

## PREPARATION OF A POLYURETHANE MEMBRANE TESTOSTERONE SENSOR AND ITS APPLICATION USING SQUARE-WAVE STRIPPING VOLTAMMETRY

Cemre Zeynep Harman<sup>1</sup>, Öznur Güngör<sup>2\*</sup>

<sup>1</sup>Inonu University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, 44280, Malatya, Türkiye

<sup>2</sup>Inonu University, Faculty of Arts and Sciences, Department of Chemistry, 44280 Malatya, Türkiye  
oznur.gungor@inonu.edu.tr

A novel electrochemical sensor for testosterone detection has been prepared by the chemical modification of a gold electrode (AuE). For the electrode modification, specific polyurethane (PU) films were synthesized from hexamethylene diisocyanate, olivetol, polyethylene glycol-100 (PEG100) and  $\beta$ -cyclodextrin. The synthesized PUs were investigated as selective films, and were used to coat a AuE surface at different concentrations and thicknesses. The testosterone responses of the modified electrodes were investigated by square-wave voltammetry (SWV). One separated cathodic SWV peak was obtained for testosterone at  $-0.390$  V, with the prepared PU-modified AuE in  $0.1$  M phosphate buffer (PB) (pH 7.2). The linearity of testosterone responses of the prepared PU modified electrode was obtained over a concentration range of  $0.1$ – $1.0$   $\mu\text{M}$  ( $R^2 = 0.995$ ). It was observed that the response of the electrode increased regularly and sensitively with increasing testosterone amount. The detection limit, relative standard deviation and sensitivity of modified electrode were found to be approximately  $5.69$  nM,  $1.669$  % and  $98.331$  %, respectively. The PU-modified AuE exhibited good selectivity and a low response time for testosterone. Therefore, the prepared testosterone sensor offers a good alternative for fast and practical testosterone determination in clinical or biomedical studies.

**Keywords:** testosterone; sensor; polyurethane membranes; square-wave voltammetry

### ПОДГОТОВКА НА ПОЛИУРЕТАНСКА МЕМБРАНА ЗА ДИЗАЈНИРАЊЕ СЕНЗОР ЗА ДЕТЕКЦИЈА НА ТЕСТОСТЕРОН И НЕГОВА АПЛИКАЦИЈА СО ПРИМЕНА НА КВАДРАТНО-БРАНОВА ВОЛТАМЕТРИЈА

Во рамките на овој труд, е развиен нов електрохемиски сензор за детекција на тестостерон, дизајниран со хемиска модификација на златна работна електрода. За модификацијата на работната електрода беа користени специфични филмови од полиуретан, синтетизирани од хексаметилен диизоцијанат, оливетол, полиетилен гликол-100 и бета-циклодекстрин. Синтетизираните полиуретански филмови беа употребени како селективни филмови и беа аплицирани во различни концентрации и дебелини за модификација на златната работна електрода. Електрохемиските својства на тестостерон на модификуваните електроди беа испитувани со употреба на квадратно-бранова волтаметрија. Притоа, на модификуваната златна електрода, во фосфатен пуфер ( $0,1$  mol/l) со pH = 7,2, беше добиен катаден пик од електрохемиската активност на тестостерон на потенцијал од  $-0,390$  V. Линеарна зависност помеѓу измерените струи на пиковите во квадратно-бранова волтаметрија и концентрацијата на тестостерон беше потврдена во концентрациското подрачје на тестостерон од  $0,1$ – $1,0$   $\mu\text{mol/l}$  ( $R^2 = 0,995$ ). Границата на детекција, релативната стандардна девијација и осетливоста на методата беа  $5,69$  nmol/l,  $1,669$  % и  $98,331$  %, соодветно. Златната електрода, модификувана со филм од полиуретан покажа добра селективност и кратко време на одговор при детекцијата на тестостерон. Соодветно на овие параметри, овој амперометриски сензор за определување

тестостерон може да се смета како добра алтернатива за брзо и евтино определување на тестостерон во клинички и биохемиски студии.

**Клучни зборови:** тестостерон; амперометриски сензор; полиуретански мембрани; квадратно-бранова волтаметрија

## 1. INTRODUCTION

Testosterone is an important hormone from the group of androgens known as the male sex hormones. In men, testosterone is primarily secreted in the testicles, along with a small amount in the adrenal glands. The organ responsible for the secretion of such hormones is the hypothalamus, which is located in the brain. Testosterone is also secreted in the ovaries of women, but in much smaller amounts than in men. It is this hormone that is responsible for physical developments such as voice deepening, hair growth, and the growth and function of the genitals in men.<sup>1</sup>

Since a significant proportion of testosterone is produced in the testicles, problems that may occur in the testicles may also affect hormone release. Problems in the hypothalamus and pituitary gland responsible for hormone production, congenital or genetic insufficient secretion of the testosterone hormone, excessive secretion of cortisol hormone secreted due to advanced age, stress, alcohol and smoking, metabolic disorders can cause insufficient sleep and decreased sleep.<sup>2</sup>

Excessive testosterone levels in the body can also cause problems. For example, this can cause unequal and excessive growth in the distribution of muscles in the body, as well as edema, increased hair growth, enlarged prostate, vascular stiffness, diabetes<sup>3,4</sup> and osteoporosis.<sup>5</sup> It may also cause increased levels of low-density lipoprotein cholesterol (LDLc), otherwise known as “bad cholesterol”.<sup>3</sup> A low testosterone level is associated with high-grade prostate cancer<sup>6</sup> and there is also a risk factor of mortality and death by cardiovascular disease.<sup>7,8</sup> These symptoms may indicate whether testosterone levels are low or high; nevertheless, it is necessary to analyze the hormone values in the blood to verify hormone levels. Thus, testosterone is an important biomarker that is used in diagnosis and treatment in the medical field.<sup>9,10</sup> Therefore, the detection and analysis of testosterone is clinically important. In clinical testosterone analysis, liquid chromatography-tandem mass spectrometry (LC-MS/MS),<sup>11,12</sup> high performance liquid chromatography-mass spectrometry (HPLC-MS),<sup>13</sup> gas chromatography-mass spectrometry (GC-MS),<sup>14</sup> ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-

MS/MS),<sup>15,16</sup> near-infrared (NIR) spectroscopy<sup>17</sup>, thin-layer chromatography (TLC)<sup>18</sup>, ultra-performance liquid chromatography-ion mobility-mass spectrometry (UPLC-IM/MS)<sup>19</sup> and liquid chromatography with UV detector<sup>20-22</sup> are generally used. These techniques are expensive, requiring expert personnel and intensive sample preparation processes. For this reason, new analysis techniques are required that can reduce appointment times, reduce the cost of analysis and perform the analysis in a shorter time. Electrochemical measurements and sensors stand out as alternative methods.<sup>23-28</sup> Some electrochemical testosterone sensor studies have been reported in the literature.<sup>29-34</sup> Levent *et al.*<sup>29</sup> investigated the redox behavior of testosterone on a glassy carbon electrode surface in the absence and presence of cationic surfactants, using the adsorptive stripping voltammetry (AdSV) technique. Liu *et al.*<sup>30</sup> used molecularly imprinted polymer/graphene oxide (MIP/GO) for the determination of testosterone. A new electrochemical sensor was developed by Moura *et al.*<sup>31</sup>, using a cobalt oxide-modified edge-plane glassy carbon electrode for *in vitro* testosterone detection. Goyal *et al.*<sup>32</sup> performed voltammetric analysis of two corticoid isomers (testosterone and epitestosterone). For detection, bare and single-walled carbon nanotube (SWNT)-modified edge plane pyrolytic graphite electrodes (EPGE) were used. Heidarimoghadam *et al.*<sup>33</sup> studied the electro-reduction behavior of testosterone at reduced graphene oxide/glassy carbon electrodes (rGO/GCE). The cationic surfactant, cetyltrimethylammonium bromide (CTAB), enhanced the reduction peak of testosterone. In another study<sup>34</sup>, the simultaneous detection of estradiol and testosterone was performed using a glassy carbon electrode/NiFe<sub>2</sub>O<sub>4</sub>-mesoporous carbon (GCE/NiFe<sub>2</sub>O<sub>4</sub>-MC) electrode.

Within the scope of this study, polyurethane (PU)-based sensors carrying  $\beta$ -cyclodextrin and olivetol groups have been developed to increase measurement sensitivity and selectivity.  $\beta$ -Cyclodextrin groups were used to increase testosterone selectivity by host-guest interaction, and olivetol groups were used to accumulate testosterone on the electrode surface. The aim of the study was to obtain a testosterone hormone sensor. For this purpose, PUs have been synthesized in order to allow testosterone to collect on a gold

electrode (AuE) surface. The synthesized PU structures were characterized by Fourier transform-infrared spectroscopy (FT-IR) and elemental analysis techniques. While the surface properties of these PU structures were determined by scanning electron microscopy (SEM) technique, their thermal properties were examined by thermogravimetric analysis (TGA), differential thermal analysis (DTA) and differential scanning calorimeter (DSC) techniques. Bare AuE surfaces were covered with the PU structures by chemical modification.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

All chemicals used in the synthesis of PU were supplied by the Sigma-Aldrich Chemical Company. VIRIGEN TESTOCAPS® (30 capsule) drug was purchased at the pharmacy. Four different supporting electrolytes citrate buffer (CB, 0.1 M, pH 7.0), acetate buffer (AcB, 0.1 M, pH 7.0), Britton-Robinson buffer (BRB, 0.1 M, pH 7.0) and PB (0.1 M, pH 7.2) were used. Cationic hexadecyltrimethylammonium bromide (HDTMA-Br) was used as one of the surfactants. All stock solutions were preserved at 4 °C when not in use and were protected from sunlight during laboratory use. Aqueous solutions were prepared with deionized water, which was further purified with a Millipore brand Elix 20.

### 2.2. Devices and equipment used in measurement

FT-IR (Mattson 1000) in the interval range of 400–4000  $\text{cm}^{-1}$  interval was used employing KBr discs to determine the polymer film structure used for the surface modification of the glassy carbon electrode. The thermal behaviors and stabilities of the synthesized PUs were determined under an air atmosphere using TGA-50 (Shimadzu, Japan) and DTA-50 (Shimadzu, Japan), with a temperature increase of 10 °C/min over a temperature range of 20–900 °C. DSC measurements were performed on a DSC-60 (Shimadzu, Japan). All samples (5 mg) were placed in sealed aluminium containers before being heated under nitrogen flow (25 ml/min) at a scanning rate of 10 °C/minute.

