engineering proceedings

MDPI

Proceeding Paper

Preliminary Studies on the Synthesis of Redox-Labelled Molecu-Larly Imprinted Nanoparticles in Sensor Development for the Quantification of Perfluoroalkyls in Water†

Marco Costa[1], Sabrina Di Masi[1*], Christopher Zaleski[2], Sergey A. Piletsky[2] and Cosimino Malitesta[1]

[1] Laboratorio di Chimica Analitica, Edificio A6, DiSTeBA, Università del Salento, Via per Monteroni 73100, Lecce, Italia; marco.costa@unisalento.it(M.C.).;sabrina.dimasi@unisalento.it(S.D.M.); cosimino.malitesta@unisalento.it (C.M.)

[2] Department of Chemistry, University of Leicester, University Rd LE1 7RH, Leicester, UK; cz155@leicester.ac.uk (C.Z.), sp523@leicester.ac.uk (S.P.)

* Correspondence: sabrina.dimasi@unisalento.it

† Presented at the 3rd International Electronic Conference on Biosensors, 8–21 May 2023; Available online: https://iecb2023.sciforum.net.

Abstract: Polyfluoroalkyl compounds (PFAS) are synthetic compounds recently classified as permanent and emerging chemicals, since their bioaccumulation in human and environment, due to the presence of carbon-fluorine functional groups. The design of novel screening tools with addressed high response time for the routine quantification of PFAS in water is highly desirable. In this work, we propose the preparation of a new voltammetric sensor-based on molecularly imprinted polymer nanoparticles (nanoMIPs) receptors for the highly sensitive and selective quantification of PFAS in water. The nanoMIPs were synthesized by the solid phase approach and immobilized onto functionalized screen-printed platinum electrode (SPPtE), chosen as the transduction element for sensor development. Dimensional characterization of nanoMIPs by Dynamic light scattering (DLS) shows small nanosized imprinted particles (<200 nm) with a polydispersity index (PDI) below 0.3. Electrochemical techniques were used for sensor preparation, characterization and to assess the analytical performance, respectively. Preliminary calibration curves of nanoMIPs based sensor in a wide range of PFAS concentration (1.5–100 ng/mL) exhibited high sensitivity towards its target.

Keywords: molecularly imprinted polymer; nanoparticles; solid phase synthesis; perfluorooctanoic acid; screen-printed electrode; voltammetric sensor

Citation: Costa, M.; Di Masi, S.; Zaleski, C.; Piletsky, S.; Malitesta, C. Preliminary Studies on the Synthesis of Redox-Labelled Molecu-Larly Imprinted Nanoparticles in Sensor Development for the Quantification of Perfluoroalkyls in Water. Eng. Proc. 2023, 35, x. https://doi.org/10.3390/xxxxx

Published: 9 May 2023

1. Introduction

Perfluorooctanoic acid (PFOA) belongs to the large class of perfluorinated chemicals (PFCs), synthetic substances that have been produced in industrial processes and for commercial purposes for over 60 years [1]. Their properties, such as oil and water repellency and resistance to heat and chemical reactions, are due to their structure, which includes a fully fluorinated carbon chain that is both hydrophobic and oleophobic, and a hydrophilic charged functional group such as carboxylic acid. For this reason, they are used in water-repellent, stain-resistant coatings for clothing, leather, upholstery, and carpets; in oil-repellent coatings for paper that comes into contact with food, in fire-fighting foams, in paints and other commercial products, and in industry as surfactants, emulsifiers, wetting agents, additives and coatings. PFOA and other PFCs produced mainly by two manufacturing processes (electrochemical fluorination, ECF, and telomerization) [1,2] are in the spotlight as environmental pollutants. Opinion concerns public continue to also grow thanks to the availability of new and recent studies that provide information on their presence in the environment, fate and transport, as well as on the effects of human exposure.

