Volume 9, Issue 4, 2019, 4090 - 4095 **ISSN 2069-5837**

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

https://doi.org/10.33263/BRIAC94.090095

Original Research Article **Open Access Journal**

Received: 26.06.2019 / Revised: 08.08.2019 / Accepted: 10.08.2019 / Published on-line: 15.08.2019

Molecularly imprinted biomimetic surface plasmon resonance sensor for hormone detection

Emir Alper Türkoğlu [1], Monireh Bakhshpour [1], Adil Denizli [1,*]

[1]Biochemistry Division, Department of Chemistry, Hacettepe University, Ankara, Turkey

*corresponding author e-mail address: ***denizli@hacettepe.edu.tr***

ABSTRACT

In this study, 17β-estradiol (E2) was performed by surface plasmon resonance (SPR) based on molecularly imprinted technique. In the first, SPR sensor was prepared by the formation of E2 imprinted poly(2-hydroxyetyl methacrylate) / (N-methacryloyl-(L)- leucine methyl esters) (PHEMALM) on the modified gold surface of SPR chip. Also, non-imprinted SPR chip was prepared by the same method at the E2 imprinted PHEMALM (E2-MIPHEMALM-SPR) chip without E2. E2-MIPHEMALM-SPR and non-imprinted (NIPHEMALM-SPR) chips were characterized with Fourier transform infrared spectrophotometry (FTIR), ellipsometry, atomic force microscopy (AFM) and contact angle measurements. After that, SPR chips were completed to SPR system to investigate kinetic properties for E2. The sensing ability of E2-MIPHEMALM-SPR chip was investigated with 20-10000 ng/mL concentrations of 17β-estradiol solutions. The E2-MIPHEMALM-SPR chip was showed more selectivity for 17β-estradiol than NIPHEMALM-SPR chip. To show the selectivity of E2-MIPHEMALM-SPR chip competitive adsorption of 17β-estradiol, cholesterol and stigmasterol were investigated. E2-MIPHEMALM-SPR chip was investigated ten times with same concentrations of 17β-estradiol solution to show reuse of the chip. The results showed that the E2-MIPHEMALM-SPR chip has high selectivity for 17β-estradiol.

Keywords: *17β-estradiol, SPR, PHEMA, molecular imprinted.*

1. INTRODUCTION

Endocrine disrupting chemicals (EDCs) are a wide range of natural and synthetic substances that have a severe effect on endocrine systems of human beings and wildlife species with the ability of manipulating endocrine functions as mimicking and/or blocking hormones [1,2]. 17β-estradiol (E2) is one of the most active EDCs [3]. This biomolecule is used in livestock production because of its anabolic characteristics [4], and in veterinary use for the infections of animals [5]. It is secreted by all mammalians and released into the water bodies [6], which leads to many changes in immune, endocrine, reproductive and nerve systems [7]. Also, E2 is used as growth assist agents to cattle to gain the weight [8]. High E2 causes high residue in animal foods that cause endocrine and reproductive effect to human body [9,10]. Therefore, it is vital to establish a sensitive and reliable method for the E2 detection [11]. Liquid chromatography and/or gas chromatography/mass spectrometry [12,13] and HPLC have been used for the detection of E2. However, these chromatographic methods cannot meet requirements in the perspectives of time [14], cost, lower sensitivity and the need for trained people [15]. Biosensors possess exceptional features in the detection of E2 [16].

Optic based SPR sensor has great attention [17] and an important analytical instrument to determine and characterize molecular interactions [18] such as protein-protein, protein-peptide, protein-lipid, protein-nucleic acid [19], DNA hybridization, antibody-antigen, peptide-metal ion [20] in real-time, high sensitivity and selectivity and without labeling [21]. SPR sensors are increasingly used for the applications of biotechnology and biomedicine [22], biopharmaceutics [23], food [24] and environmental sciences [25]. There has recently been an essential need to develop new kinds of sensors [26]. Molecular imprinting technology which is an emerging technique has been used in sensing area [27-33]. This technology is an approach to create a polymer matrix containing molecular recognition sites which are characteristic in shape/functional groups of target molecule [34]. Molecular imprinted polymers (MIPs) are unique candidates as a ligand compared with antibodies and enzymes because of their good chemical and physical stabilities in extreme conditions and at high temperatures. And MIPs also offer some properties in the cost-effective polymer preparation, high affinity to target molecule and long-term stability [35].

In this paper, we have aimed to produce 17β-estradiol imprinted PHEMALM nanofilm on SPR chip (E2-MIPHEMALM-SPR sensor) for real-time detection of 17β-estradiol. For this goal, we prepared MALM as a functional monomer in the first step. And then, E2-MIPHEMALM-SPR sensor was produced and attached to the modified golden surface of the SPR chip. Detection studies for 17β-estradiol were applied with the use of aqueous 17β-estradiol solutions in 20–10000 ng/mL concentrations. The specificity and selectivity studies of the SPR chip were determined with cholesterol and stigmasterol.

