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Measurement of Neuropeptide Y Using Aptamer-Modified Microelectrodes by Electrochemical Impedance Spectroscopy

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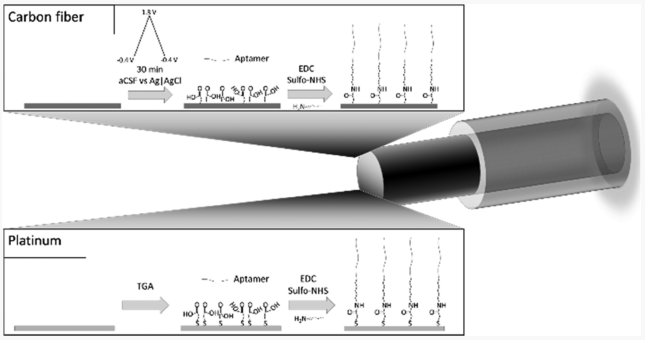
ABSTRACT: Aptamer-modified microelectrodes for Neuropeptide Y measurement by electrochemical impedance spectroscopy was described here. The advantages of using carbon fiber or platinum microelectrodes are because they are promising materials with high electrical conductivity, chemical stability, and high surface area that can be easily modified on their surface. The immobilization and biofouling were studied and compared using EIS. Moreover, the adsorption of NPY to the aptamer-modified microelectrodes was also demonstrated by EIS. Changes of $-\omega^{*}Z_{imag}$, an impedance factor that gives information of the capacitance, is directly correlated with concentrations. A widely linear range was obtained from 10 to 1000 ng/mL of NPY. This method was able to detect NPY without performing a redox reaction by adsorption at the surface of the microelectrodes, with the specificity provided by aptamer functionalization of the microelectrode surface.



INTRODUCTION

Electrochemical impedance spectroscopy (EIS) is an important electrochemical technique for the study of surface interfaces that provides useful information in terms of electron transfer and has shown high sensitivity for the detection of biomolecules.[1−3] EIS has been widely used for biosensing applications as well as the study of coatings and layers at the electrode surface; however, it has not been used in implantable microelectrodes for the measurement of neuropeptides and neurotransmitters. On the other hand, fast scan cyclic voltammetry (FSCV) has been the gold standard technique used to measure electroactive neurotransmitters with subsecond resolution due to its selectivity and excellent sensitivity.[4,5] These advantages have allowed us to understand the mechanism of neurochemical signaling dynamics in the brain. Also, enzymatic microelectrodes have been developed to measure nonelectroactive small neurotransmitters such as glutamate, GABA, and many others.[6] Every molecule for which there is an oxidase that generates H_2O_2[7] can be measured using this technique, including glucose.[8] FSCV has been an essential tool for studying the mechanism of action of many drugs, as well as the measurement of the kinetics of neurotransmitters reuptake and control.[9] Neuropeptides, a different form of neurotransmitters, are vital to the transmission and modulation of neurological signals, and their measurement needs to be sensitive for high temporal and spatial resolution.[10] They are not recycled since there is no known reuptake system for them, and they are typically released together with other neurotransmitters posing an analytical challenge.[10] Therefore, it is essential to develop a selective technique for their detection and to have a strategy to cancel out the signal exerted by other neurotransmitters. Neuropeptide Y (NPY) is formed by 36 amino acids, and it is one of the most important peptides in the human body. It is involved in the regulation of physiological functions such as anxiety,[11] memory,[12] fear,[13] and stress.[14] Their study is currently done measuring the expression of neuropeptides in vitro or using microdialysis coupled with mass spectrometry,[15] which gives temporal resolution of a few minutes. Antibodies have been used as a biological recognition element in an appropriate surface chemistry for the detection of NPY[16,17] as well as colorimetric assays.[18] Here, we report the development of a modification protocol that reproducibly binds highly selective aptamers to the surface of platinum microelectrodes and carbon microelectrodes. Decreasing the burden of biofouling of platinum microelectrodes to a similar level of carbon fiber microelectrodes as well as providing a binding site

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specific for NPY is shown. It is known that the presence of the aptamer can allow specificity and selectivity of the target molecule for sensing.[19] Aptamers have been used for the multiple detection of neurotransmitters due to their stability, high affinity, and specificity.[20] Taking advantage of the affinity of aptamers with a specific target can be a solution for detecting neuropeptides,[21] given that the structural similarity between neuropeptides makes their detection highly difficult using conventional electrochemical techniques.

