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Voltammetric investigation of β-estradiol

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Abstract

Electrooxidation of β-estradiol was investigated using a glassy carbon electrode (GCE) by cyclic voltammetry (CV), and differential pulse voltammetry (DPV). The statistical analysis of the linear relationships between concentration and peak current permits the quantitation of β-estradiol by both CV and DPV in the concentration range of 4×10^{-5}–10^{-3} M with enough precision and accuracy. A mechanism was proposed about the electrooxidation of this substance. The methods were applied to a tablet form and a transdermal therapeutic system (TTS) of this drug. The results were statistically compared with those of official high performance liquid chromatography (HPLC) method and the differences were found as insignificant.

Keywords: β-estradiol; Electrooxidation; Cyclic voltammetry; Differential pulse voltammetry; Determination

1. Introduction

Estrogenic activity is shared by many steroidal and non steroidal compounds. The most potent naturally occurring estrogen in human beings is 17β-estradiol, followed by estrone and estriol. Each of these molecules is an 18 carbon steroid.

The therapeutic use of estrogens is extremely widespread and their pharmacological actions largely reflect their physiological activities. The most common uses of these agents are hormone replacement therapy in postmenopausal women and contraception, but the specific compounds and dosages used in these two settings are different. Although oral contraceptives are used primarily to prevent pregnancy, they also have significant health benefits beyond contraception. Estrogens also affect such general body mechanisms as salt and water balance, insulin and growth-hormone secretion, carbohydrate metabolism, calcium dynamics, and cholesterol/phospholipid ratios in blood. These actions account for the side effects occasionally observed when estrogen is used in clinical medicine and for some of the maternal metabolic changes of pregnancy; they also form the basis for estrogen administration in the deficiency state following menopause. Estrogens may have an augmenting effect on breast or endometrial cancer, and for this reason their use is contraindicated in the presence of these malignancies. Naturally occurring and synthetic compounds are available for oral and parenteral uses [1,2].

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Most of the papers related to determination of β-estradiol in body fluids and pharmaceutical preparations are based on high performance liquid chromatography (HPLC) and radio immunoassay (RIA). Although these methods are sensitive and specific, they are also expensive and time-consuming. By contrast, electrochemical methods are rapid and inexpensive and generally directly applicable to many of the pharmaceutical preparations.

The electroanalytical publications about estradiols are very few, even HPLC methods with electrochemical detection [3–5] are very limited. The reason is being the typically ill defined re-

