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NRL NISE – Towards the Reagent-Less Electrochemical Detection of Polyfluoroalkyl-Compounds in Water

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14. ABSTRACT

We have been developing a prototype instrument that is based on the electrochemical detection of water contaminants (e.g. heavy metals, pesticides, industrial compounds, and polyfluoroalkyl substances) to assess the safety of drinking water. One of the main limitations of water analysis in the field for the warfighter is the dependence of chemical sensors with added reagents. Current water analysis kits are designed for company-sized units. These kits are far too large for individual warfighters and squads and include limitations such as reagent expiration, insufficient user training, and relatively long time to result. This handheld instrument will benefit warfighters by enabling the testing of water sources for drinking safety, especially if the mission excludes bulk water delivery.

For polyfluoroalkyl-compounds, the indirect electrochemical detection of perfluorooctanesulfonic acid is reported using molecularly imprinted polymers on screen printed electrodes and epitaxial graphene elecrodes. In conjuction, we also developed a unique sensor probe to eliminate the need for added buffers or salts. Our device has favorable Size, Weight, and Power characteristics; is powered by rechargeable NiMH batteries; and can be interfaced with a laptop computer or other device via a wireless Bluetooth connection. This result adds to warfighter capability by developing a hand-held, battery-powered device that can perform a set of inexpensive electrochemical assays capable of providing real-time broad-spectrum quantification of relevant water contaminants in the field.

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EXECUTIVE SUMMARY

We have been developing a prototype instrument that is based on the electrochemical detection of water contaminants (e.g. heavy metals, pesticides, industrial compounds, and polyfluoroalkyl substances) to assess the safety of drinking water. One of the main limitations of water analysis in the field for the warfighter is the dependence of chemical sensors with added reagents. Current water analysis kits are designed for company-sized units. These kits are far too large for individual warfighters and squads and include limitations such as reagent expiration, insufficient user training, and relatively long time to result. This handheld instrument will benefit warfighters by enabling the testing of water sources for drinking safety, especially if the mission excludes bulk water delivery.

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1. INTRODUCTION

We report on work to develop an electrochemical assay to detect polyfluoroalkyl substances (PFAS) in drinking water using our low cost, hand held, and battery-powered potentiostat named the CStat[1] shown in Figure. In addition to being able to detect heavy metals, herbicides, pesticides, and other toxic industrial chemicals in seawater[2-4], we have also shown that the instrument (specifically designed for rapid field assays in a handheld format) can detect PFAS in water using electrodes modified with molecularly imprinted polymers (MIPs).[5]

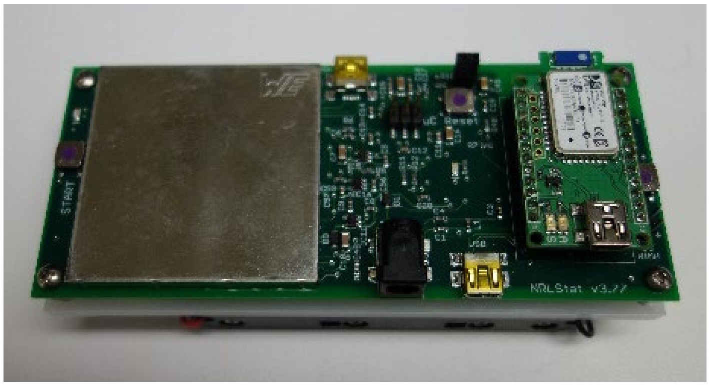

Figure 1. The CStat. The instrument in the photograph is TRL 4. The disposable electrode and its associated cable are not shown. The total weight of the CStat including 6 AA-NiMH rechargeable batteries is less than 300 g with a battery endurance of 20+ hours when running at peak output and a 100% duty cycle.