2. MATERIALS AND METHODS

2.1. Materials.

The template molecule, 17β-estradiol, cholesterol, stigmasterol, 2-Hydroxyethyl methacrylate, L-leucine methyl ester, ethylene glycol dimethacrylate, α-α'-azobisisobutylonitrile were obtained from Sigma Chemical Co. (St. Louis, USA). All other chemicals purchased from Merck A.G. (Darmstadt, Germany).

2.2. Preparation of L-leucine methyl ester functional monomer and E2-MIPHEMALM-SPR sensor. Firstly, N-methacryloyl-(L) -leucine methyl ester (MALM) monomer was briefly as

follows: L-Leucine methyl ester (5.0 g) and hydroquinone (0.2 g) were dissolved in dichloromethane (CH_2Cl_2) (100 mL) solution. The solution was cooled to 0°C. Then, triethylamine (12.7 g) was added to this solution. And then, methacroyl chloride (5.0 mL) was slowly poured onto this solution. The reaction mixture was stirred under a nitrogen atmosphere with a magnetic stirrer for 2 hours at room temperature. Finally, methacroyl chloride, which did not react, was extracted with 10% NaOH solution. The aqueous phase was removed and the residue (MALM) was dissolved in ethyl alcohol. Before the preparation of E2-MIPHEMALM-SPR chip, the gold surface on the SPR chip was immersed in ethanol/water solution (1:1, v/v) and then immersed in acidic piranha solution (3:1 H_2SO_4:H_2O_2, v/v) after that, SPR chip was washed with ethanol and deionized water (DI), respectively and then dried in vacuum oven (200 mmHg, 40ºC) for 2 h. The SPR chip was rinsed with the usage of ethanol and dried with nitrogen gas. Afterwards, 3 mM allyl mercaptan (CH_2CHCH_2SH) solution was dropped on the SPR chip and then incubated in fume hood for 12 h [32]. After that, the SPR chip was washed with ethanol and DI for five time, respectively.

E2-MIPHEMALM-SPR was obtained by polymerizing the MALM-17β-estradiol (MALM-E2) pre-complex in the room temperature. A homogeneous solution of the pre-complex was prepared by adding 700 µl toluene and 1000 µl ACN aprotic solvent mixture to the MALM-E2 pre-complex. And next step, 150 µL HEMA monomer and 470 µL EGDMA cross-linker were added to the pre-complex and dissolved for 10 minutes. After adding 10 mg of AIBN as an initiator, 1 µl of the solution was dropped onto the surface of CH_2CHCH_2SH modified SPR chip to attach 17β-estradiol imprinted onto the SPR chip. Then, polymerization of the E2-MIPHEMALM-SPR chip was initiated by UV light (100W and 365 nm) at 25ºC under nitrogen atmosphere for 40 min. After polymerization, the unreacted monomer was removed with ethanol. Stoichiometric ratio is 1:15:24 of the template molecule, monomer and cross linker. Fig. 1 was shown the steps of preparation of the E2-MIPHEMALM-SPR chip. At the end, the unreacted monomers on the E2-MIPHEMALM-SPR chip were washed with methanol solution and DI, respectively for two times.

The NIPHEMALM-SPR chip was prepared in the same way to E2-MIPHEMALM-SPR chip without the addition of 17β-estradiol into the polymer solution.

2.3. Removal of E2-MIPHEMALM-SPR sensor.

To remove of 17β-estradiol, desorption agent's glycerol solution diluted to 20% (v/v) was used. The study of template removal was carried out via batch system. E2-MIPHEMALM-SPR sensor was placed into the 20mL glycerol solution and shacked slowly in bath for 2 h. After this step, the E2-MIPHEMALM-SPR sensor was washed with DI and dried.

2.4. Surface characterization of E2-MIPHEMALM-SPR SPR sensor.

The characterization of E2-MIPHEMALM-SPR and NIPHEMALM-SPR sensors were carried out by FTIR-ATR spectrophotometer (Thermo Fisher Scientific, Nicolet iS10, Waltham, MA, USA), AFM Nanomagnetic Instruments, (Oxford, UK) to gain the surface roughness, ellipsometry (Nanofilm EP3, Goetting, Germany) to obtain the thickness of SPR sensor, and contact angle (KRÜSS DSA 100, Hamburg, Germany) measurement to determine the hydrophilicity/hydrophobicity of SPR sensor surface.

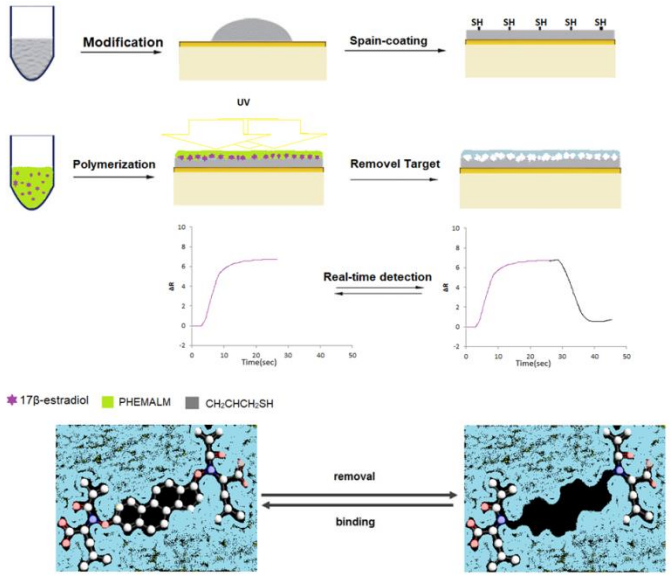

Figure 1. Schematic of preparation of E2-MIPHEMALM-SPR chip.

