



A High Sensitivity Biosensor to detect the presence of perfluorinated compounds in environment



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ABSTRACT

A novel surface plasmon resonance (SPR) optical fiber biosensor, able to bind perfluorooctanoate and perfluorooctanesulfonate compounds, is presented. In the first step, an ad hoc antibody compound has been designed, produced and tested by ELISA, then, in the second step, the gold surface of a plastic optical fiber sensor has been derivatized and functionalized with this new bio-receptor, able to bind target analytes with high affinity and selectivity. The experimental data have shown that the developed SPR optical fiber biosensor makes it possible to detect these compounds. One advantage of this approach stems from the possibility to monitor the perfluorinated compounds in the environment exploiting the remote sensing capability offered by the optical fibers. The measurements were performed in laboratory, also exploiting matrices mimicking the real environment. The limit of detection of the assay was 0.21 ppb, a value that is lower than the maximum residue limit fixed by the European Union regulations.

1. Introduction

Since 1950 perfluorooctanoate (PFOA; $C_8F_{15}COO^-$) and perfluorooctanesulfonate (PFOS; $C_8F_{17}SO^-$), belonging to the family of perfluoroalkyl and poly-fluoroalkyl substances (PFASs) were used as surfactant agents and/or as precursors in polymer production for both commercial and industrial applications.

In recent years the social and scientific interests in these compounds are considerably increased, due to their larger presence in the environment and, consequently, their potential toxicity to humans and animals. Huge consideration has been devoted to PFAS compounds and their effect on human health. PFOS and PFOA are the most extensively investigated PFASs because human exposition can occur through different pathways, and the dietary intake seems to be their main route of exposure [1].

PFAS compounds are widely distributed in the environment and they should be monitored in different micro-polluted water systems, such as river water, lake water and seawater. Also due to their remarkable chemical stability, they are considered to be inert and refractory to different chemical and microbiological treatments.

Consequently, they are persistent in the environment and can be bio-accumulative and toxic to mammalian species. In fact, the immunotoxic effects of PFASs to cellular systems and animals are largely demonstrated [2,3], and different epidemiologic research studies have demonstrated the potential effects of these chemical compounds on various human immune diseases. Recently, in vivo and in vitro studies on animal models have shown that PFASs (PFOA and PFOS) are weak environmental xeno-estrogens [4].

The scientific community has made great efforts to identify possible novel approaches for water treatment and/or detection of the PFASs in the environment. Currently, the conventional methods to PFOA determination include: high performance liquid chromatography (HPLC) [5,6], high performance liquid chromatography-mass spectrometry (HPLC-MS) [7], high performance liquid chromatography-mass spectrometry/mass spectrometry (HPLC-MS/MS) [8,9] and gas chromatograph-mass spectrometry (GC-MS) [10,11]. Furthermore, recently fluoros membrane based ion-selective electrodes [12] and a colorimetric sensor [13] for the detection of this class of compound have been described. All of the above methods are time-consuming and they often require an arduous pre-treatment step. In order to overcome these

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drawbacks, it is needed to find a simple, rapid and sensitive method for the detection of PFASs. In this scenario, a biosensor represents a valid tool to detect traces of specific molecules in different matrices. With this purpose an electrochemical biosensor for sensitive detection of PFOS was developed, based on the PFOS inhibition in the bio-catalysis process of enzymatic biofuel cell (BFC) [14].

In PFOA/PFOS detection, a very interesting perspective is the use of a platform based on optical fibers for on-site fast detection, also exhibiting the possibility of remote control. On this line of argument, we exploited a low cost SPR sensor platform based on plastic optical fibers (POFs) [15] together with a novel bio-receptor for the detection of PFOA/PFOS in aqueous medium.

POF systems are particularly advantageous due to their easily handling and installation procedures, large diameter of the fiber (a millimetre or more), low-cost and simplicity in manufacturing [16–18]. The SPR sensor based on a D-shaped POF is particularly interesting for bio/sensing applications because it works with a planar gold surface and an external medium refractive index ranging from 1.33 to 1.42, typical of the biosensors used for the selectivity detection of analytes in aqueous media by self assembling monolayers.

