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Applied Voltage Effect In Lbl Sensors While Detecting 17α-Ethinylestradiol in Water Samples †

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Abstract: The effect of applied voltage on impedance spectra, measured on sensors based on solid supports with interdigitated electrodes (IDE) covered or not with a layer-by-layer film prepared with polyethylenimine and poly(sodium 4-styrenesulfonate), was analyzed to detect 17α-ethinylestradiol(EE2) in mineral water and tap water. Results show that the sensor response is strongly affected by the voltage applied, by the presence of film, and by the water matrix meaning that electrochemical reactions are developed near of IDE. However, for low values of applied voltage the sensor response is reproducible with negligible electrochemical reactions, allowing us to conclude that 25mV is the appropriate voltage.

Keywords: voltage effect; impedance; sensor; layer-by-layer; interdigitate electrodes; electrochemical reactions;

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1. Introduction

With the advent of more modern and advanced technologies it became possible to detect chemical compounds at very low concentrations previously hidden and invisible to former analyzing methods in water bodies such as ponds, lakes, rivers, underground waters, muds or even wastewaters [1,2]. Considering that not only human lives, but also a vast majority of Earth's fauna and flora, rely deeply on the central and invaluable role that fresh and clean water plays, it is undoubtedly and alarmingly necessary to strive and succeed in finding novel ways to detect, monitor and conceivably remove these substances from water bodies. Some of these emerging contaminants are included in the category of pharmaceuticals and personal care products (PPCP), such as triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol) (TCS) which is a well-known and commonly used compound in toothpastes, shampoos, lotions due to its bacteriostatic and antimicrobial properties [3]. Amongst these PPCP there is a group of substances designated as endocrine disrupting compounds (EDC) and it is within this collection of composites that 17α-ethinylestradiol (EE2) is inserted [4].

EE2 is a synthetic hormone that is commonly used in the manufacturing of women's oral birth control pill, a widespread and rather mainstream contraceptive. This compound finds its way into wastewater through regular bodily excretions and even though this water enters wastewater treatment plants where it is treated and cleaned, it is not possible to achieve a 100% removal rate of EE2 from the water [5,6]. The then treated water still containing low concentrations of EE2 is discharged into large bodies of water such as rivers and seas where it will subsequently impact the life cycles of both fauna and flora [7–9].

Given that some PPCP and EDC effects on the environment are already known or are being object of several studies, certain degrees of precautions have been drawn. In the case of EE2, these include regulations as the European Union Decision 840 of June 5th 2018 and the Regulation of synthetic estrogen 17α-ethinylestradiol in water bodies in Europe, the United States, and Brazil, and also other measures and guidelines which hold the goal to limit and/or ban its usage [10–12].

This work is but a part of a major scope that ventures out to develop and put into practice cheaper, easier to fabricate and more user-friendly sensor devices capable of detecting and monitoring EE2 in different water bodies, while still retaining or improving the efficiency and detection limits of already existing devices of this nature. The combination of a range of sensors which would then work as a whole, such as an electronic tongue system (ET), in order to better detect pernicious molecules in water bodies is not only a viable option but also a fascinating one due to its possibilities and versatility [13]. To that end, the use of sensorial units as interdigitated electrodes (IDE) while being coupled with an impedimetric system has been demonstrated as being a useful and rather simple method to study, analyze and infer on properties and variations of a multitude of medium samples, from environmental to biomedical ones [14,15].

In the present work, by combining interdigitated gold electrodes with thin-film techniques, for instance layer-by-layer (LbL), and impedance spectroscopy the aforementioned goal of developing sensors to detect and monitor EE2 in different water bodies is made possible. In particular, the effect of different voltage levels applied to the IDE sensor devices and how it would impact the detection capabilities of EE2 in mineral water (MW) and tap water (TW) was analyzed. Although a myriad of studies and works have been conducted and performed on the subject of IDE, there were none (to the best of the authors' knowledge) that analyzed the influence of the variation of voltage levels on IDE sensors and its impact on the sensors' performance and sensitivity when detecting 17α-ethinylestradiol in different water matrices.