FTIR-ATR spectrophotometer was used to characterize the E2-MIPHEMALM-SPR sensor. For this purpose, the SPR sensor was measured in the range of 400-4000 cm^{-1} at 2 cm^{-1} resolutions. This observation was done by using AFM in tapping mode in air atmosphere. E2-MIPHEMALM-SPR sensor was placed on the sample holder by using double-side carbon strip. AFM takes measurements in high resolution (i.e., 4096 x 4096) and the experimental parameters applied were oscillation frequency (341.30 kHz), vibration amplitude (1 V_{RMS}) and free vibration amplitude (2 V_{RMS}). Sample was scanned at the scanning rate of 2 µm s^{-1} and 256 x 256 pixels' resolution to obtain view of 1 µm x 1 µm area.

Ellipsometry measurements were performed by using an auto-nulling imaging ellipsometry. The thickness measurements have been done at a wavelength of 658 nm with an angle of incidence 65º. A four-zone auto-nulling procedure, air/polymer/gold/chrome-glass, integrating over a sample area of approximately 350 µm x 350 µm followed by a fitting algorithm has been performed in the thickness analysis of layer. Contact angle of the E2-MIPHEMALM-SPR sensor was measured with sessile drop method with the using water as liquid phase.

2.5. Kinetic analyses with E2-MIPHEMALM-SPR sensor.

E2-MIPHEMALM-SPR sensor was used for the real-time detection of 17β-estradiol from aqueous solution. Therefore, a SPR system (GenOptics, SPRiLab, Orsay, France) integrating sensor immobilized onto the gold surface. In brief, all experimental procedures of the monitoring of 17β-estradiol from aqueous solution may be summarized as: the sensor was washed with 50 mL DI and 50 mL pH: 11.0 carbonate buffer. Then 17β-estradiol solutions in 20 ng/mL-10,000 ng/mL concentrations were applied to the SPR system. And the response of E2-MIPHEMALM-SPR sensor was monitored until it reached stable

frequency for 35 min. Then, 20 mL desorption agent's glycerol solution was applied with 2.0 mL/min flow-rate. After this step, E2-MIPHEMALM-SPR sensor was washed with DI and pH: 11.0 carbonate buffer, respectively. Adsorption, desorption and cleaning steps were repeated for the monitoring of each 17β-estradiol solutions in different concentrations. The real-time detection of cholesterol, stigmasterol and 17β-estradiol solutions in singular manner and mixed manner solutions such as 17β-estradiol/cholesterol, 17β-estradiol/stigmasterol, cholesterol/ stigmasterol and 17β-estradiol/cholesterol/stigmasterol in competitive manner was monitored in order to determine the specificity of E2-MIPHEMALM-SPR sensor.

3. RESULTS

3.1. Surface characterization of the SPR sensors.

In order to prove polymeric formation on the SPR chip surface, (i) the specific bands of the polymeric nanofilm were determined as carbonyl band at 1721 cm^{-1} and aliphatic -CH band at 2947 cm^{-1} (Fig. 2A). (ii) AFM image of the chip surface was given in Fig. 2B. As clearly seen from the image that a polymeric surface attached on the chip was formed because of polymerization process. As seen in the Figure, the surface deepness of the clean SPR, E2-MIPHEMALM-SPR and NIPHEMALM-SPR sensors surfaces were determined as 4.55 nm, 16.45 nm and 30.02 nm, respectively. There were shown a polymeric nanofilm on the sensor surface. (iii) Ellipsometry measurements were performed to obtain the data about sensor surface. Surface depth on the sensor surface from ellipsometry was obtained as 24.2 nm of the E2-MIPHEMALM-SPR and 29.00 nm of the NIPHEMALM-SPR sensor attached (Fig. 2C). (iv) Contact angle measurements with water were carried out to characterize the sensor surface (Fig. 2D). The contact angle values of SPR chip surface were decreased from 81.4º to 65.6º and 81.4º to 63.3º of the E2-MIPHEMALM-SPR and NPHEMALM-SPR sensors, respectively. This data in contact angle value of the chip surface stemmed from the increased hydrophilic structure of the chip surface.