The surface morphologies of the prepared PU membranes were investigated using the Leo-Evo 40xVP SEM device at 20 kV. For SEM measurements, the synthesized PU were coated on the glass surface and covered with a 100 Å gold-palladium layer by a spray coater (Bal-Tec SCB 050). The surface structures of the olivetol-based

PU structures were also examined with an optical microscope. A Soif-Upright metallurgical microscope was used for these analyses.

A Thermo Scientific STAR A-111 pH-meter was used for pH measurements. Calibration of the pH meter before each experiment was carried out with pH 7.0 (Merck 4939) and pH 4.0 (Merck 9475) buffer solutions. During the complete cleaning of the electrodes and the preparation of the solutions, a Branson 3510 model ultrasonic bath was used. Both the cells and other glass materials were first cleaned with detergent and rinsed with Millipore brand Elix 20 distilled water. Glass cells used in electrochemical analyses were kept in 6 M  $\text{HNO}_3$  solution for at least one hour, preferably overnight, then rinsed in distilled water and dried in an oven at 100 °C.

Before the electrochemical analysis, the surface of the working electrodes was cleaned by washing with distilled water. The pre-treatment depends on both the type of electrode and the composition of the test solution. To ensure the repeatability of the electrochemical coating on the AuE surface, the same surface form must be created before each experiment. Before each experiment, the AuE was cleaned mechanically. Mechanical cleaning was carried out first by cleaning with an aqueous alumina paste with a particle size of 1  $\mu\text{m}$ , 0.3  $\mu\text{m}$  and 0.05  $\mu\text{m}$ , and then sonicating in an ultrasonic bath for 1 min. The AuE was then washed with ultrapure water and dried under optimal conditions. The water used for the preparation and dilution of solutions was obtained from the Millipore brand Elix 20 model water system.

Electrochemical experiments (SWV measurements) were performed in room conditions using a potentiostat (Vertex one) controlled by a computer with appropriate software (Ivium soft) for data analysis. In addition, an electrochemical C2 cell cage of the BASi company was used, which was placed inside the cell stand to provide isolation from external electrical and magnetic effects.

Square-wave stripping voltammetry (SWSV) was used to develop an electroanalytical methodology for detecting testosterone in real samples. The optimized operating parameters for SWSV were as follows: Start potential 300 mV, end potential –1100 mV, accumulation time 20 sec, mixing speed 350 rpm, waiting time 20 sec, frequency 80 Hz. In a 10-ml single chamber voltammetric cell, a three-electrode system was used, with a platinum wire electrode (MW-1032) as the auxiliary electrode, Ag/AgCl (BASi RE-5B) in 3 M KCl as the reference electrode, and both bare (MF 2014) and polymer-modified AuE as the working electrode.

### 2.3. Preparation of testosterone stock solution

A capsule of VIRIGEN TESTOCAPS® (30 capsule) labeled as containing 40 mg testosterone undecanoate (an ester of testosterone, corresponding to 25.3 mg of pure testosterone), castor oil and propylene glycol monolaurate as excipients were used for the present analytical applications. Because of its moderate lipophilicity, the stock solution of testosterone (1.46 mM) was prepared in acetonitrile (AcN). On the day of the experiment, working solutions were prepared by diluting the stock solution with the selected supporting electrolyte.

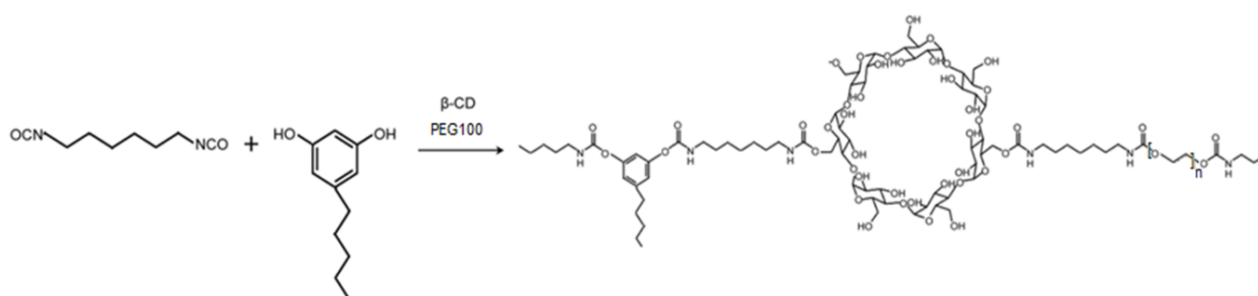
### 2.4. Synthetic urine preparation

Synthetic urine was prepared by dissolving the following in deionized water: 6.25 g of urea, 0.40 g of KCl, 0.56 g of Na<sub>2</sub>SO<sub>4</sub>, 0.25 g of NH<sub>4</sub>Cl, 0.35 g of KH<sub>2</sub>PO<sub>4</sub>, 0.28 g of CaCl<sub>2</sub>·2H<sub>2</sub>O and 0.73 g of NaCl.<sup>34</sup>

### 2.5. Olivetol-based polyurethane synthesis

Within the scope of the study, hexamethylene diisocyanate, olivetol, polyethylene glycol-100 (PEG100) and  $\beta$ -cyclodextrin were used in the synthesis of the PUs that were to be used in the modification of the electrodes. The monomer ratios and the PU structures synthesized within the scope of the study are displayed in Scheme 1.

The olivetol-based PU structures were synthesized with olivetol:PEG100: $\beta$ -cyclodextrin ratios of 0:80:20 (PU-1), 5:80:15 (PU-2), 10:80:10 (PU-3) and 15:80:5 (PU-4) mixtures of polyol in the solution medium of aliphatic diisocyanate (in THF or THF:DMF (9:1)). These structures were synthesized by refluxing at 90 °C for 12 hours. Following the reactions, the solvent was removed under vacuum from the synthesized PUs. Structural characterizations were carried out by FT-IR techniques. Thermal analyses of the polymers were provided by TGA and DSC analyses.



Polymer	Olivetol	PEG-100	$\beta$ -cyclodextrin
PU-1	0	80	20
PU-2	5	80	15
PU-3	10	80	10
PU-4	15	80	5

**Scheme 1.** The monomer ratios and PU structures

### 2.6. Modification of electrodes

For the preparation of testosterone sensors, PU was synthesized containing olivetol brushes and  $\beta$ -cyclodextrin units. The synthesized PU (0.1 g) was dissolved 1 ml of 1-methyl-2-pyrrolidone (NMP). A certain amount (2  $\mu$ l, 3  $\mu$ l, 4  $\mu$ l, or 5  $\mu$ l) of this solution was dried on the bare electrode surface for 3 hours at room temperature.

The platinum electrodes used as the auxiliary electrodes were cleaned from time to time on a bare flame, or by submerging in an ultrasonic bath of 3 M HNO<sub>3</sub> solution for 1–2 min and then washing with distilled water. After each analysis, the reference electrodes were washed with distilled water and then stored in 3 M KCl solution.

### 3. RESULTS AND DISCUSSION

#### 3.1. Characterization of olivetol-based polyurethane structures to be used in testosterone determination

Within the scope of the study, PU structures were obtained using  $\beta$ -cyclodextrin, olivetol, PEG100 and hexamethylene diisocyanate as mon-

omers. The PU structures obtained have high stability, and are easy to apply and dry. Due to its high adhesion properties, its modification to the surface is stable and durable. After the modified electrode was structurally characterized, electrochemical experiments were performed. Structural characterization processes were carried out primarily by FT-IR analysis.

