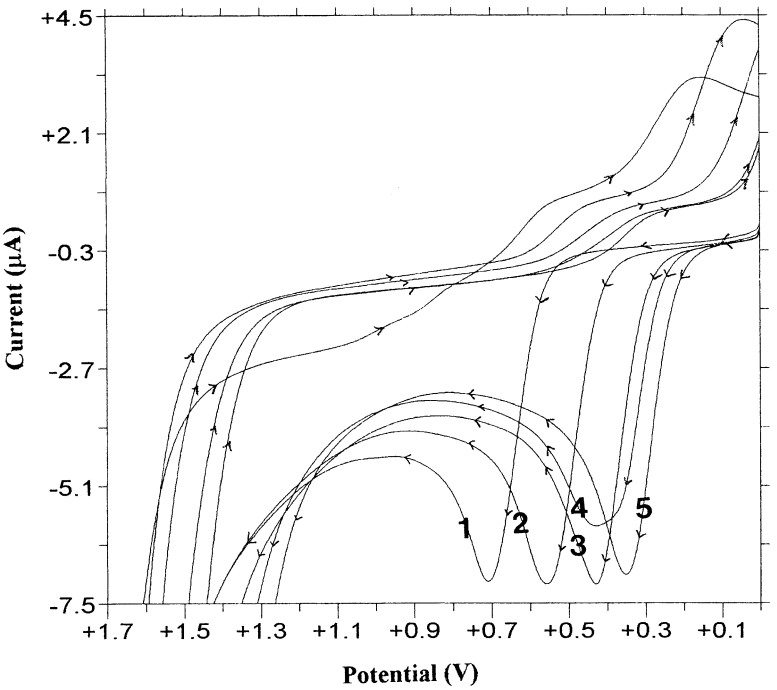


Fig. 2. CV curves obtained for of 5×10^{-4} M DES in BR buffers of pH 1, 2.88; 2, 5.37; 3, 7.25; 4, 8.13; 5, 8.86; scan rate = 50 mV/s.

MeOH/water 45/55 (v/v) mixtures were used as solvents. CV and DPV techniques were applied and the results were evaluated from the view points of quantitative analysis. CV curves were recorded in a broad scan rate interval (10–4000 mV/s). Fig. 1 shows the cyclic voltammograms, recorded in 0.05 M H_2SO_4 containing 6×10^{-4} M DES by various scan rates. At lower scan rates two oxidation steps were observed the first one was at about 0.85 V and the second one was at 1.00 V. As scan rate increased both of them shifted to positive potentials and the height of the first peak increased while the second peak's height decreased. Above 1500 mV/s only one peak could be observed. On the reverse scan two broad peaks were obtained at about 0.8 and 0.3 V at higher scan rates. The peak current (i_p) of the anodic peak at 1.0 V was found to be linearly dependent on concentration for various scan rates. In this study 25 and 50 mV/s were chosen for analytical tests. Table 1 shows the results of the statistical analysis of i_p–C relationship at 25 mV/s.

Repeatability of peak current and peak potential of this peak were tested in 0.05 M H_2SO_4 containing 10^{-4} M DES by repeating ten experiments, and relative standard deviations (R.S.D.) were found as 0.99% for both peak current and peak potential and the standard deviations (S.D.) were calculated as 1.22×10^{-2} and 1.24×10^{-2}, respectively. Multiscan curves were recorded in 0.05 M H_2SO_4 solution containing 4×10^{-4} M DES. On the oxidation branches of these curves it was observed that as scan number increased peak current decreased indicating that during first scan mainly adsorbed molecules took place in the electrode reaction, at the following scans gradually, diffused molecules became major reactant.

The effects of both pH and scan rate were studied in BR buffers in the pH range of 2.88–8.86. Fig. 2 shows the voltammograms taken in BR buffers having different pH values, as an example a scan rate of 50 mV/s was chosen. It is seen that, on the oxidation curves the peak potential shifted to less positive region with the

