

Biosensors for β 17-estradiol detection based on graphene quantum dots (GQDs)/conducting polymer and laccase modified platinum/gold electrodes

Kamila Sychalska, Sylwia Baluta, Agnieszka Świst, Joanna Cabaj*

Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

*E-mail: joanna.cabaj@pwr.edu.pl

Received: 14 October 2019 / Accepted: 24 December 2019 / Published: 10 March 2020

A convenient electrochemical sensing pathway was investigated for β 17-estradiol determination based on graphene quantum dots (GQDs)/conducting polymer and laccase modified platinum (Pt)/gold (Au) electrodes. The miniature estradiol biosensors were created through the immobilization of laccase on the surface of an electroactive layer of an electrode coated with GQDs or with electroconducting polymer – poly(4-(5-hexylthiophen-2-yl)-2,6-bis(5-(selenophen-2-yl)thiophen-2-yl)pyridine). This sensing arrangement utilized the catalytic oxidation of β 17-estradiol to a reactive oxygen agent, and then to oligomeric structures. The process of hormone determination was based on the redox reaction using the presence of the laccase enzyme. Under optimized conditions, the constructed systems demonstrated a convenient degree of sensitivity – $0.25\text{--}0.75 \mu\text{A mM}^{-1}\text{cm}^{-2}$ - selectivity in a wide linear range ($0.1\text{--}120 \times 10^{-6}$ M) with a detection limit of ca. $1 \mu\text{M}$. Moreover, the method was successfully applied for hormone determination in the presence of interfering compounds (ascorbic acid, L-cysteine, uric acid).

Keywords: biosensor, EDCs, hormones, GQDs, laccase, semiconductive polymer, voltammetry techniques

1. INTRODUCTION

Endocrine disrupting compounds (EDCs) belong to the group of exogenous substances, such as polychlorinated dibenzodioxins (PCDD), polychlorinated biphenyls (PCB), substances contained in pesticides (biocides), phthalates, and alkylphenols, which all have a significant influence on metabolic processes. Additionally, EDCs also include natural and synthetic hormones (which are ingredients of pharmaceutical products) [1-3].

Estrogens and gestagens belong to the group of female sex hormones, which ensure the proper development and functioning of reproductive organs. The amount of estrogens (Fig. 1) (estrone – E1,

β 17-estradiol - E2, estriol – E3 and estetrol – E4) in living organisms depends on a wide range of environmental factors, such as age, diet, general health, and the season [4]. These compounds have always been present in the environment (mostly aquatic), however, the first report about their presence in surface water and their influence on the aquatic environment appeared in the 80s of the last century [5]. Currently, we can observe an increase in interest of researchers regarding inherence EDCs in aquatic systems and their effect on all living organisms. In most cases, water pollution comes from the medical industry, which is connected with the presence of synthetic equivalents (17 α -ethinylestradiol – EE2, diethylstilbestrol – DES or mestranol – MES) in the water environment. This presence is due to the high usage of oral contraceptives by females. Such drugs are produced in two forms - of one or two components, namely estrogen and progesterone in various proportions, depending on the phase of the contraceptive [1-5].

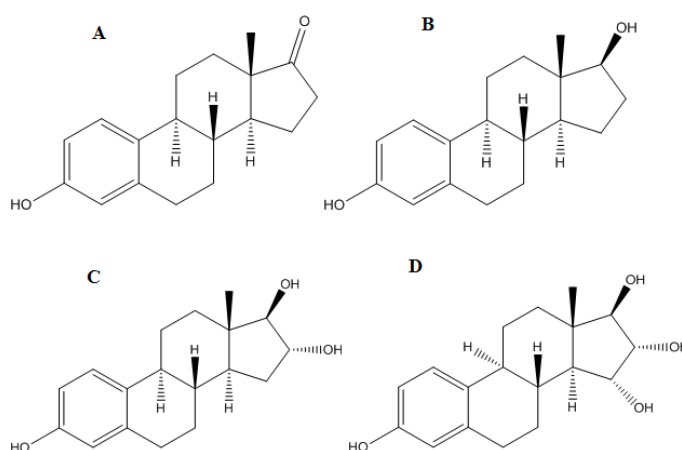


Figure 1. Chemical structure of estrogens (A – estrone (E1), B – β 17-estradiol (E2), C – estriol (E3) and D – estetrol (E4))

In medicine, estrogens are used in hormone replacement therapy (HRT), which aims to supplement estrogen deficiency around the menopausal period. This method is recommended for the relief of vasomotor symptoms, bone disorders (osteoporosis), vaginal dryness, urogenital atrophy and cardiovascular diseases. In the case of male treatment, the described drugs, which are based on hormones, are used as adjuncts in the treatment of prostate cancer [6].

In animal husbandry, estrogen hormones are very often administered as feed additives (so-called „growth promoters”) to accelerate growth and increase the meat mass of cattle, poultry and pigs [7].

Drug metabolites may be excreted with wastewater into the environment in two ways - in a free and bound form. In recent years, an increasing concern regarding the detection of sensitive and selective estrogen hormones has been observed, which is due to the presence of these substances in not only sewage and surface waters, but also in groundwater, and what is particularly worrying – in drinking water [7,8]. This kind of contamination can be related to the interference in the gender structure of fish and lower species, but also to their survival rates. It is very important to measure current levels of hormones in surface waters, and how they can affect the water environment. This is

an essential reason for the constant monitoring of water before and after big urban agglomerations, as pollution of surface waters can pose a great danger for both humans and the environment [9].

The negative impact of EDCs on the environment was first observed 30 years ago. This impact was first seen as irregularities in the reproductive processes in aquatic organisms, especially fish, and was based on the feminization of males [10]. These compounds affect the induction of vitellogenin, reduce egg production and the male gonadosomatic index (GSI), induce obesity, and reduce the sexual behavior of males. Reproductive disorders and the development of organisms caused by exposure to estrogens are also manifested by decreased fertility, dystocia (so-called “heavy births”), premature births, shortened pregnancy, miscarriages and ovulation disorders. It should be emphasized that in the case of EE2, these phenomena are already observed at concentrations determined in the environment at the level of 0.05 – 10 ng/L. According to many authors, there is a possibility of bioaccumulation of these compounds in aquatic organisms, and thus also a likelihood of them reaching through the food chain to higher organisms [11-13].

Literature has reported different methods for hormone determination, such as high performance liquid chromatography (HPLC) [14], capillary electrophoresis [15], spectrophotometry-UV [16], or gas chromatography combined with mass spectroscopy (GC-MS) [16]. However, commonly used techniques for the determination of EDCs, such as HPLC or GC-MS, are time-consuming, very expensive and often inaccurate. Electrochemical biosensors have recently gained popularity because of a number of advantages such as their low cost, fast response, ease of use and real-time analysis [17]. Electrochemical biosensors for estradiol detection are widely described in the literature, however, there is a lack of information about enzyme-based sensors, and what is more, a lack of reports connected with enzyme-based electrochemical sensors [18,19].