3.2. Kinetic studies with E2-MIPHEMALM-SPR sensor

Several studies have already been established on E2 determination in various environments. Auberšek and colleagues found 4.2 ng/L in hospital wastewater and 2.4 ng/L-12.7 ng/L E2 in samples taken from the wastewater treatment plant [36]. McAvoy has determined 7.22 ng/L and 0.557 ng/L E2 for samples taken from the wastewater treatment plant and the garbage collection site, respectively by gas chromatograph-mass spectrometry [37]. Numerous studies have been carried out on the determination of E2 with biosensors. Hui-Bin and colleagues have determined E2 from human urine at concentrations of 0.75-135 μg/mL [38]. In another study, the fluorescent sensory system determined E2 at 30-5000 ng/mL that used MIP technique in liquid chromatography [39]. Jia and co-workers were used magnetic nanoparticles as amplifying element for detection of E2 in the SPR system. The magnetic nanoparticles via signal amplification protein A were conjugated with anti-estradiol monoclonal antibody. This method was resulted high sensitively for E2 detection in the range of 1.95-2000 ng/mL [40]. The E2-MIPHEMALM-SPR sensor was prepared for real-time monitoring of the interactions between the recognition sites that nanofilm produced by molecularly imprinted approach for the target analyte and E2 from aqueous solution. The SPR sensor has interacted with aqueous E2 solutions in different concentrations in the range of 20-10000 ng/mL (Fig. 3A). As seen from Figure, each step including equilibration–adsorption–desorption–regeneration for a new analysis was run in 40 min for E2 molecules. The increase in E2 concentration also triggered an increased reflectivity (ΔR) in sensor.

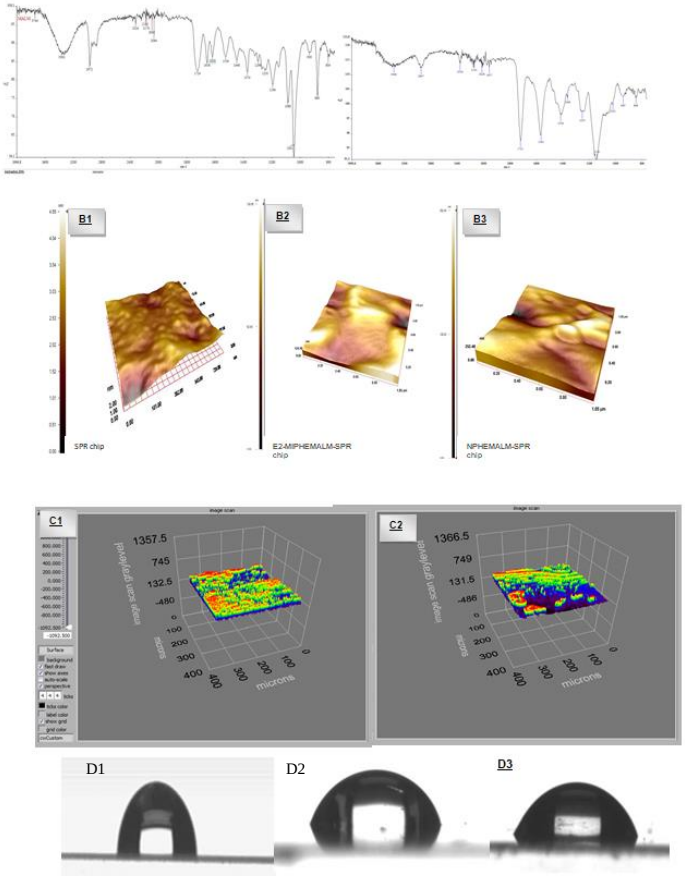

Figure 2. A. FTIR-ATR spectrophotometer of L-leucine methyl ester monomer and E2-MIP polymer. **B1.** AFM images of the cleaned, **B2.** E2-MIPHEMALM-SPR and **B3.** NPHEMALM-SPR chips surface, **C1.** Ellipsometry images of E2-MIPHEMALM-SPR and **C2.** Ellipsometry images of NPHEMALM-SPR chips. **D1.** Contact angle images of the clean, **D2.** E2-NIPHEMALM-SPR and **D3.** E2-MIPHEMALM-SPR chips surface.

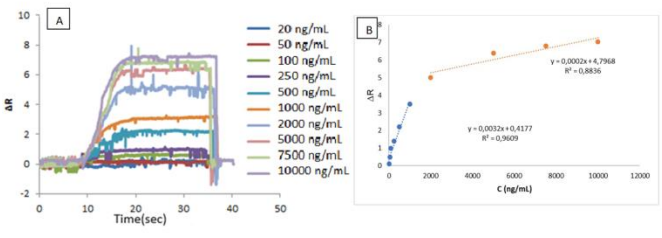

Figure 3. A. Response of E2-MIPHEMALM-SPR sensor for E2, experimental conditions: pH: 11.0 carbonate buffer, 50mL, temperature: 25˚C, concentrations range: 20-10000 ng/mL and 2.0 mL/min flow-rate, **B.** Calibration curve of different concentration.

Fig. 3B illustrates the concentration dependency of E2-MIPHEMALM-SPR sensor for aqueous E2 solutions. As expected, an increase in concentration also caused an increase in sensor response. The binding tendencies of E2-MIPHEMALM-SPR sensor to E2 molecules have high linearity and affinities with regression constants as 96% in the range of 20-100 µg/mL and 88.3% in the range of 200-10000 ng/mL.