Adsorption of NPY is measured using carbon fiber and platinum microelectrodes with EIS, resulting in a novel strategy for the detection of neuropeptides when using microelectrodes with a form factor that can be used for implantation. EIS allows monitoring changes produced by interaction of biomolecules with the electrode with high sensitivity, allowing that those changes can be measured as well, due to the affinity of the biomolecules to the electrode surface.[22] Moreover, we are proposing the use of $-\omega^{*}Z_{imag}$ as a better representation of EIS for this specific method of measuring NPY. The use of carbon fiber microelectrodes has been the gold standard for the measurement of neurotransmitters due to their known surface chemistry and interaction with monoamine neurotransmitters[23] as well as easy preparation and use under several conditions.[24] However, the low selectivity of carbon fiber in addition to the difficulty to modify their surface in a consistent and homogeneous way makes them a bad candidate for the measurement of neuropeptides using recognition molecules. Also, it is known that platinum-iridium electrodes have been the gold standard for deep brain stimulation due to the stability of platinum within the body,[25] which makes microelectrodes made with these metals an important target to aim for future applications. Platinum, however, microelectrodes are not planar and very prone to biofouling, and they readily oxidize at the potentials needed for dopamine oxidation;[26] therefore, it was required to study their biofouling to understand their potential use in the measurement of neuropeptides. In this work, we immobilized an aptamer[27] on the surface of the microelectrodes using the carboxylic acid functionalization on carbon fiber microelectrodes and thioglycolic acid-modified platinum microelectrodes to analytically measure NPY using impedance measurements and to study the properties of the modified layer of microelectrodes.

The advantages of using metals for detection of neurotransmitters[28] are the possibility of using them at higher scan rates, high surface area, the fact that they have higher activity to some neurotransmitters, and their versatility to be modified to enhance their sensitivity is of great interest for use in biomolecule detection.[4,29] Platinum microelectrodes have been used because they are commonly used for microfabrication for electrochemical studies and they are easy to modify using different molecules and nanoparticles to increase the sensitivity detection of neurotransmitters.[30] Platinum, among other properties, possesses beneficial surface properties that produce a strong bond to thiol groups, making it very versatile for a variety of biosensing applications. However, the electrochemical properties of platinum differ greatly from those of carbon fibers, which are the most common material used for neurotransmitter biosensing. Therefore, it is of utmost importance to understand the interaction between aptamers and their target on platinum using EIS. This study uses platinum microelectrodes and compares them to carbon fiber microelectrodes to measure Neuropeptide Y using different electrochemical techniques. It is expected that this would open the door for the development of neuropeptide biosensing using microelectrodes.

EXPERIMENTAL SECTION

Fabrication of Platinum and Carbon Fiber Microelectrodes. Platinum and carbon fiber microelectrodes used in this work had a diameter of ≈25 and 7 μm, respectively, and were sealed with the borosilicate glass capillary (1.5 mm × 0.86 mm) for platinum (1.0 mm × 0.5 mm) for carbon fiber using a micropipette puller. A single platinum wire or carbon fiber (Thornel T-300) was aspirated into the glass capillary using vacuum with a filter to avoid the fibers reaching the motor. Then, microelectrodes were placed in the micropipette puller (Narishige PC-10) to obtain the electrode. After that, the platinum microelectrode with the tip exposed approximately 15−80 μm was put back into the micropipette puller to seal the platinum tip in the borosilicate capillary. The carbon fiber exposed was physically cut using a razor blade to a length of 50−100 μm. Microscopic observation with water immersion was used to ensure that a seal between the glass and the carbon fiber or platinum was present to avoid leaking of the solution into the capillary that would change the electroactive area. The electrical connection was made with silver paint and a silver-plated copper wire that was connected to the potentiostat for measurements. Finally, the electrodes were soaked in deionized water and isopropanol and placed in an ultrasonic bath to clean possible debris on their surface. Carbon fiber microelectrodes were oxidized in artificial cerebrospinal fluid (aCSF), and platinum microelectrodes were cleaned electrochemically in 0.5 M H_2SO_4 vs Ag|AgCl at 100 mV/s for 300 cycles.

Reagents. Electrochemical characterization of platinum and carbon fiber was carried out in aCSF at pH 7.4 prepared as reported previously.[3] Neuropeptide Y (GenScript) also diluted in aCSF for the preparation of standard solutions. The master solution was prepared fresh daily at a concentration of 5 μg/mL and was used to prepare the different standard solutions in the electrochemical cell by addition to the initial 15 mL of aCSF. Therefore, the volume of the electrochemical cell used was 15 mL. The detection of NPY was done using different concentrations by adding NPY at the end of each run to prepare target concentrations between 10 and 1000 ng/mL[31] to understand the behavior of the microelectrodes at different concentrations. The electrochemical cell was stirred for 1 min without disturbing the electrodes after adding each aliquot of NPY and waiting until the solution was stagnant after stirring to avoid mass transfer due to convection.