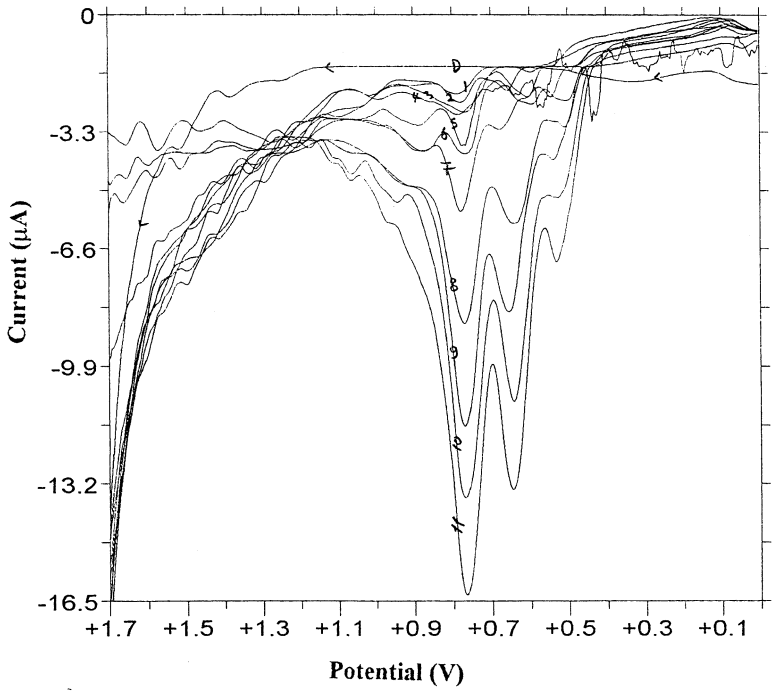


Fig. 3. DPV curves of DES in 0.05 M H_2SO_4 MeOH:water (45:55 v/v) obtained in the concentration range of 1, 1×10^{-5} M; 2, 2×10^{-5} M; 3, 4×10^{-5} M; 4, 6×10^{-5} M; 5, 8×10^{-5} M; 6, 1×10^{-4} M; 7, 2×10^{-4} M; 8, 4×10^{-4} M; 9, 6×10^{-4} M; 10, 8×10^{-4} M; 11, 1×10^{-3} M DES.

increase in pH. Between pH 2.88 and 5.37 at the scan rates lower than 200 mV/s, peaks were observed on oxidation and reduction branches, at higher scan rates and higher pH values although oxidation peaks were clearly observed, reduction peaks disappeared. At all the scan rates peak current decreased when pH increased up to 7.25. The highest anodic peak currents were obtained in 0.05 M H_2SO_4 and BR buffer of 2.88. For analytical and mechanistic studies 0.05 M H_2SO_4 was chosen as supporting electrolyte.

DPV tests were performed in two solvent systems namely ACN/water (9/1, v/v) and MeOH/water (45/55, v/v). Optimum conditions were found as 20 mV/s scan rate, 50 mV pulse amplitude, 17 ms pulse width and 200 ms pulse period. In MeOH/water mixture containing 0.05 M H_2SO_4, DPV curves of DES in the concentration range of 10^{-5}–10^{-3} M are seen in Fig. 3. On these curves three well defined peaks at 0.77, 0.65 and 0.55 V are observed. Linear peak current–concentration relationships were obtained for the ones at 0.77 and 0.65 V, but as the peak current of the former is higher, this peak was used for analytical purpose. When ACN/water mixture was used as solvent peak potentials and the shapes of the voltammograms changed, and a good resolution was not obtained, only a main peak at about 0.92 V appeared, the current of which is linearly changed with concentration. The peak potentials in ACN/water (9/1, v/v) mixture shifted to more positive potential region. The statistical analysis of peak current–concentration relationships for DPV curves obtained in both solvent systems are given in Table 1. As can be seen from this table the linearity range obtained in MeOH/water mixture is broader than in ACN/water solvent system. Repeatability of DPV peak current and peak potential was tested by repeating ten experiment in 10^{-4} M DES. The R.S.D. was

calculated to be 1.07% with a S.D. of 1.44×10^{-2} for peak current, and 1.09% with a S.D. of 1.57×10^{-2} for peak potential.

Using CV curves obtained in 6×10^{-4} M DES in ACN/water (9/1, v/v) containing 0.05 M H_2SO_4 in the scan rate range of 10–4015 mV/s. i_p–ν (rate) and i_p–$\nu^{1/2}$ relationships were examined.

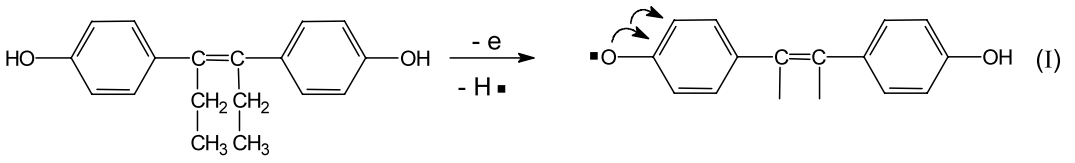
The plot of i_p–ν is not a line while i_p–$\nu^{1/2}$ relationship results two lines with different slopes for 10–30 and 50–200 mV/s represented by the equations of $i_p = 8.79 \times 10^{-6} + 1.18 \times 10^{-4}\nu^{1/2}$ and $i_p = 2.15 \times 10^{-5} + 5.99 \times 10^{-8}\nu^{1/2}$, respectively. This reveals that the reaction was diffusion controlled but the mechanism was rather complicated and changed depending on the scan rate.