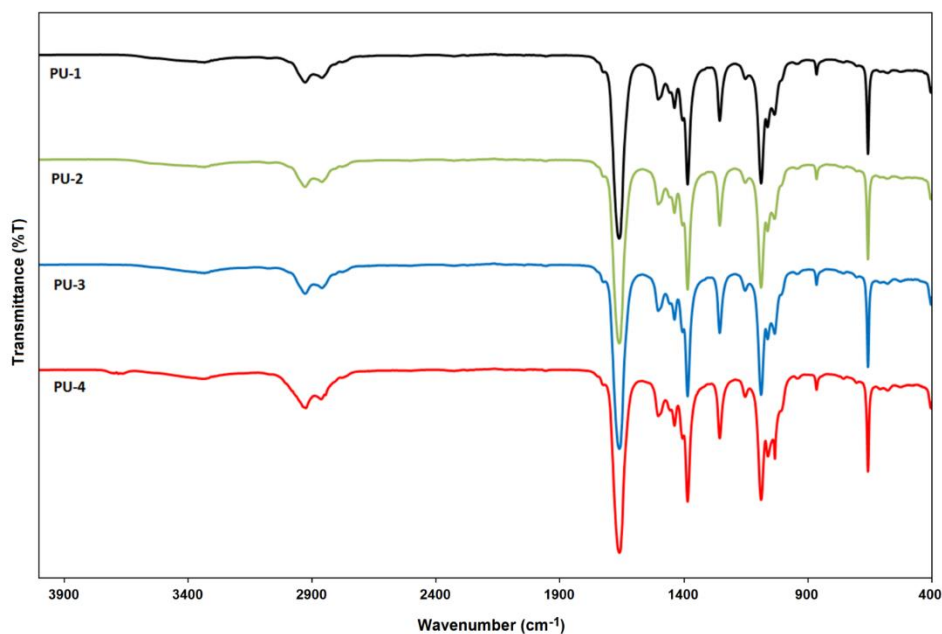


Fig. 1. FTIR spectra of olivetol-based PUs

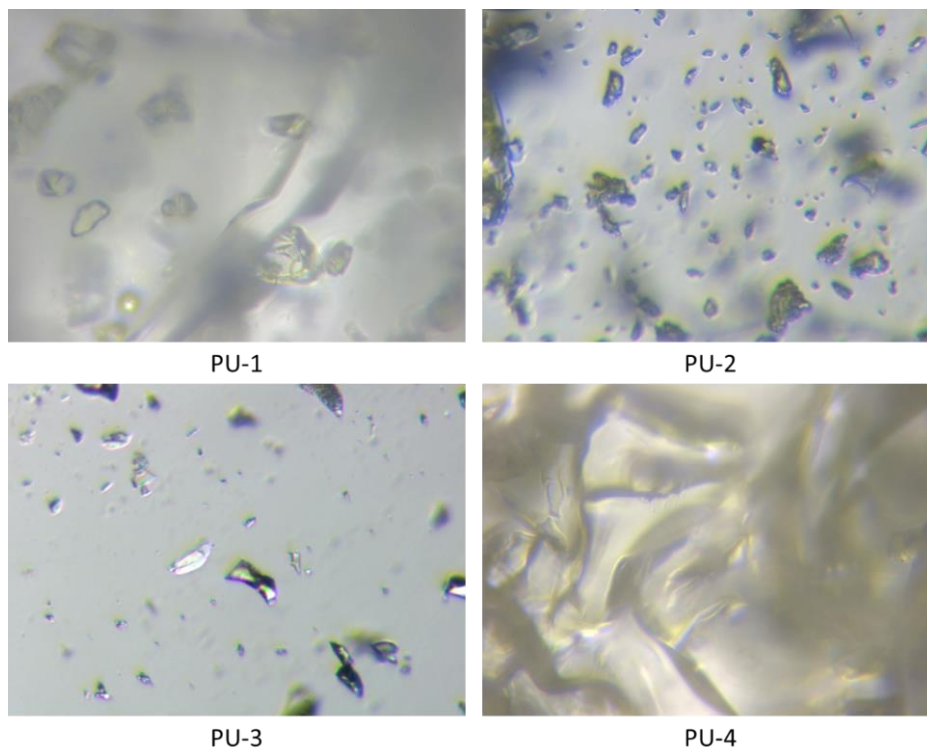


Fig. 2. Optical microscope images of olivetol-based PU membranes



Figure 1 shows the FT-IR analysis of the PU structures. In these analyses, the basic peaks of the classical PU structure can be clearly observed. First, a hydrogen bonding structure peak was observed in the region of  $3650\text{--}3100\text{ cm}^{-1}$  and an aliphatic C–H stretching vibration was observed in the region of  $2850\text{--}2950\text{ cm}^{-1}$ . A C–O stress vibration was observed around  $1700\text{ cm}^{-1}$ , while a C–C stress vibration was observed around  $1500\text{ cm}^{-1}$ . A C–O–C stress vibration at  $1100\text{ cm}^{-1}$  was clearly visible, which is associated with the  $\beta$ -cyclodextrin units connected to the structure. The final important peak in the PU structure was at  $825\text{ cm}^{-1}$ , which corresponds to the PU aromatic C–H stretching vibration. Overall, the C=O, C–N, C–O–C, C–N–H peaks and aliphatic C–H peaks seen in Figure 1 confirm the PU structure.

Figure 2 shows the optical microscope images of these structures. From these images it was determined that the PUs exist as transparent, stable

and homogeneous films. Furthermore, no cracks and fractures were observed in the polymeric film structures. More detailed morphologies were confirmed by SEM analysis.

In the SEM analysis presented in Figure 3, homogeneous and non-fractal film structures can be observed, which are evenly distributed on the surface. The homogeneity of the film structures reveals that the synthesized PUs are suitable for electrode modification. Thus, PU structures were coated on electrode surfaces and used in electrode surface modifications. Following these modifications, the morphology of the film structure on the surface was probed by SEM analysis, where it could be observed that the smooth electrode surface increased the surface area, which became more caved and rough, following the application of the polymers. This increase in the area of the electrode surface can be clearly observed at 2500-fold magnification.

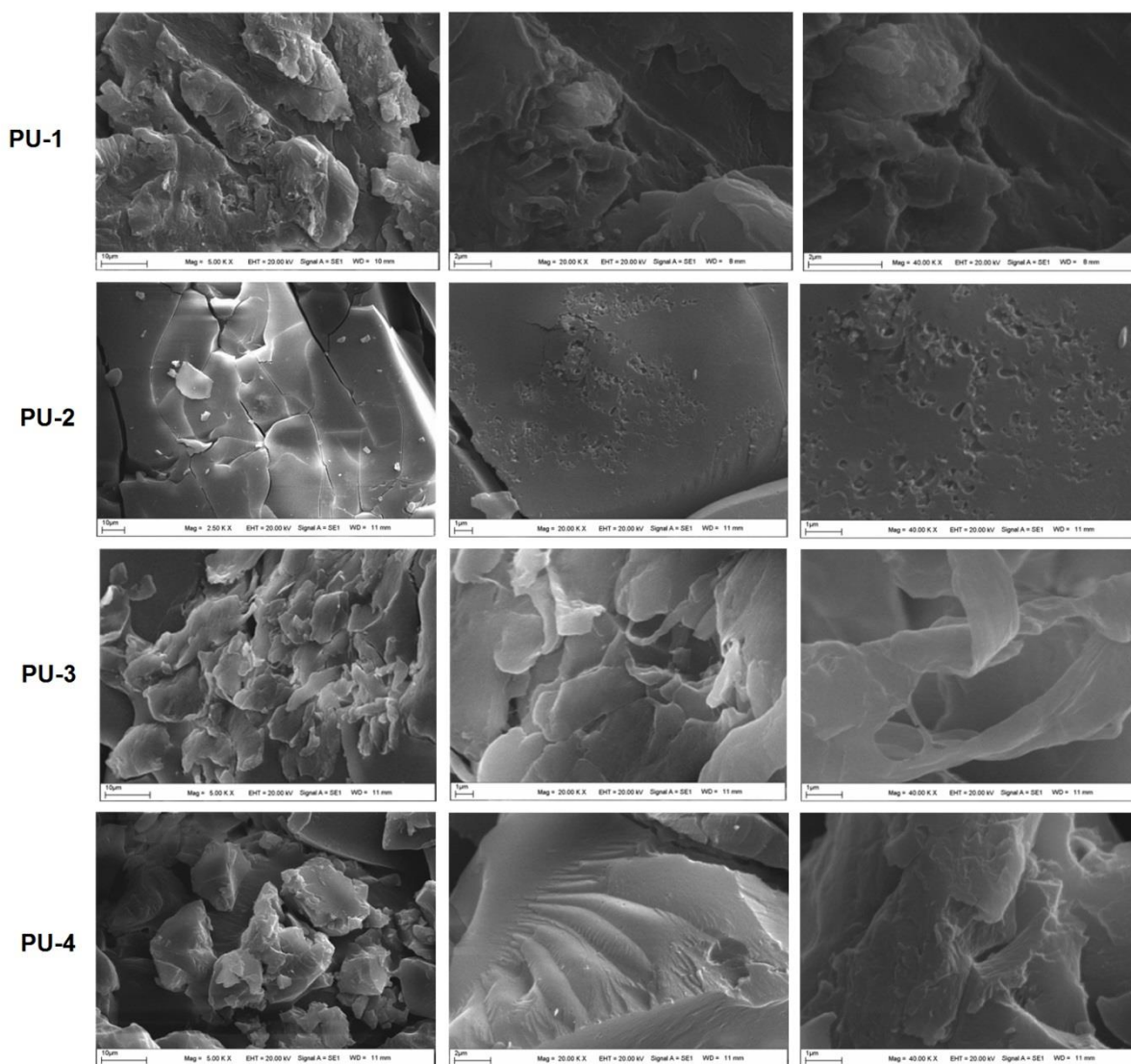


Fig. 3. SEM images of olivetol-based PU structures (20 KV)

The TGA thermograms of olivetol-based polymeric films are shown in Figure 4. These thermograms showed two major mass losses. The first weight loss (200–365 °C) resulted from the degradation of soft segments in the PU structure. The second weight loss (370–590 °C) was caused by the degradation of hard segments and the thermo-oxidative degradation of the polymeric structure. All of these findings were consistent with the DTA thermograms shown in Figure 5. In general, three main exothermic peaks were observed in the DTA thermograms of the obtained olivetol-based PUs. The first exotherm, in the range of 240–340 °C, derives from the degradation of the PEG units of the

main polymer chain. The second exotherm, in the range of 340–460 °C, originated from the degradation of the cyclodextrin units of the PU structure. The third exotherm peak, observed at 470–630 °C, is due to the degradation of the olivetol units, which contain aromatic units and are more stable than the other units. According to the DTA thermograms, the four different polymer structures showed similar degradation properties, and the onset of the thermal degradation of all of the PUs started at ~240 °C. Therefore, it can be concluded that the thermal stability of the PU structures is also around 240°C.