Electrochemical Measurements. All electrochemical measurements were done using a three-electrode electrochemical cell with platinum and carbon fiber as the working electrodes, a platinum wire as the counter electrode, and an Ag|AgCl reference electrode (3 M NaCl-filled solution). A Reference 600+ Gamry potentiostat was used for electrochemical measurements, and all data were collected using Gamry Instruments Framework software. All potentials in this work are expressed versus Ag|AgCl. An amplitude of 10 mV, an initial frequency of 5 MHz, and a final frequency of 10 Hz were used for EIS. Before the experiments, potentiostat and cables were calibrated using the potentiostat's calibration procedure. Gamry's original cables were used with the included separators that are designed to reduce mutual inductance. We did not see any inductive behavior during the impedance measurements.

Aptamer Immobilization. Figure 1A,B shows the modification of both microelectrodes, platinum (Figure 1A)

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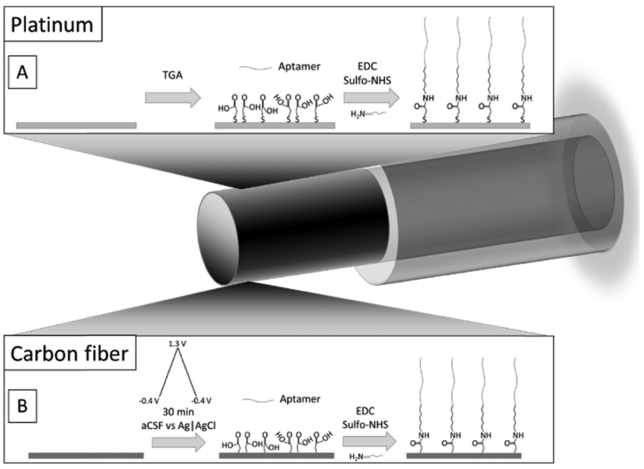

Figure 1. Scheme of surface modifications in (A) platinum and (B) carbon fiber microelectrodes.

and carbon fiber microelectrodes (Figure 1B). Carbon fiber microelectrodes were treated with a triangular waveform between −0.4 and 1.3 V at 1.0 V/s for 30 min to oxidize their surface and add oxygen-containing functionalization. The modification of the Pt microelectrodes was different due to the possibility to form Pt−S strong bond using thiol-terminated molecules. Therefore, Pt microelectrodes were exposed to a 5.0 μM thioglycolic acid solution in ethanol for 24 h at 4 °C to generate a thiol−metal bond that produced the availability of carboxyl groups for the carbodiimide cross-linking reactions. After adsorption of thioglycolic acid to the surface of the platinum microelectrodes, carboxyl groups were available to continue the addition of the aptamer to their surfaces. Then, both microelectrodes were exposed to 1.0 μM solutions of a single-stranded DNA aptamer (5′-NH_2-C_6-AGC AGC ACA GAG GTC AGA TGC AAA CCA CAG CCT GAG TGG TTA GCG TAT GTC ATT TAC GGA CCT ATG CGT GCT ACC GTG AA-3′) that recognizes NPY with an affinity of 295 ± 28 × 10^{-9} M,[27,31] in the same solution with 5 μM EDC for 24 h at 4 °C. This functionalization in both microelectrodes allowed us to have a selective interaction with the analyte of interest, in this case NPY, without the need of a redox reaction but only with its adsorption.

Statistics. GraphPad Prism 8.0 was used for the analysis of the statistics. All data are provided as mean ± SD, and the significance is defined as $P \leq 0.05$.

RESULTS AND DISCUSSION

Surface Modification of Microelectrodes. The electrochemical analysis of biomolecules can be affected directly by their adsorption to the electrode surface, giving a weak detection signal and affecting the performance of the biosensors or electrochemical techniques. Previous reports have established that surface modifications or coatings can decrease the biofouling of the electrode by providing a physical and chemical barrier between fouling agents and the electrode surface.[32] In this work, we took advantage of surface modifications to decrease the biofouling of platinum microelectrodes to resemble the behavior of carbon fiber under the same circumstances.[33] Decreasing or avoiding biofouling is the first step required to use platinum microelectrodes to be able to measure any biomolecule in biological samples such as measuring NPY using electrochemical techniques. In this work, we aimed to decrease the biofouling of platinum to a degree similar to carbon fiber microelectrodes since it is impossible to completely avoid this process. Having biofouling in platinum microelectrodes similar to carbon fiber microelectrodes will provide us an excellent standpoint from where to start developing their use in biological fluids.

