3D PRINTING TECHNOLOGIES FOR ELECTROCHEMICAL SENSING: 17-ALPHA-ETHINYLESTRADIOL DETECTION

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Abstract:

Monitoring of trace organic contaminants in aquatic media has become a major concern today. A 3D-printed device combined MIP-SPE extraction and electrochemical detection was developed for the determination of ethinyl estradiol (EE); one of endocrine-disrupting compounds (EDCs).

The proposed method was applied for the detection of EE in river water samples in order to monitor its concentration. A linear response was obtained for analyte in the concentration range from 6.6 to 1064 $\mu g.mL^{-1}$, with a quantification limit of 20.0 $\mu g.mL^{-1}$.

Keywords: Ethinyl estradiol, 3D printing, Electrochemical sensor, Water analysis

1. INTRODUCTION

Ethinyl estradiol (EE) is a synthetic estrogenic hormone and the active ingredient of many oral contraceptive formulations. EE was described as one of the EDCs, these are compounds interfere with the balance of the endocrine system [1]. EDCs can bind to the endocrine receptors either to activate, mimic or interfere with the action of endogenous hormones. If EE flows into the surface waters through drainage, it has been reported to promote the feminization of wildlife, affect human health and there are recent concerns about its impact on the ecosystem.

In order to evaluate the effect of EE on the ecosystem and human health, it is necessary to monitor EE in the environmental water. Up to now, different analytical methods were reported for the determination of EE including spectrophotometry [2], high performance liquid chromatography with fluorescence detection [3], or mass spectrometry detection[4]. Electrochemical methods were also reported for the determination of EE in different matrices either pharmaceutical formulations [5] or environmental samples.

Moreover, the analysis of compounds at low concentrations in complex matrices requires a sensitive analytical method and an efficient sample preparation procedure in order to extract and pre-concentrate the analytes at trace levels. SPE (Solid phase extraction) is a sample preparation technology that uses solid particle, chromatographic packing material, usually contained in a cartridge type device, to chemically separate the different components of a sample. The intent is to simplify complex sample matrices, purify the analytes of interest, fractionate a complex mixture of active compounds, or concentrate any trace levels of analytes

In the last decade, Molecularly Imprinted Polymers (MIPs) have been developed to selectively adsorb contaminants from environmental waters. MIPs are cross-linked polymers, which are synthesized in the presence of a template molecule, a monomer and a porogen solvent. Cross-linkers enclose the pre-polymerisation complex formed by the functional monomer and the template. After polymerization, removal of the template leaves cavities with geometrical and functional properties able to specifically recognize the template molecule and its analogues.

Therefore, in this study, we attempted to develop a 3D printed device based on MIP-SPE selective extraction followed by electrochemical analysis. Within the proposed method, various parameters such as linearity range, sensitivity parameters, reproducibility, robustness and selectivity were examined. Additionally, it was applied for detection of EE in river water sample.

2. EXPERIMENTAL SECTION

2.1. Chemicals, reagents, materials

17-alpha-ethinylestradiol was purchased from Aldrich. Ethanol, methanol, acetonitrile, and reagents were of analytical-grade quality and were purchased from.

SPE with Molecularly imprinted polymer specifically designed for estrogens recognition (product code: AFFINIMIP Estrogens) was provided by Polyintell (Val de Reuil, France).

2.2. Instrumentation

The electrochemical measurements were performed with a CHI1222B coupled to a microcomputer containing CHI software. A conventional three-electrode system was used. A modified glassy carbon electrode (GCE) was used as the working electrode. A platinum wire was employed as the counter electrode and $Ag/AgNO_3$ electrode served as the reference electrode.

2.3. CAD and 3D PRINTING

Free CAD was used as computer aided design. A Formlab stereolithography and a Polyjet Objet30 were used for 3D printing devices. The resin used are biocompatible resin, and RGD525 respectively.

2.4. Calibration curve

A stock solution of ethinyl estradiol (EE) (2 mmol L^{-1}) was prepared.
The calibration curve was obtained by successive additions from this stock solution in triplicate analysis at ten different levels of concentration in mineral water.