In this paper, a comparison of two different electrochemical biosensor systems for the detection of β 17-estradiol is presented. The key step in the biosensors design is the immobilization of biological material (in this case, the enzyme) on a suitable matrix, which should not only effectively bind the biomolecule on its surface, but also prevent the weakening of its catalytic activity and ensure stability during measurements for as long as possible. In this work, we want to compare two different semi-conductive layers on two different electrode surfaces: a traditional, widely used polymer (with platinum electrode); and a nanomaterial (graphene quantum dots – GQDs, with gold electrodes). GQDs do not require long synthesis, or toxic and expensive materials, which is in contrast to polymer synthesis. We have proved, despite the wide range of nanomaterial advantages, that polymer is a better matrix for enzyme binding, and that it improves the working of a biosensor system and obtains better results for a semi-conductive polymer-based measuring biosystem. Such biosensors can be a good prototype for the construction of a device for the detection of β 17-estradiol for environmental protection or the medical industry.

2. EXPERIMENTS

2.1. Reagents and materials

Laccase (from *Cerrera unicolor*, EC 1.10.3.2, ≥ 10 U/mg) β 17-estradiol, as well as tetrabutylammonium tetrafluoroborate (TBA-TFB) from Sigma-Aldrich Co. Dichloromethane, citric

acid (CA), Tris, HCl, KH_2PO_4 , NaH_2PO_4 , CH_3COOH and CH_3COONa were purchased from POCH. Monomer 4-(5-hexylthiophen-2-yl)-2,6-bis(5-(selenophen-2-yl)thiophen-2-yl)pyridine was synthesized according to the previous procedure [20]. All buffers, which were needed in order to wash unbound proteins, were prepared according to obligatory standards. All chemicals were analytically pure and used without any further purification.

2.2. Apparatus and synthesis

2.2.1. Synthesis and characterization of Graphene Quantum Dots

Graphene quantum dots (GQDs), because of their biocompatibility, chemical inertness, high mechanical strength, semi-conducting properties and strong fluorescence, belong to the most interesting group of carbon-based nanoparticles [21]. One of their most attractive properties is the fact that they are a carbonaceous material, which therefore means they are sufficiently available and have low toxicity. They are also soluble in various solvents and can be equipped with functional groups on their surface. These properties have resulted in the use of GQDs in research on photovoltaic devices, biosensors, carriers of anticancer drugs, and especially in bioanalysis and cell imaging [21,22].

The GQDs were synthesized with the use of pyrolysis of citric acid (CA). According to literature [22] (Fig. 2), 4 g of citric acid can be pyrolyzed at 200°C . The obtained orange liquid was added dropwise into 100 mL of NaOH solution (2 mg/100 mL) at pH 10. The received GQDs were stored at 4°C . Atomic force microscopy (AFM) and Fourier transform infrared spectroscopy (FTIR) were used to analyze the properties of the GQDs. AFM was used to characterize their size and shape. The measurements were carried out in tapping mode (AFM NanoScope V Veeco) and were executed at air-ambient conditions (25°C and 35% relative humidity). The cutting edge TESP type, 10 nm size and scanning rate of $3\ \mu\text{m/s}$. For investigation of the creation of GQDs from CA, FTIR (Nicolet iS10 Spectrometer) spectroscopy was used.

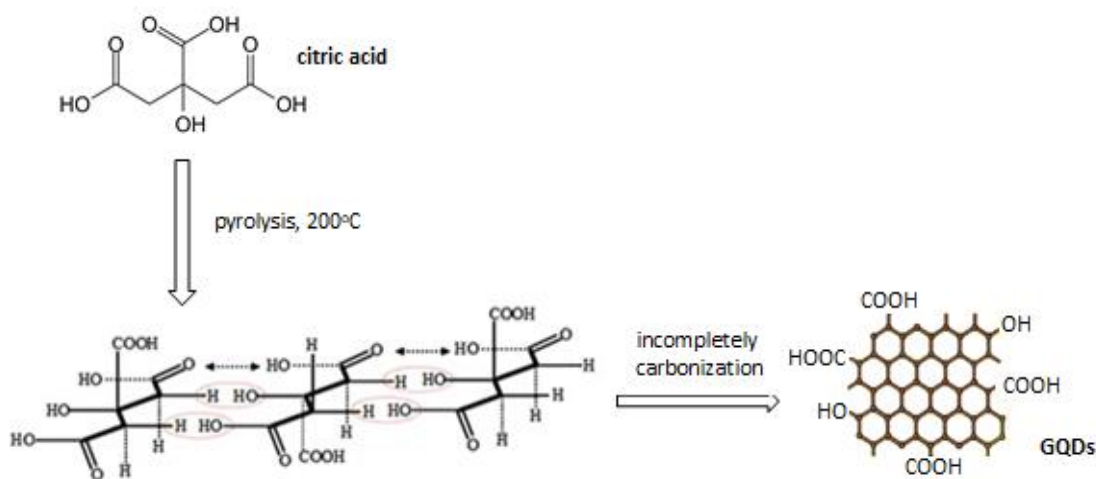


Figure 2. Synthesis of Graphene Quantum Dots [based on 22]

2.2.2. Synthesis of 4-(5-hexylthiophen-2-yl)-2,6-bis(5-(selenophen-2-yl)thiophen-2-yl)pyridine

The key reaction during the monomer synthesis was Suzuki condensation, which has been shown to be very useful for the formation of a carbon-carbon bond. The first step was the aldol reaction, the product of which was used to synthesize the bromide according to the Kröhnke reaction mechanism. The synthesized product was a substrate for the Suzuki reaction. Cross-coupling between reagents allowed for the formation of a compound that was used for the last stage of monomer synthesis, which was formed by the elimination of bromine from carbon. The synthesis process is described in detail in [20].