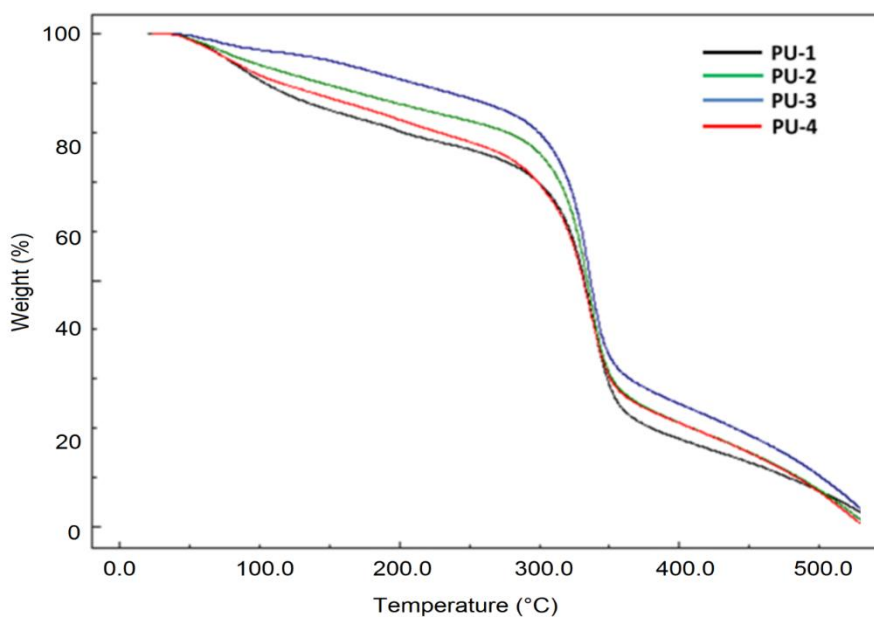


Fig. 4. TGA thermograms of olivetol-based PUs

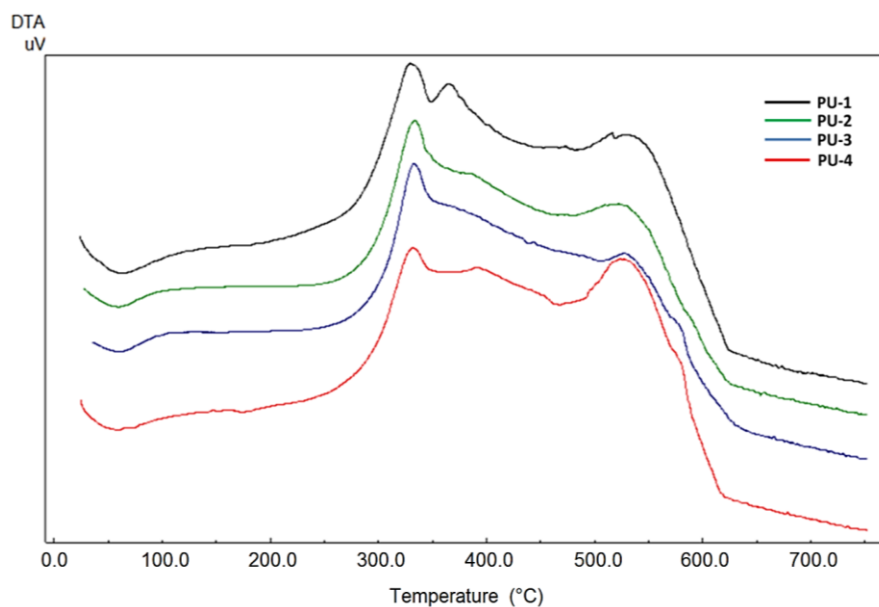


Fig. 5. DTA thermograms of olivetol-based PUs

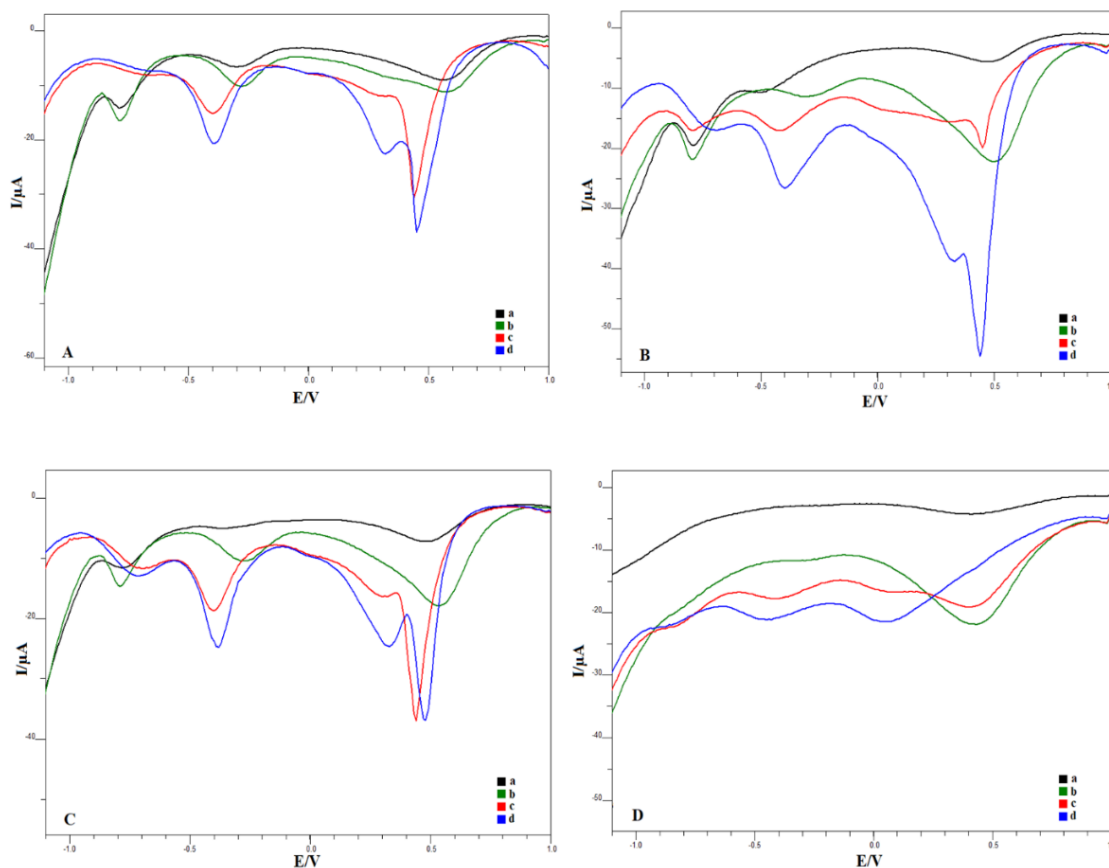
### 3.2. Electrochemical measurements

The operating parameters for SWV were as follows: Start potential 0 mV, end potential  $-1100$  mV, pulse amplitude 10 mV, frequency 80 Hz, equilibration time 2 s, and current range 100  $\mu$ A. As a result of voltammograms recorded under these parameters, PB (0.1 M, pH 7.2) was selected as the most suitable electrolyte. After selection of the electrolyte, 4  $\mu$ l of the four different olivetol-added polymers (PU-1, PU-2, PU-3 and PU-4) were deposited on the AuE, as shown in Figures 6(A), 6(B), 6(C) and 6(D). Voltammograms were obtained using two concentrations of testosterone, in backgrounds and with modified electrodes, and in the presence and absence of the cationic surfactant, HDTMA-Br. Surfactants are substances with both hydrophilic and lipophilic groups, which can be arranged in the direction of the surface of the solution, causing the surface tension to be significantly reduced. They facilitate emulsification by lowering the surface tension of their environment, allowing the control of foam formation. Thus, they play a very important role not only in dis-

solving organic compounds, but also in the field of surface modified electrodes. By using surfactants in electroanalytical studies, an increase in the sensitivity and the selectivity of the measurements is obtained.<sup>35</sup>

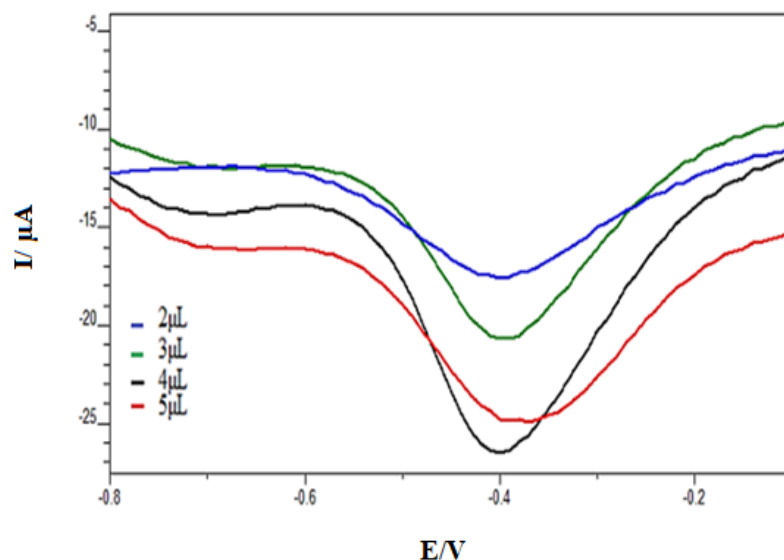
Electrolyte background voltammograms were first recorded with electrodes modified by PU-1, PU-2, PU-3 and PU-4. Subsequently, the appropriate testosterone solution (0.025 or 0.050  $\mu$ M) was added to the medium, followed by surfactant, and their voltammograms were taken separately. As shown in Figure 6, the peak current of the PU-3-doped polymer was higher than the others, and therefore PU-3 was used to conduct the rest of the study. To obtain the optimal thickness of the PU-3 polymer, various amounts (2  $\mu$ l, 3  $\mu$ l, 4  $\mu$ l and 5  $\mu$ l) of PU-3 were deposited on the AuE, which was then dried at room temperature.

According to the voltammograms shown in Figure 7, the polymer was thickest when 4  $\mu$ l of PU-3 was deposited, thus it was deemed appropriate to use the electrode modified in this way for the remainder of the study.



**Fig. 6.** A) PU-1, B) PU-2, C) PU-3, and D) PU-4-modified voltammograms obtained with AuE (a: Background; b: 0.025  $\mu$ M testosterone; c: 0.025  $\mu$ M testosterone + 3 mM HDTMA-Br; d: 0.050  $\mu$ M testosterone + 3 mM HDTMA-Br.)





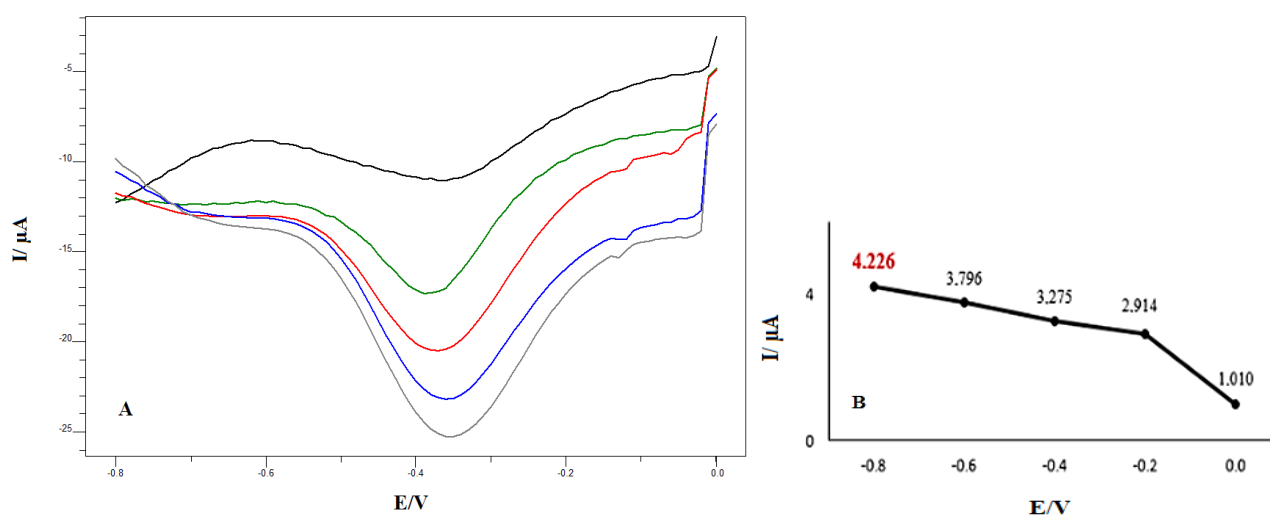
**Fig. 7.** Voltammograms of the modified electrode obtained by depositing four different volumes of PU-3 on the AuE surface with 0.050  $\mu\text{M}$  testosterone and 3 mM HDTMA-Br additive

Optimization of SWV parameters that might affect the current response of the analyte is an important step in developing the voltammetric procedure. For this reason, parameters such as the deposition potential, stripping potential, deposition time and pulse amplitude were optimized during this study. The SWV method was used in parts of the study up to this stage. From this stage onwards, the SWSV method, which is the same as the SWV method, was used to examine the effect of the accumulation potential, the stripping potential and the accumulation time (except the accumulation step, which is an extension of the SWV method).