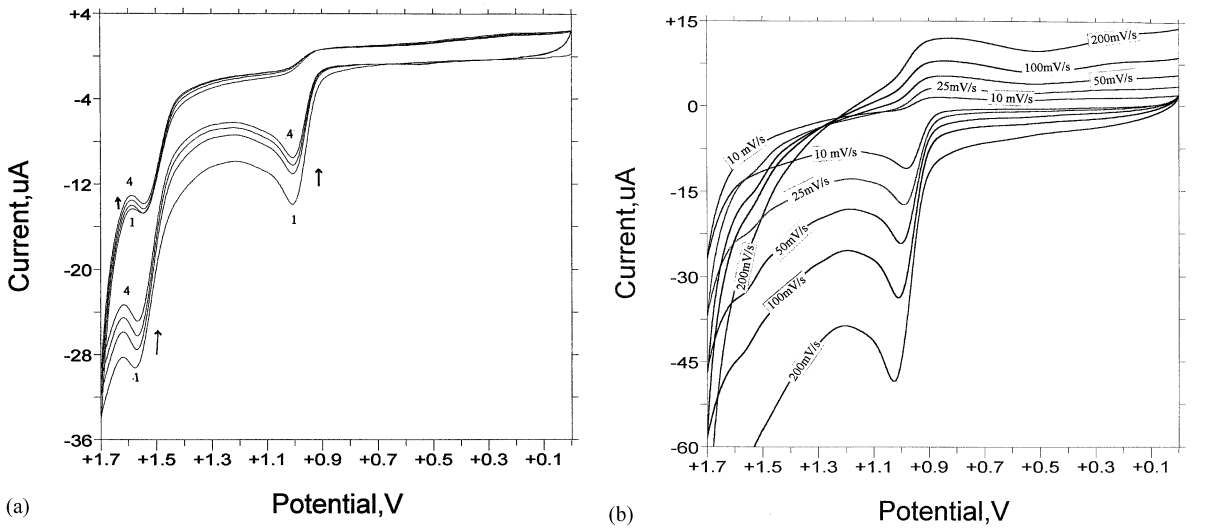


Fig. 1. (a) Multi scan voltammograms of 5×10^{-4} M β-estradiol taken in 0.05 M H_2SO_4 methanol/water 9:1 (v/v), scan rate, 50 mV s^{-1}. (b) Voltammograms of 8×10^{-4} M β-estradiol obtained in 0.05 M H_2SO_4 and methanol/water 9:1 (v/v) at various scan rates.

Table 1
Statistical analysis of calibration plots of β-estradiol obtained at various scan rates in H_2SO_4 solution

Scan rate (mV s^{-1})	Linearity range (M)	Detection limit (M)	i_p–C relationship	S.E. of slope (μA M^{-1})	S.E. of intercept (μA)
10	4×10^{-5} -1×10^{-3}	2.02×10^{-5}	$i_p = 2.04 \times 10^{-7} + 1.22 \times 10^{-2}C$ ($r = 0.999$)	1.00×10^{-4}	5.00×10^{-8}
25	4×10^{-5} -1×10^{-3}	2.45×10^{-5}	$i_p = 4.62 \times 10^{-7} + 1.83 \times 10^{-2}C$ ($r = 0.999$)	1.70×10^{-4}	6.74×10^{-8}
50	6×10^{-5} -1×10^{-3}	4.00×10^{-5}	$i_p = 7.98 \times 10^{-7} + 2.44 \times 10^{-2}C$ ($r = 0.999$)	2.55×10^{-4}	9.70×10^{-8}
100	6×10^{-5} -1×10^{-3}	4.01×10^{-5}	$i_p = 1.23 \times 10^{-6} + 3.30 \times 10^{-2}C$ ($r = 0.999$)	3.24×10^{-4}	1.70×10^{-7}
200	1×10^{-4} -1×10^{-3}	8.78×10^{-5}	$i_p = 2.31 \times 10^{-6} + 4.45 \times 10^{-2}C$ ($r = 0.999$)	8.08×10^{-4}	6.70×10^{-7}