2.2.3 Modification of electrodes

Due to the construction of two different bio-systems, two different modified electrodes were prepared – platinum and gold (Fig. 3A). Choosing an electrode to be modified is an important element in the construction of bio-platforms for the sensitive determination of an analyte. The working electrode is a polarizable electrode, and forms the basis for the measurements. Depending on the sign of voltage applied, it may be a cathode (negative potential) or anode (positive potential). To use a working electrode in the field of positive potentials, noble metals (e.g. Pt, Au, Ag) or carbon electrodes (like glassy carbon) are equally used. Two types of conductive compounds were used to modify the surface of the electrodes: polymer and graphene quantum dots. The platinum electrode (Pt, Ø 3 mm, manufactured by BASi), as well as gold electrode (Au) were prepared before modification, according to the procedure described in [23]. The prepared electrodes were modified with laccase (System A), as well as a thin layer of polymer of 4-(5-hexylthiophen-2-yl)-2,6-bis(5-(selenophen-2-yl)thiophen-2-yl)pyridine (Fig. 3B) using the cyclic voltammetry technique. The platinum electrode was chosen for modification with polymer to obtain the best results (electropolymerization of a monomer occurs with a higher efficiency when the Pt electrode is used). 0.1 mM of the monomer was dissolved in 10 mL of dichloromethane. In order to obtain a thin layer of polymer film on the surface of the PE, for the three electrode system, 10 measurement cycles were carried out in the potential range of 0 – 1.6 V with a scanning rate of 50 mV/s. The gold electrode (Ø 3 mm, manufactured by BASi) (System B) was modified through the physical adsorption of GQDs on its surface and covalent cross-linking for 24h. It is generally known that nanomaterials create strong bonding between noble metals, however the strongest bonding occurs when the gold electrode is used. In both cases, the immobilization process was the same - the laccase was immobilized on the surface of the modified electrode by physical adsorption and covalent cross-linking with glutaraldehyde. Laccase solution (1.5 mg/ml) was prepared in a phosphate-citrate buffer. The immobilization process was carried out for 2 hours by the injection of 40 µL of laccase solution to the electrode's surface. 40 µL of glutaraldehyde was added for 10 min to crosslink the enzyme. The unbound protein was washed by repeatedly plunging the electrode into the following buffers: two times for 15 minutes in phosphate buffer at pH 7.0, two times for 15 minutes in acetate buffer at pH 5.2, and for 45 minutes in Tris-HCl buffer at pH 7.2. The prepared enzyme-modified electrodes were stored in a phosphate-citric buffer at an optimal pH (5.2) at 4°C.

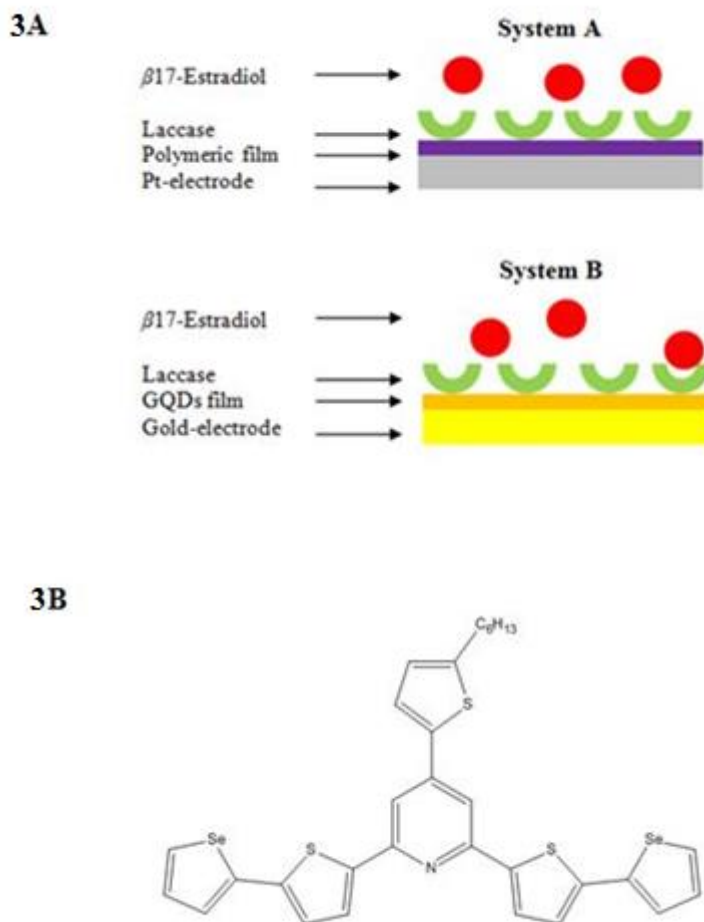


Figure 3. Scheme of system A and system B (3A) and the structure of 4-(5-hexylthiophen-2-yl)-2,6-bis(5-(selenophen-2-yl)thiophen-2-yl)pyridine (3B) [20]

2.2.4 Electrochemical measurements

Research was carried out for two systems: A: which consisted of a modified monomer and laccase platinum electrode, and system B: which consisted of a gold electrode coated with GQDs and laccase. Determination of $\beta 17$ -estradiol was executed using the CV technique with a potentiostat/galvanostat AUTOLAB PGSTAT 128N with GPES software. All measurements were executed using a standard three-electrode system in a 8 mL cell. The platinum electrode (modified with polymer and enzyme) and the gold electrode (modified with GQDs and enzyme) were used as working electrodes, together with a coiled platinum wire as the auxiliary electrode and a silver-silver chloride as the reference electrode (Ag/AgCl). CV was carried out by repeated potential scanning in the range 0.6 – 1.0 V with a scan rate of 50 mV/s in the presence of different concentrations of $\beta 17$ -estradiol dissolved in 0.1 M of phosphate buffer with a pH = 6.0 (0.1 – 120 μ M). All electrochemical measurements were carried out at room temperature and in air-opened conditions.

2.2.5 Interferences study

To investigate the influence of interfering species, to each β 17-estradiol standard solution (0.1 – 120 μ M) the most common interferences were added: ascorbic acid (AA), L-Cysteine (CYS), Uric acid (UA) and testosterone (TES) in a concentration of 50 μ M. The above-mentioned species were mixed each time with β 17-estradiol solution in a volume ratio of 1:1.

3. RESULTS NAD DISCUSSION

3.1. Characterization of GQDs

New synthesized GQDs were analyzed using an atomic force microscope (AFM). Fig. 4 A,B confirms the monodisperse nature and spherical shape of the obtained particles, which had a diameter of around 5 nm. These values are characteristic for nanocrystalline materials and confirm the structure of graphene. What is important is that the monodisperse nature of GQDs allows the attachment of proteins, e.g. enzymes on their surface [17]. The creation of characteristic functional groups in the GQDs synthesized from CA, which are necessary for adsorption of the enzyme (laccase) on the surface of GQDs, was proved using the FT-IR technique (Fig. 5 A,B). Because of the presence of an aqueous environment, the samples were made on glass plates using the layer-layer technique. The FT-IR spectrum of CA presents the characteristic peaks at 1126 and 1219 cm^{-1} , which correspond to C=O in the =COOH group. The same characteristic signals are present at 1325 and 1489 cm^{-1} on the GQDs' spectrum. At the same time, peaks derived from the CA carbonyl group (1700-1750) were not recorded on the GQDs' spectrum. The presence of a broad absorption peak at 2885-3392 cm^{-1} on the GQDs indicates the presence of the -OH group, which is derived from water molecules. The spectrum (Fig. 5A) confirms the creation of the GQDs, which were synthesized using CA. The characteristic peak belongs to complex GQDs-laccase and shows that the enzyme is successfully immobilized on the surface of the GQDs.