Biofouling of Carbon Fiber and Platinum Microelectrodes. Different materials have been used as microelectrodes for biosensing applications, which have attracted much attention in the biochemical field, for the great affinity,

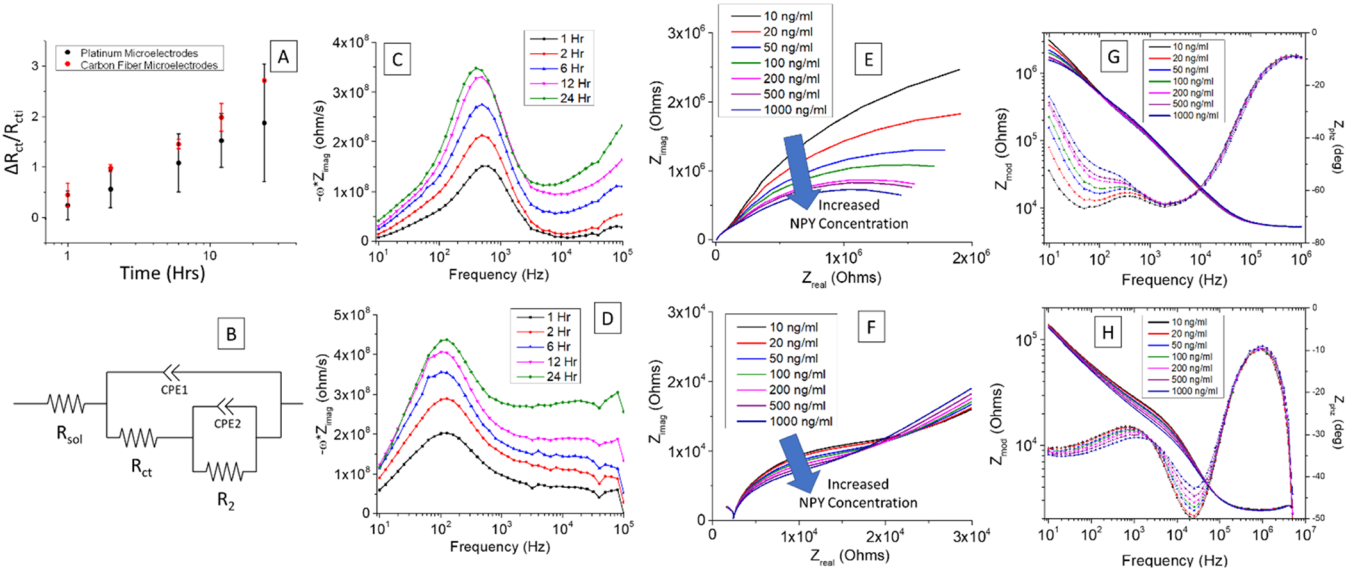

Figure 2. (A) Change in the charge transfer resistance (R_{ct}) of aptamer-modified platinum (black) and carbon fiber (red) microelectrodes due to biofouling. (B) Circuit model used to calculate the charge transfer resistance. Electrochemical impedance spectroscopy of the change in $-\omega^*Z_{imag}$ of aptamer-modified (C) carbon fiber and (D) platinum microelectrodes after exposed to 1 × 10^5 cells/mL PC12 extracts. Electrochemical impedance spectra of (E: Nyquist, G: Bode) carbon fiber and (F: Nyquist, H: Bode) platinum microelectrodes, with the blue arrow showing the increase of NPY concentration. Experiments were done in a 5 mM K_3FeCN_6/K_4FeCN_6 solution in aCSF buffer at 0.2 V vs AglAgCl.