3.3. Selectivity of E2-MIPHEMALM-SPR sensor.

Selectivity of E2-MIPHEMALM-SPR sensor was determined by their responses to cholesterol, stigmasterol and 17β-estradiol solutions in singular manner and mixed manner solutions such as 17β-estradiol/cholesterol, 17β-estradiol/stigmasterol, cholesterol/stigmasterol and 7β-estradiol/cholesterol/stigmasterol in competitive manner cholesterol and stigmasterol, which are similar in size/shape and molecular weight with 17β-estradiol. The concentration of cholesterol, stigmasterol and 17β-estradiol were kept constant as 1000 ng/mL. The NIPHEMALM-SPR SPR sensor was applied in the same manner for determining the non-selective binding. Fig. 4A shows the responses of SPR sensor systems for cholesterol and stigmasterol were lower than 17β-estradiol. Also, NIPHEMALM-SPR sensor caused low values when cholesterol, stigmasterol and 17β-estradiol introduced to the SPR sensor in both E2-MIPHEMALM-SPR and NIPHEMALM-SPR sensors (Fig. 4B).

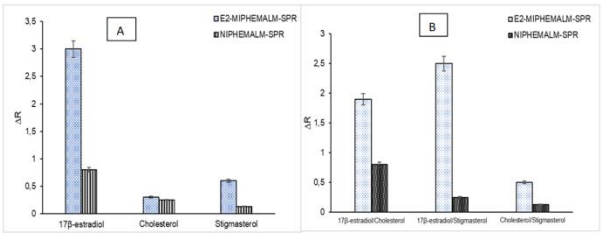

Figure 4. Selectivity of E2-MIPHEMALM-SPR and NIPHEMALM-SPR sensors for **A.** 17β-estradiol, Cholesterol, Stigmansterol, and **B.** 17β-estradiol/Cholesterol, 17β-estradiol/Stigmansterol and Cholesterol/Stigmansterol.

The ΔR values of E2-MIPHEMALM-SPR sensor were obtained as 3, 0.3, 0.6 for 17β-estradiol, cholesterol and stigmasterol, respectively. The ΔR values of NIPHEMALM-SPR sensor were obtained as 0.8, 0.25, 0.13 for E2, cholesterol and stigmasterol. E2-MIPHEMALM-SPR sensor was more selective with respect to the aqueous solutions of 17β-estradiol, cholesterol and stigmasterol. The excellent characteristics of special cavities of 17β-estradiol were showed higher responses against 17β-estradiol. As seen in Table 1, 17β-estradiol imprinted chip was obtained higher responses against other molecules. SPR sensor response was investigated of E2-MIPHEMALM-SPR and NIPHEMALM-SPR sensors that k showed the selectivity coefficient versus competing molecules for E2 and k' for relative selectivity coefficient for E2-MIPHEMALM-SPR sensor versus NIPHEMALM-SPR sensor.

Table 1. Selectivity Coefficients of E2-MIPHEMALM-SPR and NIPHEMALM-SPR sensors.

	E2-MIPHEMALM-SPR		NIPHEMALM-SPR		
	ΔR	k	ΔR	k	k'
17β-estradiol	3.00	-	1.05	-	-
Stigmasterol	0.60	5.00	0.25	4.20	1.20
Cholesterol	0.30	10.00	0.13	8.07	1.23

3.4. Mathematical analysis of kinetic data

In order to describe the detection system between E2-MIPHEMALM-SPR sensor and 17β-estradiol and to analyze the interaction kinetic, five models such as association kinetic analysis and Scatchard, Langmuir, Freundlich and Langmuir-Freundlich models were carried out biosensing data (Table 2).

Association kinetic analysis $d\Delta R / dt = k_a C (\Delta R_{max} - \Delta R) - k_d \Delta R$ (1)

Scatchard $\Delta R_{ex} / [C] = K_A(\Delta R_{max} - \Delta R_{eq})$ (2)

Langmuir $\Delta R = \{\Delta R_{max} [C] / K_D + [C]\}$ (3)

Freundlich $\Delta R = \Delta R_{max} [C]^{1/n}$ (4)

Langmuir-Freundlich $\Delta R = \{\Delta R_{max} [C]^{1/n} / K_D + [C]^{1/n}\}$ (5)

The Langmuir isotherm model was the best adapted to surfaces, that shows the 17β-estradiol binding properties in the SPR sensor surface are homogeneously distributed, monolayer, co-energetic and with minimal lateral interaction. (R^2 = 0.9985).

Table 2. Kinetic and Isotherm Parameters.