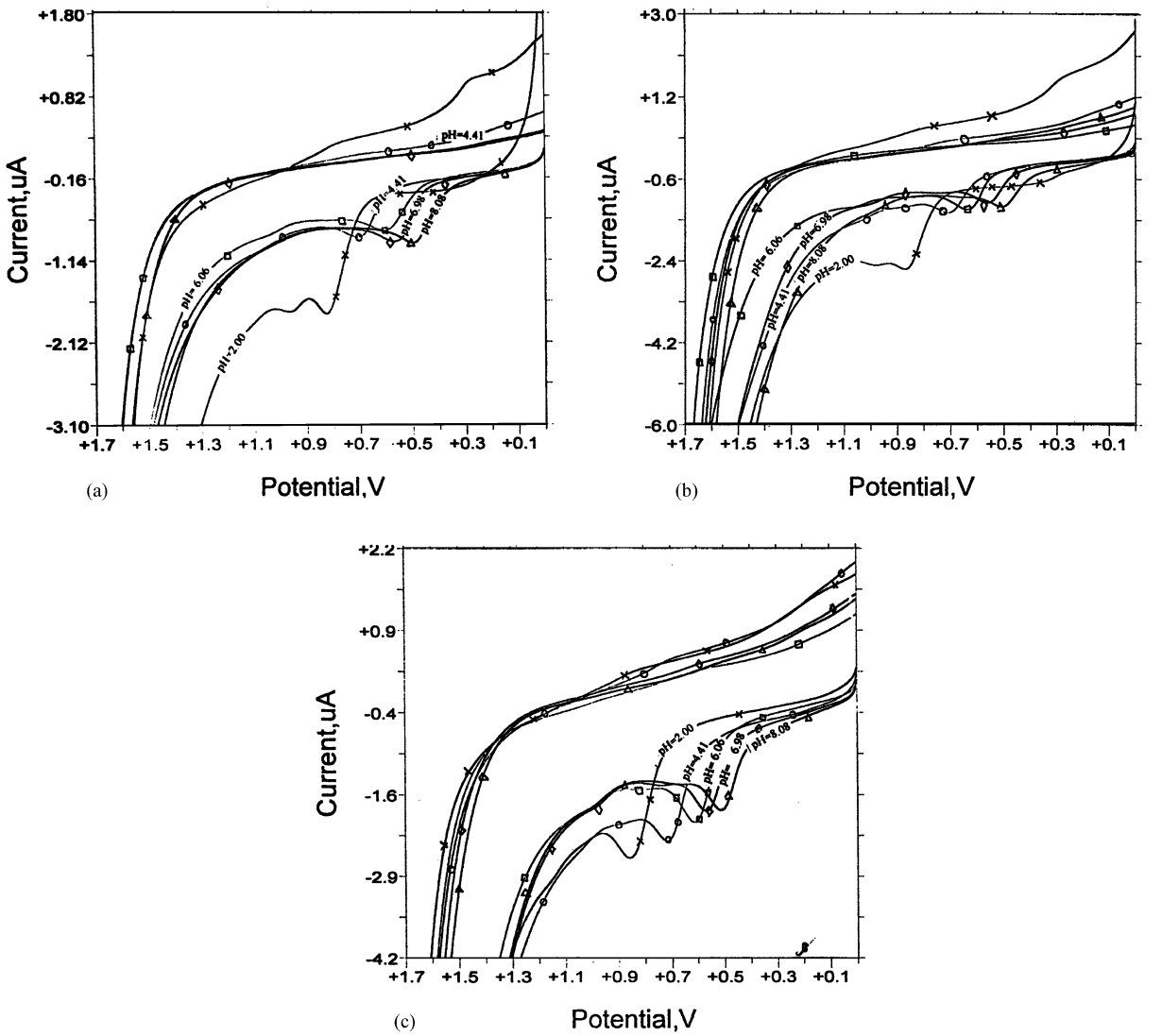
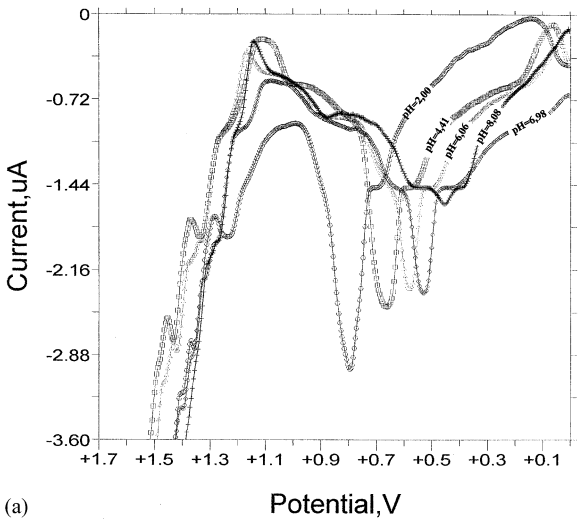


Fig. 2. Voltammograms taken in BR buffer solutions having different pH values for 1×10^{-4} M β-estradiol, scan rate (a) 10 mV s^{-1}; (b) 25 mV s^{-1}; (c) 50 mV s^{-1}, pH, $X \rightarrow 2.00$; $O \rightarrow 4.41$; $\square \rightarrow 6.06$; $\diamond \rightarrow 6.98$; $\Delta \rightarrow 8.08$.