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sensitivity, and greater surface area[34,35] as well as the diverse chemical and physical behavior that can be obtained tailoring their properties for specific needs. Carbon fiber microelectrodes have been used for the detection of neurotransmitters due to their biocompatibility and their low surface biofouling[36] when they are cycled to a potential high enough that renews the surface. Metals, however, although can be easily modified on their surface, having better modification coverage, are known to be more prone to biofouling, as discussed previously. In this case, we aimed to compare the biofouling of surface-modified platinum and carbon fiber microelectrodes using EIS[37] in vitro to ensure that their surface modification using aptamers provided similar possibilities compared to carbon fibers. In this way, given the particular case in which aptamers are used, platinum would be a similar option to carbon fibers from the biofouling standpoint. To produce biofouling in the surface of the microelectrodes, bovine serum albumin is commonly used;[38] however, the use of cell extracts provides a more comprehensive mix of biological molecules to test both surfaces.[39] Therefore, PC12 cell extracts were used for this comparison. Figure 2 shows the interaction of platinum microelectrodes and carbon fiber microelectrodes already modified with single-stranded DNA aptamer and exposed to PC12 extracts to test the biofouling of their surface. This experiment was conducted to show the ability of these different materials to be used as biosensors. Aptamer-modified platinum and carbon fiber microelectrodes (n = 3) were exposed for 1, 2, 6, 12, and 24 h to a homogenized extract solution of 1×10^5 cells/mL PC12 cells in RPMI-1640 culture medium with fetal bovine serum and horse serum. Then, the aptamer-modified microelectrodes were placed in a three-electrode cell with a solution of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ to test the change in the electron transfer due to the surface blockage produced by biofouling. It is expected that biofouling reduces the activity of this redox reaction with increasing charge transfer resistance (R_{ct}). EIS data were obtained from 10 Hz to 5 MHz at 0.2 V vs Ag|AgCl using an amplitude of 10 mV. The charge transfer resistance was calculated, and its change with time was used to analyze the variation of surface blockage due to biofouling and plotted versus time in Figure 2A. Figure 2B shows the circuit used to model the EIS of the microelectrodes, which was chosen in this particular case because of the use of the redox couple $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$.[40] Aptamer modification in both materials after exposing to the cell extracts showed changes in R_{ct} with time; however, these changes showed a significant deviation between them when analyzed for different microelectrodes compared between them giving high error bars. The change in R_{ct} was attributed to the chemical changes in the surface of the microelectrodes produced by the interactions between the highly diverse mixture of biological molecules of the cell extract with the surface of the microelectrodes that cover the surface reducing the electron transfer and redox reaction. When electrodes with a high surface area are exposed, the imperfections at different points in the materials are averaged and more reproducible values are obtained. However, such imperfections found on the surface of the platinum with small surface area decrease the possibility to have reproducibility in the electroactivity of two electrodes with similar length exposed. Metal microelectrodes are typically avoided because of the biofouling produced by biomolecules when exposed to biological tissue. We studied the biofouling produced when platinum microelectrodes are modified with aptamers, and they were compared to carbon fiber to show that aptamer-modified platinum microelectrodes are a viable substrate to be used in biological applications regarding to biofouling.

Observing the percentual changes of R_{ct} with time for both materials, in these studies, the average change in R_{ct} was not statistically significant (p = 0.47, unpaired t-test) when testing the change in R_{ct} due to biofouling. This result means that the change in the surface produced due to biofouling in platinum microelectrodes is similar to the change produced on the surface of carbon fiber microelectrodes when exposed to cell extract solutions and measured using EIS. To provide context for the values obtained, the average of the initial value of R_{ct} for platinum microelectrodes was 2.8 ± 1.2 MΩ, and that for carbon fiber microelectrodes was 1.3 ± 0.4 MΩ. It is important to indicate that these experiments were done exposing the aptamer-modified microelectrodes to the cell extracts, which is different from the use of platinum microelectrodes when cyclic voltammetry techniques are used, in which the oxidation of neurotransmitters can polymerize in the surface blocking it. However, given that this work is done with the goal of using aptamers as the recognition molecule for the biosensing target, no oxidation is expected to take place at the potentials at which the microelectrodes will be held for target adsorption.

To understand the changes produced by biofouling to the microelectrodes, we subtracted the EIS spectra obtained before the exposure to the cell extracts to the data obtained after 1, 2, 6, 12, and 24 h. As seen in Figure 2, carbon fiber (Figure 2C) and platinum (Figure 2D) microelectrodes have similar changes in their impedance due to biofouling. The main change is seen at lower frequencies, while platinum microelectrodes have the highest change at ca. 100 Hz, in carbon fiber microelectrodes the maximum is located at higher frequencies at around 500 Hz. Both materials, however, increased the capacitance at all frequencies, a pattern that was seen in all of the electrodes that were run. One of the advantages of platinum is that the increase in capacitance is constant at frequencies higher than about 1 kHz. For measurement at these frequencies, a well-characterized change in capacitance that is constant at different frequencies in a specific range, allows for a predictable behavior that can be taken into consideration when a biomolecule is being measured. Moreover, the change in capacitance was about 0.5×10^8 Ω/s after the first hour with a maximum of less than 3.0×10^8 Ω/s after 24 h, which are not only predictable but significantly smaller than the change produced by the presence of NPY at different concentrations as will be shown later. We hypothesize that the difference between both materials is given by the different homogeneity of the surface modification in platinum vs carbon fiber microelectrodes. Platinum surface is homogeneously modified while carbon fiber microelectrodes have areas in which the modification was successful due to the presence of carboxyl groups while other areas remain with the bare carbon surface exposed. This difference in surface modification can be seen in the Nyquist plots in Figure S1C,D where carbon fiber microelectrodes present more than one time constant while platinum microelectrodes only present one constant, which is due to the non-uniform current distribution at the electrode surface.[40] Moreover, the lower frequencies that are impacted in platinum are related to limitation in mass transport, which is expected given the high charge transfer seen in metal microelectrodes compared to carbon fiber.

