Article

Detecting Traces of 17α-ethinylestradiol in Complex Water Matrices

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Abstract: The detection of hormones, pharmaceutical and personal care products (PPCPs), in aqueous complex media as water bodies is an urgent and mandatory matter because of its harmful impact on the environment. This work presents a sensor device which is able to detect trace of the synthetic hormone 17α-ethinylestradiol (EE2), in a range of concentration of 10^{-15} to 10^{-9} M in both mineral and tap waters. These types of water were studied due to their different degrees of complexity, which allow to attain a better understanding of EE2's behavior and response in different media. The sensor body consists of solid supports having interdigitated electrodes without and with a thin film deposited on it, and device response is evaluated through impedance spectroscopy. The thin films were prepared by the layer-by-layer technique from aqueous solutions of polyethylenimine (PEI) and poly(sodium 4-styrenesulfonate) (PSS) polyelectrolytes. Results demonstrated that it is possible to discriminate clearly the different waters used and to detect EE2, obtained from the second principal component (PC2) as a result of the application of principal component analysis method, with a sensitivity of -0.072 ± 0.009 and -0.44 ± 0.03 per decade of concentration, for mineral and tap water, respectively. Detection limits values were found to be of 8.6 fM (2.6 pg/L) and of 7.5 fM (22.2 pg/L) for TW and MW, respectively and are lower than the ones found in literature. Moreover, the achieved constant proportionality between the PC2 values for each EE2 concentration reveals that one can use this methodology to quantify EE2 concentration in unknown aqueous complex matrices.

Keywords: PPCP; EE2; hormone; impedance; sensor; interdigitated electrodes; PEI; PSS; LbL films

1. Introduction

17α-ethinylestradiol (EE2) is a man-made synthetic female hormone that even at extremely low concentrations has a strong biological impact, due to have a rather high estrogenic action (approximately 10 times higher than estrogens naturally produced by the human body) [1, 2]. This compound is commonly used in treatments aimed at osteoporosis, hormone imbalance, infertility issues and is one of the main components present in female birth control pills [3]. Due to its main uses, this hormone is a pharmaceutical and personal care product (PPCP) and given its strong biological effects and consequences it is also labeled as one of the more predominant and active endocrine disruptors (EDC) present in the environment [4]. When faced with environmental risks brought upon by compounds such as this, relevant policies and legislations must be passed and used to create watchlists, limit usage and/or even ban certain molecules in order to preserve the environment and population health quality in general. In the European Union Decision 840 of 5 June 2018 several pernicious compounds, among which EE2 is included, were placed in a watch list with the goal of conducting further studies and analysis on their impact on the environment [5]. Even though this hormone can be considered somewhat stable, its widespread usage and ceaseless build-up in the environment, namely in lakes, ponds, muds, rivers, underground waters have led to several documented cases of toxic and harmful consequences on both fauna and flora [6, 7].

Thus, arose the need to tackle this issue by developing, studying and put into practice new sensors that are better cut out for the detection and monitoring of molecules such as EE2, whilst venturing to achieve an efficient and effective systems based on the electronic tongue concept [8 and references therein]. The sensor system proposed here is based on the measurement of impedance spectra of carried out on a set of sensor units made of solid supports, with interdigitated electrodes bare and with a thin film, prepared by the layer-by-layer technique using aqueous solutions of polyethylenimine (PEI) and poly(sodium 4-styrenesulfonate) (PSS) polyelectrolytes. The choice of these polyelectrolytes was based in previous results attained for the detection of other PPCP molecule, triclosan [9, 10, 11], and because of these films have been well investigated in literature.

2. Materials and Methods

The chemicals polyethylenimine (PEI), poly(sodium 4-styrenesulfonate) (PSS) and 17α-ethinylestradiol (EE2), provided by Sigma Aldrich. Thin films of PEI/PSS were prepared onto ceramic substrates with interdigitated gold electrodes (IDE) (200μm/200μm), acquired from Metrohm DropSens, by the layer-by-layer (LbL) technique [12, 13].