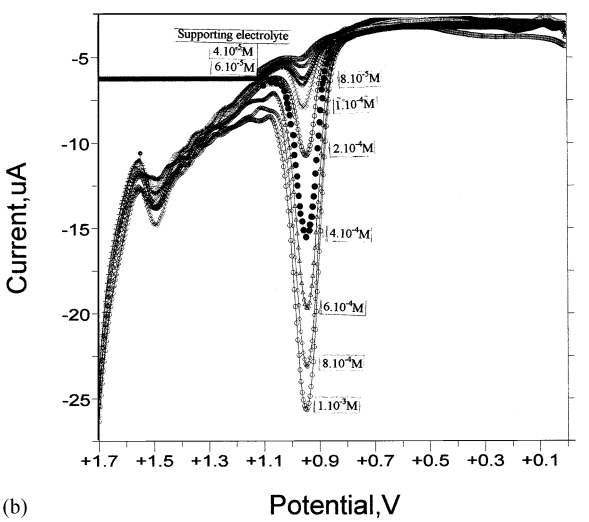
sponses of this type of compounds. Duan J.P. et al. investigated adsorptive and electrochemical behaviours of estradiol valerate at a mercury electrode [6]. Cathodic stripping of β-estradiol was publish by Shengshui et al. [7]. Patriarche et al. [8] investigated polarographic behaviours of some compounds covering estrogens. Estradiol and estriol were determined polarographically by Hu et al. [9]. Maria I. Parades et al. [10] developed a differential pulse polarographic (DPP) method for the determination of β-estradiol. Woodson et al. investigated estrogens polarographically [11]. This compound has not been investigated anodically in detailed. The only anodic voltammetric method, related to β-estradiol, we met in literature, was about anodic DPV of four estrogens including β-estradiol [12].

Table 2
The peak potential–pH relationship for peak I at various scan rates

Scan rate (mV s^{-1})	Equation of the linear relationship	r (%)
10	$Y = 0.9386 - 5.530 \times 10^{-2} X$	99.9
25	$Y = 0.9846 - 6.037 \times 10^{-2} X$	99.7
50	$Y = 0.9698 - 5.750 \times 10^{-2} X$	99.9



(a)



(b)

Fig. 3. (a) DPV curves obtained for 1×10^{-4} M β-estradiol in BR buffer solutions having different pH values. (b) DPV curves obtained in 0.05 M H_2SO_4 methanol/water 9:1 (v/v) having various β-estradiol concentration.

In the present work, electrooxidation of β-estradiol was studied using a glassy carbon electrode (GCE) by cyclic voltammetry (CV), and differential pulse voltammetry (DPV) and the effects of pH, supporting electrolyte and solvent systems were investigated in detail in order to throw light on the reaction mechanism and to propose a method for the determination of this substance.

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 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.

2.2. Reagents

Standard β-estradiol, was supplied by Sigma. All other reagents were of analytical grade. All the solutions were prepared using doubly distilled water. A stock solution of 10^{-3} M β-estradiol was prepared in 9:1 (v/v) methanol/water. Standard solutions were prepared using this stock solution and contained 9:1 (v/v) methanol/water. 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 glassy carbon (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.

Table 3
Characteristics of β-estradiol DPV calibration plots

Medium	Concentration range (M)	Slope (A/M)	S.E. of slope (A/M)	Intercept (A)	S.E. of intercept (A)	Correlation coefficient (r)
0.05 M H_2SO_4 (methanol/water 9:1 (v/v))	4×10^{-5}–1×10^{-3}	1.98×10^{-2}	1.05×10^{-3}	8.68×10^{-7}	5.20×10^{-7}	0.992

Table 4
Comparative studies for β-estradiol formulations

Analysis techniques	LCV		DPV		HPLC	
Formulations[a]	Tablet	TTS	Tablet	TTS	Tablet	TTS
Mean (mg)[b]	1.982	7.940	1.994	7.970	1.982	7.970
R.S.D. (%)	0.55	0.38	0.61	0.55	0.38	0.43
Calculated t value	0.000[c]	1.408[c]	1.448[c]	0.071[c]		
t, theoretical ($P=0.05$)	2.306	2.306	2.306	2.306		

[a] Tablet, 2000 mg per tablet; TTS, 8000 mg per strip.
[b] Each value is the mean of five experiments.
[c] NS, not significant.

2.4. Analysis of pharmaceutical dosage forms

2.4.1. Tablets

Ten tablets of β-estradiol (contained 2 mg β-estradiol and 1 mg norethisterone acetate per tablet) were accurately weighed and finely powdered. The correct amount of powder was dissolved in the supporting electrolyte and by stirring this solution for about 15 min, a stock solution of 10^{-3} M was prepared. All the test solutions were obtained by diluting this stock solution. The results were compared with those obtained by high performance liquid chromatography (HPLC) given in USP XXIII.

