Proceeding Paper

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

Paulo M. Zagalo [1,*], **Cátia Magro** [2] , **Paulo A. Ribeiro** [1] **and Maria Raposo** [1,*]

1   Laboratory of Instrumentation, Biomedical Engineering and Radiation Physics (LIBPhys-UNL), Department of Physics, NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal; pfr@fct.unl.pt

2   Department of Physics, NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal; c.magro@fct.unl.pt

*   Correspondence: p.zagalo@campus.fct.unl.pt (P.M.Z.); mfr@fct.unl.pt (M.R.)

†   Presented at the 1st International Electronic Conference on Chemical Sensors and Analytical Chemistry, 1–15 July 2021; Available online: https://csac2021.sciforum.net/.

Abstract: The effect of the applied voltage on impedance spectra, measured on sensors based on solid supports with interdigitated electrodes (IDE) that are either 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. The results show that the sensor response is strongly affected by the applied voltage, the presence of film, and the water matrix, meaning that electrochemical reactions develop near the IDE. However, for low values of applied voltage, the sensor response is reproducible with negligible electrochemical reactions, allowing us to conclude that 25 mV is the appropriate voltage.

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

1. Introduction

With the advent of more modern and advanced technologies, it has become possible to detect chemical compounds at very low concentrations. These compounds were previously hidden and invisible to former methods of analysis 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 toothpaste, shampoos and lotions due to its bacteriostatic and antimicrobial properties [3]. Amongst these PPCPs, 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].

More specifically, EE2 is a synthetic hormone that is commonly used in the manufacturing of women's oral birth control pills, 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 water [5,6]. The treated water that still contains 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 the object of several studies, a certain degree of precautions has been taken. In the case of EE2, these include regulations such 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 aim to limit and/or ban its usage [10–12].

This work is but a part of a major venture that aims 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), to better detect harmful 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) coupled with an impedimetric system has been demonstrated as being a useful and rather simple method to study, analyze and infer the properties and variations of a multitude of medium samples, from environmental to biomedical ones [14,15].

IDE's versatility of being deployable in a myriad of applications without the added labor and expenses of modifying their core geometry and/or configuration has catapulted these devices into wide use in a vast array of fields and areas, also allowing for the possibility of lab-on-chip systems based on IDE. These types of sensors present further features and advantages that make them coveted and chosen by researchers worldwide in their works, such as the advent of more powerful and precise technological advances that paved the way for the production of increasingly smaller electrodes, at highly competitive costs due to processes as mass-fabrication [16–18].

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 IDEs, none (to the best of the authors' knowledge) have+ 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 the way various water complexities 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 [19]. 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]$_5$).