In order to investigate the effect of the accumulation potential measurements were carried out by stripping at 10 mV potential, after accumulating

for 10 seconds at 0.0, -0.2, -0.4, -0.6 and -0.8 V potentials. The voltammograms of the measurements made are shown in Figure 8 (A) and the graphic expressing the measurement results is shown in Figure 8 (B). As can be seen in the said voltammograms and graph, the deposition process has a positive effect on the electrochemical behaviour of testosterone. Deposition at a potential of -0.8 V provided the optimal testosterone response.

Testosterone (0.050  $\mu\text{M}$ ) was accumulated for 10 seconds at -0.8 V potential on the modified AuE, before the effect of the stripping potential was investigated. The electrochemical behaviour of testosterone was investigated by stripping at 0.0, -0.1, -0.2, -0.3 and -0.4 V potentials. The optimal stripping potential was determined to be 0.0 V.



**Fig. 8.** The effect of accumulation potential on testosterone (0.050  $\mu\text{M}$ ) SWSV peak current, voltammograms (A), expressed graphically (B)

In order to determine the effect of the accumulation time, the accumulation process was performed on the modified AuE at  $-0.8$  V potential for 5, 10, 15, 20, 25 and 30 s, and then measurements were made by stripping at  $0.0$  V potential using the SWSV method. As can be understood from the examination of the voltammograms in Figure 9 (A), and also from the graph in Figure 9 (B) expressing the results, the optimal accumulation time was determined to be 20 s. The decrease in the peak current during further accumulation indicates that the amount of testosterone accumula-

tion on the electrode surface reached its limit value, thus exceeding its capacity.

SWSV measurements with pulse amplitudes in the range of 10–150 mV (10, 20, 30, 50, 75, 100, 125 and 150 mV) were performed to determine the effect of pulse amplitude on the electrochemical behaviour of the prepared modified electrode for testosterone ( $0.050 \mu\text{M}$ ). The SWSV peak current for testosterone increased up to 100 mV pulse amplitude and decreased after this value. For this reason, it was decided that optimal pulse amplitude value for testosterone analysis was 100 mV.

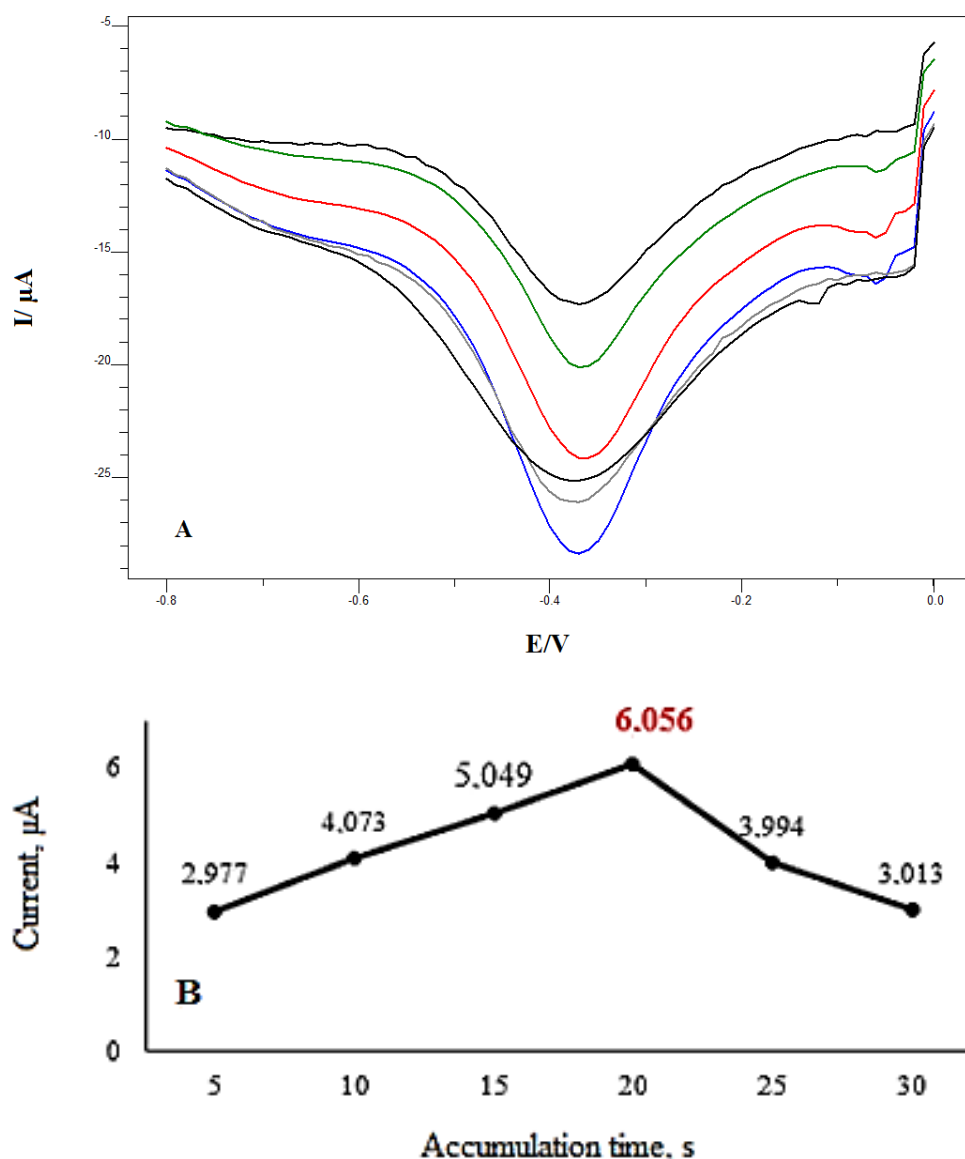


Fig. 9. The effect of accumulation time on testosterone ( $0.050 \mu\text{M}$ ) SWSV peak current, voltammograms (A), expressed graphically (B)

### 3.3. Stability, repeatability and sensitivity of the prepared electrode

The electrochemical behaviour of testosterone at 0.2  $\mu\text{M}$  concentration was investigated by the SWSV method using the modified AuE, prepared under the optimal conditions. The voltammetric responses obtained using ten different modified electrodes show very good reproducibility. Three consecutive measurements were made on

each modified electrode. The voltammograms obtained from the measurements are shown in Figure 10 (A). Using voltammograms and the data in the bar graph in Figure 10 (B), the standard deviation was calculated as 0.268 and the relative standard deviation as 1.669 %. In Figure 10 (B), each bar shows the result of three measurements. Considering these values, it was observed that the method is reproducible, stable and sensitive (98.331 %).