Langmuir		Freundlich		Langmuir-Freundlich	
ΔR_{max}, µg/ml	**7.28**	ΔR_{max}, µg/ml	2.356	ΔR_{max}, µg/ml	2.78
K_D, ml/µg	**0.1354**	1/n	0.6436	1/n	0.1150
K_A, µg/ml	**7.39**	R^2	0.9629	K_D, ml/µg	1.69
R^2	**0.9985**			K_A, µg/ml	0.592
				R^2	0.9749

3.5. Reproducibility of E2-MIPHEMALM-SPR sensor.

Molecularly imprinted polymers possess several advantages compared with other bio-ligands used as recognition elements. Ten equilibration-adsorption-regeneration cycles were applied with the use of aqueous 17β-estradiol solution under the same conditions (1.0 µg/mL, 2.0 mL/min. flow rate, 25ºC). E2-MIPHEMALM-SPR sensor has exhibited a good reproducibility without decrease during the ten cycles.

4. CONCLUSIONS

The specific detection of 17β-estradiol molecules is challenging and it is crucial for clinical chemistry. In this study, we were prepared E2-MIPHEMALM-SPR chip for detection of 17β-estradiol. E2-MIPHEMALM-SPR sensor has been successfully used for the real-time detection of 17β-estradiol from aqueous solution by SPR method. The selectivity studies show 3.0, 0.3, 0.6 ΔR values for E2, cholesterol and stigmasterol, respectively. E2-MIPHEMALM-SPR sensor was more selective of 17β-estradiol. According to these values, the imprinting technique enables the biorecognition of 17β-estradiol in SPR sensor. The excellent characteristics of special cavities of 17β-estradiol were showed higher responses against 17β-estradiol. E2-MIPHEMALM-SPR sensor was showed high sensitivity, real-time monitoring, low detection concentration and low volume sample against other methods. Also, 17β-estradiol detection was easy to perform and can be a cost-effective solution and reusability.