Their hazardousness is not only due to their effects on our organism, but also to their persistence, being very resistant to typical environmental degradation processes due to their carbon-fluorine bonds, which are among the strongest in organic chemistry [3]. Their presence has been detected in environmental matrices around the world, including finished drinking water, surface water, groundwater, air, sludge, soil, sediment, and indoor dust, biota, and ice caps [4]. The production and use of PFOA is currently being phased out, but environmental contamination and human exposure to PFOA is expected to continue into the foreseeable future because it is persistent and arises from precursor compounds such as the degradation of compounds such as fluorotelomer alcohols (FTOH) used in industry and various products [2,5]. PFOA concentrations ranging from 12.6 ng/L to 287 ng/L were detected by UPLC-MS /MS [6]. Although HPLC/HRMS is still an accurate analytical method that can detect a wide range of analytes, it has practical disadvantages, such as high cost and lack of user experience in handling and interpreting the data, etc. Therefore, portable, sensitive, and highly selective sensors capable of detecting low concentrations of PFOA in real matrices are desirable. Biosensors and chemical sensors offer rapid measurements and responses, easy-to-use platforms, portability, low-cost fabrication, and high sensitivity [7,8]. Molecular imprinted polymers (MIPs) have been widely used as synthetic receptors in sensors development [9,10] and provide the high sensitivity and selectivity of biological receptors, while their polymeric nature makes them resistant to harsh conditions [11]. The use of MIPs in the form of nanoparticles (nanoMIPs) allows for the production of analyte-compliant cavities, thus possessing higher specificity and overall performance than traditional techniques [12]. The regular distribution of analyte binding sites also leads to efficient mass transfer, which enables rapid detection of the analyte. In addition, nanoMIPs can be easily synthesized and integrated into a variety of transducers, including electrochemical devices. In this work, we present the preparation of a new electrochemical sensor based on nanoMIPs for the quantification of PFOA in water. The nanoMIPs were prepared by solid-phase approach, in the presence of a mixture of functional monomers, cross-linker agents and the target. The electroactivity of the particles is achieved by introducing a redox marker (ferrocene) into the polymer system, which imparts signaling properties [13]. The resulting PFOA-nanoMIPs sensor showed good performance, stability and high performance.

2. Materials and Methods

2.1. Materials

Glass Beads, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), acetic anhydride, acetonitrile (AcN), acetone, itaconic acid (ITA), 2-Hydroxyethyl methacrylate (HEMA), Ferrocenylmethyl methacrylate (FcMMA), Ethylene Glycol Dimethacrylate (EGDMA), Trimethylolpropane trimethacrylate (TRIM), Pentaerythritol tetrakis (3-mercaptopropionate) (PETMP), Benzyl diethyldithiocarbamate, Dimethylformamide (DMF), ethanol (EtOH), 3-aminopropyltriethoxysilane (APTES), phosphate buffer solution (PBS) tablet, perfluorooctanoic acid (PFOA).

2.2. Apparatus

Polymerization of nanoMIPs was performed using a UV-lamp (0.5 W cm−1, 4× 15 W, Philips HB171/A, Germany). For hot wash step, was used heating jacket linked with variable power supply (TENMA, Bench Power Supply). Dynamic light scattering (DLS) was employed to measure the hydrodynamic diameter of the particles using a Zetasizer Nano (Malvern Instruments Ltd, UK). For these measurements, 100 μL solution of nanoparticles in 800 μL of water at 25 °C was previously sonicated for 1 min using an Ultrasonic bath (Hilsonic Ultrasonic Cleaner, 20–100 kHz, 220 V). The electrochemical measurements were performed using screen-printed platinum electrodes (SPPtE, Dropsens DRP-550) composed of three-electrode configuration on a planar ceramic support (3.3 × 1 cm) and they consisted of a platinum disk-shaped working electrode (4 mm diameter), a platinum electrode as counter electrode and silver electrode as reference electrode purchased from Metrohm, UK. The electrochemical measurements were performed by using a Potentiostat (PalmSens4, Netherlands). PSTrace 5 was the software to control the instrument and data acquisition.