2.4.2. Transdermal therapeutic system (TTS) 100 strips

Five TTS 100 strips (each strip contained 8 mg β-estradiol) were taken by carefully removal of protective cover. They were treated with 9:1 (v/v) methanol/water and 0.05 M H_2SO_4 as supporting electrolyte and mechanically stirred for about 30 min. Then filtered and pieces of strips on the filter paper were washed and all the filtrant and washings were taken into a 500 ml volumetric flask and completed to volume with methanol/water mixture 9:1 (v/v) containing 0.05 M H_2SO_4. Thus a stock of β-estradiol was obtained. All the test solutions were prepared by diluting this stock with supporting electrolyte.

2.5. Analysis of β-estradiol in serum

Trichloroacetic acid, sulphuric acid and acetonitrile were tested in order to precipitate serum proteins. Acetonitrile was found as the proper precipitant because when this substance was used in small volumes the precipitation was successfully completed. Serum samples (0.5 ml) are added to 10 ml screw-capped centrifugation tubes and β-estradiol was added from a stock solution followed by 0.5 ml acetonitrile addition and the volume was completed to 10 ml with methanol/water mixture 9:1 (v/v) containing 0.05 M H_2SO_4. After centrifugation (10 min) the upper solution filtrated and filtrate was transferred to voltammetric cell.

3. Results and discussion

The electrooxidation of β-estradiol was investi-

Table 5
Recoveries of β-estradiol from serum by CV and DPV techniques

Analysis techniques	Amount added (μg)	Amount found (μg)	Recovery (%)
CV scan rate 50 (mV s^{-1})	27.24	26.43	97.03 ± 0.4
DPV	108.96	109.50	100.50 ± 0.4

gated in 0.05 M H_2SO_4 solution and BR buffers covering the pH range of 2–8. The effect of scan rate (v) was also tested in a broad interval (10–3000 mV s^{-1}).

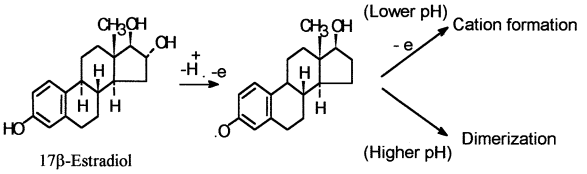
Cyclic voltammograms of 5×10^{-4} M β-estradiol obtained in 0.05 M H_2SO_4 (Fig. 1(a)) showed two anodic peaks at 1.0 and 1.58 V and two cathodic peaks at 1.56 and 0.92 V. At the second and following scans, peak currents of both of the oxidation peaks decreased, because at the first scan mainly adsorbed molecules took part in the electrode reaction but at the following scans as the time for the establishment a new adsorption equilibrium was not enough, surface concentration decreased. In Fig. 1(b), voltammograms showing the effect of the scan rate are given. As scan rate increased peak potentials shifted to more positive values and peak currents increased. The shift of the first peak potential is larger up to 50 mV s^{-1} and becomes smaller for higher scan rates. The shift of the second peak is more pronounced. The peak current of the first anodic peak is linearly dependent on β-estradiol concentration for all the scan rates given in Fig. 1(b), but as the scan rate increased the linear region becomes narrower. For analytical purpose best results were obtained with a scan rate of 50 mV s^{-1} (Table 1). Reproducibilitiy for CV curves obtained with a scan rate of 50 mV s^{-1} was tested by repeating ten experiments in 1×10^{-4} M β-estradiol. The relative standard deviation (R.S.D.) was calculated to be 0.99% with a standard deviation (S.D.) of 1.35×10^{-2} for peak current, and 0.87% with a S.D. of 4.08 for peak potential. The effect of pH was investigated in BR buffers at various scan rates (Fig. 2). At pH 2 two anodic peaks were observed at 0.82 V (peak I) and 0.95 V (peak II). When scan rate increased peak II shifted to more positive potentials and became broader, while the shift at peak I was not at this degree. On the reverse scan two cathodic steps were observed at 1.55 and 0.90 V which were clearly seen at the scan rates higher than 10 mV s^{-1}. When pH increased the first anodic peak (peak I) shifted to less positive potentials and peak II nearly disappeared. (Fig. 2(a–c)).