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NPY Measurement Using EIS with Carbon Fiber and Platinum Microelectrodes. It has been widely reported that EIS can be used to study the adsorption of biomolecules on the surface.[3,41,42] We first tested the measurement of NPY concentration using carbon fiber and platinum microelectrodes using 5 mM K_3FeCN_6/K_4FeCN_6 redox probe with EIS. In this case, all measurements were done at the half-wave potential of the redox reaction at 0.2 V vs AglAgCl. Figure 2E,G shows the Nyquist and Bode plots, respectively, of different concentrations of NPY in aptamer-modified carbon fiber microelectrodes. Two different time constants were seen with the major change attributable to NPY concentration seen at low frequencies. Given the inhomogeneities of the microelectrodes, we expect that the reaction of the redox probe at the surface of the carbon fiber in places where no aptamer is present would yield the reaction seen at higher frequencies, while the areas where aptamers are attached would provide the reaction seen at low frequencies. Figure 2F,H, on the other hand, shows the Nyquist and Bode plots, respectively, of different concentrations of NPY in aptamer-modified platinum microelectrodes. In this case, only one semicircle was seen showing a more homogeneous modification of the surface with only one time constant. Moreover, the Bode plot shows a homogeneous change at higher frequencies than carbon fiber. Comparing both materials, the homogeneity of the modifications produced in platinum microelectrodes with the immobilization of aptamers ensures a better coverage, which in turn translate to higher sensitivity.

Given that these redox probes are not present in the human body, it is of utmost importance to study the measurement of the target biomolecules in systems that resemble more the chemistry found in biological fluids. Therefore, we also tested aptamer-modified carbon fiber and platinum microelectrodes directly in aCSF without the redox probe to make sure that we were able to observe a change with NPY concentration.

Figure 3 shows the comparison between the measurement of NPY at different concentrations (A) with and (B) without aptamers on the surface of platinum microelectrodes and (C) with and (D) without aptamers on the surface of carbon fiber microelectrodes. These experiments were run in aCSF without any additional redox probe at different potentials using a three-electrode cell with AglAgCl as the reference electrode and a platinum wire as the counter electrode. Figure 3 only shows the experiments done applying a potential of −0.4 V vs AglAgCl to demonstrate the differences; however, all potentials studied are shown in Figure S6 for platinum and Figure S8 for carbon fiber microelectrodes. All potentials showed similar features. It is important to clarify that there is one of the datapoints (60 Hz) where noise is always seen due to the AC mains frequency. It is important to clarify that Z refers to the impedance of the entire system and not of any specific component of the model circuit. In simple systems where only one electrochemical reaction exists, $-\omega^*Z_{imag} = 1/C$, where C is the surface capacitance.[3] This allowed us to visualize the changes produced by the adsorption of NPY either to the bare microelectrode surface in a nonspecific manner or to the aptamer in modified microelectrodes at different frequencies. Nyquist plots, on the other hand, are not suitable for this analysis due to the frequency-dependent properties of the aptamers since these plots hinder this variable (or makes this variable more difficult to visualize). In these plots, we are showing $-\omega^*Z_{imag}$ instead of Z_{imag} because in the case that only one reaction is taking place, $-\omega^*Z_{imag}$ equals the