PFAS are suspected to be responsible for a wide range of long-term illnesses, but have proven to be very difficult to detect without the use of centralized reference laboratories. Although these compounds are not electrochemically active, recent studies have reported that detection of PFAS in aqueous samples is possible with appropriately modified electrodes with molecular imprinted polymers.[6-8]

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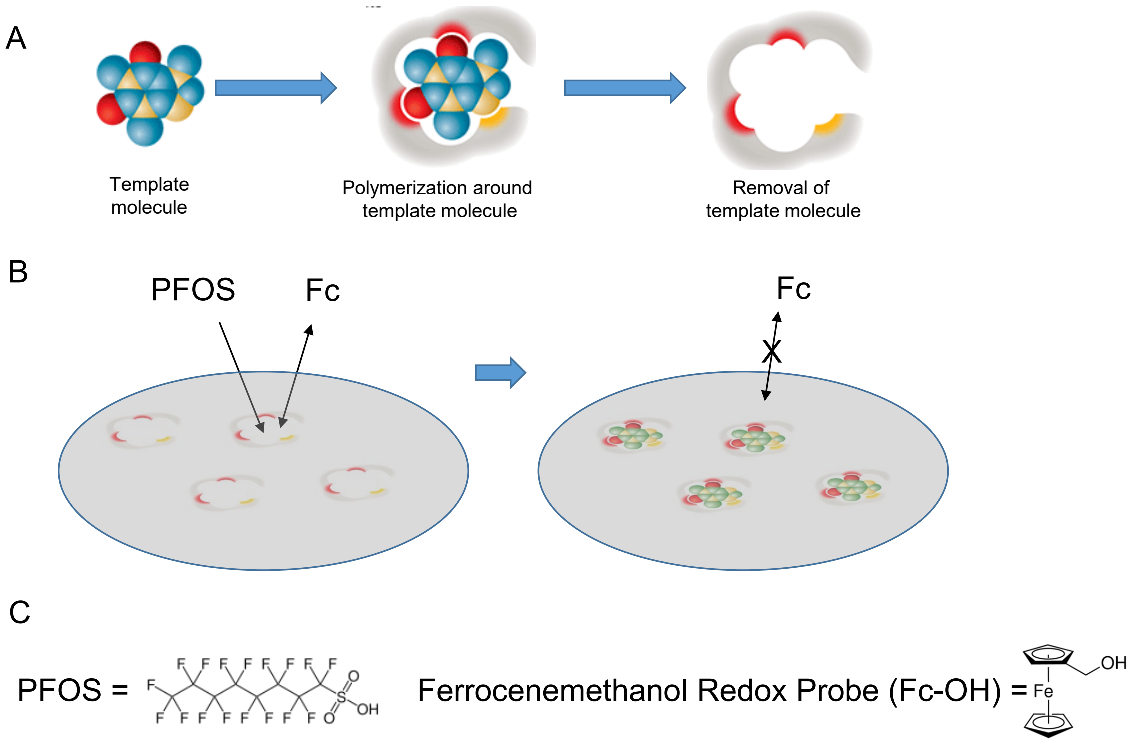

Figure 2. A schematic of molecular imprinted polymers on electrodes showing the strategy of creating a MIP with a template molecule (**A**) and the use of a MIP at an electrode surface for detection (**B**). The chemical structures of PFOS (the analyte to be detected) and Fc-OH (the working redox probe) are shown in panel **C**.

Our strategy focuses on the use of molecular imprinted polymers.[9-11] As shown in Figure 2A, the process begins with a selected imprintable polymer (one example being phenylenediamine) that are incubated with the target of interest and then electro-polymerized onto a working electrode, and finally followed by embedded target removal with solvent. The result is an imprinted electrode that is sensitive to adsorption of the target compound. Similar to enzymes, target selectivity is based on its shape, charge, polarity and surface characteristics.

Because the polymer is an insulator, the only electrochemically useable portions of the electrode are those that were imprinted. In the absence of target, oxidation and reduction of a redox probe like ferrocene can take place within the imprinted regions (Figure 2 B). In the presence of target, some or all of the imprinted regions will be blocked. The amount of blocked region is based on the target adsorption isotherm; this can be quantitatively measured by monitoring the activity of a redox probe like ferrocene.