2. Materials and Methods

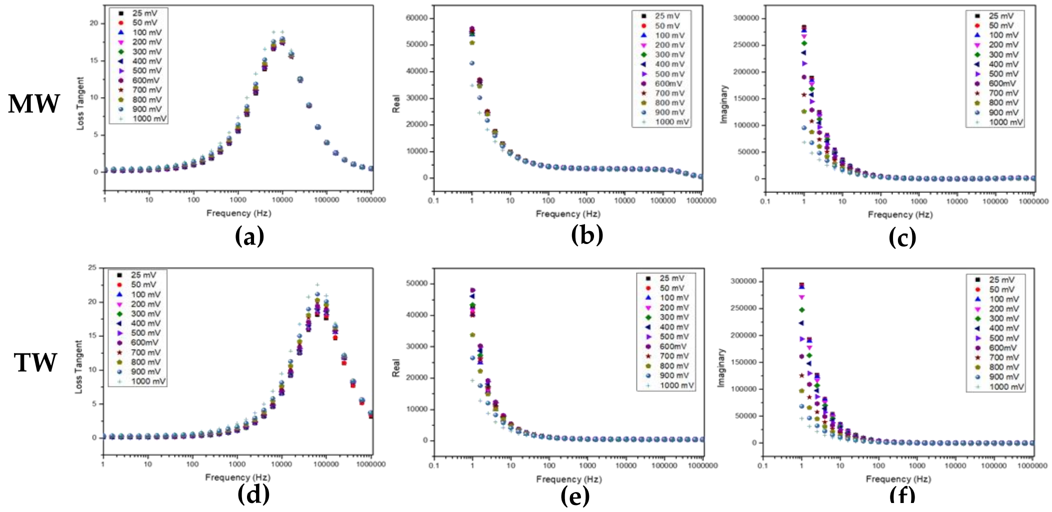
In the course of the experimental work conducted for this study, gold IDE (200 µm/200 µm) deposited onto ceramic substrates were used as sensor devices to detect 17α-ethinylestradiol (EE2) in two different water matrices with distinct complexity levels. The water samples used were a commercial Portuguese mineral water (MW) (pH = 5.7 ± 0.3) and tap water (TW) (pH = 6.8 ± 0.1). These matrices were chosen to observe how various water complexities would interact and impact these electrical measurements performed as well as the detection of the hormone. Regarding the IDE, for each of the matrices two distinct types of sensors were prepared: naked (uncoated) IDE and thin-film IDE. The thin-films deposited onto the IDE were prepared through the technique of Layer-by-Layer (LbL), where the alternate deposition of polyelectrolytes (positive and negative) builds up to the formation of thin film bilayers [16]. The positive polyelectrolyte used was polyethylenimine (PEI) and its negatively charged counterpart was poly(sodium 4-styrenesulfonate) (PSS), prepared using aqueous solutions with a 10^{-2}M concentration of both polyelectrolytes. Through this technique, thin-films of PEI/PSS with 5 bilayers were produced ($[PEI/PSS]_5$). This type of film was chosen since it is viable as demonstrated in in previous works conducted where several types of thin film combinations were studied to ascertain which one (or ones) would be the most suitable to use in experiments that aim to detect (and possibly monitor) noxious compounds in water bodies [14]. These films have shown to be the most promising among all of the thin films that were analyzed, particularly as the complexity and pH of the water matrices increased. The reason being that these factors have a deep impact on this thin film's stability, namely on the sulfonate group present within the chemical composition and structure of the outer layer (PSS), which achieves a more stable behaviour when in the presence of pH of approximately 7 and above [17].

Solutions of both MW and TP were spiked with a concentration of 10^{-12}M of EE2. Subsequently, both types of sensors were immersed in these solutions and electrical measurements were conducted whilst applying varying AC voltages to the IDE sensors: 25 mV, 50 mV, 100 mV, 200 mV, 300 mV, 400 mV, 500 mV, 600 mV, 700 mV, 800 mV, 900 mV and 1000 mV. The aforementioned electrical measurements were performed using a Solartron 1260 Impedance Analyzer, with a frequency range of [1 - 1M] Hz.

Both polyelectrolytes (PEI and PSS) and EE2 standards were purchased from Sigma-Aldrich (Darmstadt, Germany).