Repeatability of the curves obtained in the test solutions using the same stock revealed the stability of β-estradiol. The peak potential–pH relationship for peak I was found to be linear for a broad scan rate interval (Table 2). Best results were obtained in acidic solutions, regarding the repeatability. 0.05 M H_2SO_4 was chosen as supporting electrolyte. For peak I, peak current was found to be linearly dependent on the square root of scan rate. ($i_p = 3.73 \times 10^{-7} + 4.85 \times 10^{-5}\ v^{1/2}$, $r = 99.8\%$ for 4.10^{-4} M β-estradiol). This means that the reaction is diffusion controlled.

The logarithm of peak current ($\log i_p$)—logarithm of scan rate ($\log v$) relationship was also linear, $\log i_p = -6.32 + 0.49 \log v$, $r = 99.9\%$ for 10^{-4} M β-estradiol; for 6×10^{-4} M β-estradiol up to 200 mV s^{-1} $\log i_p = -5.36 + 0.43 \log v$ $r = 99.9\%$ and for 200–3000 mV s^{-1} $\log i_p = -6.73 + 0.96 \log v$ $r = 87.0\%$. These equations reveal that at lower concentrations and lower scan rates the reaction is diffusion controlled but when concentration and scan rate increase the effect of adsorption becomes clear. When logarithm of current at 0.98 V on the curves obtained in 0.05 M H_2SO_4 solution having β-estradiol in the concentration range of 4×10^{-5}–10^{-3} M was plotted against logarithm of concentration, a line was obtained ($\log i = -2.05 + 0.95 \log C$).

Tafel lines were drawn with a scan rate of 10 mV s^{-1} beginning from a steady-state potential and from the slope, $n\alpha$ was calculated to be 0.5. From these kinetic parameters the reaction seems to be first order according to β-estradiol and $[H^+]$

and proceeds with one electron transfer. A mechanism in accordance with these data can be as follows:



Phenoxyl radical is rather stable in acidic media as seen in Fig. 2a for the curve of pH 2, the peak at 0.95 V may due to further oxidation of this radical. At higher pH the radicals form dimers. This mechanism is in accordance with the literature [13–15]. In Fig. 3(a) differential pulse voltammograms, which were obtained in BR solutions are given. Sharp peak at 0.80 V in pH 2 buffer shifted to less positive values as pH increased. In Fig. 3(b) differential pulse voltammograms obtained in 0.05 M H_2SO_4 solutions having different concentration of β-estradiol are seen. Evaluation of these curves revealed that quantitative determination of β-estradiol could be made by DPV and the optimum conditions were found as methanol/water 9:1 (v/v) having 0.05 M H_2SO_4, 20 mV s^{-1} scan rate, 50 mV pulse amplitude, 17 ms sample width 50 ms pulse width and 200 ms pulse period. Under these conditions peak current of DPV curves are linearly dependent on concentration. Statistical treatment of this dependence is given in Table 3. The detection limit was found as 1.21×10^{-5} M. Reproducibility for DPV peak current and peak potentials was tested by repeating ten experiments in 10^{-4} M β-estradiol. The R.S.D. was calculated to be 0.99% with a S.D. of 1.48×10^{-2} for peak current, and 0.89% with a S.D. of 4.98 for peak potential.

The applicability of the LCV and DPV methods for the assay of a simple dosage form was examined by analysis of a tablet form and a TTS. The results confirm the suitability of the proposed method for the accurate and sensitive analysis of β-estradiol. The LCV and DPV results were compared with those of official high performance liquid chromatographic (HPLC) methods [16] by means of Student's *t*-test at 95% confidence level and no significant difference was found between them (Table 4).

It was also shown that the DPV method could be applied for the determination of β-estradiol in human serum. The accuracy and sensitivity of the method was tested by means of recovery test (Table 5). At the peak potential no interference related to conjugates was observed. Although the quantitation limit seemed to be high for the determination of natural β-estradiol in serum with a simple GCE, researches in our laboratory have been continued for the development a more sensitive sensor.

In conclusion the proposed CV and DPV methods have the advantages of being rapid, simple, directly applicable to tablet and TTS form of β-estradiol and inexpensive when compared with official HPLC method and applicable to serum.

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