The films consisted of 5 PEI/PSS bilayers ($(PEI/PSS)_5$), were prepared from 10^{-2} M monomeric concentration aqueous solutions of each polyelectrolyte and 30 seconds of adsorption time in each polyelectrolyte solution. To investigate the response of the sensor units when exposed to the hormone solutions spiked/doped with different concentrations of EE2 (0 M, 10^{-15} M, 10^{-14} M, 10^{-13} M, 10^{-12} M, 10^{-11} M, 10^{-10} M, 10^{-9} M) were prepared with different water matrices with increasing complexity, namely, MilliQ ultrapure water (UW), mineral water (MW) and tap water (TW)

For the electrical characterization, two separate types of sensor units were used: bare (no film) IDE and thin film IDE. The electrical measurements were conducted with both sensor units immersed in EE2 solutions ranging from the lowest (0 M) to the highest (10^{-9} M) concentration and by measuring the impedance spectra at the sensors terminals using a Solartron 1260 Impedance Analyzer, in a frequency range of 1 Hz to MHz and AC signal voltage of 25 mV. These measurements were performed in triplicate. The electrical impedance spectra data features were treated by the Principal component analysis (PCA) method in order to reduce the data size and to obtain a new space of orthogonal components to verify if different concentration patterns can be observed.

3. Results

Figure 1 shows the loss tangent spectra for UW (a and b), MW (c and d) and TW (e and f) analyzed with bare (a, c, e) and $(PEI/PSS)_5$ thin-film (b, d, f) IDE sensors when immersed in solutions with different EE2 concentrations.

When analyzing the spectra displayed in Figure 1, it is possible to observe that as the water matrix becomes more complex the loss tangent curves become more coherent, i.e. there is an evolution towards an ordered sequence between the curves for each concentration, which becomes more apparent in Figure 1(f) where a film coated $(PEI/PSS)_5$ sensor was immersed in the complex water in this work (tap water). Repeated measurements also revealed that tangent-loss curves are more reproducible in more complex solutions.

Principal Component Analysis (PCA) of loss tangent spectra measured with both types of sensors when immersed in the solutions of UW, MW and TW with different EE2 concentrations presence are depicted in the plots of Figure 2 (a) and (b).

The PCA plots of Figure 2 provide valuable information regarding the sensors' capabilities to discriminate each EE2 concentration and to infer about the possibility of the eventual occurrence of clusters and if there are any particular patterns or tendencies to be observed. In this case, the sensors' ability to discriminate and classify the different doped water matrices is clearly demonstrated by the PCA plot of Figure 2 (a), obtained from measurements carried out in all three types of water. However, based on the loss tangent spectra present in Figure 1, the need arose to compare side by side the behavior of the more complex matrixes, MW and TW, without the influence of UW which yielded inconclusive results that could not be used in a comparison with the other waters. This comparison is depicted in Figure 2 (b) depicts, allowing to verify that although both MW and TW waters do present a similar behavior, the sensors units are shown to display a much more intense change in response and sensitivity to TW which is the most complex matrix of the three that are studied in this work.