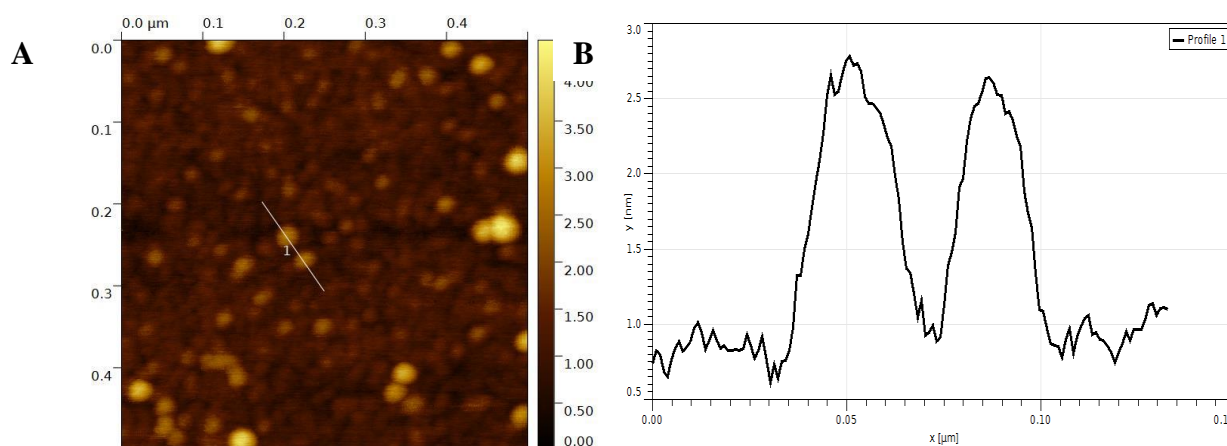


Figure 4. Representative AFM image (A) and profile (B) of the obtained GQDs and their profile in tapping mode (scanning rate of 3 μ m/s). The obtained GQDs have an average dimension c.a. 5.0 nm

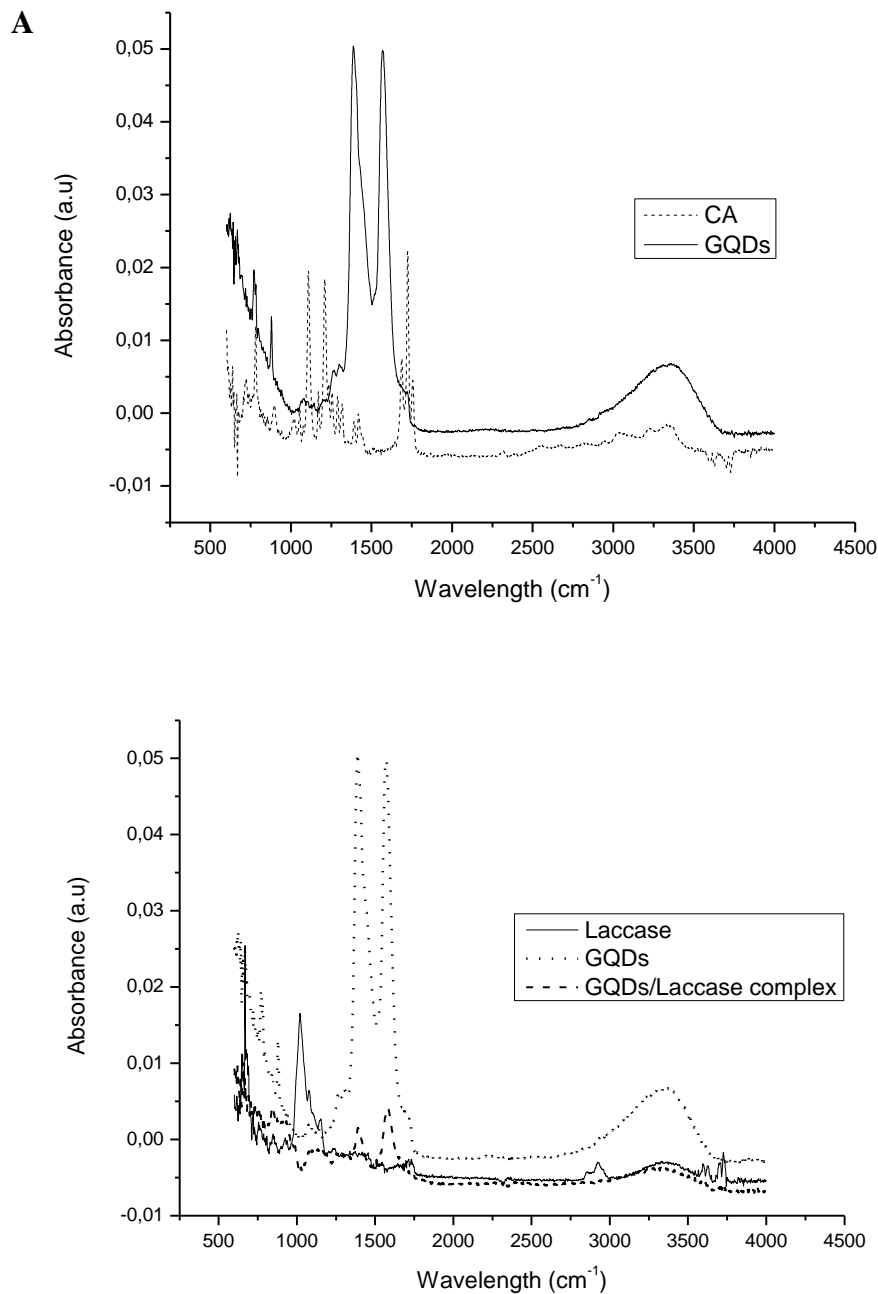


Figure 5. FT-IR spectrum of the CA and GQDs created from the CA (A), laccase, GQDs and GQDs/laccase complex (B), laser frequency = 15798.0 cm⁻¹

3.2. Modification of electrodes

Electrodeposition of the monomers was provided with the cyclic voltammetry method, which allows a controlled thin layer of polymer film on the surface of the platinum electrode to be obtained. The Ag/AgCl electrode was used as a reference electrode. Surface modification with an enzyme, and a platform for anchoring the protein is a key step during the construction of biosensors. The enzymatic activity depends on the chemical species used, which are a matrix for the immobilization of a

biocatalyst. On the presented voltamperogram (Fig. 6), one visible peak corresponding to the oxidation reaction (anodic peak) is observed. During the process, an increase in peak values is visible with each subsequent cycle. This demonstrates an effective electropolymerization of the 4-(5-hexylthiophen-2-yl)-2,6-bis(5-(selenophen-2-yl)thiophen-2-yl)pyridine, which is connected with electrochemical synthesis of the monomer.