In this work, we developed and characterized a new SPR-POF biosensor to detect traces of PFOA/PFOS in seawater samples. For this purpose, the gold surface of the SPR-POF chip was chemically modified through the formation of a self-assembling monolayer (SAM) using the α -lipoic acid compound, as reported in Cennamo [19]. After this step, the functionalized gold surface was derivatized with an ad hoc produced mono-specific antibody against the PFOA [20].

The obtained results showed that the SPR-POF biosensor is able to sense PFOA/PFOS at a concentration less than 0.21 ppb. This result could be considered of interest when compared it to the detection limit of PFASs obtained by using different approaches as reported in Oughena et al. [21] and Trojanowicz et al. [22].

2. Materials and methods

2.1. Materials

N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), 3,3',5,5'-Tetramethylbenzidine (TMB), bovine serum albumin (BSA; fraction V), Protein A Sepharose resin, EAH-Sepharose resin and α -lipoic acid were purchased from Sigma-Aldrich (Sigma-Aldrich S.r.l. Milan, Italy). All other chemicals were commercial samples of the purest quality.

2.2. BSA-PFOA conjugates preparation

The antigen was prepared by reacting PFOA (0.8 mg/mL) with bovine serum albumin (8 mg/mL) in the presence of 4 mg/mL 1-Ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride (EED) in PBS buffer at pH 7.0. The reaction was run for 30 min at 37 °C, then, the mix was centrifuged at 350 g for 10 min and the supernatant desalted, using a PD10 column to remove un-bound PFOA. Mass spectrum shows that about 95% of the proteins have been derivatized with an average increase in the molecular mass of 2 kDa (66–68 kDa). As the molecular mass of PFOA is 414, this implies that, on average, about 5 PFOA molecules are bound to each BSA.

2.3. Antibody anti-PFOA production and purification

For the immunization, after extracting pre-immune serum, rabbits were intra-dermally injected with 200 μ g antigen (BSA-PFOA) in complete Freund adjuvant, and again, after 21 and 28 days, with 100 μ g antigen in incomplete Freund adjuvant. After 35 days, the animals were bled, the sera separated and subjected to the subsequent assays, such as ELISA tests. The obtained sera were used for the purification of total IgGs anti-PFOA. For this purpose a protocol according to Varriale

used [20]. In brief, 2.0 mL sample of anti-serum obtained from the two rabbits was diluted in 1:1 ratio with binding buffer (50 mM Tris-HCl, pH 7.4) and Protein A resin (Sigma) was applied. The wash steps were done using binding buffer and the total fraction of IgGs from resin was eluted by using a strong pH change (50 mM Glycine, pH 3.0). At the end of the purification process the purity of total IgGs was evaluated by SDS-PAGE analysis.

2.4. Affinity column preparation of PFOA-EAH Sepharose 4B and mono-specific antibody anti-PFOA purification

With the aim to purify a mono-specific anti-PFOA, an affinity column was prepared by conjugating of the PFOA to EAH Sepharose 4B. The protocol used is in accordance with the manufacturer's instructions. In brief, 1.2 mL sample of the resin was washed with H₂O at pH 4.5 (160 mL), with 0.5 M NaCl (100 mL), and again with H₂O at pH 4.5 (100 mL). The Sepharose resin was finally packed into a polystyrene column (10 mL, BIORAD) suspended in 2.0 mL of H₂O at pH 4.5 and the resulting suspensions were gently shaken. In the meantime, 2.8 mM of PFOA were diluted in 2 mL of ethanol and EDC (in H₂O pH 4.5) to a final concentration of 0.1 M. The reaction solution was mixed and incubated for 10 min at room temperature and then overnight at 4 °C. The solution of PFOA-EDC was added to the slurry resin and incubated with gentle shaking for 2 h at room temperature. The slurry resin solution was extensively washed with H₂O at pH 4.5, 0.5 M NaCl in 50% ethanol (15 mL). The slurry resin in 50% ethanol was previously treated with 15 mL of 0.1 M AcOH at pH 4.0, 0.5 M NaCl in 50% ethanol (blocking buffer) and later with 0.1 M Tris-HCl at pH 7.0, 0.5 M NaCl in 50% ethanol (wash buffer). After this step, the resin was washed with the blocking buffer and incubated for 30 min at room temperature. Afterward, the slurry resin solution was treated with 15 mL of the washing buffer and then with 15 mL of the blocking buffer. Finally, the resin was equilibrated with 10 mL of sodium phosphate buffer 20 mM, pH 7.4. The total IgGs obtained from the purification step of Protein A Sepharose was loaded from the EAH-PFOA resin produced. The purification protocol used is similar to the one used in Varriale [20]. After the loading step, the column was washed extensively with sodium phosphate buffer, pH 7.4 in order to remove the un-specific binding of the IgGs with the EAH-PFOA resin. The mono-specific IgGs was eluted by strong pH changing (Glycine 0.1 M pH 3.0) and the purity of the obtained mono-specific antibody was evaluated through the SDS-PAGE test.