2.5. MIP-SPE extraction

The MIP-SPE sorbent was first conditioned with 5 mL acetonitrile and 5 mL UP water. Fortified mineral water samples were applied (10 ml or 100 ml) and then washed with 5 mL UP water and 5 mL UP water/MeOH (60:40, v/v). Target EE were eluted with 3 mL MeOH.

2.6. Method Application in River Sample

The water sample of the river was collected upstream of the OISE river. This sample of water was enriched with standard EE solution. Small and high water volumes, from 10 mL to 100 mL, can be preconcentrated.

3. DESCRIPTION OF THE WORK

3.1. Additive manufacturing of the device

Additive manufacturing, refer also to "3D printing", is the process of joining materials to create objects based on digital models, using computer aided design or CAD (**Figure 1**A). 3D printing is a process whereby a 3D design is turned into a real object. First, software is used to slice the 3D design into layers, and then the design is printed layer by layer on a 3D printer. A support structure is created, where needed, in order to deal with overhangs and cavities.
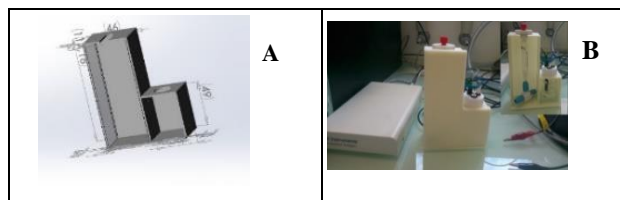

Figure 1. (A) CAO (B) device with a potentiostat

Two types of 3D printing technology were used: the first one is polyjet and the second one is stereolithography (SLA). Objet's patented PolyJet inkjet technology works by jetting photopolymer materials in ultra-thin layers (thickness less than 50 µm) onto a build tray, layer by layer until the model is completed. Each photopolymer layer is cured by UV light immediately after being jetted, producing fully cured models that can be handled and used immediately. The gel-like support material, which is specially designed to support complicated geometries, can easily be removed by hand and water jetting. In SLA, the process takes place in a large tank and begins when a layer of liquid polymer is spread over a platform. This machine then uses a computer controlled laser to draw the first layer onto the surface of a liquid polymer, which hardens when struck by the laser. The model is then lowered and the next layer is then drawn directly on top of the previous one. This is repeated until the model is finished. When the object is complete, it is raised out of the tank via the supporting platform with the excess liquid flowing away. Stereolithography is the oldest 3D Printing technology
The first device, Figure 1B, is composed of two compartments, one allowing the positioning of the SPE cartridge, and a second allowing the electrochemical detection.

3.2. Electrochemical characterization

The electrochemical measurements were performed with a conventional three-electrode system. A glassy carbon electrode (GCE) was used as the working electrode. A platinum wire was employed as the counter electrode and $Ag/AgNO_3$ electrode served as the reference electrode. $LiBF_4$ was used as supporting electrolyte. Initially, three different electroanalytical techniques were evaluated, namely cyclic voltammetry (CV), linear voltammetry (LV), and differential pulse voltammetry (DPV), with the objective of ascertaining which technique presented the highest sensitivity for the determination of EE using the printed device. The technique that presented a higher sensitivity for EE determination using the sensor under study was differential pulse voltammetry. The response of the analyte of

interest is shown in Figure 2 (the LV overlaps the CV and is therefore not represented).
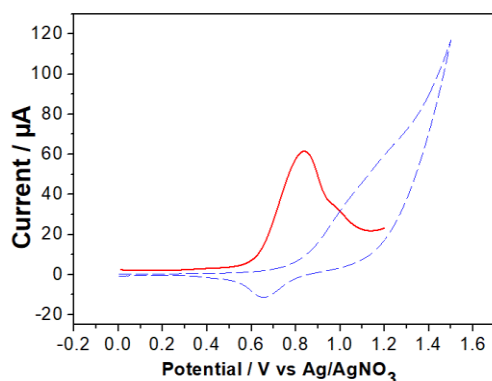

Figure 2. CV (— —) and DPV (——) of a solution of mineral water doped with EE after SPE extraction and using electrochemical sensing.