2.3. Preparation of nanoMIPs

Procedure for nanoMIPs preparation was adapted from [14]. Briefly, glass beads (30 g) were activated with 1 M NaOH and then silanized in dry toluene to obtain -NH_2-bearing beads. After, perfluorooctanoic acid (PFOA) was immobilized on silanized glass beads (PFOA-GB) as shown in Figure 1, via EDC/NHS activation mechanism of template. For this step, the solution was composed by 150mM of EDC, 225 mM of NHS, 50 mM of analyte. The reaction time was 2 hours under stirring and then, was dried under vacuum, until a powder was obtained. Next, unreacted primary amino groups were blocked via Acetic anhydrous (1.5 mL) in AcN (58 mL) reaction, under stirring for 30 minutes. The mixture was wash with dry acetone and dried under vacuum condition again. The perfluorooctanoic acid-imprinted polymer nanoparticles (PFOA-nanoMIPs) were synthesized by solid phase synthesis using photo-polymerization in the presence of PFOA-GB. The nanoMIPs polymerization mixture comprised following: FcMMA (0.05 g, 3.5 mmol) as redox marker, Benzyl diethyldithiocarbamate as iniferter (0.150 g, 12.5 mmol), PETMP (0.36 g, 1.5 mmol) as chain-transfer agent, EGDMA (0.648 g, 65.3 mmol) and TRIM (0.648 g, 38.3 mmol) as cross-linkers. ITA (0.436 g, 67 mmol) and HEMA (0.067 g, 67 mmol) were selected as functional monomers using computational chemistrywere selected as functional monomers. All polymerization components were dissolved in 50 mL of acetonitrile. The mixture was vortex vigorously until complete dissolution, then sonicated and degassed with nitrogen for 30 min. Afterwards, the dry beads (powder) were added to a flat bottom wide crystallizing dish by adding the mixture solution. It was shaken until complete incorporation and contact was obtained. To complete the polymerization, the mixture was exposed to UV radiation for 3 min and then, stored in the fridge. The charging-temperature elution step (cold and hot wash) was adopted to elute PFOA from the polymeric structure.

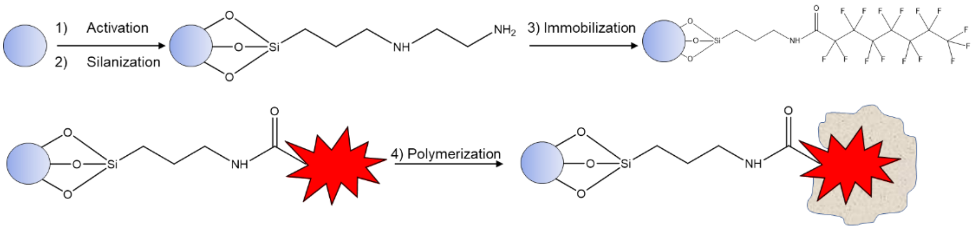


Figure 1. General scheme of polymerization protocol.

Prior to the cold-wash, nanoMIPs were placed in DMF in the freezer for 1 hour and the glass beads, in powder form, were placed in a syringe. Both ACN and DMF were passed through the syringe, for elution of unreacted monomers and low-affinity nanoMIPs For the hot wash, 100 mL of EtOH were placed in a hot bath (70-80°C) and were used it. At the syringe, was applied a warm by a heating jacket linked with variable power supply. Hot EtOH was passed through into the syringe for elution of high-affinity nanoMIPs. The solution EtOH with nanoMIPs was collected into a round bottom flask and then the solution has been pre-concentrated with rotary evaporation, with the pressure fix at 175 mbar and the temperature at 60°C.

2.4. Preparation and electrochemical characterization of the PFOA-nanoMIPs sensor

Prior to functionalization, the SPPtE was cleaned using ultrapure water. To perform functionalization, 5 µL of 3-aminopropyltriethoxysilane (APTES) solution at a concentration of 5.0% v/v (dissolved in ethanol) was dropped on the surface of the working electrode, for 3 times, and then dried at 150 °C for 1 h in an oven. After this procedure, the working electrode was rinsed with ultrapure water to eliminate the excess of APTES. Finally, nanoMIPs were immobilized by dropping 100 μL of a solution comprising 20 μL of EDC/NHS (1:1.5) and 80 μL of nanoMIPs. The incubation time was performed for 40 minutes. Finally, the developed sensor (PFOA-nanoMIPs sensor) was rinsed with water to remove not adsorbed nanoMIPs from the electrode surface. Electrode modification was electrochemically studied using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Specifically, the electrochemical characterization was performed for bare SPPtE, APTES /SPPtE and PFOA-nanoMIPs/ APTES/SPPtE, respectively, in 5 mM PBS pH 7.2 and in KCl 0.1 M solution including 10 mM ferrocyanide-ferricyanide redox probe.