Since one of the main limitations of water analysis in the field for the warfighter is the dependence on sensors with additive reagents, we are developing a molecular toolbox based on ionic liquids focusing at the liquid/liquid interface to eliminate this need. Ionic liquids (IL) are salts of cations and anions having irregular structures causing them to remain in liquid solution at room temperature.[12] Typical electrochemical detection schemes are based on buffered water using simple salts as electrolytes; however, the use of ionic liquids can give an advantage for electroanalytical techniques due to their low vapor pressure, high stability and large voltage window. The chemical parameter space for ILs is quite large which presents an opportunity to exploit a variety of chemical properties of the solvent for enhanced chemical sensors. In this report, we demonstrate that the CStat can detect PFAS in spiked water samples using a working electrode modified with MIPs in conjugation with an electrochemical probe design based on ILs that eliminates the need for the addition of buffers or reagents.

2. EXPERIMENTAL

2.1 Materials

The potassium salt of heptadecafluorooctanesulfonic acid (PFOS), o-phenylenediamine (o-PD) (8 mg tablets from SigmaFast), ferrocene (Fc), ferrocenemethanol (Fc-OH), and 1-Hexyl-3-methylimidazolium hexafluorophosphate (HMIM-PF_6) were purchased from Sigma-Aldrich (St. Louis, MO). Screen-printed carbon electrodes and the nylon membranes were purchased from Metrohm USA Inc (Riverview, Fl). Epitaxial graphene on SiC was acquired from a previous study.[2]

2.2 Molecularly Imprinted Polymers and PFOS detection

A custom-built potentiostat (the CStat v3.79), used for all assays reported in this study, has been demonstrated previously for electrochemical detection of multiple compounds, including nitrogen-containing explosives, heavy metals, herbicides, pesticides, and industrial chemicals.[2-4] Electropolymerization at a working electrode was performed with a 2:1 (v/v) mixture of 0.1 M acetate buffer (pH = 5.8) and methanol containing 10 mM o-PD and 1 mM PFOS. A potentiostat from CH instruments was used for the electropolymerization. Cyclic voltammograms were recorded for 25 cycles in a potential window of 0 − 1 V vs Ag/AgCl at a scan rate of 200 mV/s.

To remove the PFOS, the imprinted electrodes were soaked in 50:50 water: methanol solution with slight shaking for 1 hr. X-ray photoelectron spectroscopy (XPS) was performed using a Thermo Scientific Nexsa instrument with a monochromatic Al-Kα source (1486.68 eV) and 400 μm spot size. Survey scans were collected using 200 eV pass energy and 1 eV step size, and high resolution scans of the C 1s, O 1s, N 1s, F 1s, and Au 4f peaks were acquired using 20 eV pass energy and 0.15 eV step size.

For the traditional approach, PFOS detection was performed in PBS buffer with the MIP electrodes using redox probe ferrocenemethanol. Parameters used for square wave voltammetry were a square wave frequency of 17.5 Hz, and a potential range from 0 to 0.5 V vs. Ag/AgCl. Dose response curves were generated by measuring the absolute net peak current decrease of the ferrocenemethanol probe as a function of PFOS concentration.

For the reagent-less approach a probe is pre-fabricated using a solution of Fc in HMIM-PF_6 made by adding 4 mg of ferrocene (Fc) to 100 μL of HMIM-PF_6 in a small Eppendorf tube and mixed by a vortexer. To remove excess Fc from the HMIM-PF_6, the sample was centrifuged for 5 minutes at 6,000 rpm and decanted to generate a saturated solution of Fc in HMIM-PF_6, which was slightly yellow. The compact probe was made by placing a nylon membrane with adhesive (Metrohm USA Inc) on a carbon screen-printed electrode and then adding 10 or 20 μL of the Fc solution in HMIM-PF_6. The compact probe was then introduced to 10 mL of DI water and electrochemical measurements were made with the CStat. PFOS was added from a stock solution in MeOH and the sample was allowed to stir with a magnetic stir bar for 5 minutes at 300 rpm before each measurement. Dose-response curves were generated by measuring the absolute net peak current decrease of the ferrocene probe as a function of PFOS concentration.