5. REFERENCES

1. Liu, Z.H.; Kanjo, Y.; Mizutani, S. Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment—physical means, biodegradation, and chemical advanced oxidation: A review. *Sci. Total. Envir.* **2009**, *407*, 731-748, https://doi.org/10.1016/j.scitotenv.2008.08.039.
2. Rogers, J.A.; Metz, L.; Yong, V.W. Review: Endocrine disrupting chemicals and immune responses: A focus on bisphenol-A and its potential mechanisms. *Mol. Immunol.* **2013**, *53*, 421-430, https://doi.org/10.1016/j.molimm.2012.09.013.
3. Jobling, S.; Nolan, M.; Tyler, C.R.; Brighty, G.; Sumpter, J.P. Widespread sexual distruption in wild fish. *Envir. Sci. Technol.* **1998**, *32*, 2498-2506, https://doi.org/10.1021/es9710870**.**
4. Jiang, X.; Waterland, M.; Blackwell, L.; Wu, Y.; Jayasundera, K.P.; Partridege, A. Sensitive determination of estriol-16-glucuronide using surface plasmon resonance sensing. *Steroids* **2009**, *74*, 819-824,**.**
5. Volpe, G.; Fares, G.; Quadri, F.D.; Draisci, R.; Ferretti, G.; Marchiafava, C.; Moscone, D.; Palleschi, G. A disposable immunosensor for detection of 17β-estradiol in non-extracted bovine serum. *Analytica. Chim. Acta.* **2006**, *572*, 11-16, https://doi.org/10.1016/j.aca.2006.05.008**.**
6. Chaisuwan, N.; Xu, H.; Wu, G.; Liu, J. A highly sensitive differential pulse anodic stripping voltammetry for determination of 17β-estradiol (E2) using CdSe quantum dots based on indirect competitive immunoassay. *Biosens. Bioelectron.* **2013**, *46*, 150-154, https://doi.org/10.1016/j.bios.2013.02.041**.**
7. Golden, R.J.; Noller, K.L.; Titus-Ernstoff, L.; Kaufman, R.H.; Mittendorf, R.; Stillman, R.; Reese, E.A. Environmental endocrine modulators and human health: An assessment of the biological evidence. *Crit. Rev. Toxicol.* **1998**, *28*, 109-227, https://doi.org/10.1080/10408449891344191.
8. Gao, R.; Cui, X.; Hao, Y.; Zhang, L.; Liu, D.; Tang, Y. A highly-efficient imprintedmagnetic nanoparticle for selective separation and detection of 17β-estradiolin milk. *Food. Chem.* **2015**, *194*, 1040–1047, https://doi.org/10.1016/j.foodchem.2015.08.112.
9. Li, R.; Liu, Y.; Yan, T.; Li, Y.; Cao, W.; Wei, Q.; Du, B. A competitive photoelectrochemical assay for estradiol based on in situ generatedCdS-enhanced TiO2. *Biosens. Bioelectron.* **2015**, *66*, 596–602, https://doi.org/10.1016/j.bios.2014.12.002**.**
10. Blumberg, B. Endocrine disrupting chemicals and disease susceptibility. *J. Steroid Biochem.* **2011**, *127*, 204–215, https://doi.org/10.1016/j.jsbmb.2011.08.007**.**
11. Huang, K.J.; Liu, Y.J.; Zhang, J.Z.; Cao, J.T.; Liu, Y.M. Aptamer/Au nanoparticles/cobalt sulfide nanosheets biosensor for 17β-estradiol detection using a guanine-rich complementary DNA sequence for signal amplification. *Biosens. Bioelectron.* **2015**, *67*, 184-191, https://doi.org/10.1016/j.bios.2014.08.010.
12. Heub, S.; Tscharner, N.; Monnier, V.; Kehl, F.; Dittrich, P.S.; Follonier, S.; Barbe, L. Automated and portable solid phase extraction platform for immuno-detection of 17β-estradiol in water. *J. Chromatogr. A.* **2015**, *1381*, 22-28, https://doi.org/10.1016/j.chroma.2014.12.076**.**
13. Monerris, M.J.; Arévalo, F.J.; Fernández, H.; Zon, M.A.; Molina, P.G. Developmentof a very sensitive electrochemical immunosensor for the determination of17β-estradiol in bovine serum samples. *Sens. Actuators. B.* **2015**, *208*, 525–531, http://dx.doi.org/10.1016/j.snb.2014.11.048**.**
14. Fan, L.; Zhao, G.; Shi, H.; Liu, M. A simple and label-free aptasensor based on nickel hexacyanoferrate nanoparticles as signal probe for highly sensitive detection of 17β-estradiol. *Biosens. Bioelectron.* **2015**, 68, 303-309, https://doi.org/10.1016/j.bios.2015.01.015**.**
15. Xin, T.B.; Chen, H.; Lin, Z.; Liang, S.X.; Lin, J.M. A secondary antibody format chemiluminescence immunoassay for the determination of estradiol in human serum. *Talanta.* **2010**, *82*, 1472-1477, https://doi.org/10.1016/j.talanta.2010.07.023
16. Vanschoenbeek, K.; Vanbrabant, J.; Hosseinkhani, B.; Vermeeren, V.; Michiels, L. Aptamers targeting different functional groups of 17β-estradiol. *J. Steroid. Biochem. Mol. Biol.* **2015**, *147*, 10-16, https://doi.org/10.1016/j.jsbmb.2014.10.013**.**
17. Li, Y.; Yan, Y.; Lei, Y.; Zhao, D.; Yuan, T.; Zhang, D.; Cheng, W.; Ding, S. Surface plasmon resonance biosensor for label-free and highlysensitive detection of point mutation using polymerizationextension reaction. *Colloids. Surf. B.* **2014**, *120*, 15-20, https://doi.org/10.1016/j.colsurfb.2014.04.007**.**
18. Bakhshpour, M.; Piskin, A.K.; Yavuz, H.; Denizli, A. Quartz crystal microbalance biosensor for label-free MDA MB 231 cancer cell detection via notch-4 receptor. *Talanta.* **2019**, *204*, 840-845, https://doi.org/10.1016/j.talanta.2019.06.060.
19. Couture, M.; Zhao, S.S.; Masson, J.F. Modern surface plasmon resonance for analytics and biophysics. *Phys. Chem. Chem. Phys.* **2013**, *15*, 11190-11216, https://doi.org/10.1039/c3cp50281c.
20. Safran, V.; Göktürk, I.; Derazshamshir, A.; Yılmaz, F.; Sağlam, N.; Denizli, A. Rapid sensing of Cu+2 in water and biological samples by sensitive molecularly imprinted based plasmonic biosensor. *Microchem. J.* **2019**, *148*, 141-150, https://doi.org/10.1016/j.microc.2019.04.069**.**
21. Gür, S.D.; Bakhshpour, M.; Denizli, A. Selective detection of Escherichia coli caused UTIs with surface imprinted plasmonic nanoscale sensor. *Mater. Sci. Eng. C.* **2019,** *104*, 109869-76, https://doi.org/10.1016/j.msec.2019.109869**.**
22. Bakhshpour, M.; Özgür, E.; Bereli, N.; Denizli, A. Microcontact imprinted quartz crystal microbalance nanosensor for protein C recognition. *Colloids. Surf. B.* **2017**, *151*, 264-270, https://doi.org/10.1016/j.msec.2019.109869**.**
23. Thillaivinayagalingam, P.; Gommeaux, J.; McLoughlin, M.; Collins, D.; Newcombe, A.R., Biopharmaceutical production: Application of surface plasmon resonance biosensors. *J. Chromatogr. B.* **2010**, *878*, 149-153, https://doi.org/10.1016/j.jchromb.2009.08.040**.**
24. Wei, W.; Nong, J.; Zhu, Y.; Zhang, G.; Wang, N.; Luo, S.; Chen, N.; Lan, G.; Chuang, C-J.; Huang, Y. Graphene/Au-Enhanced Plastic Clad Silica Fiber Optic Surface Plasmon Resonance Sensor. *Plasmonic.* **2018**, *13*, 483–491. https://doi.org/10.1007/s11468-017-0534-0.
25. Chen, X.; Xi, L.; Li, C. Surface Plasmon Resonance Sensor Based on a Novel D-Shaped Photonic Crystal Fiber for Low Refractive Index Detection. *IEEE.* **2018**, *10*, 6800709. https://doi.org/10.1109/JPHOT.2018.2790424.
26. Zhou, C.; Zou, H.; Li, M.; Sun, C.; Ren, .; Li, Y. Fiber optic surface plasmon resonance sensor for detection of E. coli O157:H7 based on antimicrobial peptides and AgNPs-rGO. *Biosens. Bioelectron.* **2018**, *117*, 347-353. https://doi.org/10.1016/j.bios.2018.06.005.
27. Saylan, Y.; Akgönüllü, S.; Yavuz, H.; Ünal, S.; Denizli, A. Molecularly Imprinted Polymer Based Sensors for Medical Applications. *Sensors.* **2019**, *19*, 1279-98, https://doi.org/10.3390/s19061279.
28. Zhao, Y.; Lei, M.; Liu, S.X.; Zhao, Q. Smart hydrogel-based optical fiber SPR sensor for pH measurements. *Sens. Actuators. B. Chem.* **2018**, *261*, 226-232, https://doi.org/10.1016/j.snb.2018.01.120**.**