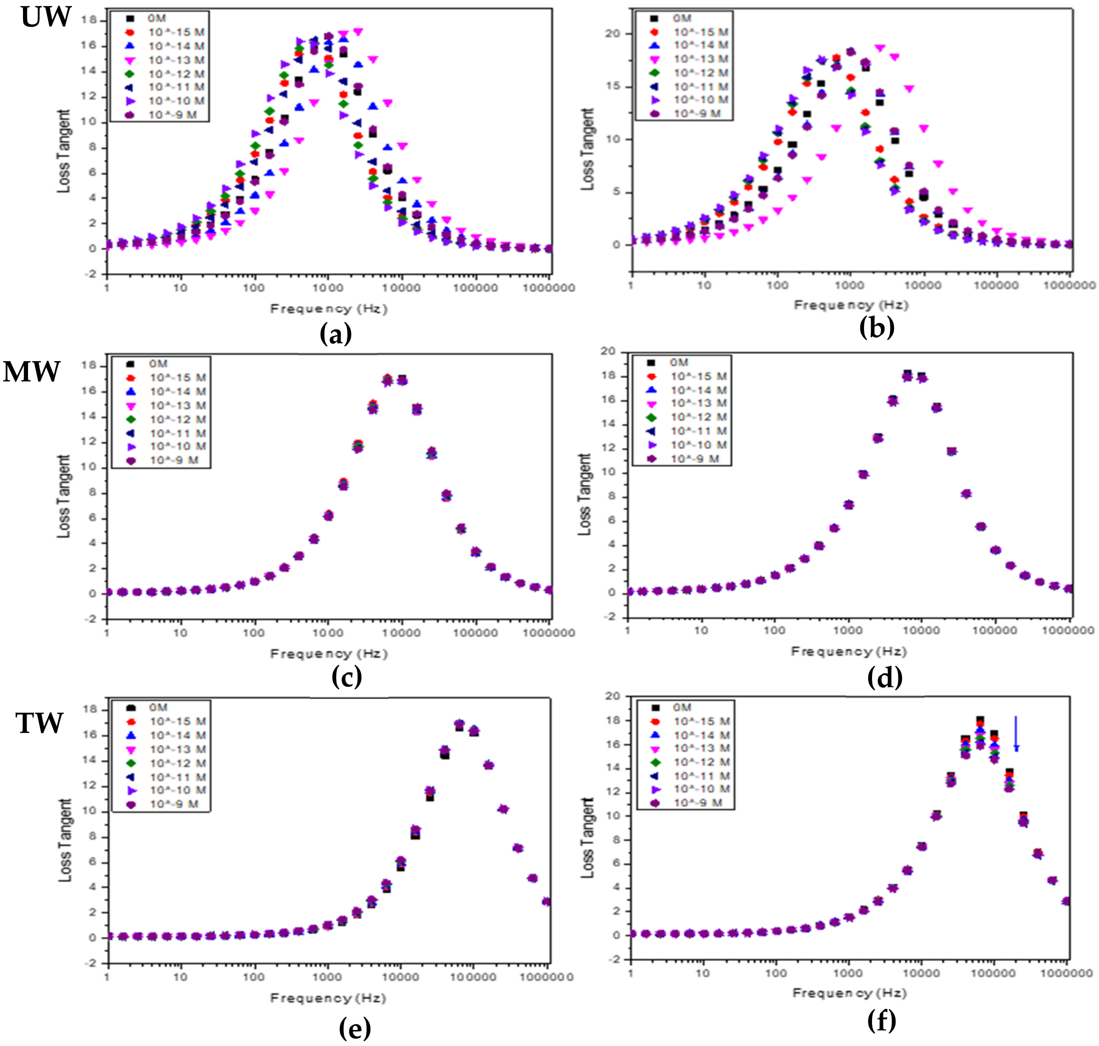
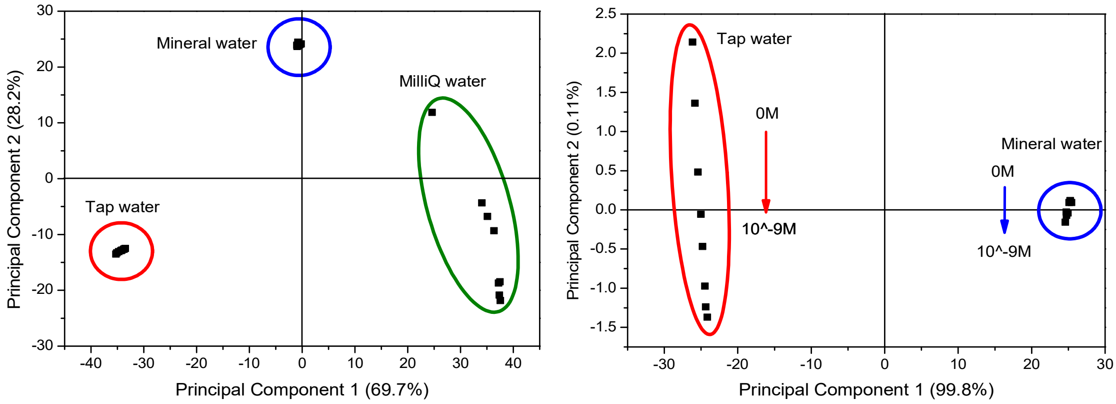


Figure 1. Loss tangent spectra of the sensor devices when immersed in water solutions spiked with different EE2 concentrations: **(a)** naked in UW, **(b)** $(PEI/PSS)_5$ in UW, **(c)** naked in MW, **(d)** $(PEI/PSS)_5$ in MW, **(e)** naked in TW, **(f)** $(PEI/PSS)_5$ in TW.