Solutions of both MW and TW 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 those coated with a $(PEI/PSS)_5$ LbL film, respectively, while the sensors were 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.
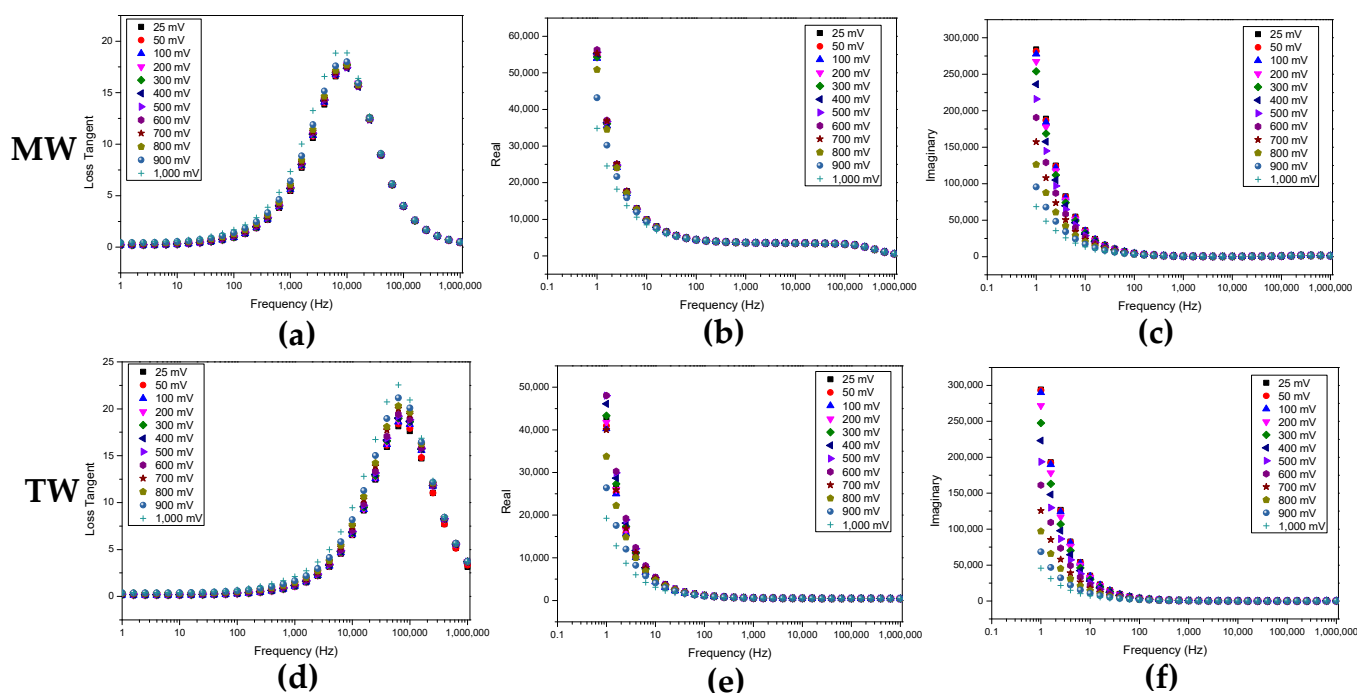

Figure 1. Comparison of derived information from impedance experimental data as functions of frequency for uncoated sensors after immersion in solutions spiked with EE2 in MW and TW: (**a**,**d**) Loss tangent; (**b**,**e**) Real; (**c**,**f**) Imaginary.

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 performed, the degree of distinction and separation between the various voltage values 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.
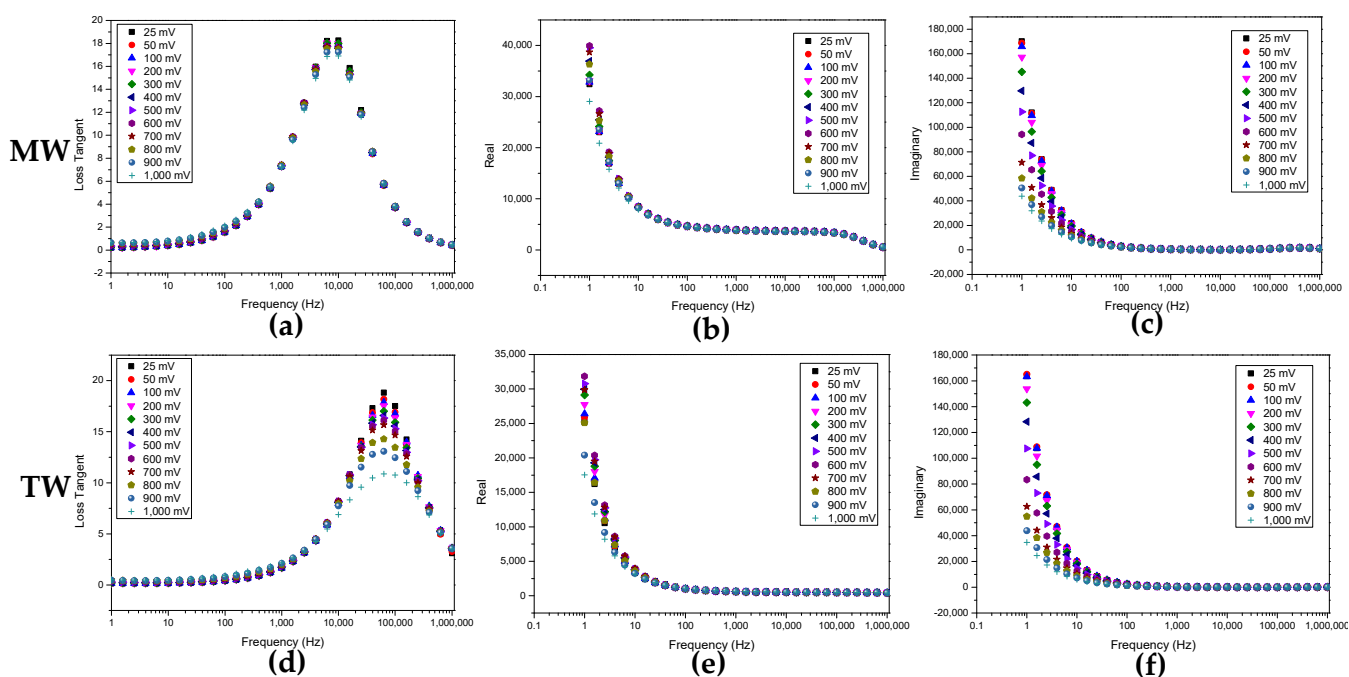
Figure 2. Comparison of derived information from impedance experimental data as functions of frequency for (PEI/PSS)₅ sensors after immersion in solutions spiked with EE2 in MW and TW: (**a**,**d**) Loss tangent; (**b**,**e**) Real; (**c**,**f**) Imaginary.