3. RESULTS AND DISCUSSION

Molecularly imprinted polymers (MIPs) at the surface of working electrodes were created as reported from the chemical literature for PFOS by the electropolymerization of o-phenylenediamine (o-PD) using cyclic voltammetry in a 0.1 M acetate buffer (pH = 5.8) at a molar ratio of 10:1 o-PD to PFOS. On thin film gold electrodes, XPS studies showed that the template molecule, PFOS, was contained in the electropolymerized film and approximately 2/3 of the PFOS in the MIP film could be removed in a 50% mixture of MeOH and water (data not shown).

To test for PFOS detection using the MIPs in both the traditional and reagent-less configurations, we used either Fc-OH (for the traditional assay) or Fc (for the reagent-less-probe) as the redox probe. Fc base compounds are traditionally used as electrochemical redox probes since the Fc group can be easily oxidized and reduced at assessable potentials in water displaying nearly ideal electrochemistry. In the traditional assay, PBS was used as the sample matrix, and for the reagent-less-probe, distilled water was used as the sample matrix.

3.1 MIPs with added redox probe

As shown in Figure 3A, detection of PFOS was successful using the o-PD MIPs for PFOS at screen-printed carbon electrodes in a PBS matrix. As the PFOS concentration increased, more of the binding sites generated by the polymerization of molecularly imprinted o-PD were bound by PFOS, which simultaneously showed a decrease in the electrochemical signal for the Fc-OH redox probe as the electrode became more blocked. However, only a portion of the redox probe was blocked which suggests there are defect sites at the polymer/electrode interface. Screen printed electrodes tend to have a roughness factor sufficient to explain incomplete blockage of the electrode.[8] The linear range from the dose response curve was estimated to be between 100 to 200 nM with a LOD = 100 nM (3 x s/n).

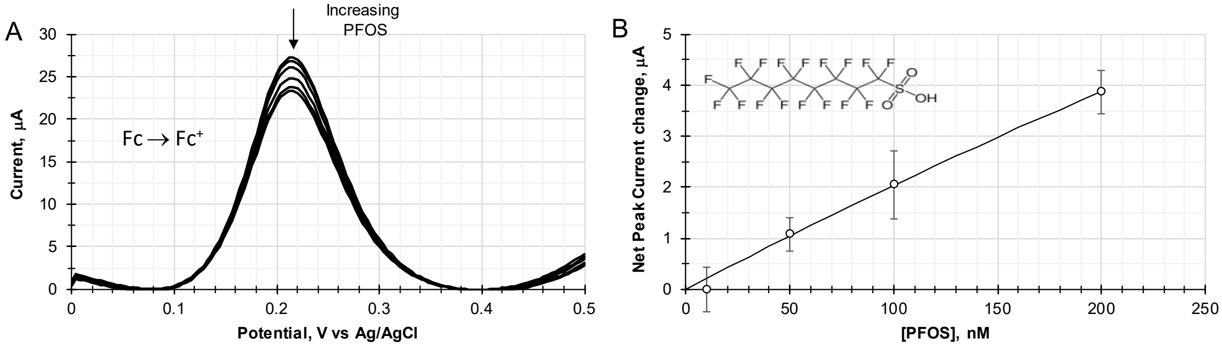

Figure 3. Electrochemical detection of PFOS using an o-PD based MIP at a carbon screen-printed electrode. **A.** Square wave voltammograms of Fc-OH showing the change with the additions of PFOS. **B.** The dose response curves of net peak current vs. [PFOS]. Experimental parameters: Square wave frequency = 17.5 Hz, Square wave amplitude 25 mV, Step = 4 mV, Sample matrix = PBS.

3.2 MIPs at Graphene

Since for screen-printed carbon electrodes, there was a significant signal response of the redox probe at polymer defect sites giving a large background current, we investigated MIPs on epitaxial hydrogen-intercalated, quasi-freestanding bilayer graphene (QFSBLG) to see if a flatter electrode would reduce the amount of polymer defect sites, thus decreasing the background current.