2.5. ELISA test

The antibody titer was determined using indirect ELISA assay. We used the general procedure reported by Pennacchio [23]. To avoid interference by the protein carrier in the polyclonal antibody detection process, PFOA was conjugated to the glutamine-binding protein (GlnBP) isolated from *E. coli*, and purified according to Staiano [24]. GlnBP-PFOA (2 mg/mL), diluted 1/200, was dissolved in coating buffer at pH 9.5 (25 mM carbonate/bicarbonate) and was deposited on coat 96-well micro-plates surface in a range of concentrations from 1.2 ng/mL to 1.7 ng/mL GlnBP with same concentration was dissolved in coating buffer and used as control sample. The plate was incubated overnight at 4 °C. After this incubation, it was washed three-times with PBS buffer (0.1 M) containing 0.05% Tween (PBS-T), pH 7.4 and blocked for 1 h at room temperature with a solution of 1% milk in PBS-T buffer. The wells were washed several times with PBS-T after each step, incubated with mono-specific anti-PFOA antibodies at 25 °C for 1 h and subsequently with horseradish peroxidase-conjugated anti-rabbit IgG antibodies (diluted 1:12,000). This solution was incubated for 1 h at room temperature. The enzyme substrate TMB was added, and the colour reaction was quenched after 5 min by the addition of 2.5 M HCl. The absorbance value at 450 nm was measured, plotting the reciprocal of the antibody dilution against absorbance.

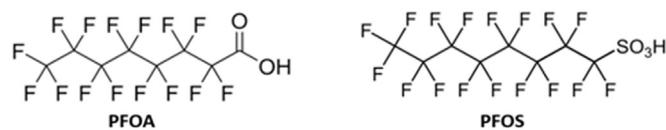


Fig. 1. Structures of most common PFASs compounds, (A) perfluorooctanoate acid (PFOA) and (B) perfluorooctanesulfonic acid (PFOS).

2.6. Optical platform and experimental setup

The optical sensor platform is based on surface plasmon resonance (SPR) in a D-shaped POF, with a buffer layer between the exposed POF core and the thin gold film. This optical platform is realized by removing the cladding of POF (along half circumference), spin coating a buffer layer on the exposed core and finally sputtering a thin gold film. The sensing region is about 10 mm in length. The buffer layer proposed in this work is the photoresist Microposit S1813, with a refractive index greater than the one of the POF core. This buffer layer improves the performances of the SPR sensor [15].

In the visible range of interest, the refractive indices of the optical materials are about 1.49 RIU for POF core (PMMA), 1.41 RIU for cladding (fluorinated polymer) and 1.61 RIU for buffer layer (Microposit S1813). The size of the POF is 980 μm of core and 20 μm of cladding (1 mm in diameter), whereas the multilayer on D-shaped POF presents a thickness of the buffer layer of about 1.5 μm and a thin gold film of 60 nm. The planar gold surface can be employed for depositing a bio-receptor layer, as we will explain in the next section. In this case the selectivity detection of the analyte is possible. The experimental configuration based on simple and low-cost components is composed by a halogen lamp (HL-2000-LL, manufactured by Ocean Optics, Dunedin, FL, USA) exhibiting a wavelength emission range from 360 nm to 1700 nm, as the light source, the SPR-POF biosensor and a spectrometer (FLAME-S-VIS-NIR-ES, manufactured by Ocean Optics, Dunedin, FL, USA), with a detection range from 350 nm to 1023 nm, connected to a PC.