2.5. Characterization of nanoMIPs

The nanoMIPs size was evaluated by dynamic light scattering (DLS). DLS is an optical and non-destructive measurement technique for the spectroscopic characterization of disperse systems. It evaluates high-frequency fluctuation in scattered light. The result about DLS analysis is the intensity-weighted distribution of the particles hydrodynamic equivalent diameter, or hydrodynamic diameter.2.6 PFOA sensing

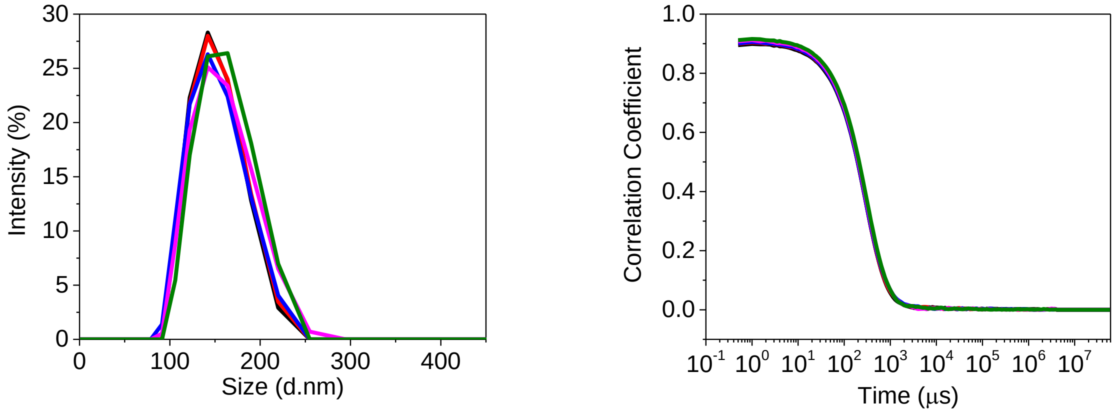
The electrochemical performances of the PFOA-nanoMIPs sensor towards PFOA were recorded using differential pulse voltammetry (DPV) measurements (in triplicate), in the potential range between − 0.15 V and + 0.4 V (*vs* Ag reference), modulation amplitude of 0.2 V, step potential of 4.95 mV, equilibration time of 5 s, and scan rate of 50 mV/s. Cyclic Voltammetry (CV) were recorded in the potential range from -0.1 V to + 0.8 V (*vs* Ag reference), step potential of 10 mV, scan rate of 50 mV/s. The analytical signals of developed sensors were collected by exposure to different increased concentration of PFOA (1.5 –100 ng/mL) dissolved in phosphate buffer solution (PBS) (5 mM, pH = 7.4), allowing the specific interaction between the cavities of nanoMIPs and its target. Incubation time was 10 min. After each measurement, the electrode surface was gently washed with ultrapure water.

3. Results and Discussions

The polymerization of MIPs based on nanoparticles produced a sensitive receptor for that template, showing high performance and wide margins for improvement. Through the charge given by the FcMMA, it was possible to measure the peak (about 0.1 V) which current intensity decreases with increasing analyte concentration.