4. Discussion

Upon the close analysis of the spectra in Figure 1, as the solutions evolve from a simple matrix, UW, to a more complex and “crowded” water, TW, a right shift takes place in the curves consistent with an increase of the conductivity of the medium which translates into a stronger response in the higher frequencies’ region. It is also relevant to note that the measurements conducted for UW do not allow for an ordered distinction between the different concentrations while for MW there is a clear improvement albeit still with poor sensitivity regarding the EE2 concentration level. However, for TW it is possible to observe that not only there is an ordered sequence from lowest (0 M) to highest (10^{-9} M) EE2 concentration, as illustrated by the arrow in Figure 1 (f), but there is also a clear separation among them (higher sensitivity) which was further explored in Figure 2 plots.



(a) **(b)**

Figure 2. PCA plots for EE2 concentrations (10^{-9} M to 0 M) for the bare and $(PEI/PSS)_5$ sensors : **(a)** all three types of water used; **(b)** mineral water and tap water. The colored circular and oval lines surround the PCA achieved data associated to the different waters.

Building upon what is stated in the previous section, in Figure 2 (a), as highlighted by the colored visual aids, there is an unmistakable and tangible distinction between the water samples as it can be seen from the formation of effective clusters for MW and TW. Regardless, since the clustering observed for UW was not as successful as for the other waters (not even displaying a sequence or order), an additional PCA graph (Figure 2 (b)) was plotted with only MW and TW as to go in more deeply these two more responsive mediums. It becomes apparent that the sensors are not only being able to discriminate between each type of water, in the case of TW an outstanding separation (and greater than perceived for MW) can be observed that follows an ordered sequence from the lowest to the highest concentration along the Principal Component 2's axis. It is also relevant to note that sensors' sensitivity is higher at lower concentrations (10^{-15} M to 10^{-12} M) and as the concentration is seen increase, it seems to build-up to a saturation point (close to 10^{-9} M).

Moreover, linear behaviors are achieved when Principal Component 2 (PC2) values, from data of Figure 2(b), are plotted as a function of logarithm of concentration as seen in plots of Figure 3.

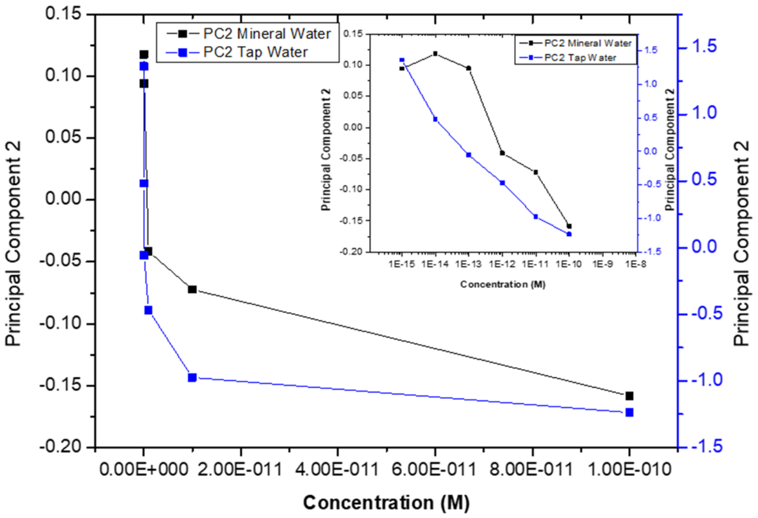


Figure 3. Plots of Principal Component 2 calculated values for mineral and tap waters as a function of logarithm of the sample's concentrations, excluding 10^{-9} M due to its saturation effect. In the inset are presented the same graphs in logarithm scale of the concentration. The lines between the plotted points are only guidelines.

In addition, by plotting in the same graph the Principal Component 2's axis for both waters as function of the concentration of EE2, as shown in Figure 4, one can verify a constant proportionality between the PC2 values achieved for the two types of water attained at each EE2 concentration. This proportionality between the PC2 values for all the EE2 concentrations reveals that one can use this methodology to quantify EE2 concentration in unknown aqueous complex matrices.