4. Discussion

By delving deeper into the analysis of Figures 1 and 2, in the spectra of loss tangent for TW (Figures 1d and 2d), it becomes possible to infer 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 an 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 the case for 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 observable in Figure 1a,d from lowest (25 mV) to highest (1000 mV) to the one displayed in Figure 2a,d which transitions from highest to lowest. This behavior change 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 conducted, with regard to an 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 sensor, (LbL films on IDE) and emerging compounds such as triclosan and EE2 [20–23]. This type of film ([PEI/PSS]₅) was chosen since it is viable, as demonstrated 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 been found 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 the structure of the outer layer (PSS),

which achieves more stable behaviour when in the presence of pH of approximately 7 and above [23,24].

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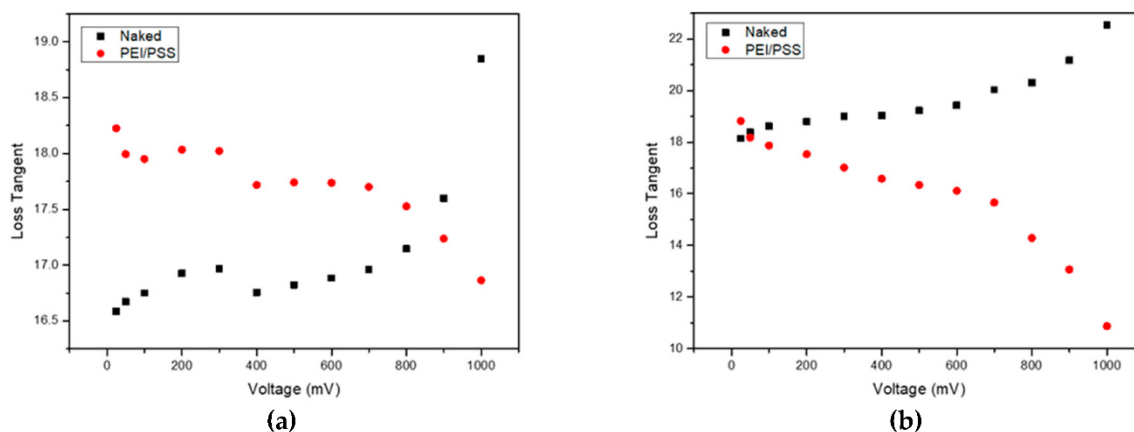

Figure 3. Plots of loss tangent values at the peak vs voltage for both types of IDE used (naked and [PEI/PSS]$_5$) for: (**a**) mineral water; (**b**) tap water.