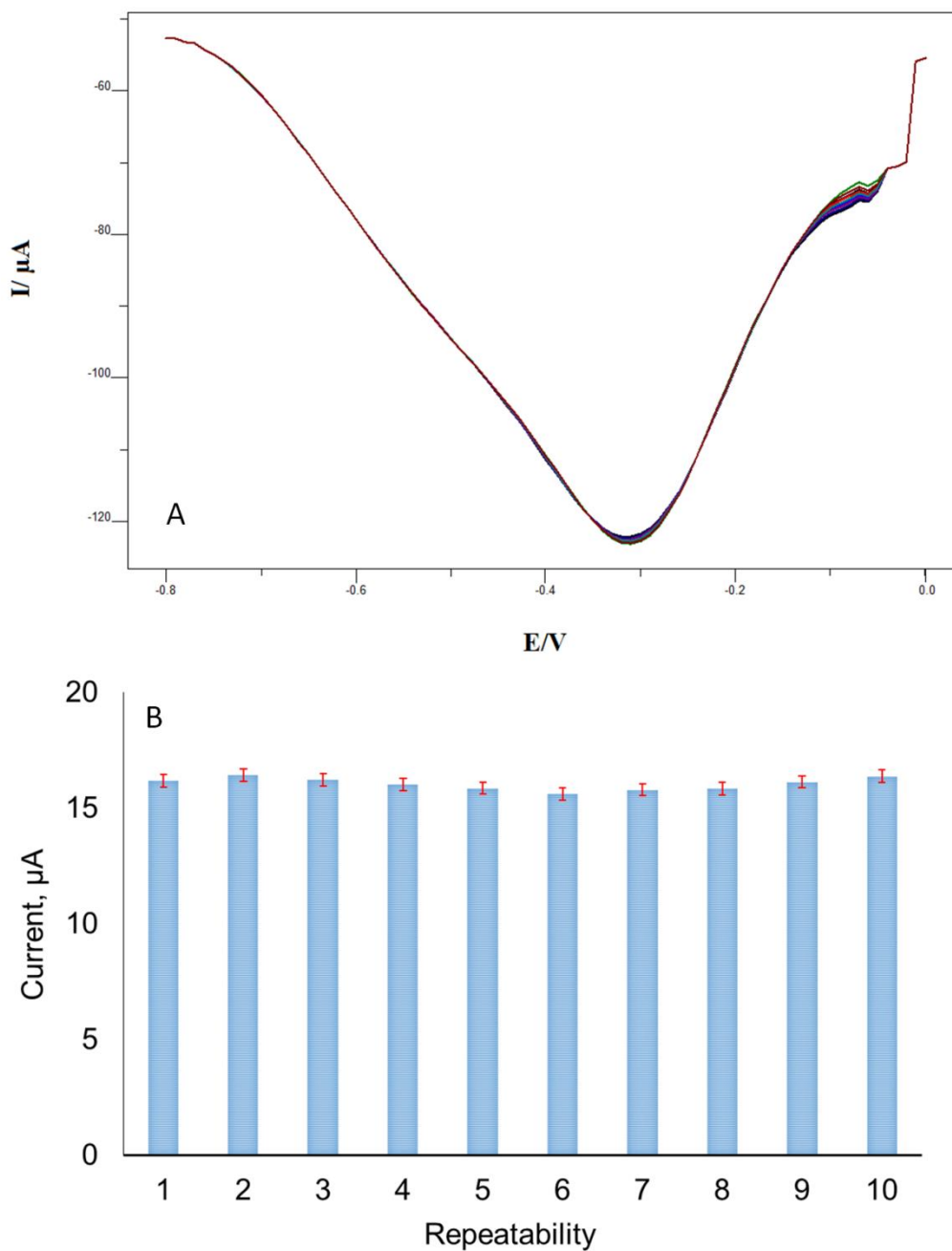


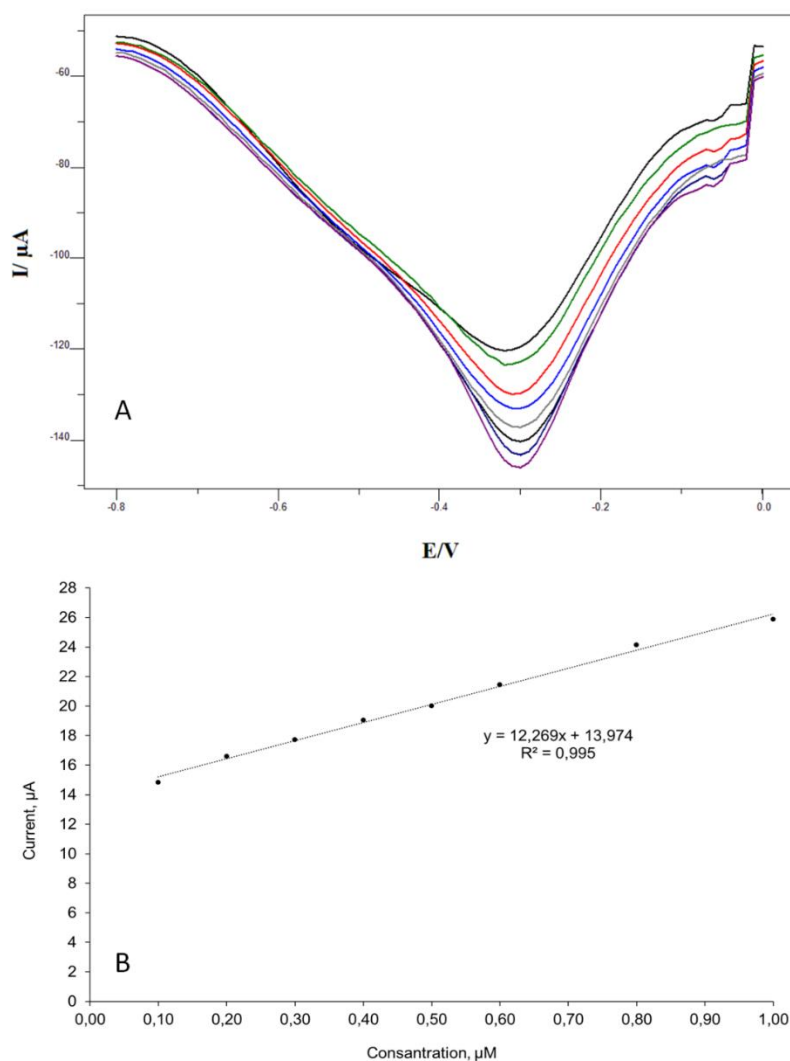
Fig. 10. Reproducibility of voltammograms (A) and testosterone results (B) of modified AuE

### 3.4. Calibration curve and statistical analysis

Voltammograms were obtained as a result of the measurements made by the SWSV method, in the testosterone concentration range of 0.1–1.0  $\mu\text{M}$  (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1.0  $\mu\text{M}$ ), under the optimal conditions displayed in Figure 11.

The correct equation for the calibration graph was calculated as  $y = 12.269x + 13.974$ , with

a linearity of  $R^2 = 0.995$ . Using the data obtained from the calibration graph, the LOD calculated according to the  $3s/m$  formula is 5.69 nM, and the LOQ calculated according to the  $10s/m$  formula is 19 nM. In these equations,  $s$  is the standard deviation of the oxidation peak currents, and  $m$  is the slope of the calibration graph.



**Fig. 11.** SWSV responses of 0.1–1.0  $\mu\text{M}$  testosterone on PU/AuE (A), calibration curve (B)

### 3.5. Testosterone sensing mechanism

Within the scope of the study, a modified electrode was developed for the detection of testosterone, which is an important hormone for the human body and its correct functioning. This electrode is designed for the dose control of testosterone-based drugs and for the detection of testosterone in bodily fluids, such as blood and urine. In particular, the electrode surface is covered with a polymeric film that is molecularly designed to ac-

cumulate testosterone molecules on the electrode surface and be sensitive to this molecule. For the electrode modification, the structure of the polymeric film is PU, which has high adhesion, selective permeability and chemical stability.<sup>24,36–38</sup> In the synthesis of the PU structure, a mixture of olivetol, PEG100 and cyclodextrin in different proportions was used as polyol monomer, in addition to aliphatic diisocyanate. In this design, the cyclodextrin structure provides sensitivity to testosterone molecules,<sup>39–41</sup> the olivetol structure pro-

vides the accumulation of testosterone molecules on the electrode surface with its hydrophobic structure,<sup>42</sup> and the PEG100 structure provides good film properties, high adhesion to the electrode surface, and high reproducibility.<sup>43,44</sup> The PU film structure synthesized with all these structural features provided the accurate and sensitive measurement of testosterone molecules in drug dose control. The testosterone molecules in the drug structure is usually the sole component and therefore there is no interferant effect, and only an accurate and sensitive measurement is needed. In bodily fluids, there are some steroids with similar molecular structures to testosterone.<sup>45</sup> Using the prepared PU-modified AuE in 0.1 M PB (pH 7.2) provided one separated cathodic SWV peak for testosterone at  $-0.390$  V. Among the steroids of similar structures, only testosterone responds at this potential value ( $-0.390$  V) and therefore there is no interferant effect in the readings. Overall, the developed modified AuE exhibited good selectivity and low response times for testosterone. Thus, this prepared modified electrode offers a good alternative for

fast and practical testosterone determination within pharmaceutical and medical applications.

### 3.6. Analysis of a real sample

The developed PU-modified AuE was used to determine the testosterone concentration in synthetic urine, using the standard addition method. First, a solution of synthetic urine was prepared. One ml of this prepared urine solution was taken and diluted to 5 ml with PB (pH 7.2) (1:4), before the pH of this prepared solution was adjusted to 7.2. After taking SWS voltammograms of each of the three serial solutions that were prepared in this way, 0.07 and 0.1  $\mu\text{M}$  testosterone was added, respectively, and measurements were performed using the SWSV method after each addition. The percentage yields of recovery were calculated (using the concentrations calculated from the measurements), and it can be seen in Table 1 that the recovery efficiency is 95.25 % following the addition of 0.07  $\mu\text{M}$  testosterone, and 98.72 % following the addition of 0.1  $\mu\text{M}$  testosterone.

Table 1

*Determination of testosterone concentration in synthetic urine samples (n = 3)*

Sample	Standard added ( $\mu\text{M}$ )	Total found ( $\mu\text{M}$ )	RSD (%)	R (%)
Synthetic urine	0.07	$0.0677 \pm 0.003$	4.47	95.25
Synthetic urine	0.1	$0.0987 \pm 0.001$	0.64	98.72

This sensor was compared with other sensors in the literature (Table 2). In this study, the prepared modified electrode provides accurate, sensitive, selective, repetitive and rapid testosterone quantification in drug dose control in the pharmaceutical field, and in the detection of the amount of testosterone present in bodily fluids. Therefore, it can be said that this study is compatible with the studies in the literature. A review of the literature indicated that this is the first use of a AuE in such a process.

Recently, some electrochemical sensor studies have been performed for the fast, sensitive and reproducible measurement of testosterone in the clinical and biomedical fields. In a study by Levent *et al.*<sup>29</sup>, the determination of testosterone in a surfactant environment with a voltammetric technique was performed on a glassy carbon electrode without any modifications, except for the addition of a cationic surfactant to the solution. Quantitative and sensitive detection of testosterone at a glassy carbon electrode (GCE) in the presence of CTAB was investigated using square-wave adsorptive stripping voltammetry (SW-AdSV). Liu *et al.*<sup>30</sup> have

determined testosterone levels at femtomolar to micromolar levels *via* electrochemical impedance spectroscopy (EIS) measurements. They developed a highly effective electrochemical biosensor based on a nanosized molecularly imprinted polymer (MIP) film on the surface of graphene-oxide sheets. For detecting testosterone, Moura *et al.*<sup>31</sup> studied an edge plane glassy carbon electrode, modified with cobalt oxide, by cyclic voltammetry (CV). An electron transfer mechanism of testosterone using a modified electrode with a cobalt oxide film was proposed, which suggested the formation of an anion-radical in the carbonyl group (C=O) of the steroid structure. In another study, Goyal *et al.*<sup>32</sup> have described an extremely sensitive electroanalytical procedure for the simultaneous determination of testosterone and epitestosterone levels based on osteryoung square-wave voltammetry utilizing SWNT-modified EPPGE. The detection of the testosterone at a reduced graphene oxide/glassy carbon electrode (rGO/GCE) in the presence of CTAB was investigated by the use of stripping voltammetry

by Heidarimoghadam *et al.*<sup>33</sup> The sensitivity of the rGO/GCE was demonstrated by the use of testosterone in both biological fluids and in testosterone drugs. These recent studies show the importance of testosterone as a biomarker and the need for sensors to be developed in this field. Thus, in this study, we have prepared a PU-modified AuE for the detection of testosterone with SWSV.