3.1. Dynamic Light Scattering (DLS) characterization

Prior to DLS analysis, the prepared nanoMIPs solution was sonicated for 2 min. Subsequently, nanoMIPs were diluted (1:10) in water and analysed. DLS results displayed a diameter of 138 ± 27.80 nm for nanoMIPs, as shown in Table 1 and Figure 2. The polydispersity indexes (PDIs) provide information about the uniformity and mono-dispersive nature of nanoparticles. The PDIs of nanoMIPs was 0.137, which indicates a homogeneity and uniform distribution of polymer nanoparticles

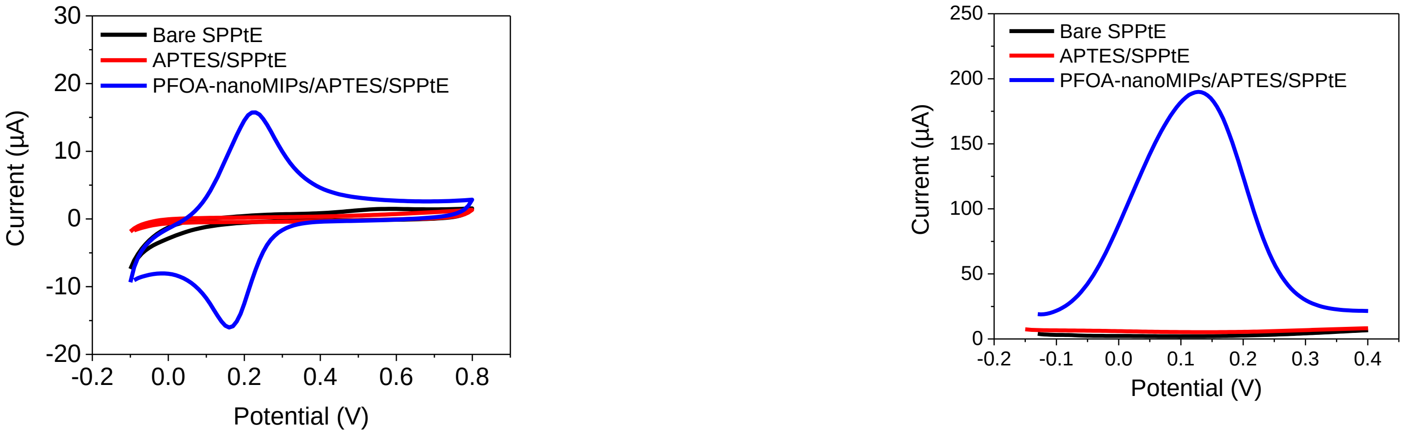


(**a**) (**b**)

Figure 2. Dynamic Light Scattering analysis of the PFOA-nanoMIPs showing (**a**) particle size distribution and (**b**) the plot of correlation coefficient *vs* time.

3.2. Electrochemical characterization

The electrochemical characterization for bare SPPtE, APTES/SPPtE and PFOA-nanoMIPs/APTES/SPPtE is presented in Figure 3, which shows CV and DPV measurements, respectively.



(**a**) (**b**)

Figure 3. Electrochemical characterization by (**a**) CV (fifth cycle) and (**b**) DPV in PBS solution for (black) bare screen-printed platinum electrode; (red) functionalized APTES/SPPtE, (blue) after PFOA-nanoMIPs immobilization. DPV condition: (i) Potential range: −0.15 to + 0.4 V; (ii) scan rate: 50 mV s^{-1}; (iii)potential step: 4.95 mV, (iv) modulation amplitude: 0.2V, (v) time pulse: 20 ms. CV condition: (i) Potential range: −0.1 to + 0.8 V; (ii) scan rate: 50 mV s^{-1}; (iii)potential step: 10 mV.

Bare SPPtE exhibited fewer electrochemical activity in PBS solution, as expected. After functionalization with APTES, the electrode retained its poor electroactivity. Very interestingly, after PFOA-nanoMIPS immobilization, a reversible redox process was obtained, with oxidation and reduction peaks located at + 0.21 V and -0.195 V, respectively. The aforementioned redox activity is due to the presence of ferrocene-methylmetacrylate moieties into the backbone of nanoMIPs, which was opportunely added during the polymerization stage. The presence of those redox labels can provide a sophisticated and enhanced sensitivity of the receptor towards its target, which is not electroactive at the bare SPPtE (results not shown). Results were confirmed also by DPV measurements, were a high-peak current at + 0.12 V (now chosen as the electrochemical response of the nanoMIPs) was attributed to the presence of ferrocene, confirming the successfully immobilization of PFOA-nanoMIPs.