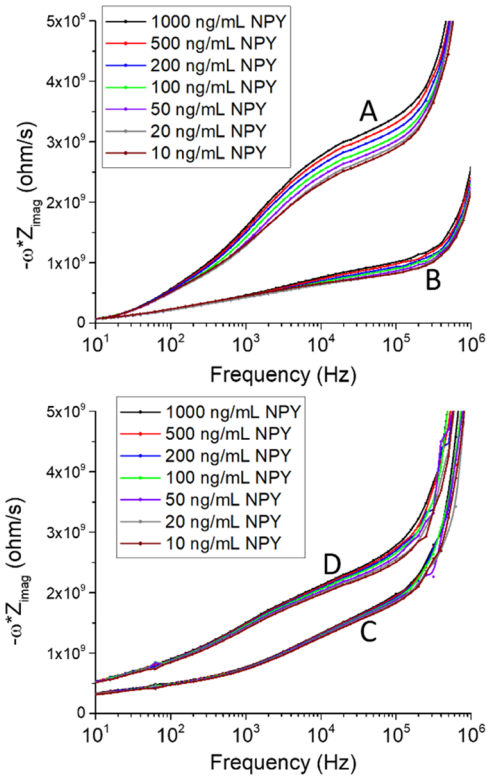

Figure 3. Electrochemical impedance spectra ($-\omega^*Z_{imag}$) of different NPY concentrations at −0.4 V vs AglAgCl potentials in (A) aptamer-modified and (B) bare platinum microelectrodes, in addition to (C) aptamer-modified and (D) bare carbon fiber microelectrodes. Other applied potentials for platinum can be found in Figure S6, and those for carbon fiber can be found in Figure S8. All experiments were done in aCSF buffer.

capacitance of the surface. There was no clear difference between the behavior of the platinum and carbon fiber microelectrodes across different potentials (Figures S6 and S8, respectively). However, the results change when comparing platinum microelectrodes with aptamers (Figure 3A) versus without aptamers (Figure 3B), as well as carbon fiber microelectrodes with aptamers (Figure 3C) versus without aptamers (Figure 3D). In Figure 3, it is clearly seen the change produced by the aptamer immobilization in the spectra when the adsorption of NPY is specific (with aptamers) or nonspecific (without aptamers). There was a much higher degree of change in the surface capacitance produced by the specific adsorption of NPY when the aptamer modification was done. This was seen notably starting at frequencies of 100 Hz and higher when the aptamers were present while no change was seen in the capacitance of the bare platinum microelectrodes up to 1 kHz or higher. However, the most significant change between concentrations is observed at frequencies higher than 4 kHz where the capacitance reaches a plateau. Nonmodified platinum microelectrodes showed significantly less change in $-\omega^*Z_{imag}$ with NPY concentration, which can be attributed to the slow adsorption rate of NPY without the presence of the aptamer. This slow adsorption is produced in all metals and is not selective to NPY but most peptides due to their intrinsic properties.[43] Having the aptamer not only provides selectivity for faster adsorption of NPY but also makes the biofouling of the surface of the electrode closer between platinum and carbon fibers, as shown in Figure 2A.

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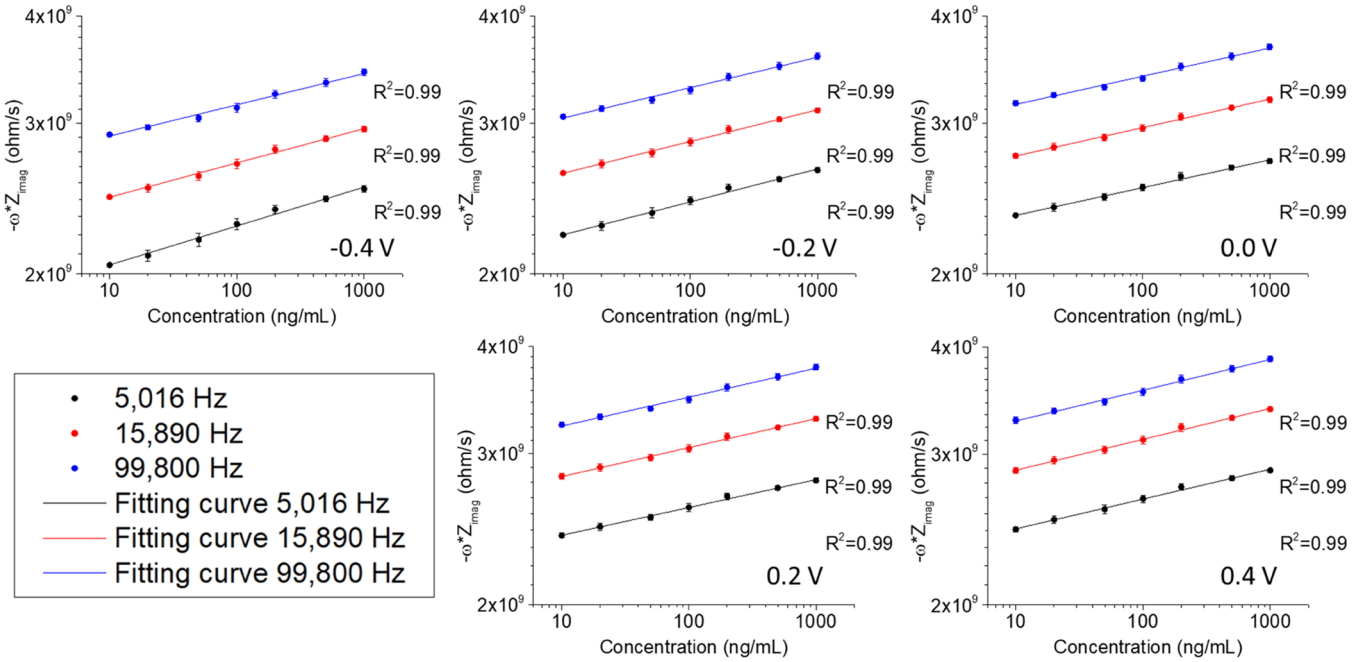