29. Perçin, I.; Idil, N.; Bakhshpour, M.; Yılmaz, E.; Mattiasson, B.; Denizli, A. Microcontact Imprinted Plasmonic Nanosensors: Powerful Tools in the Detection of Salmonella paratyphi. *Sensors.* **2017**, *17* 127-140, http://dx.doi.org/10.3390/s17061375**.**
30. Rifat, A.A.; Haider, F.; Ahmed, R.; Mahdiraji, G.A.; Adikan, F.R.M.; Miroshnichenko, A.E. Highly sensitive selectively coated photonic crystal fiber-based plasmonic sensor. *Optic. Lett.* **2018**, *43*, 891-894. https://doi.org/10.1364/OL.43.000891.
31. Zhang, L.; Zhu, C.; Chen, C.; Zhu, S.; Zhou, J., Wang, M.; Shang, P. Determination of kanamycin using a molecularly imprinted SPR sensor. *Food. Chem.* **2018**, *266*, 170-174. https://doi.org/10.1016/j.foodchem.2018.05.128.
32. Liu, Z.; Yang, X.; Zhang, Y.; Zhang, Y.; Zhu, Z.; Yang, X.; Zhang, J.; Yang, J.; Yuan, L. Hollow fiber SPR sensor available for microfluidic chip. *Sens. Actuators. B.* **2018**, *265*, 211-216. https://doi.org/10.1016/j.snb.2018.03.030.
33. Ahmad, O.S.; Bedwell, T.S.; Esen, C.; Garcia-Cruz, A.; Piletsky, S.A. Molecularly Imprinted Polymers in Electrochemical and Optical Sensors. *Trends in Biotechnology*. **2019**, *37*, 294-309. https://doi.org/10.1016/j.tibtech.2018.08.009.
34. Bakhshpour, M.; Yavuz H.; Denizli, A. Controlled release of mitomycin C from PHEMAH–Cu(II) cryogel membranes, *Artif. Cell. Nanomed. B.* **2018**, *46*, 946-954. https://doi.org/10.1080/21691401.2018.1439840.
35. Rezaei, B.; Rahmanian, O.; Ensafi, A.A. An electrochemical sensor based on multiwall carbon nanotubes and molecular imprinting strategy for warfarin recognition and determination. *Sens. Actuators. B. Chem.* **2014,** *196*, 539-545, https://doi.org/10.1016/j.snb.2014.02.037.
36. Avberšek, M.; Sömen, J.; Heath, E. Dynamics of steroid estrogen daily concentrations in hospital effluent and connected waste water treatment plant. *J. Envir. Monitor.* **2011**, *13*, 2221-2226, https://doi.org/10.1039/c1em10147a**.**
37. McAvoy, K. Occurence of estrogens in wastewater treatment plant and waste disposal site water samples. *Clear waters*. **2008**, *38*, 28-34**.**
38. Hui-Bin, W.; Jin-Ming, L.; Dan-Ning, W.; Li-Xia, Z.; Zhen-Jia, L.; Xi-Tang, Y. Detection of 17β-Estradiol in River Water and Human Urine by Highly Sensitive Chemiluminescence Enzyme Immunoassay. *Chinese. J. Anal Chem.* **2007**, *35*, 320-324, https://doi.org/10.1016/S1872-2040(07)60037-1**.**
39. Rachkov, A.; McNiven, S.; El'skaya, A.; Yano, K.; Karube I. Fluorescence detection of β-estradiol using a molecularly imprinted polymer. *Anal. Chimica. Acta* **2000**, *405*, 23-29, https://doi.org/10.1016/S0003-2670(99)00743-6.
40. Jia, Y.; Peng, Y.; Bai, J.; Zhang, X.; Cui, Y.; Ning, B.; Cui, J.; Gao, Z. Magnetic nanoparticle enhanced surface plasmon resonance sensor for estradiol analysis, *Sens. Actuators. B. Chem.* **2018**, *254*, 629-635, https://doi.org/10.1039/c7an00469a.