The SPR transmission spectra, normalized to the reference spectrum achieved with air as the surrounding medium, are obtained using the Matlab software and the resonance wavelength was extracted for the analytical [15].

2.7. Immobilization process on the chip surface

The surface of the POF chip was sequentially cleaned with: (1) milli-Q water (3 times for 5 min) and (2) 10% of ethanol solution in milli-Q water (3 times for 5 min). The surface of the chip was pre-treated before the covalently immobilization of mono specific antibody and the procedure consists of three different steps: (1) thiol film production, (2) derivatization of the surface by EDC/NHS (3) antibody against PFOA/PFOS immobilization.

In the first step the gold chip was immersed in freshly prepared

solution of α -lipoic acid dissolved in a solution of pure ethanol 10% in water at the final concentration of 40 mM and incubated at 25 $^{\circ}\text{C}$ for 18 h. After this period of incubation, the gold-coated substrate surface was washed with milli-Q water and incubated 20 min at room temperature with a mixture of EDC/NHS at the final concentration of 20 mM and 50 mM respectively, dissolved in potassium phosphate buffer 100 mM, pH 5.5. The final step was the incubation of the surface with a solution msAb anti-PFOA 2.0 mg/mL (100 μL) for 2 h at room temperature dissolved in sodium phosphate buffer 20 mM, pH 7.5. The chips were, at the end of this treatment, washed with sodium phosphate buffer 20 mM, pH 7.5 and finally dried with a nitrogen flow.

2.8. Binding experiments

Experimental results were collected by the SPR-POF biosensor and the setup previously illustrated. After each addition of the sample (solution with different concentration of the analyte), we have used a standard measuring protocol based on these three steps: first, incubation step for bio-interaction between analytes and receptor (for 10 min at room temperature); second, washing step with PBS (buffer); third, recording step for the spectrum (when buffer is present as bulk).

This protocol is necessary in order to measure the shift of the resonance determined by the specific binding (analyte/receptor interaction) on the sensing surface, and not by bulk refractive index changes or by non-specific binding between gold surface and analyte. We have tested the binding between the SPR-POF-biosensor's receptor and the PFOA in the range from 0.0 to 100 ppb, in 20 mM sodium phosphate buffer pH 7.4 (PBS). An analogous experiment was performed in a solution 460 mM NaCl (a solution very similar to seawater as regard the ionic strength).

3. Results and discussion

3.1. The novel bio-receptor: preliminary investigation and selectivity

PFOA is a low molecular weight compound (Fig. 1A) used together with other perfluoroalkyl and poly-fluoroalkyl compounds as a surfactant agent in polymer production for both commercial and industrial applications.

Since PFOA compound is too small to elicit any immunological response if injected in animals, we used the following strategy to produce high-affinity polyclonal antibody against this compound: PFOA compounds were covalently attached to an immunological protein carrier (BSA). In Fig. 2 is reported the scheme of conjugation reaction between BSA and the PFOA. The reaction between the carboxyl group of PFOA and amino reactive groups of the carrier was performed at room temperature and pH 6.0, using the EDC/NHS conjugation protocol. The obtained BSA-PFOA conjugate was employed to produce a high-affinity antibody, using a standard protocol of immunization. Then the monospecific antibodies against PFOA (msAb-PFOA) were purified from the