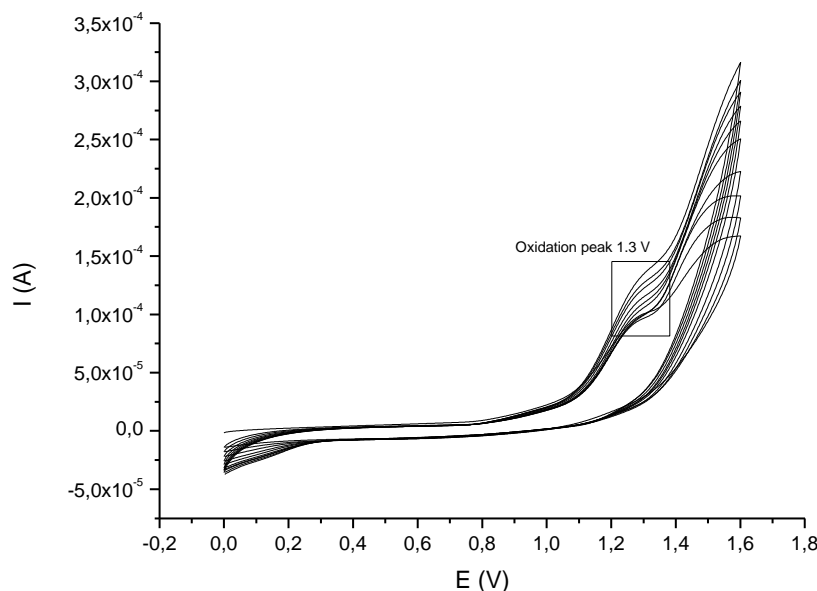


Figure 6. CV-scan of the 4-(5-hexylthiophen-2-yl)-2,6-bis(5-(selenophen-2-yl)thiophen-2-yl)pyridine electropolymerization and deposition on the Pt electrode surface with a scan range: 0 – 1.6 V, scan rate: 50 mV/s, for 10 cycles via Ag/AgCl as the reference electrode

3.3. Electrochemical measurements

Many natural and synthetic estrogens are amenable to oxidation through the catalytic action of oxidative enzymes such as the fungal laccase *Trametes versicolor* [24]. Laccase (Lac) (EC 1.10.3.2) is a polyphenol oxidase that belongs to a large family of enzymes, known as “Multicopper Oxidases” (MCOs). Laccase catalyzes the oxidation of a various range of compounds, mainly aromatic, e.g. belonging to phenols and anilines containing easily expressive moieties. This process takes place with the participation of atmospheric oxygen (Fig. 7). The mechanism of reactions catalyzed by laccase is based on the formation of the product. This is a result of the oxidation of four molecules of the substrate to the radicals, as well as a reduction of the oxygen to two molecules of water. This process can be run in three ways: direct oxidation of the substrate, oxidation of the substrate with the participation of a mediator, or during the coupling reactions [25,26]. As shown in Fig. 8, the laccase oxidation of $\beta 17$ – estradiol generates oxygen radicals that can delocalize to carbon – located radicals. Coupling of these reactive intermediates led to C-C or C-O dimers, which can be oxidized to oligomers and polymers [27].

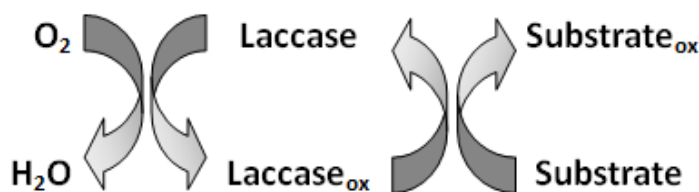


Figure 7. Scheme of reaction catalyzed by Laccase [according to 26]

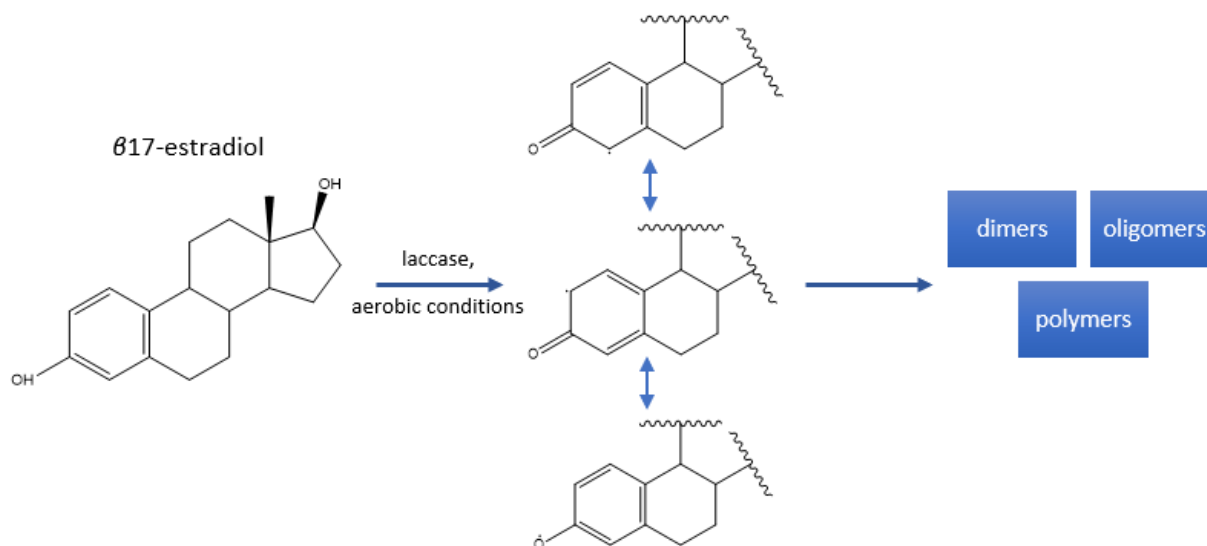


Figure 8. Laccase-mediated oxidation of $\beta 17$ – estradiol [according to 27]

The first of the presented systems is the heterocyclic semiconductive compound-based biosensor. Conducting polymers (CPs) are an interesting alternative to organic polymers, which are mostly insulators. A characteristic feature of CPs is the presence of conjugated π electrons (a system that has conjugated bonds $C=C$), thanks to which they exhibit unique electronic properties, such as low optical transition energy, low ionization potential and high electron affinities [28]. Conducting polymers arouse wide interest as materials that act as matrix binding molecules and are used to increase the stability, speed and sensitivity of various biomedical devices [23]. What is more, CPs are cheap, easy to synthesize and universal, because their properties can be easily modulated by physical changes (such as pH, temperature), even after their synthesis [29,30]. The first bio-system (A) for hormone determination is the system using the Pt electrode modified with poly[4-(5-hexylthiophen-2-yl)-2,6-bis(5-(selenophen-2-yl)thiophen-2-yl)pyridine] and laccase in the presence of a wide range of concentrations of $\beta 17$ -estradiol (0.1 – 120 μM).