The technique that presented a higher sensitivity for EE determination using the sensor under study was DPV, which presents a strong oxidation peak at 0.7 V vs Ag/AgNO$_3$.

As shown in Figure 3 (left), in differential pulse voltammetry sequence, several parameters can be adjusted, such as pulse width, amplitude, or pulse period.
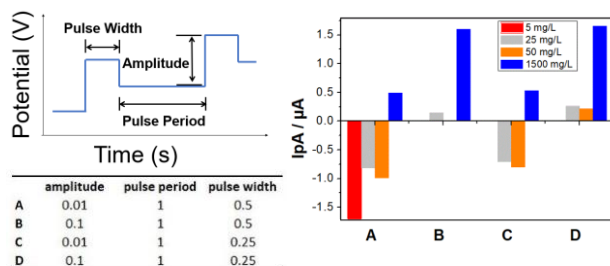

Figure 3. Optimization of DPV parameters.

These settings were varying in order to optimize the electrochemical signal and to find the best conditions. At each sequence, the current Ipa was measured and compared (Figure 3, right). The best conditions were found to be 0.1V for the amplitude, 1s for the pulse period and 0.25s for the pulse width.

3.3. Validation of analytical method

The validation of analytical methodologies is extremely important because it ensures that the analytical methodology is accurate, precise, linear, and stable over a specific range of analysis [6].

Linearity

The standards were prepared at ten different levels of concentration in mineral water. The results can be observed in Figure 4, which shows the analytical curve.
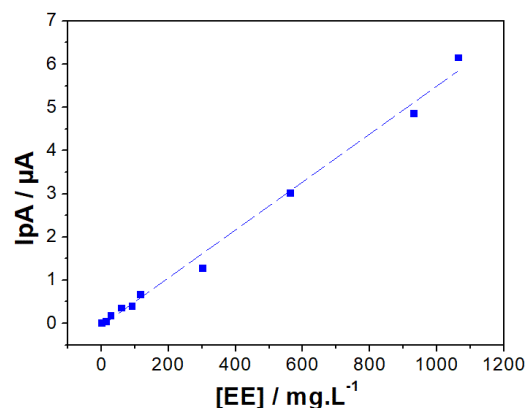

Figure 4. Analytical curve constructed from the voltammogram

The MIP-SPE-electrochemical sensor showed linearity in the concentration range of 0 to 1064 µg.mL-1, with a linear correlation coefficient of 0.9966 and a sensitivity of 0.0055 µA.mL.µg $^{-1}$, which is a larger dynamic range than most sensors reported in the literature.[7]

Limit of detection and limit of quantification

With the analytical curve, it is possible to establish other important parameters as the limit of detection (LD) and the limit of quantification (LQ) for the validation of the proposed methodology (Table 1).

Table 2. Limit of detection and quantification of the printed device for Ethinyl estradiol (blank n=9)

Parameters	Result
LD	6.6 µg.mL^{-1}
LQ	20.0 µg.mL^{-1}

LD and LQ of the printed device for Ethinyl estradiol are respectively 6.6 µg.mL^{-1} and 20.0 µg.mL^{-1}, which is, at least, 100 times larger than previously reported sensors [7].

Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment and takes into account more changes than repeatability.