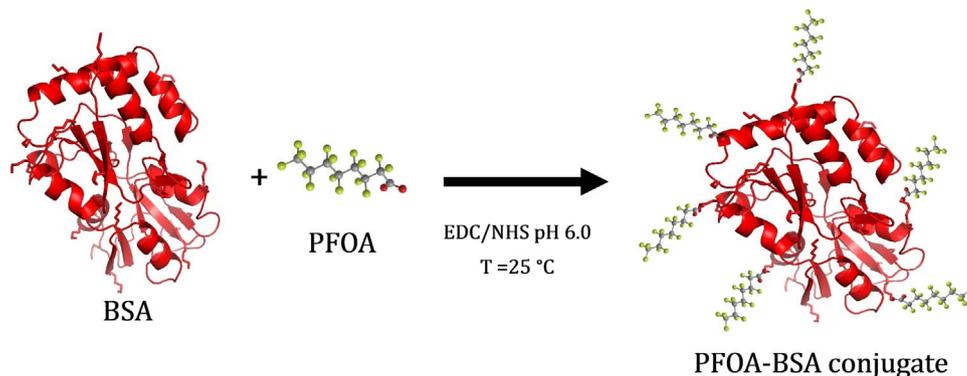


Fig. 2. Scheme of conjugation reaction between the BSA and PFOA, the reaction was performed at RT, at pH 6.0 for 2 h.

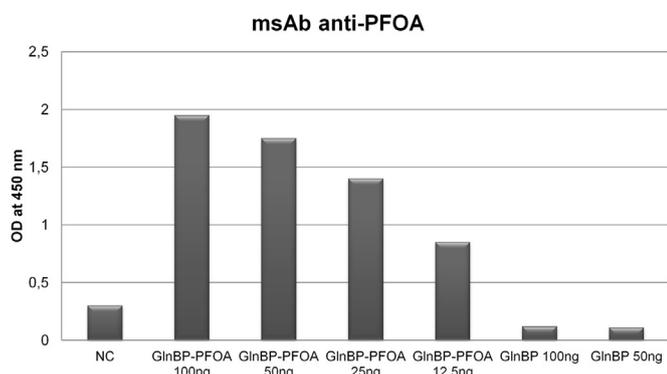


Fig. 3. Indirect ELISA test results obtained using msAb anti-PFOA. The assay was performed in the Tris-borate buffer in the presence of 0.005% Tween and 1% milk. Temperature was set at 25 °C. The dilution of anti PFOA was 1:12,000.

total IgGs fraction by an affinity chromatography using PFOA-EAH-Sephacrose-4B resin. The purity of different samples, obtained from the chromatography step, was analyzed by SDS-PAG. Their molar

concentration was determined spectrophotometrically, and the pooled samples of antibody solutions were tested by Dot blot experiments according to Di Giovanni [25]. The results show a response to antibody binding only for the conjugate GlnBP-PFOA. Contrary a negative response was registered for GlnBP and BSA, confirming the specificity and selectivity of the antibodies versus the PFOA compound and not versus the carrier used in the immunization processes (data are not shown). To estimate the titer of the msAb against the PFOA, an indirect ELISA test was performed. In order to avoid interference by the carrier protein in the msAb detection process, the PFOA molecule was conjugated to the GlnBP isolated from *E. coli*. Microplate wells were coated with different concentrations of antigens GlnBP-PFOA and reacted with serially diluted mono-specific antibodies against PFOA. Fig. 3 shows the results of the ELISA tests as a bar histogram in which the absorbance value at 450 nm is plotted against different concentrations of coated GlnBP-PFOA. No signal was registered from non-coated wells. The results show a positive signal when the msAb dilution is up to 1 in 12,000.

In order to test the antibody selectivity, the ELISA test was also performed using as potential antigens different molecules that are known to be co-pollutants in the seawater, (e.g naphthalene) and no