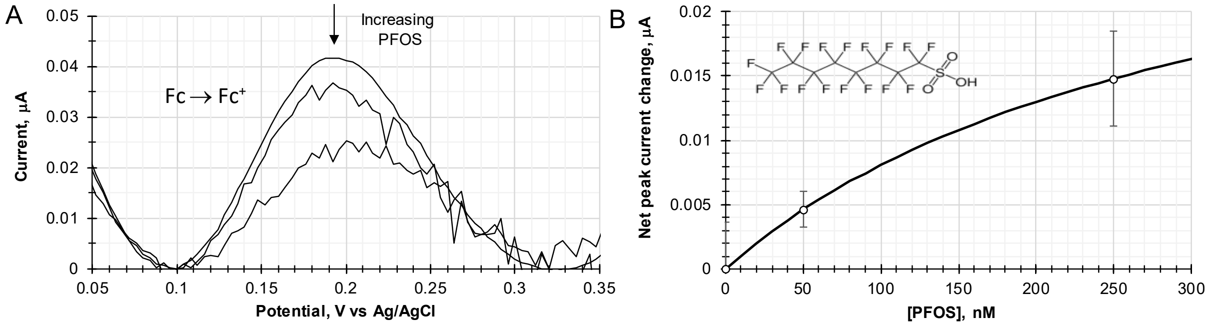

Figure 4. Electrochemical detection of PFOS using an o-PD based MIP at epitaxial hydrogen-intercalated, quasi-freestanding bilayer graphene (QFSBLG). **A**. Square wave voltammograms of Fc-OH showing the change with the additions of PFOS. **B**. The dose response curves of net peak current vs. [PFOS]. Experimental parameters: Square wave frequency = 17.5 Hz, Square wave amplitude = 25 mV, Step = 4 mV, Sample matrix = PBS.

The results of MIP modified QFSBLG for the electrochemical response in the voltammetry of the Fc-OH at the MIP modified electrode is shown in Figure 4. Increasing amounts of PFOS behave as a Langmuir binding curves with a decrease in current as the PFOS binds the MIP and blocks the electrode. In the case of the QFSBLG compared to screen printed carbon, there is a larger percent decrease in current with increasing PFOS which suggests a decrease in defect sites. For continued work in the future, we will look for a reduction in polymer defects on the graphene electrodes using atomic force microscopy (AFM) and/or scanning electron microscopy (SEM)/ energy dispersive x-ray spectroscopy (EDS).

3.3 MIPs on carbon with reagent-less design

We have initiated work to make the electrochemical probe reagent-less in order for the sensor to be useful in the field. In other words, we want the sensing probe to be free from the user having to add buffers or reagents to the sample. Our concept is shown in Figure 5. In Figure 5A, a typical electrochemical cell with three electrodes is shown. In the past several years, the three individual electrodes have been manufactured into screen-printed electrodes from which our compact probe reagent-less design is based (Figure 5B). The heart of the reagent-less probe design is the use of hydrophobic ionic liquids in a thin film on the working electrode to provide the underlining electrochemical response both as the electrolyte and as the component containing the redox probe, which interrogates the MIP. The structural variation of ILs is shown in Figure 5C, with the different cations and anions having chemical R groups that can range from hydrophilic to hydrophobic moieties. This design keeps the electrolyte and redox probe from leaching into the water sample, and at the same time, allows the PFOS to partition into the thin film as shown in Figure 5D.