3.3. Effect of APTES concentration

To allow maximization of nanoMIP immobilisation, the functionalization of SPPtE was investigated. Basically, the effect of APTES concentration (0.5, 1, 2.5, 5% v/v) on electrochemical response of nanoMIPs was studied by CV and DPV measurement (Figure 4). As visible from DPV measurements in PBS solution, the electrochemical responses of nanoMIPs increases in the intensity peak current by increasing APTES concentration, which can be more accommodate the receptor onto the electrode surface. For that reason, 5 % of APTES was chosen as the optimum condition for electrode functionalization.

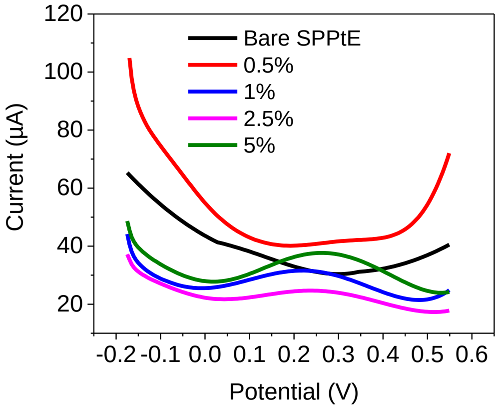
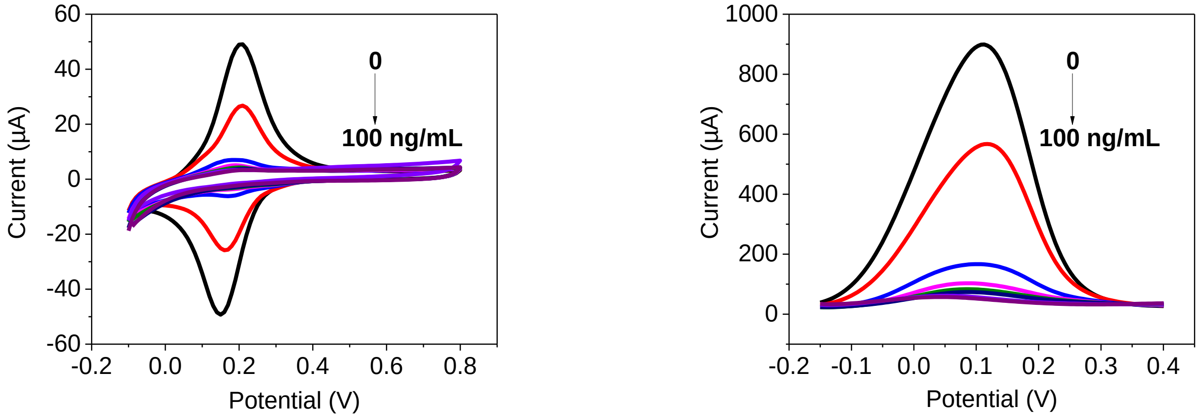


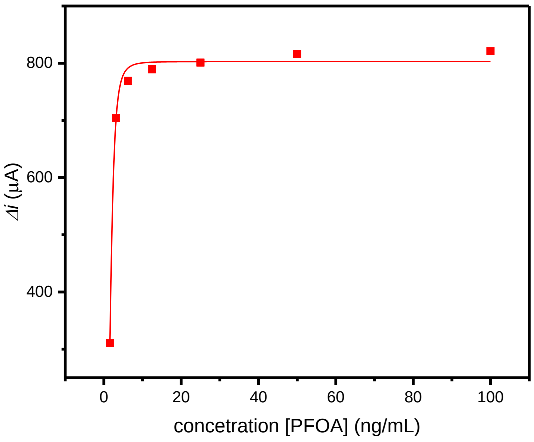
Figure 4. Electrochemical characterization by DPV in PBS solution for (black) bare screen-printed platinum electrode; (pink) 0.5%, (blue) 1%, (green) 2.5%, (purple) 5% of APTES in EtOH v/v. DPV condition: (i) Potential range: −0.175 to + 0.6 V; (ii) scan rate: 50 mV s−1; (iii)potential step: 4.95 mV, (iv) modulation amplitude: 0.2V, (v) time pulse: 20 ms. CV condition: (i) Potential range: −0.1 to + 0.8 V; (ii) scan rate: 50 mV s−1; (iii)potential step: 10 mV.