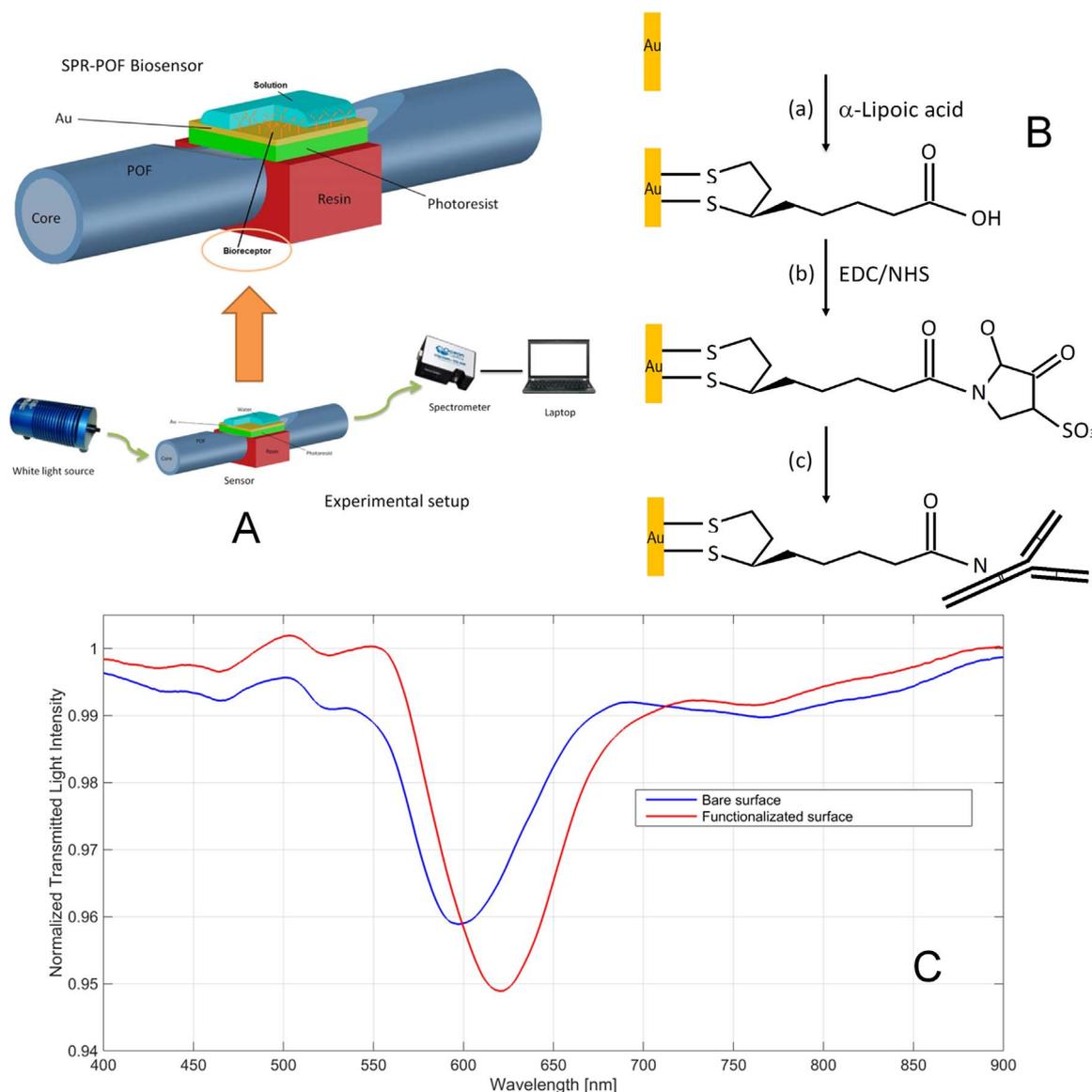


Fig. 4. (A) Optical biosensor system based on POF-SPR platform. (B) Functionalization process of the gold surface. (C) Resonance spectra acquired in buffer solution obtained before and after the functionalization process with msAb anti-PFOA. Both measurements were obtained by dropping 50 μ L of 20 Sodium phosphate buffer pH7.4 over the sensing surface, with and without the receptor layer.