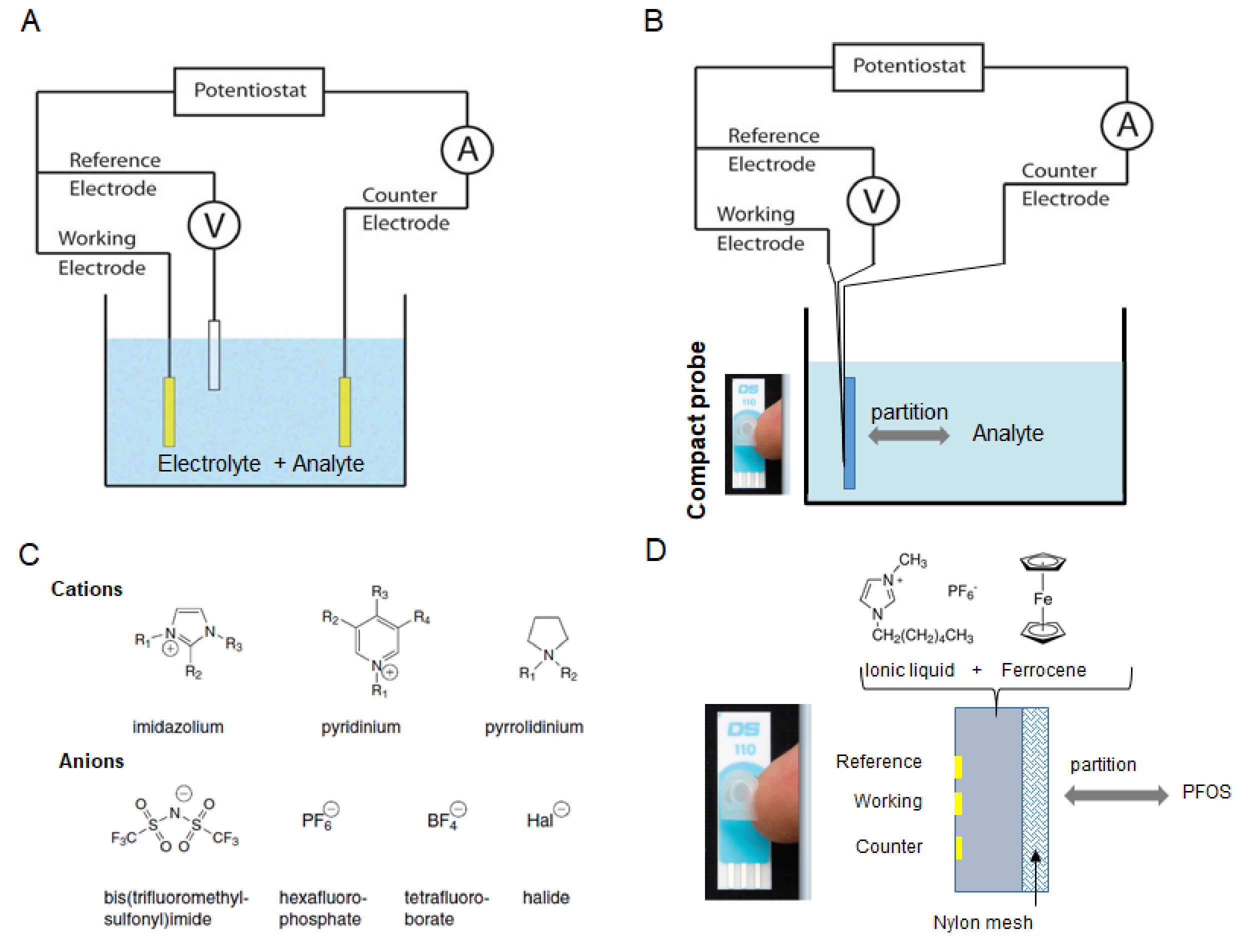

Figure 5. The reagent-less designed compact probe. **A.** A typical electrochemical cell with three electrodes. **B.** The schematic of the compact probe design. **C.** Various cations and anions of ILs. **D.** Details of the compact probe used in a thin layer design.

We constructed the compacted probe by placing a nylon membrane with adhesive (Metrohm USA Inc) on a carbon screen-printed electrode and then added 10 or 20 μL of HMIM-PF_6 saturated with Fc to the membrane. A well-defined and stable square wave voltammogram of Fc is shown in Figure 6A and 6B in stirring DI water. With the addition of PFOS, the MIP sites become blocked and the Fc current peaks in the voltammograms decrease. The net decrease can then be fit to a

Langmuir isotherm typical of a surface binding event. One note of interest is the increase in response to PFOS for the probe containing 20 μL vs 10 μL of IL.