From these plots, one can surmise, for both MW and TW, that by increasing the voltage that is applied to each IDE device, a decrease in the polarization of the thin film sensors occurs, 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 effect could be due to a combination of factors, namely the sensors becoming more prone to external noise at higher voltages and possible structural damage on either the IDE Au layer or the (PEI/PSS)$_5$ thin film itself, or of both simultaneously. These results are in accordance with the results observed by Magro et al. [14] which demonstrated the IDE are damaged by electrical measurements, inducing irreproducibility in the electrical measurements, which emphasizes the necessity of the presence of a thin film covering the IDE and the 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, namely, 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 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 was also found to change with the increase of the applied voltage with an opposite behavior of that achieved with the uncoated electrodes. This points to the increase in chemical reactions with an increase in the electric field between the electrodes. These reactions differ 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 the IDE electrodes. However, for low values of applied voltage the sensor is reproducible, and the electrochemical reactions are negligible. Moreover, the chemical reactions are dependent on the presence and type of the LbL film deposited on the electrodes. Finally, one can conclude that, to avoid chemical reactions, the applied voltage should be reduced to 25 mV when

one can use this technique to characterize this kind of sensor. However, further studies and analyses should be conducted to investigate this subject further, while also striving to optimize the experimental system used.

References

1. Houtman, C.J. Emerging contaminants in surface waters and their relevance for the production of drinking water in Europe. *J. Integr. Environ. Sci.* **2010**, *7*, 271–295. [CrossRef]
2. Wintgens, T.; Salehi, F.; Hochstrat, R.; Melin, T. Emerging contaminants and treatment options in water recycling for indirect potable use. *Water Sci. Technol.* **2008**, *57*, 99–107. [CrossRef] [PubMed]
3. Marques, I.; Magalhâes-Mota, G.; Pires, F.; Sério, S.; Ribeiro, P.A.; Raposo, M. Detection of traces of triclosan in water. *Appl. Surf. Sci.* **2017**, *421*, 142–147. [CrossRef]
4. Water Quality Association. PPCP & EDC Fact Sheet, (Ccl Iii). 2014. Available online: https://www.wqa.org/Portals/0/Technical/Technical%20Fact%20Sheets/2014_Ps-PCPs-EDCs.pdf (accessed on 1 March 2020).
5. Johnson, A.C.; Williams, R.J. A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works. *Environ. Sci. Technol.* **2004**, *38*, 3649–3658. [CrossRef] [PubMed]
6. Thomas, K.V.; Hurst, M.R.; Matthiessen, P.; Waldock, M.J. Characterization of estrogenic compounds in water samples collected from United Kingdom estuaries. *Environ. Toxicol. Chem.* **2001**, *20*, 2165–2170. [CrossRef] [PubMed]
7. Heberer, T. Tracking persistent pharmaceutical residues from municipal sewage to drinking water. *J. Hydrol.* **2002**, *266*, 175–189. [CrossRef]
8. Alistair, B.A. *Boxall. New and Emerging Water Pollutants Arising from Agriculture*; OECD: York, UK, 2012.
9. Adeel, M.; Song, X.; Wang, Y.; Francis, D.; Yang, Y. Environmental impact of estrogens on human, animal and plant life: A critical review. *Environ. Int.* **2017**, *99*, 107–119. [CrossRef] [PubMed]
10. Vella, K. Commission Implementing Decision (EU) 2018/840 of 5 June 2018. *Off. J. Eur. Union* **2018**, *141*, 9–12.
11. da Cunha, D.L.; Camargo da Silva, S.M.; Bila, D.M.; da Mota Oliveira, J.L.; de Novaes Sarcinelli, P.; Larentis, A.L. Regulation of the synthetic estrogen 17α-ethinylestradiol in water bodies in Europe, the United States, and Brazil. *Cad. De Saúde Pública* **2016**, *32*, e00056715. [CrossRef]
12. Nagpal, N.; Meays, C. *Water Quality Guidelines for Pharmaceutically-Active-Technical Appendix*; Ministry of Environment of British Columbia Province: Victoria, BC, Canada, 2009; pp. 1–86.
13. Magro, C.; Mateus, E.; Raposo, M.; Ribeiro, A.B. Overview of electronic tongue sensing in environmental aqueous matrices: Potential for monitoring emerging organic contaminants. *Environ. Rev.* **2019**, *27*, 202–214. [CrossRef]
14. Magro, C.; Zagalo, P.; Pereira-da-Silva, J.; Pires Mateus, E.; Branco Ribeiro, A.; Ribeiro, P.; Raposo, M. Polyelectrolyte Based Sensors as Key to Achieve Quantitative Electronic Tongues: Detection of Triclosan on Aqueous Environmental Matrices. *Nanomaterials* **2020**, *10*, 640. [CrossRef] [PubMed]
15. Ibrahim, M.; Claudel, J.; Kourtiche, D.; Nadi, M. Geometric parameters optimization of planar interdigitated electrodes for bioimpedance spectroscopy. *J. Electr. Bioimpedance* **2013**, *4*, 13–22. [CrossRef]