Using the anodic curves obtained in the concentration range of 2×10^{-5}–6×10^{-4} M DES with a scan rate 10 mV/s, log i (at 1.00 V)–log C relationship was obtained as $\log i = 0.92 \log C - 1.75$, ($r = 0.99$) indicating a line, the slope points a degree of the reaction about 1.

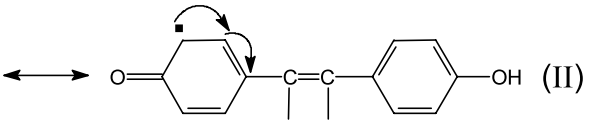
For 5×10^{-4} M DES in BR buffers having the pH values of 2.88, 5.38, 7.25, 8.13 and 8.86 anodic voltammograms were obtained by different scan rates, by means of these curves peak potential–pH relationship for all the scan rates were found linear with the slopes about 0.059 V which can be interpreted as one proton per electron takes place in the reaction.

Considering all of the results given above and taking into account for the investigations related to the oxidation mechanisms of phenols [19,21–23] the mechanism given below has been proposed for the electrooxidation of DES.

First oxidation step:



DES Aryloxy radical



Phenoxonium radical

Second oxidation step:

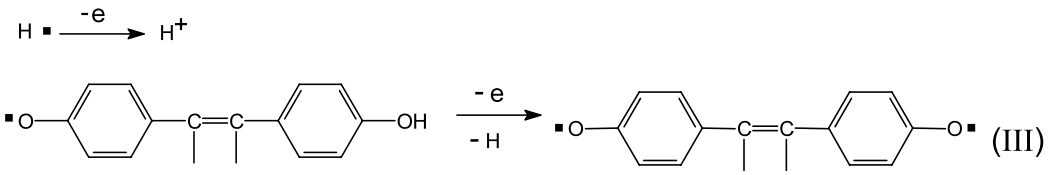


Table 2
Recoveries of DES from serum by CV and DPV techniques

Techniques	Scan rate (mV/s)	Added amount	Found amount (μg)	% Recovery	Mean % recovery
CV	10	1.00×10^{-4} M (26.84 μg)	29.25	109.00	97.27 ± 1.73% ($n = 4$)
CV	10	2.00×10^{-4} M (53.68 μg)	49.12	91.51	
CV	10	4.00×10^{-4} M (107.36 μg)	100.65	93.75	
CV	10	6.00×10^{-4} M (161.04 μg)	152.72	94.83	
CV	25	1.00×10^{-4} M (26.84 μg)	27.65	103.02	92.90 ± 0.12% ($n = 4$)
CV	25	2.00×10^{-4} M (53.68 μg)	50.73	94.50	
CV	25	4.00×10^{-4} M (107.36 μg)	95.28	88.75	
CV	25	6.00×10^{-4} M (161.04 μg)	137.42	85.33	
CV	50	1.00×10^{-4} M (26.84 μg)	28.45	106.00	96.71 ± 9.29% ($n = 4$)
CV	50	2.00×10^{-4} M (53.68 μg)	54.42	101.01	
CV	50	4.00×10^{-4} M (107.36 μg)	98.23	91.50	
CV	50	6.00×10^{-4} M (161.04 μg)	142.25	88.33	
CV	100	1.00×10^{-4} M (26.84 μg)	26.03	96.98	97.64 ± 6.37% ($n = 4$)
CV	100	2.00×10^{-4} M (53.68 μg)	55.83	104.01	
CV	100	4.00×10^{-4} M (107.36 μg)	103.87	96.75	
CV	100	6.00×10^{-4} M (161.04 μg)	149.50	92.83	
DPV		1.00×10^{-4} M (26.84 μg)	23.35	87.00	91.47 ± 4.47% ($n = 4$)
DPV		2.00×10^{-4} M (53.68 μg)	49.65	92.49	
DPV		4.00×10^{-4} M (107.36 μg)	100.11	93.24	
DPV		6.00×10^{-4} M (161.04 μg)	150.04	93.16	

Third oxidation step can be oxidation of phenolic compounds formed by the chemical reactions of (II).

Séquaris et al. [19] proposed a similar mechanism but as a single step two electron process.

4. Application of CV and DPV methods to serum

It was shown that CV and DPV methods could be applied for the determination of DES in human serum. The accuracy and sensitivity of the methods were tested by means of recovery tests (Table 2).

In conclusion this investigation revealed that DPV and CV methods could be applied for the rapid, simple, accurate and specific determination of DES and the methods were readily applicable to serum.

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