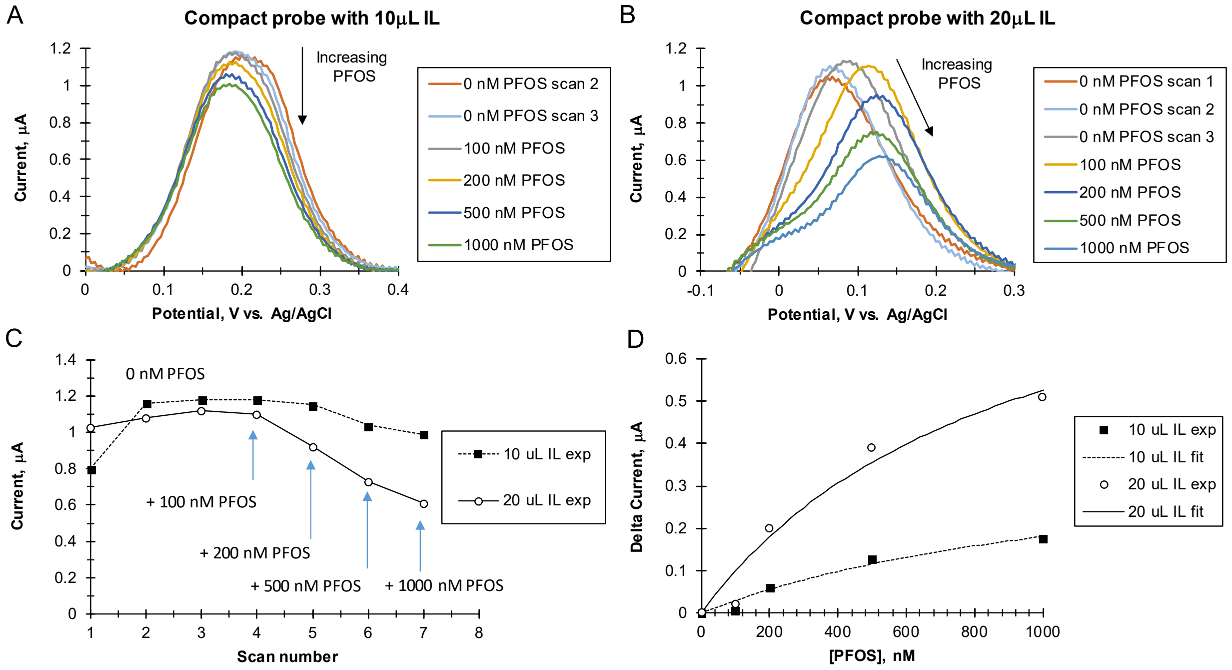

Figure 6. Electrochemical detection of PFOS using an o-PD based MIP at a carbon screen-printed electrode with a reagent-less compact probe design. **A.** Square wave voltammograms of the compact probe using 10 μL of IL showing the change with addition of PFOS. **B.** Square wave voltammograms of the compact probe using 20 μL of IL showing the change with addition of PFOS. **C.** Net peak current of the redox probe vs. scan number with the additions of PFOS. **D.** The dose response curves of net peak current vs. [PFOS]. Experimental parameters: Square wave frequency = 17.5 Hz, Square wave amplitude 25 mV, Step = 4 mV, Sample matrix = distilled water.

4. CONCLUSION

Using an electrochemical strategy, we have shown that a small custom-built potentiostat in conjugation with an electrochemical probe design based on ILs can be used for the detection polyfluoroalkyl-compounds in water. This compact probe design eliminates the need for the addition of buffers or reagents. While the initial work seems promising, we note that the reagent-less compact probe design has not been optimized. Questions remain which include; what amounts and concentrations of ILs and Fc are needed in the probe to maximize the response to PFOS? Moreover, how robust will the probe be to field conditions? Further work is required to advance this technology, and we will be expanding the scope of the chemicals that we can detect using the MIPs with ionic liquids for a reagent-less design both in water and even in the vapor phase. We envision a final device will have excellent SWaP characteristics; which can run on rechargeable NiMH batteries, and be interfaced with a computer.

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