16. Mukhopadhyay, S.C. Sensing and Instrumentation for a Low Cost Intelligent Sensing System. In Proceedings of the SICE-ICASE International Joint Conference, Busan, Korea, 18–21 October 2006; pp. 1075–1080.

17. Mukhopadhyay, S.C.; Gooneratne, C.P.; Demidenko, S.; Sen Gupta, G. Low cost sensing system for dairy products quality monitoring. In Proceedings of the 2005 International Instrumentation and Measurement Technology Conference, Ottawa, ON, Canada, 16–19 May 2005; pp. 244–249.

18. Van Gerwen, P.; Laureyn, W.; Laureys, W.; Huyberechts, G.; Op De Beeck, M.; Baert, K.; Suls, F.; Sansen, W.; Jacobs, P.; Hermans, L.; et al. Nanoscaled interdigitated electrode arrays for biochemical sensors. *Sens. Actuators B* **1998**, *49*, 73–80. [CrossRef]

19. MacKay, S.; Hermansen, P.; Wishart, D.; Chen, J. Simulations of interdigitated electrode interactions with gold nanoparticles for impedance-based biosensing applications. *Sensors* **2015**, *15*, 22192–22208. [CrossRef] [PubMed]

20. Pereira-da-Silva, J.; Zagalo, P.M.; Magro, C.; Pinto, I.; Ribeiro, P.A.; Raposo, M. Detection and Adsorption of Triclosan on Sensors Based on PAH/PVS Thin-Films. In Proceedings of the 2019 IEEE 6th Portuguese Meeting on Bioengineering (ENBENG), Caparica, Portugal, 6–8 September 2019; IEEE: Piscataway, NJ, USA, 2019; pp. 1–4.

21. Pereira-da-Silva, J.; Zagalo, P.; Magalhães-Mota, G.; Ribeiro, P.; Raposo, M. Adsorption of Triclosan on Sensors Based on PAH/PAZO Thin-Films: The Effect of pH. In *Computational Intelligence Methods for Bioinformatics and Biostatistics*; Raposo, M., Ribeiro, P., Sério, S., Staiano, A., Ciaramella, A., Eds.; Springer: Cham, Switzerland, 2020; pp. 319–325.

22. Zagalo, P.M.; Magro, C.; Pereira-da-Silva, J.; Bouchikhi, B.; Bari, N.E.; Ribeiro, P.A.; Raposo, M. Detection of Triclosan in Tuned Solutions by pH and Ionic Strength Using PAH/PAZO Thin Films. *Proceedings* **2019**, *15*, 25. [CrossRef]

23. Zagalo, P.M.; Ribeiro, P.A.; Raposo, M. Detecting Traces of 17α-Ethinylestradiol in Complex Water Matrices. *Sensors* **2020**, *20*, 7324. [CrossRef] [PubMed]

24. Smith, R.N.; McCormick, M.; Barrett, C.J.; Reven, L.; Spiess, H.W. NMR studies of PAH/PSS polyelectrolyte multilayers adsorbed onto silica. *Macromolecules* **2004**, *37*, 4830–4838. [CrossRef]