The most important advantages of this modified electrode are its easy manufacturability, short detection time, and accurate and reproducible measurement results. HDTMA-Br has been used as a cationic surfactant to increase the cathodic current signal of testosterone and increase the measurement sensitivity.

Table 2

*The comparison of the properties of the newly developed sensor with other sensors in the literature*

Classical methods	Electrode/Modifier sensors	Linear concentration range, M	LOD, M	Ref
SW-AdSV	GCE	$10 \times 10^{-9} - 70 \times 10^{-9}$	$1.18 \times 10^{-9}$	(26)
Cyclic voltammetry	MIP/GO	$1.0 \times 10^{-15} - 1.0 \times 10^{-6}$	$4.0 \times 10^{-16}$	(27)
Cyclic voltammetry	GCE/Cobalt oxide	$3.3 \times 10^{-7} - 2.0 \times 10^{-6}$	$1.6 \times 10^{-7}$	(28)
OSWV	SWNT/EPPGE	$5.0 \times 10^{-9} - 1000 \times 10^{-9}$	$2.8 - 4.1 \times 10^{-9}$	(29)
Stripping voltammetry	rGO/GCE	$2.0 \times 10^{-9} - 210 \times 10^{-9}$	$1.0 \times 10^{-10}$	(30)
Square wave stripping voltammetry	PU/AuE	$1.0 \times 10^{-7} - 1.0 \times 10^{-6}$	$5.69 \times 10^{-9}$	This study

**SW-AdSV:** square-wave adsorptive stripping voltammetry,

**MIP/GO:** molecularly imprinted polymer/graphene-oxide,

**OSWV:** osteryoung square-wave voltammetry,

**SWNT/EPPGE:** single-wall carbon nanotubes/edge plane pyrolytic graphite electrode,

**rGO/GCE:** reduced graphene oxide/glassy carbon electrode.

Today, testosterone hormone disorder is a very important problem. As a result, it is necessary to determine the correct testosterone level in patients suffering with this problem and then to apply the appropriate dose of treatment. Thus, it is necessary to measure testosterone levels with an easy and accurate method. For this reason, we have developed a sensor that can be used in the measurement of testosterone based on the adsorptive accumulation in different percentages of olivetol-added PU-modified AuE. For this purpose, four different PU membranes, comprised of different olivetol ratios, were investigated. Prepared PUs were characterized by FT-IR, SEM and thermal analysis techniques. Furthermore, PU films were formed by dropping a solution of the PU on a bare glassy carbon electrode, in order to prepare the testosterone selective electrode. The effects of the scan rate on the reduction peak of testosterone at the AuE were checked by SWSV in PB. The reduction peak shifted slightly toward more negative potentials as the scan rate increased a typical behavior of irreversible electrochemical reactions. Testosterone selectivity properties of PU membrane-based electrodes were examined by the SWSV technique.

#### 4. CONCLUSIONS

In this work, olivetol-based PU films were synthesized from  $\beta$ -cyclodextrin, olivetol, PEG100 and hexamethylene diisocyanate, using a solution

polymerization technique. These PU structures were characterized by FT-IR spectroscopy and thermal analysis techniques. The synthesized PUs exhibit high chemical resistance, good adhesion and flexibility. The PU films were formed by casting the film on the electrode surface. The surface structures and morphologies of the coated PU films were determined by SEM and optical microscope analysis. Furthermore, it was also examined whether these PU films could be used as a membrane for the voltammetric determination of testosterone. One important cathodic SWV peak was obtained for testosterone at  $-0.390$  V, when a prepared olivetol-based PU-modified AuE in  $0.1$  M PB (pH 7.2) was used. The linearity of the testosterone responses of the modified electrode was obtained over a concentration range of  $0.1-1.0$   $\mu$ M ( $R^2 = 0.995$ ). The detection limit, the relative standard deviation and the sensitivity of olivetol-based PU-modified electrode were found to be approximately  $5.69$  nM,  $1.669$  % and  $98.331$  %, respectively. The voltammetric results indicate that the PU-based electrodes can be used as a sensor for the rapid determination of testosterone with the good repeatability, sensitivity and selectivity.

**Acknowledgment:** This project was financially supported by TÜBİTAK — Directorate of Science Fellowships and Grant Programmes (BİDEB) (2209 A).



## REFERENCES

- (1) Monerris, M. J.; D'Eramo, F.; Arévalo, F. J.; Fernández, H.; Zon, M. A.; Molina, P. G., Electrochemical immunosensor based on gold nanoparticles deposited on a conductive polymer to determine estrone in water samples. *Microchemical Journal*, **2016**, *129*, 71–77. <https://doi.org/10.1016/j.microc.2016.06.001>
- (2) Bassil, N.; Alkaade, S.; Morley, J. E., The benefits and risks of testosterone replacement therapy: a review. *The Clin. Risk Manag.* **2009**, *5*, 427–448. <https://doi.org/10.2147/tcrm.s3025>
- (3) Wang, C.; Jackson, G.; Jones, T. H.; Matsumoto, A. M.; Nehra, A.; Perelman, M. A.; Swerdloff, R. S.; Traish, A.; Zitzmann, M.; Cunningham, G., Low testosterone associated with obesity and the metabolic syndrome contributes to sexual dysfunction and cardiovascular disease risk in men with type 2 diabetes. *Diabetes Care* **2011**, *34* (7), 1669–1675. <https://doi.org/10.2337/dc10-2339>
- (4) Selvin, E.; Feinleib, M.; Zhang, L.; Rohrmann, S.; Rifai, N.; Nelson, W. G.; Dobs, A.; Basaria, S.; Golden, S. H.; Platz, E. A., Androgens and diabetes in men: results from the Third National Health and Nutrition Examination Survey (NHANES III). *Diabetes Care* **2007**, *30* (2), 234–238. <https://doi.org/10.2337/dc06-1579>
- (5) Fink, H. A.; Ewing, S. K.; Ensrud, K. E.; Connor, E. B.; Taylor, B. C.; Cauley, J. A.; Orwoll, E. S., Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men. *J Clin Endocrinol Metab.* **2006**, *91* (10), 3908–3915. <https://doi.org/10.1210/jc.2006-0173>
- (6) Lane, B. R.; Stephenson, A. J.; Galluzzi, C. M.; Lakin, M. M.; Klein, E. A., Low testosterone and risk of biochemical recurrence and poorly differentiated prostate cancer at radical prostatectomy. *Urology* **2008**, *72* (6), 1240–1245. <https://doi.org/10.1016/j.urology.2008.06.001>
- (7) Rosano, G. M.; Cornoldi, A.; Fini, M., Effects of androgens on the cardiovascular system. *J Endocrinol Invest.* **2005**, *28* (3), 32–38. PMID: 16042358
- (8) Araujo, A. B.; Dixon, J. M.; Suarez, E. A.; Murad, M. H.; Guey, L. T.; Wittert, G. A., Endogenous testosterone and mortality in men: A systematic review and meta-analysis. *J Clin. Endocrinol. Metab.* **2011**, *96* (10), 3007–3019. <https://doi.org/10.1210/jc.2011-1137>
- (9) Herold, D. A. Fitzgerald, R. L., Immunoassays for testosterone in women: better than a guess?. *Clin Chem.* **2003**, *49* (8), 1250–1251. <https://doi.org/10.1373/49.8.1250>
- (10) Taieb, J.; Mathian, B.; Millot, F.; Patricot, M. C.; Mathieu, E.; Queyrel, N.; Lacroix, I.; Delpero, C. S.; Boudou, P., Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography-mass spectrometry in sera from 116 men, women, and children. *Clin Chem.*, **2003**, *49* (8), 1381–1395. <https://doi.org/10.1373/49.8.1381>
- (11) Turpeinen, U.; Linko, S.; Itkonen, O.; Hämäläinen, E., Determination of testosterone in serum by liquid chromatography-tandem mass spectrometry. *The Scandinavian Journal of Clinical & Laboratory Investigation*, **2008**, *68* (1), 50–57. <https://doi.org/10.1080/00365510701496496>
- (12) Wang, Y.; Gay, G. D.; Botelho, J. C.; Caudill, S. P.; Vesper, H. W., Total testosterone quantitative measurement in serum by LC-MS/MS. *Clin Chim Acta* **2014**, *436*, 263–267. <https://doi.org/10.1016/j.cca.2014.06.009>
- (13) Blasco, M.; Carriquiriborde, P.; Marino, D.; Ronco, A. E.; Somoza, G. M., A quantitative HPLC-MS method for the simultaneous determination of testosterone, 11-ketotestosterone and 11- $\beta$  hydroxyandrostenedione in fish serum. *J Chromatogr B* **2009**, *877* (14–15), 1509–1515. <https://doi.org/10.1016/j.jchromb.2009.03.028>
- (14) Vierhapper, H.; Nowotny, P.; Waldhäusl, W., Estimation by gas chromatography-mass spectrometry with selected ion monitoring of urinary excretion rates of 3 $\alpha$ -androstenediol during/after I.V. administration of 13-labelled testosterone in man. *Journal of Steroid Biochemistry* **1988**, *29* (1), 105–109. [https://doi.org/10.1016/0022-4731\(88\)90383-4](https://doi.org/10.1016/0022-4731(88)90383-4)
- (15) Perez, H. L.; Wang, S.; Szapacs, M. E.; Yang, E., Development of a highly sensitive and selective UPLC/MS/MS method for the simultaneous determination of testosterone and 5 $\alpha$ -dihydrotestosterone in human serum to support testosterone replacement therapy for hypogonadism. *Steroids* **2008**, *73* (6), 601–610. <https://doi.org/10.1016/j.steroids.2008.01.018>
- (16) Wang, G.; Hsieh, Y.; Cui, X.; Cheng, K. C.; Korfmacher, W. A., Ultra-performance liquid chromatography/tandem mass spectrometric determination of testosterone and its metabolites in in vitro samples. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2215–2221. <https://doi.org/10.1002/rcm.2580>
- (17) Fountain, W.; Dumstorf, K.; Lowell, A. E.; Lodder, R. A.; Mumper, R. J., Near-infrared spectroscopy for the determination of testosterone in thin-film composites. *Journal of Pharmaceutical and Biomedical Analysis* **2003**, *33* (2), 181–189. [https://doi.org/10.1016/S0731-7085\(03\)00345-5](https://doi.org/10.1016/S0731-7085(03)00345-5)
- (18) Ibayashi, H.; Nakamura, M.; Murakawa, S.; Uchikawa, T.; Tanioka, T.; Nakao, K., The determination of urinary testosterone using thin-layer chromatography and gas chromatography. *Steroid* **1964**, *3* (5), 559–568. [https://doi.org/10.1016/0039-128X\(64\)90087-X](https://doi.org/10.1016/0039-128X(64)90087-X)
- (19) Atwal, G. K.; Reynolds, J. C.; Mussell, C.; Champarnaud, E.; Knapman, T. W.; Ashcroft, A. E.; O'Connor, G.; Christie, S. D. R.; Creaser, C. S., Determination of testosterone and epitestosterone glucuronides in urine by ultra-performance liquid chromatography-ion mobility-mass spectrometry. *Analyst* **2011**, *136*, 3911–3916. <https://doi.org/10.1039/C1AN15450H>
- (20) Konieczna, L.; Plenis, A.; Oledzka, I.; Kowalski, P.; Baczek, T., Optimization of LC method for the determination of testosterone and epitestosterone in urine samples in view of biomedical studies and anti-doping research studies. *Talanta* **2011**, *83* (3), 804–814. <https://doi.org/10.1016/j.talanta.2010.10.044>
- (21) Ng, B. H.; Yuen, K. H., Determination of plasma testosterone using a simple liquid chromatographic method. *J Chromatogr. B* **2003**, *793* (2), 421–426. [https://doi.org/10.1016/S1570-0232\(03\)00326-X](https://doi.org/10.1016/S1570-0232(03)00326-X)
- (22) Loi, V.; Vertzoni, M.; Vryonidou, A.; Phenekos, C., Development and validation of a simple reversed-phase high-performance liquid chromatography method for the determination of testosterone in serum of males. *J Pharm. Biomed. Anal.* **2006**, *41* (2), 527–532. <https://doi.org/10.1016/j.jpba.2005.11.021>
- (23) Titretir Duran, S.; Ayhan, N.; Aksoy, B.; Köytepe, S.; Paşahan, A., Preparation of triaminotriazinebased poly-