In Fig. 9, the results obtained for system A are presented, and as can be observed, the signals precisely respond to the given $\beta 17$ -estradiol concentration. The peaks rise proportionally as a result of an increasing concentration of the studied hormone species.

One of the most important parameters affecting the correct operation of a biosensor is the limit of detection (LOD) and linear range. LOD is the lowest concentration of analyte that can be examined

with statistical certainty using the presented technique. The LOD was calculated using the following equation:

$$LOD = 3.29 \cdot \frac{\sigma_B}{b} \quad (1)$$

where σ_B is the standard deviation of the population of blank responses, and b is the slope of the regression line [31]. The limit of detection for system Pt/Polymer/Lac is 9.94×10^{-7} [M]. This value is desirable for hormone detection in ground water, where the level of dangerous concentration is even around 0.01 $\mu\text{g/L}$ (2.8 μM) [32].

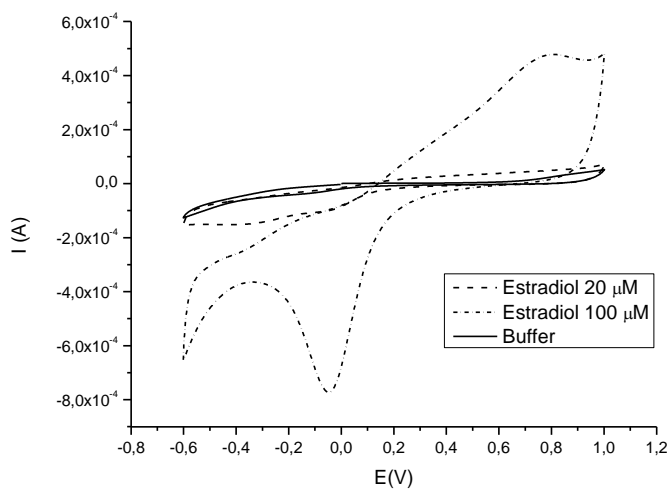


Figure 9. System A – Pt/Polymer/Lac. Representative CV-scans of the biosensor system via only the buffer and hormone (20 and 100 μM) under the applied potential in the range: -0.6 – 1.0 V, scan rate 50 mV/s, for 10 cycles, vs. Ag/AgCl (0.1 M)

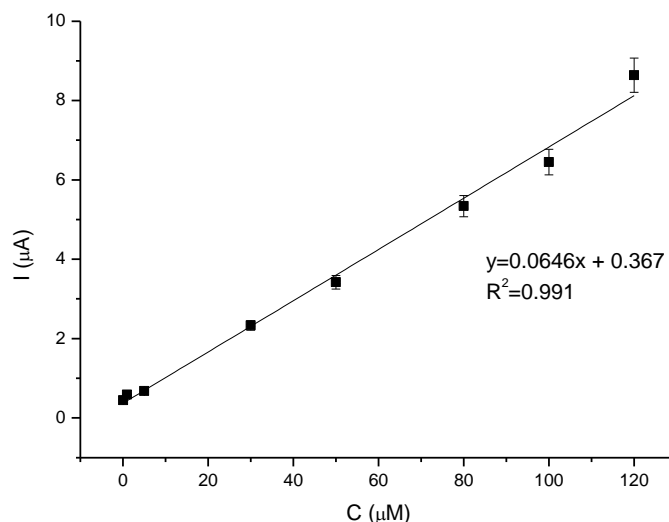


Figure 10. System A. Relationship between the given concentration of the hormone and current. Limit of detection: 102 nM, linear range: 0.1 – 120 μM

The sensitivity of the constructed system is equal to $0.752 \mu\text{A mM}^{-1} \text{cm}^{-2}$. The limit of quantification (LOQ) was also calculated (using equation 2) and is equal to 1.51×10^{-6} [M].

$$LOQ = 5 \cdot \frac{\sigma_B}{b} \quad (2)$$

where σ_B is the standard deviation of the population of blank responses, and b is the slope of the regression line [31]. Figure 10 demonstrates the linear response to β 17-estradiol in the tested range of concentrations. The concentration response was linear in the range 0.1 - 120 μ M. Additionally, the R^2 factor for the described system has an excellent value equal to 0.99.

The next examined biosensor (system B – gold electrode modified with GQDs and laccase) is the system constructed using graphene quantum dots as a matrix for enzyme immobilization. From the obtained data (Fig. 10,11), it can be observed that this system presents lower values for β 17-estradiol determination, which may be related to the fact that the GQDs do not permit as good enzyme catalytic activity as in the case of using the polymeric matrix (system A). The peaks shown in Fig. 11 represent signals obtained for Gold/GQDs/Lac system responded to given β 17-estradiol concentration (in range 0.1 – 120 μ M). The signals increased proportionally with an increasing concentration of the examined hormone derivative.

The limit of detection for this system is calculated as before, using equation 1, and was found to be 1.51×10^{-6} [M]. The limit of quantification, using equation 2, was calculated and is equal to 2.13×10^{-6} [M]. Sensitivity for this system equals $0.248 \mu\text{A mM}^{-1} \text{cm}^{-2}$.