These factors are constant within a day (i.e. behave systematically within day timescale) but are not constant over a longer time period and thus behave as random in the context of intermediate precision. The analytical curve was performed on 3 different days with the same standards (Figure 5).
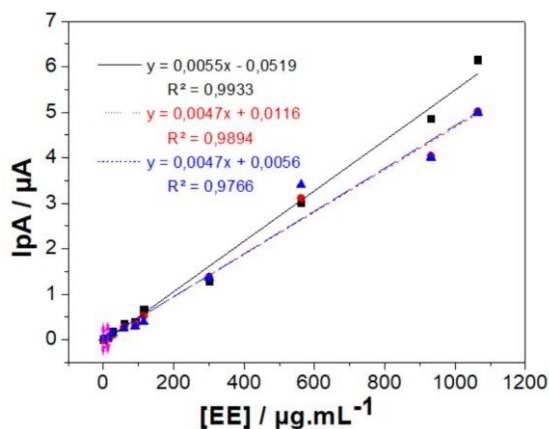
Figure 5. Intermediate precision (——)Day 1 (— -) Day 2 (— —) Day 3

As can be seen from Figure 5, the slope of the regression line for Day 1 (D1) is 0.0055, the slopes of the regression line for Day 2 (D2) and Day 3 (D3) are 0.0047. The slopes were compared with the aid of statistical tests (F- and t-test).

Table 3. Values

	D1-D2	D2-D3	D1-D3
F	1.122	2.263	2.540
t	11.210	0.397	-8.275

The null hypothesis, H_0 : the slopes are equal, cannot be rejected for D2-D3, but must be rejected for D1-D2 and D1-D3. There is a significant difference between day 1 and day 2 or 3.

Accuracy, precision, selectivity and stability

Accuracy and repeatability were measured by spiking three river water (Oise) with 150 mg L^{-1} of EE.
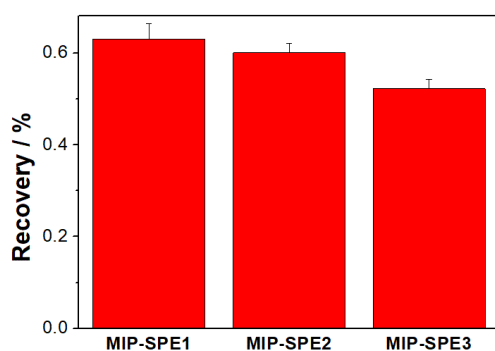

Figure 6. Average recovery ± SD (n= 2) of EE in the elution fraction obtained on 3 different MIP (sample volume 100mL).

The recovery of EE in the elution fraction was 58 ± 6%. SD values between 2 and 3% (n=2) also indicate the good repeatability of this MIP-SPE-electrochemical sensor.

Considering the importance and necessity of the determination of EE in complex real samples, studies were carried out on the selectivity of the proposed method investigating the behavior of possible interfering chemical species, which in the case of EE are organic pollutants with phenol group, such as 4-nonylphenol (4-NP) and bisphenol-A (BPA), both present in water samples.
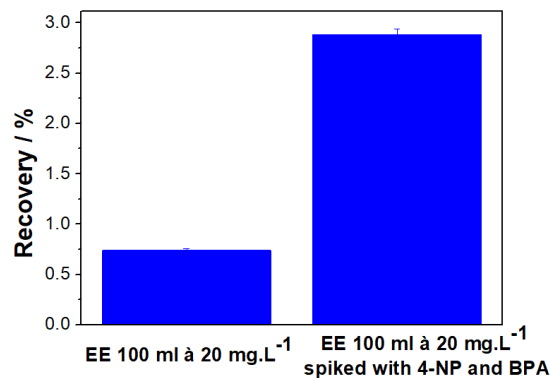

Figure 7. Average recovery ± SD (n= 2) of EE in river sample spiked with and without 4-NP and BPA.

According to the results obtained in Figure 7, the sensor underwent significant interference by 4-NP and BPA. Indeed, the commercial MIP-SPE is designed for estrogens recognition, and not only for EE.

4. SUMMARY

A 3D-printed device combined MIP-SPE extraction and electrochemical detection was developed for determination of ethinyl estradiol in river samples. A linear response was obtained for analyte in the concentration range from 6.6 to 1064 µg.mL^{-1}. High water volumes, up to 100 ml, can be preconcentrated with a small loss of EE (recoveries about 60%). Some improvements are needed to enhance the selectivity of the sensor. Finally, a 2nd generation of the device is in conception.

5. REFERENCES

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