3. Results

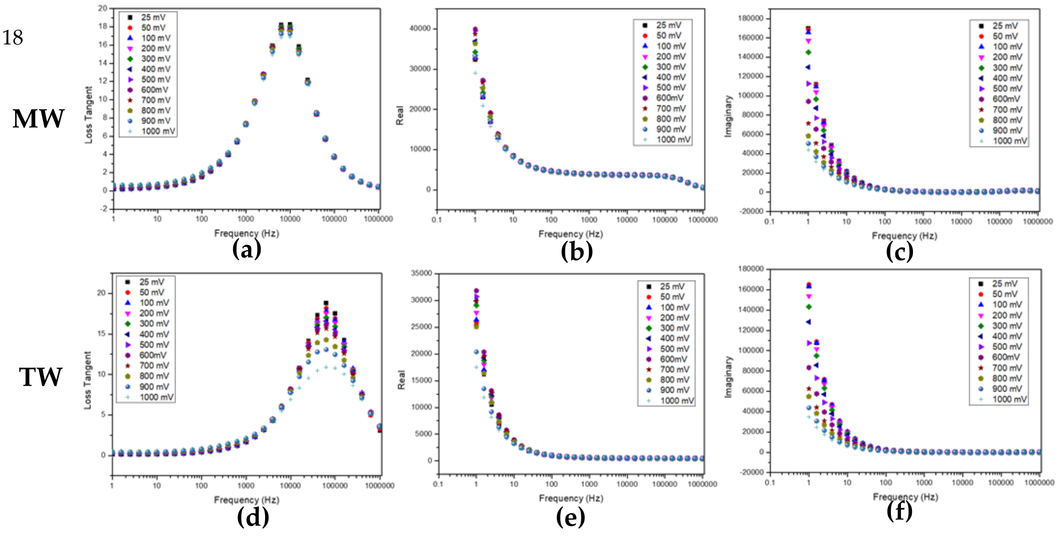
Figures 1 and 2 show the loss tangent, real, and imaginary spectra for the uncoated sensors and coated with a $(PEI/PSS)_5$ LbL film, respectively, while the sensors are immersed in aqueous MW and TW solutions spiked with a fixed concentration of 10^{-12} M of EE2. The voltage applied on the IDE sensors was then sequentially altered from an initial value of 25 mV up to a maximum value of 1000 mV.



(a) (b) (c)

(d) (e) (f)

Figure 1. Comparison of derived information from impedance experimental data as functions of frequency for uncoated sensors after immersion in solutions spiked with EE2 in MW and TW: (a) and (d) Loss tangent; (b) and (e) Real; (c) and (f) Imaginary.



(a) (b) (c)

(d) (e) (f)

Figure 2. Comparison of derived information from impedance experimental data as functions of frequency for $(PEI/PSS)_5$ sensors after immersion in solutions spiked with EE2 in MW and TW: (a) and (d) Loss tangent; (b) and (e) Real; (c) and (f) Imaginary.

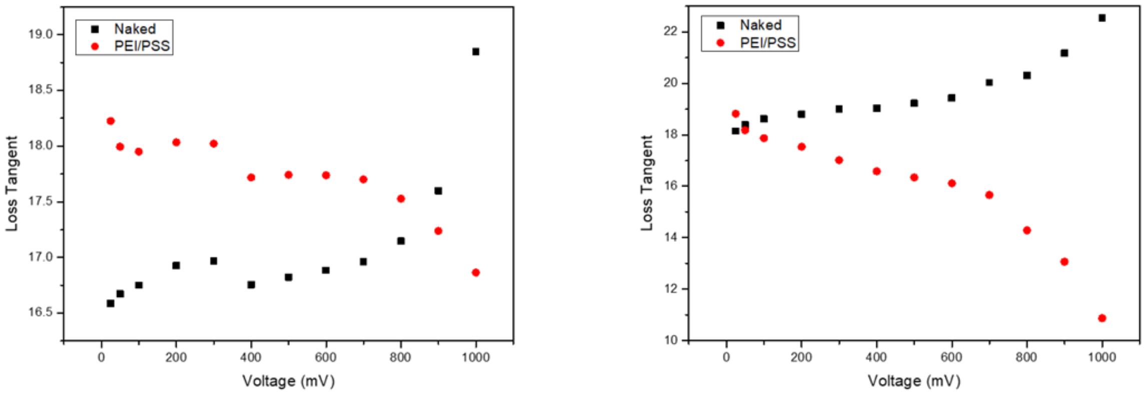
Through an analysis of the plots shown in Figures 1 and 2, the roles that different types of water play become apparent. In both figures, when the transition from mineral water to tap water is conducted, the degree of distinction and separation between the various voltage values that were being applied to the sensors greatly increases while also displaying a higher tendency to achieve an ordered sequence between voltages. Although this behavior can be observed in all spectra above, it is particularly prominent in the loss tangent spectra.