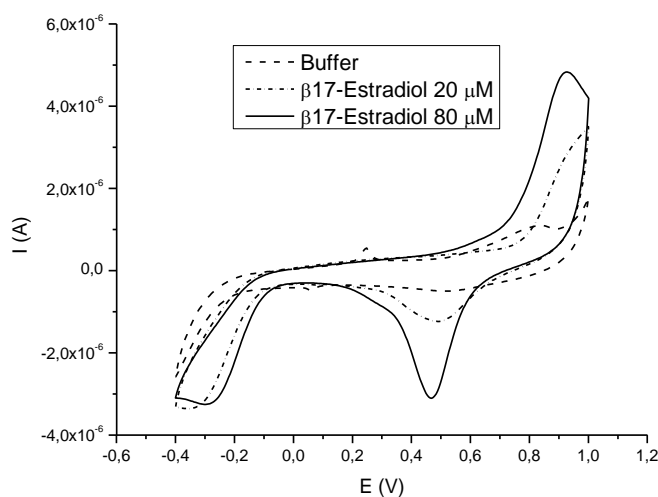


Figure 11. System B – Gold/GQDs/Lac. Representative CV-scans of the biosensor system via only the buffer and hormone (20 and 100 μ M) under the applied potential in the range: -0.4 – 1.0 V, scan rate 50 mV/s, for 10 cycles, vs. Ag/AgCl (0.1 M)

Fig. 12 presents the linear response of system B to β 17-estradiol in the concentration range 5.0 – 50 μ M. As can be observed, the constructed system, based on GQDs/Lac, shows the linear in the investigated concentration range with the R^2 factor equal to 0.99.

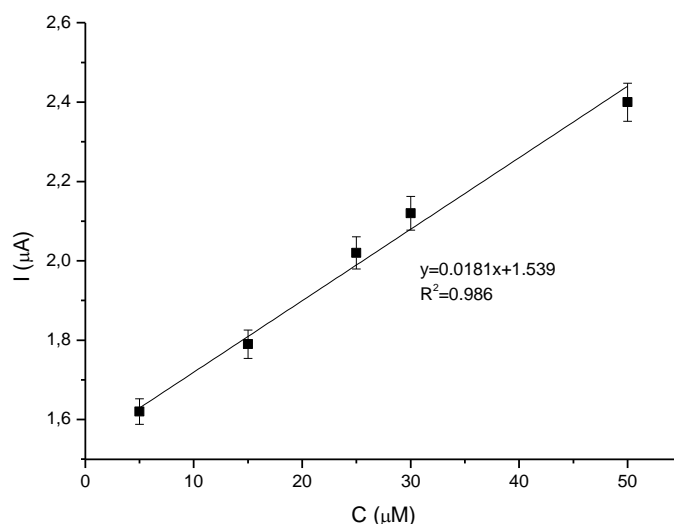


Figure 12. System B. Relationship between the given concentration of the hormone and current (sensor response). Linear range: 5.0 - 50 μM . Limit of detection: 1,51 μM .

3.3.1. Comparison of measuring systems

Table 1 presents the most important analytical parameters of both biosensors .

Table 1 Analytical validation of the presented biosensors

	Linear range	LOD	LOQ	R ²	Slope [$\mu\text{A } \mu\text{M}^{-1}$]
System A	0.1 – 120 μM	994 nM	1.51 μM	0.99	0.065±2.4 E-04
System B	5 – 50 μM	1.51 μM	2.13 μM	0.95	0.013±1.1 E-04

As can be observed by the parameters presented in Table 1, better results were obtained in the biosensor system A based on the platinum electrode modified with semiconducting polymer and laccase. This fact is connected with the presence of a more stable and rich in functional groups material - 4-(5-hexylthiophen-2-yl)-2,6-bis(5-(selenophen-2-yl)thiophen-2-yl)pyridine. On the surface of the GQDs there is less space for protein anchoring, which is due to the small size of this nanomaterial and the fact that it does not have much polymer space for laccase immobilization. Due to this, the polymer is a better matrix, which permits a higher enzymatic catalytic activity and improves the working of the whole biosensing system. Moreover, it can be observed that both systems have good analytical characteristics – an interesting linear range, as well as a low detection limit. These results are comparable with some reported systems for the detection of these substances [Table 2].

Table 2 Reported electrodes for 17β -estradiol determination

Sensing platform	Biological component	Detection method	LOD	Ref.
Pt/MWNTs/GCE	X	SWV	$1,8 \times 10^{-7} \text{ mol L}^{-1}$	33
PEDOT/AuNPs	17β -estradiol binding aptamers	SWV	0,02 nM	34
GR/PANI/HRP-GO-Ab	Anti 17β -estradiol antibody, horseradish peroxidase	DPV	0,02 ng mL ⁻¹	35
RGO-DHP/GCE	X	CV/ ESI	$7.7 \times 10^{-8} \text{ mol L}^{-1}$	36
GR/OMC/CPE	X	SWV	$2.0 \times 10^{-9} \text{ mol L}^{-1}$	37

3.4. Interferences study

The selectivity and specificity of a biosensor are very important parameters in the context of the correct design of bio-devices. It means that a biosensor should not be subjected to interference by other hormones or biomarkers while in use. Human body fluids (plasma and urine) contain a wide range of interfering compounds, such as the above-mentioned ascorbic acid and uric acid. Our results present some species that could have an influence on the obtained signals during the conducted measurements, such as: ascorbic acid (AA), uric acid (UA), L-cysteine (CYS) and testosterone (TES).

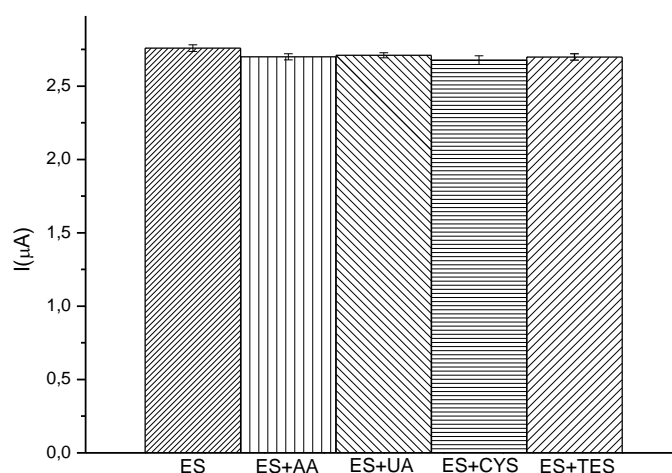
**Figure 13.** The influence of interfering substances (50 μM) on $\beta 17$ -estradiol determination

Figure 13 shows the effect of compounds that could potentially interfere with signals generated during measurements, which include: ascorbic acid (AA), uric acid (UA), L-Cysteine (CYS) and testosterone (TES). Individual interfering compounds were added at a concentration of 50 μM to 1, 50, and 100 μM of 17β -estradiol solution to study the effects of these compounds when in excess, balance, and deficiency. Each of the tested interfering compounds had a slight effect (<6.4%) on the peak

current of the samples relative to the blank. In addition, the use of laccase in the constructed system allows effective testing of lower 17β -estradiol concentrations ($<10\ \mu\text{M}$), and the interfering compound does not have a significant impact on the measurement process due to the selective nature of the enzyme. Consequently, the biosensor showed adequate selectivity for the determination of 17β -estradiol.