3.4. Analytical assessment of PFOA-nanoMIPs sensor

The electrochemical sensing of PFOA on developed PFOA-nanoMIP sensor was investigated by DPV and CV measurements, respectively. Sensor was exposed to distinct PFOA concentrations ranging between 1.5 and 100 ng/mL in PBS solution (Figure 5).



(**a**) (**b**)



(**c**)

Figure 5. (a) CV and (b) DPV measurements performed for PFOA-nanoMIPs sensor after the exposure to (blank, 0) 1.5, 3, 6, 12, 25, 50, 100 ng/mL of PFOA dissolved in 5 mM PBS. DPV condition: (i) Potential range: −0.15 to + 0.4 V; (ii) scan rate: 50 mV s−1; (iii)potential step: 4.95 mV, (iv) modulation amplitude: 0.2V, (v) time pulse: 20 ms. CV condition: (i) Potential range: −0.1 to + 0.8 V; (ii) scan rate: 50 mV s^{-1}; (iii) potential step: 10 mV. (c) Related calibration curve fitted with Hill isotherm of PFOA-nanoMIPs sensor.

As shown in Figure 5a, the electroactivity of nanoMIPs greatly decreased with increasing concentration of PFOA. The latter was most visible by DPV measurements (Figure 5b), which can be explained as the interaction of PFOA with the selective cavities. The redox properties of ferrocenylmethyl methacrylate give electroactive properties to nanoMIPs, used for electrochemical sensing of non-electroactive molecule. The selective interaction of the electrode modified with nanoMIPs with the non-electroactive target molecule causes a decrease in the redox current of the ferrocene groups, proportional to the PFOA concentration. This can be attributed to the effect of the non-electroactive analyte on the redox processes of ferrocene. The attenuation of the PFOA binding current results in a restriction of the counter ions that access the anchored redox probe to balance the charge, thus hindering the transfer of electrons [15] Particularly, the platform resulted very sensitive towards PFOA, with a slope of 0.578 µA/ng mL^{-1} and a linear working range between 0 and 6 ng mL^{-1}. The limit of detection, LOD, calculated as 3s/*m* where s was the standard deviation of the intercept and *m* is the slope of the regression plot, was 0.84 ng mL^{-1}. Saturation of cavities was reached at 50 ng/mL, due to the occupancy of cavities. In these cases, the optimal mathematical model, which introduces the nonlinearity, is the sigmoidal model, therefore, the curve fitting was performed using the saturation link of the nonlinear Hill function equation [16](Figure 5d). The proposed sensor shows high sensitivity and affinity with the analyte. These preliminary results currently encourage us to conduct further experiments with respect to lower concentrations, selectivity properties, and application to real water sample, which we will report shortly.

4. Conclusions

Preliminary results on the preparation of voltammetric sensor based on nanoMIPs for PFOA determination in water were reported. Preparation of nanoMIPs by solid-phase approach in the presence of redox label, such as ferrocenylmethyl methacrylate, provided highly sensitive electroactive receptor for further advanced uses in sensing purposes. The developed platform was assessed towards increased concentration of PFOA in standard solution of PBS, exhibiting highly sensitive properties. Further experiments to evaluate more properties of the sensor are currently under study.

Author Contributions: "Conceptualization, S.D.M., C.Z., C.M., S.P.; methodology, M.C., C.Z.; formal analysis, M.C.; investigation, S.D.M.,M.C.,C.Z.,C.M.,S.P.; data curation, M.C.,S.D.M.; writing—original draft preparation, M.C.; writing—review and editing, S.D.M.,C.Z.,C.M.,S.P.; supervision, S.D.M.,C.Z.,C.M.,S.P. All authors have read and agreed to the published version of the manuscript."

Funding: This research was funded by the Ph.D. program entitled "Green Analytical Chemistry: development of molecularly imprinted polymers for emerging pollutants" (CUP:F85F21005750001) of "Dottorati su tematiche Green del PON R&I 2014-2020".