Figure 4. Calibration curves plotted in log−log scale for the detection of NPY using electrochemical impedance spectroscopy at different frequencies and potentials in platinum microelectrodes vs Ag|AgCl. All experiments were done in aCSF buffer.

Moreover, when comparing platinum to carbon fiber microelectrodes, a completely different directional change was observed in capacitance between the different concentrations of NPY, which is probably due to a different modification mechanism.

In the case of carbon fiber microelectrodes (Figure 3C,D), unlike platinum, a lower degree of change is seen in the capacitance with the aptamer immobilization compared to the bare microelectrodes, and this change is observed in the entire range of frequencies. The coverage of aptamers in carbon fibers is expected to be lower than platinum because of the number of binding sites. While platinum atoms can bind to the sulfur in the TGA to immobilize the aptamers, only carboxyl groups in the carbon fiber are able to form the carbodiimide cross-linking. This difference in coverage allows the aptamers bound to the carbon fibers to be in any direction, and it is known that single-stranded DNA have high affinity to interact with graphitic structures along the entire oligomer.[44,45] Therefore, it is expected that the single-stranded DNA aptamer is lying flat against the carbon fiber surface without its active binding site available for NPY to bond but covering the microelectrode surface decreasing the surface area where nonspecific interactions can occur. This, in turn, lowers the change in the capacitance seen by the nonspecific binding of NPY to the carbon fiber surface due to a decrease in the surface area available. Moreover, the fact that the capacitance shifts along the entire frequency range in the same manner with or without aptamers suggests that the mechanism for NPY-to-carbon fiber bonding is the same with or without aptamers but with a decreased surface area.

After obtaining the impedance at different frequencies using EIS, calibration curves were plotted and analyzed for platinum (Figure 4) microelectrodes with error bars in each point (n = 3). We chose these bias potentials to see if there was any difference comparing positive and negative charge on the electrode surface. NPY is supposed to have one positive charge at a pH of 7.4; therefore, we wanted to understand to what extent the surface charge may change its adsorption. We found that there was not a pronounced change in the calibration curves from −0.4 to 0.4 V vs Ag|AgCl except for a slight increase in the capacitance with voltage. When plotting $-\omega^*Z_{imag}$ vs concentration of NPY in log−log scale for the data obtained with platinum microelectrodes, we can observe that the calibration curves at each of the five potentials tested followed a linear relationship at the three chosen frequencies: 5016, 15 890, and 99 800 Hz. This linear relationship is due to the roughness of the microelectrodes instead of a planar surface, which follows the Freundlich adsorption isotherm model, as shown in a previous study by our group.[3]

CONCLUSIONS

Aptamer-modified microelectrode biosensors for the measurement of NPY by EIS were developed. To develop these biosensors, biofouling was compared between carbon fiber and platinum microelectrodes up to 24 h using cell extracts. We showed that aptamer-modified platinum microelectrodes behave similarly to carbon fiber microelectrodes in the fouling of their surface, which provides hope for the use of metals with biological fluids given their better coverage when using surface modifications. Using a redox probe, the change in impedance with NPY concentration was measured and easily observed. Moreover, the immobilization of the aptamer on the platinum exhibited a wide linear working range that was measured as low as 10 ng/mL in aCSF without the redox probe showing great potential for the use of these biosensors in biological fluids. It is expected that these changes in the impedance, directly related to concentrations, can be applied to determine NPY levels in cells and model organisms in the future. The use of EIS and the aptamer modification in microelectrodes and aptamers could have great potential for the analysis of NPY in vivo.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c03719.

Electrochemical and physical characterization of microelectrodes and their modifications; electrochemical study of diffusion process in platinum microelectrodes; and electrochemical impedance spectroscopy of carbon fiber microelectrodes in the presence of NPY (PDF)

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Notes

The authors declare no competing financial interest.

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