- imide-modified electrodes and their use for selective detection of catechin in green tea samples. *Polymer Bulletin* **2019**, *77* (10), 5065–5082. <https://doi.org/10.1007/s00289-019-03005-5>
- (24) Alışık, F.; Burç, M.; Titretir Duran, S.; Güngör, Ö.; Cengiz, M. A.; Köytepe, S., Development of Gum Arabic based polyurethane membrane modified electrodes as voltammetric sensor for the detection of phenylalanine. *Polymer Bulletin* **2021**, *78*, 4699–4719. <https://doi.org/10.1007/s00289-021-03605-0>
- (25) Mirčeski, V.; Gulaboski, R., Recent achievements in square-wave voltammetry (a review). *Maced. J. Chem. Chem. Eng.* **2014**, *33* (1), 1–12. <http://dx.doi.org/10.20450/mjccce.2014.515>
- (26) Gulaboski, R.; Mirčeski, V., Application of voltammetry in biomedicine – recent achievements in enzymatic voltammetry. *Maced. J. Chem. Chem. Eng.* **2020**, *39* (2), 1–14. <http://dx.doi.org/10.20450/mjccce.2020.2152>
- (27) Taşkın, İ.; Güngör, Ö.; Titretir Duran, S., Voltammetric determination of forfenicol by using poly(3-methylthiophene) modified glassy carbon electrode. *Polymer Bulletin* **2021**, *78*, 4721–4741. <https://doi.org/10.1007/s00289-021-03732-8>
- (28) Burc, M.; Asma, D.; Titretir Duran, S., Development of voltammetric melanin sensor with 2,5-dimethylfuran modified platinum electrode, *Macedonian Journal of Chemistry and Chemical Engineering* **2021**, *40* (2), <https://doi.org/10.20450/mjccce.2021.2399>
- (29) Levent, A.; Altun, A.; Yardım, Y.; Şentürk, Z., Sensitive voltammetric determination of testosterone in pharmaceuticals and human urine using a glassy carbon electrode in the presence of cationic surfactant. *Electrochimica Acta* **2014**, *128*, 54–60. <https://doi.org/10.1016/j.electacta.2013.10.024>
- (30) Liu, W.; Ma, Y.; Sun, G.; Wang, S.; Deng, J.; Wei, H., Molecularly imprinted polymers on graphene oxide surface for EIS sensing of testosterone. *Biosensors and Bioelectronics* **2017**, *92*, 305–312. <https://doi.org/10.1016/j.bios.2016.11.007>
- (31) Moura, S. L.; De Moraes, R. R.; Dos Santos, M. A. P.; Pividorib, M. I.; Lopes, J. A. D.; De Lima Moreira, D.; Zucolotto, V.; Dos Santos, J. R. Júnior, Electrochemical detection in vitro and electron transfer mechanism of testosterone using a modified electrode with a cobalt oxide film. *Sensors and Actuators B: Chemical* **2014**, *202*, 469–474. <https://doi.org/10.1016/j.snb.2014.05.104>
- (32) Goyal, R. N.; Gupta, V. K.; Chatterjee, S., Electrochemical investigations of corticosteroid isomers — testosterone and epitestosterone and their simultaneous determination in human urine. *Analytica Chimica Acta* **2010**, *657* (2), 147–153. <https://doi.org/10.1016/j.aca.2009.10.035>
- (33) Heidarimoghadam, R.; Akhavan, O.; Ghaderi, E.; Hashemi, E.; Mortazavi, S. S.; Farmany, A., Graphene oxide for rapid determination of testosterone in the presence of cetyltrimethylammonium bromide in urine and blood plasma of athletes. *Materials Science and Engineering: C* **2016**, *61*, 246–250. <https://doi.org/10.1016/j.msec.2015.12.005>
- (34) Tanrikut, E.; Özcan, İ.; Sel, E.; Köytepe, S.; Kuyumcu Savan, E., Simultaneous electrochemical detection of estradiol and testosterone using nickel ferrite oxide doped mesoporous carbon nanocomposite modified sensor. *Journal of the Electrochemical Society*, **2020**, *167* (8), 087509. <https://doi.org/10.1149/1945-7111/ab927f>
- (35) Vittal, R.; Gomathi, H.; Kim, K. J., Beneficial role of surfactants in electrochemistry and in the modification of electrodes. *Adv. Colloid Interface Sci.* **2006**, *119* (1), 55. <https://doi.org/10.1016/j.cis.2005.09.004>
- (36) Güngör, Ö.; Paşahan, A.; Cengiz, M. A.; Köytepe, S.; Seçkin, Turgay, Fructose-based polyurethane membranes: synthesis, characterization, and their use as voltammetric pH electrode. *International Journal of Polymeric Materials and Polymeric Biomaterials* **2015**, *64*, 563–569. <https://doi.org/10.1080/00914037.2014.996705>
- (37) Titretir Duran, S.; Paşahan, A.; Ayhan, N.; Güngör, Ö.; Cengiz, M. A.; Köytepe S., Synthesis, characterization of guar-containing polyurethane films and their non-enzymatic caffeic acid sensor applications. *Polymer-Plastics Technology and Engineering* **2017**, *56* (16), 1741–1751. <https://doi.org/10.1080/03602559.2017.1289403>
- (38) Kuyumcu Savan, E.; Paşahan, A.; Aksoy, B.; Güngör, Ö.; Köytepe, S.; Seçkin, T., Preparation and properties of selective polyurethane films and their use for the development of biomedical dopamine sensor. *International Journal of Polymeric Materials and Polymeric Biomaterials* **2016**, *65* (8), 402–408. <http://dx.doi.org/10.1080/00914037.2015.1129952>
- (39) Liu, F.; Kildsig, D. O.; Mitra, A. K., Beta-cyclodextrin/steroid complexation: effect of steroid structure on association equilibria. *Pharm Res.* **1990**, *7* (8), 869–873. <https://doi.org/10.1023/a:1015973218303>. PMID: 2235884.
- (40) Luo, M.; Hua, Y.; Liang, Y.; Han, J.; Liu, D.; Zhao, W.; Wang, P., Synthesis of novel  $\beta$ -cyclodextrin functionalized S, N codoped carbon dots for selective detection of testosterone. *Biosensors and Bioelectronics* **2017**, *98*, 195–201. <https://doi.org/10.1016/j.bios.2017.06.056>
- (41) Cai, W.; Sun, T.; Liu, P.; Chipot, C.; Shao, X., Inclusion mechanism of steroid drugs into  $\beta$ -cyclodextrins. Insights from free energy calculations. *The Journal of Physical Chemistry B* **2009**, *113* (22), 7836–7843. <https://doi.org/10.1021/jp901825w>
- (42) Chanphai, P.; Agudelo, D.; Vesper, A. R.; Bérubé, G.; Tajmir-Riahi, H. A., Effect of testosterone and its aliphatic and aromatic dimers on DNA morphology. *International Journal of Biological Macromolecules* **2017**, *95*, 850–855. <https://doi.org/10.1016/j.ijbiomac.2016.09.090>
- (43) Ates, B.; Koytepe, S.; Karaaslan, M. G.; Balcioglu, S.; Gulgen, S., Biodegradable non-aromatic adhesive polyurethanes based on disaccharides for medical applications. *International Journal of Adhesion and Adhesives* **2014**, *49*, 90–96. <https://doi.org/10.1016/j.ijadhadh.2013.12.012>
- (44) Balcioglu, S.; Parlakpınar, H.; Vardi, N.; Denkbas, E. B.; Karaaslan, M. G.; Gulgen, S.; Taslidere, E.; Koytepe, S.; Ates, B., Design of xylose-based semisynthetic polyurethane tissue adhesives with enhanced bioactivity properties. *ACS Applied Materials & Interfaces* **2016**, *8* (7), 4456–4466. <https://doi.org/10.1021/acsami.5b12279>
- (45) Holst, J. P.; Soldin, O. P.; Guo, T.; Soldin, S. J. Steroid hormones: relevance and measurement in the clinical laboratory. *Clinics in Laboratory Medicine* **2004**, *24* (1), 105–118. <https://doi.org/10.1016/j.cll.2004.01.004>