4. Discussion

By delving deeper into the analysis of Figures 1 and 2, in the spectra of loss tangent for TW (Figure 1d and Figure 2d), it becomes possible to extrapolate that the increase in conductivity of the water medium translates itself in a right shift of the curves towards higher frequencies, which in turn underlines an increase in signal intensity for that range of frequency. Building upon what was stated in the previous section, there is a clear and evident improvement regarding the distinction and separation of the different voltage values not only when transitioning from MW to a medium richer and densely filled with electric charges as is TW, but also by adding the (PEI/PSS)$_5$ thin film coating onto the surface of the IDE sensors. There is one more interesting behavior to note, which is the inversion of the voltage ordered sequence present in Figure 1a and d from lowest (25mV) to highest (1000 mV) to the one displayed in Figure 2a and d which goes from highest to lowest. This change in behavior is most likely due to the addition of the thin film coating, given that it introduces other electrochemical reactions on the IDE surface.

To the best of the authors' knowledge this type of study has not yet been done, the analysis of how LbL thin films behave when subjected to different applied voltages while detecting PPCP or EDC such as in this case with EE2. However, the need to perform this experiment arose from the lack of information regarding this subject while conducting experimental works within our research group that are directly related to both this kind of sensors (LbL films on IDE) and emerging compounds such as triclosan and EE2 [18–22].

Figure 3 presents curves that illustrate the behavior of the maximum loss tangent and the applied voltage to each IDE upon the collection of data through impedance spectroscopy.



(a) **(b)**

Figure 3. Plots of loss tangent values at the peak vs voltage for both types of IDE used (naked and [PEI/PSS]$_5$) for: (a) mineral water; (b) tap water.

From these plots, one can surmise, for both MW and TW, that by increasing the voltage that is applied to each IDE device there is a decrease in the polarization of the thin film sensors, which results in these sensor devices exhibiting a poorer signal response to the external electrical stimuli. It was also possible to observe that the higher the voltage, the less reproducible the sensors tend to be. This resulting effect could be a conjunction of factors, namely the sensors becoming more prone to external noise at higher voltages and possible structural damages on either the IDE Au layer or the (PEI/PSS)$_5$ thin film itself, or both simultaneously. These results are in accordance with the observed by Magro et al [14] which demonstrated the IDE are damage by the electrical measurements inducing irreproducibility in the electrical measurements, being always necessary the presence of a thin film covering the IDE and use of low voltage to measure the impedance spectra.

5. Conclusions

This work set out to study and understand how the effect of varying applied voltage to IDE sensors influences their overall responses while detecting EE2 in complex water matrices. For this purpose, two types of sensor devices consisting of ceramic solid supports coated with Au IDE were used: naked sensors (no thin film) and (PEI/PSS)$_5$ thin film sensors. From the impedance spectra it was possible to observe that by increasing the complexity of the water matrix, in this case going from MW to TW, the sensors exhibit, while detecting EE2, a strong dependence with the applied voltage. Furthermore, by depositing a thin film of (PEI/PSS) with 5 bilayers, the abovementioned sensors' spectra also change with the increase of the applied voltage with an opposite behavior of the achieved with the uncoated electrodes. This points to the increase of chemical reactions with the increase of the electric field between the electrodes. These reactions are different depending on whether the electrodes are covered by the thin film or not. Therefore one can conclude that the sensor impedance response is strongly affected by the voltage applied and by the water matrix meaning that electrochemical reactions are developed near of IDE electrodes. However, for low values of applied voltage the sensor is reproducible, and the electrochemical reactions are negligible. Moreover, the chemical reactions are dependents of the presence and type of the LbL film deposited on the electrodes. Finally, one can conclude to avoid chemical reactions the applied voltage should be reduced to 25 mV when one can use this technique to characterize this kind of sensors. However, further studies and analysis should be conducted to infer on this subject, while also striving to optimize the experimental system used.

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