Informed Consent Statement: Not applicable.
Data Availability Statement: Data will be available under request.

Acknowledgments: The authors would like to thank Alvaro Garcia Cruz for his experimental support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lindstrom, A.B.; Strynar, M.J.; Libelo, E.L. Polyfluorinated compounds: past, present, and future. *Environ. Sci. Technol.* **2011**. *45*,7954–7961.
2. Buck, R.C.; Franklin, J.; Berger, U.; Conder, J.M.; Cousins, I.T.; De Voogt, P.; Jensen, A.A.; Kannan, K.; Mabury. S.A.; van Leeuwen, S.P. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr. *Environ. Assess. Manag*. **2011**., *7*, 513–541.
3. Vaalgamaa, S.; Vähätalo, A. V.; Perkola, N.; Huhtala, S. Photochemical reactivity of perfluorooctanoic acid (PFOA) in conditions representing surface water. *Sci. Total. Environ*. **2011**, *409*, 3043–3048.
4. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol. Sci*. **2007**, *99*, 366–394.
5. Butt, C. M.; Berger, U.; Bossi, R.; Tomy, G.T. Levels and trends of poly-and perfluorinated compounds in the arctic environment. *Sci. Total. Environ*. **2010**, *408*, 2936–2965.
6. Nakayama, S.F.; Strynar, M.J.; Reiner, J.L.; Delinsky, A.D., Lindstrom, A.B. Determination of perfluorinated compounds in the Upper Mississippi River Basin. *Environ. Sci. Technol.* **2010**, *44*, 103–4109.
7. Dincer, C.; Bruch, R.; Costa-Rama, E.; Fernández-Abedul, M.T.; Merkoçi, A.; Manz, A.; Urban, G.A.; Güder, F., Disposable sensors in diagnostics, food, and environmental monitoring. *Adv. Mater.* **2019**, *31*, 1806739.
8. Naresh, V.; Lee, N. A review on biosensors and recent development of nanostructured materials-enabled biosensors. *Sensors* **2021**, *21*,1109.
9. Xiong-Hui, M.A.; Jian-Ping, L.I.; Chao, W.A.N.G.; Guo-Bao, X.U. A review on bio-macromolecular imprinted sensors and their applications. *Chin. J. Anal. Chem*. **2016**, *44*, 152–159.
10. Kadhem, A.J.; Gentile, G.J.; Fidalgo de Cortalezzi, M.M. Fidalgo de Cortalezzi, Molecularly Imprinted Polymers (MIPs) in Sensors for Environmental and Biomedical Applications: A Review. *Molecules* **2021**, *26*, 6233.
11. Ahmad, O.S.; Bedwell, T.S.; Esen, C.; Garcia-Cruz, A.; Piletsky, S.A. Molecularly imprinted polymers in electrochemical and optical sensors. *Trends Biotechnol*. **2019**, *37*, 294–309.
12. Haq, I., et al., Determination of sitagliptin in human plasma using a smart electrochemical sensor based on electroactive molecularly imprinted nanoparticles. Nanoscale Adv. **2021**, *3*, 4276–4285.
13. Garcia-Cruz, A.; Ahmad, O.S.; Alanazi, K.; Piletska, E.; Piletsky, S.A. Generic sensor platform based on electro-responsive molecularly imprinted polymer nanoparticles (e-NanoMIPs). *Microsystems Nanoeng*. **2020**, **6**, 83.
14. Canfarotta, F.; Poma, A.; Guerreiro, A.; Piletsky, S. Solid-phase synthesis of molecularly imprinted nanoparticles. *Nat. Protoc.* **2016**, *11*, 443–455.
15. Khor, S.M.; Liu, G.; Fairman, C.; Iyengar, S.G.; Gooding, J.J. The importance of interfacial design for the sensitivity of a label-free electrochemical immuno-biosensor for small organic molecules. *Biosens. Bioelectron*. **2011**, *26*, 2038–2044.
16. Ko, S.;Gunasekaran, S. Analysis of cheese melt profile using inverse-Hill function. *J. Food Eng*. **2008**, *87*, 266–273.