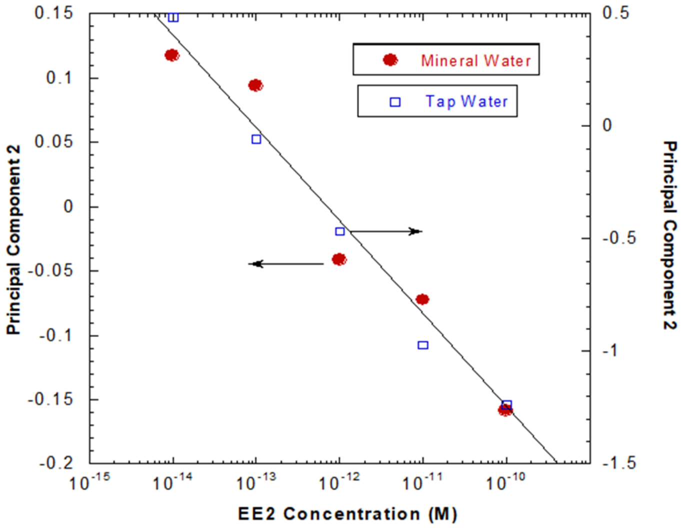


Figure 4. Plot illustrating the constant proportionality achieved when the sensor's responses attained in both waters are plotted as a function of EE2 concentration.

Table 1. Comparison between sensors present in published works versus this work.

Detection Technique	Sensor	Detection Limit	References
Differential Pulse Voltammetry	Au/Fe3O4@TA/MWNT/GCE	3.3 nM	[14]
Electrochemical Detection	PVP/Chi/rGO_Laccase	0.15 pmol/L	[15]
Adsorptive Stripping Voltammetry	HDME	14.8 μg/L at -0.23 V	[16]
		9.7 μg/L at -1.20 V	
	SPCE	182 μg/L	
	SPCNTE	191 μg/L	
Square Wave Voltammetry	LbL FTO-(Chi/CNT)$_3$	0.009 μmol/L	[17]
Impedance Spectroscopy	LbL (PEI/PSS)$_5$ (from 0 M to 10^{-9} M)	7.5 fM (22.2 pg/L) for Mineral Water	This work
		8.6 fM (2.6 pg/L) for Tap Water	

Notwithstanding the fact that both curves display a fairly similar behavior, detailed in the insert in Figure 3, it is possible to verify that for TW it presents a more linear tendency, which is a far more complex matrix than MW, and in turn directly impacts the sensitivity of the sensors in their ability to detect, separate and discriminate between the concentrations analyzed. This linear regime allows us to calculate the sensor sensitivity. Values of -0.072 ± 0.009 and -0.44 ± 0.03 per decade of concentration, are achieved for the sensor system sensitivity with respect to EE2 in mineral and tap water, respectively, as well as detection limits of 7.5 x 10^{-15} M and 8.6 x 10^{-15} M in MW and TW, respectively. For better understanding the obtained results, a comparison of this sensor system with other EE2 sensors of referred in the literature was conducted. Table 1 summarizes the results achieved by the different type of EE2 sensors present in literature and different measurement techniques. From the results displayed in this table, one can state that the type of sensor proposed in this work presents a lower detection limit when compared the other sensors which reached limits as low as pg/L. This confirms that these sensors, as well as the measurement technique, should be further used to develop an optimized EE2 sensor. One expects that a sensors array with some of the sensor units having specificity to EE2 will allow to develop a sensitive, cheap and easy to handle sensor which allow the detection in complex aqueous matrices.

5. Conclusions

Through this work IDE based sensor system were investigated consisting of bare or with a $(PEI/PSS)_5$ thin film IDE towards the detection of EE2 in complex water media, regarding sensitivity, ability to discriminate and classify different water matrices and their overall response to the conducted impedance measurements. These sensors systems revealed an increasingly better response as the complexity of the water under scrutiny increases, which may lead to promising future studies with wastewater or even river water samples. It was also possible to observe that the IDE sensors with the $(PEI/PSS)_5$ thin film coating show a better sensitivity when exposed to the same conditions and samples as their bare(no film) counterpart. Through the different studies and results obtained one can conclude that the developed sensors IDE based systems are able to classify and discriminate between different water types distinguishing differing concentrations and demonstrate an ordered sequence of concentrations.

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