4. CONCLUSIONS

In summary, rapid, sensitive, selective and simple biosensors for the detection of 17β -estradiol were developed using two different systems: system A, based on PE/Polymer/Lac, and system B, based on Gold/GQDs/Lac. The electrochemical measurements were carried out using cyclic voltammetry techniques. System A was able to detect 17β -estradiol in the linear range from 0.1 to $120\ \mu\text{M}$, with a detection limit of 994 nM. In the case of system B, the linear range was found to be 5.0 - $120\ \mu\text{M}$, with a detection limit of $1.51\ \mu\text{M}$. The modified electrodes were active for 60-80 cycles. The comparison of both sensing systems shows that the use of polymer as a matrix for the immobilization of enzymes causes a significant increase in sensitivity. The characteristics of both systems show a suitable, easy-to-prepare and long-term method for hormone monitoring. The method can also be recommended as a future bio-tool for diagnostic or environmental purposes. Both biosensor systems were constructed in as simple a way as possible and are cost-effective. Our presented preliminary studies may lead to the creation of a sensitive, fast, biocompatible and selective bio-device that is suitable for single-use, disposable *in vitro* or *in situ* applications.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Wroclaw University of Science and Technology for their financial support (10401/0194/17).

References

1. F. Lahnsteiner, B. Berger, M. Kletzi, T. Weismann, *Aquat. Toxicol.*, 79 (2006) 124.
2. H. Segner, K. Caroll, M. Fenske, C.R. Janssen, G. Maack, D. Pascoe, C. Schafers, G.F. Vandenberg, M. Watts, A. Wenzel, *Ecotox. Environ. Saf.*, 54 (2003) 302.
3. D. DeCantazaro, *Horm Behav*, 68 (2015) 103.
4. V. Gabet, C. Miegé, P. Bados, M. Coquery, *Trends Anal. Chem.*, 26 (2007) 1113.
5. J. Carpintero, J.B. Quintana, I. Rodriguez, A.M. Carro, R.A. Lorenzo, R. Cela, *J. Chromatogr. A*, 1056 (2004) 179.
6. M.D. Hernando, M. Mezcua, M.J. Gómez, O. Malato, A. Aguera, A.R. Fernandez-Alba, *J. Chromatogr. A*, 1047 (2004) 129.
7. A.C. Belfroid, A. Van der Horst, A.D. Vethaak, A.J. Schafer, G.B.J. Rijs, J. Wegener, W.P. Cofino, *Sci. Total Environ.*, 225 (1999) 101.
8. E. Vulliet, L. Wiest, R. Baudot, M.F. Grenier-Laustalot, *J. Chromatogr. A*, 1210 (2008) 84.
9. J.E. Harries, T. Runnalls, E. Hill, C.A. Harris, S. Maddix, J.P. Sumpter, C.R. Tyler, *Environ. Sci. Technol.*, 34 (2000) 3003.
10. J. Parrott, B.R. Blunt, *Environ. Toxicol.*, 20 (2005) 131.
11. S. Orn, H. Holbech, T.H. Madsen, L. Norrgren, G.I. Petersen, *Aquat. Toxicol.*, 65 (2003) 397.

12. L. Mills, C. Chichester, *Sci. Total Environ.*, 343 (2005) 1.
13. A. Penalyer, E. Pocurull, F. Borrull, R.M. Marce, *J. Chromatogr. A*, 964 (2002) 153.
14. L. Nováková, P. Solich, L. Matysová, J. Sícha, *Anal. Bioanal. Chem.*, 379 (2004) 781.
15. B. Yilmaz, Y. Kadioglu, *Arab. J. Chem.*, 10 (2013) 1422-1428.
16. K. Prokai-Tatrai, D. Bonds, L. Prokai, *Chromatographia*, 71 (2010) 311.
17. J.D. Fernstrom, M.H. Fernstrom, *J. Nutr.*, 137 (2007) 1539.
18. E. Eltzov, A. Kushmaro, R.S. Marks, *Endocrine-Disrupting Chem. Food*, 8 (2009) 183.
19. W. Nawrot, K. Drzozga, S. Baluta, J. Cabaj, K. Malecha, *Sensors*, 18 (2018) 2357.
20. M.P. Pander, P. Data, R. Turczyn, M. Lapkowski, A. Swist, J. Soloducho, *Electrochim. Acta*, 210 (2016) 773.
21. J. Peng, W. Gao, B.K. Gupta, Z. Liu, R. Romero-Aburto, L. Ge, L. Song, L.B. Alemany, X. Zhan, G. Gao, *Nano Lett.*, 12 (2012) 844.
22. J. Prakash, P. Sutradhar, M. Saha, *J. Nanostructure Chem.*, 7 (2017) 85.
23. S. Baluta, A. Lesiak, J. Cabaj, *Electroanal.*, 30 (2018) 1773.
24. T. Shraddha, R. Shekher, S. Sehgal, M. Kamthania, A. Kumar, *Enzyme Res.*, 2011 (2011) 1.
25. K.K. Sharma, R.C. Kuhad, *Ind. J. Microbiol.* 48 (2008) 309.
26. J. Polak, A. Jarosz-Wilkolazka, *Biotechnologia*, 4 (2007) 82.
27. S. Nicotra, A. Intra, G. Ottolina, *Tetrahedron Asymmetry*, 15 (2004) 2927.
28. R. Ravichandran, S. Sundarrajan, J.R. Venugopal, S. Mukherjee, S. Ramakrishna, *J. R. Soc. Interface*, 7 (2010) 559.
29. R. Balint, N.J. Cassidy, S.H. Cartmell, *Acta Biomater.*, 10 (2014) 2341.
30. J.W. Schultze, H. Karabulut, *Electrochim. Acta*, 50 (2004) 1739.
31. E. Desimoni, B. Brunetti, *Electroanalysis*, 25 (2013) 1645.
32. D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, *Environ. Sci. Technol.*, 36 (2002) 1202.
33. X. Lin, Y. Li, *Biosens. Bioelectron.*, 22 (2006) 253.
34. R.A. Olowu, O. Arotiba, S.N. Mailu, T.T. Waryo, P. Baker, E. Iwuoha, *Sensors*, 10 (2010) 9872.
35. J. Li, S. Liu, J. Yu, W. Lian, M. Cui, W. Xu, J. Huang, *Sens. Actuators B*, 188 (2013) 99.
36. B.C. Janegitz, F.A. dos Santos, R.C. Faria, V. Zucolotto, *Mater. Sci. Eng. C*, 37 (2014) 14.
37. Y. Zhu, X. Liu, J. Jia, *Anal. Methods*, 7 (2015) 8626.

© 2020 The Authors. Published by ESG (www.electrochemsci.org). This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).