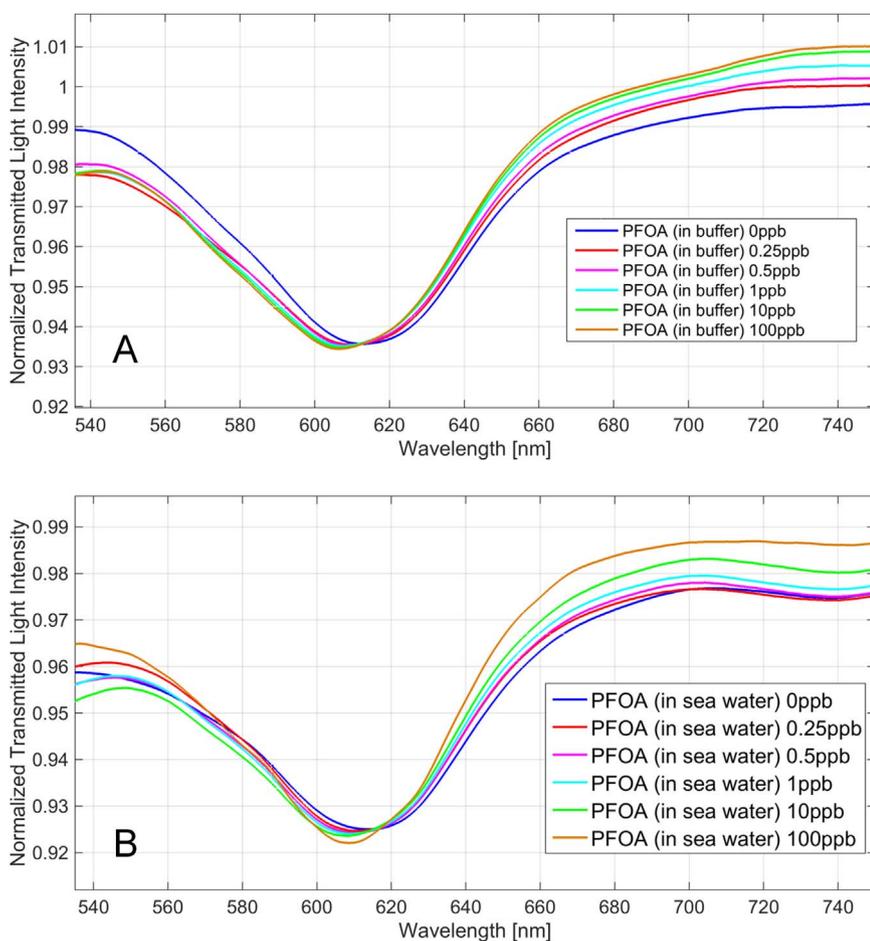


Fig. 5. SPR spectra acquired to PFOA detection. (A) SPR spectra obtained at different concentrations of PFOA in buffer solution (0–100 ppb). (B) SPR spectra for the same PFOA concentrations in 460 mM NaCl.

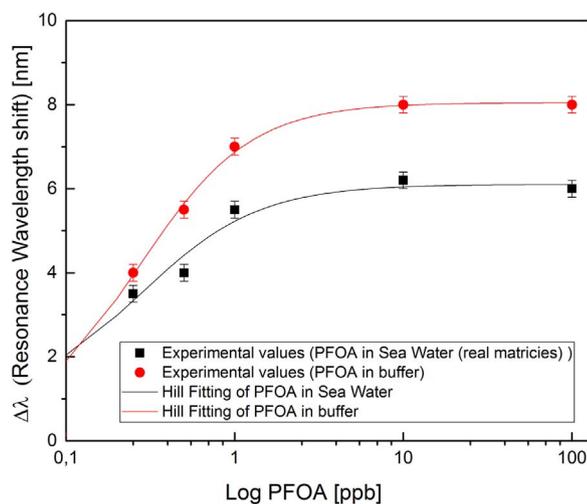


Fig. 6. Plasmon resonance wavelength shift ($\Delta\lambda$) versus concentration of PFOA (ppb), in semi-logarithmic axes, and Hill Fitting of the experimental values.

binding phenomena were registered. This result indicates a good selectivity of the bio-receptor for the PFOA/PFOS compounds.

In Fig. 4 is shown the SPR-POF biosensor system (Fig. 4A) and the strategy adopted for derivatization of the gold surface of the SPR-POF platform (Fig. 4B). We have applied a modified version protocol described in [19]. In particular, as shown in Fig. 4B, in order to obtain a smart surface able to bind the PFOA compound, the gold surface was treated sequentially with a solution of α -lipoic acid (a), EDC/NHS (b) and finally with mono-specific antibodies against the PFOA (c). The

immobilization of the antibody on the sensor surface is confirmed by SPR curves obtained by using the SPR-POF platform, directly. Fig. 4C shows the SPR transmission spectra before and after the functionalization with antibody anti-PFOA (normalized to the reference spectrum) acquired in the presence of PBS buffer. This experimental result shows a shift in the SPR transmission spectrum (the resonance wavelength increases) in the presence of the same bulk refractive index, before and after the functionalization procedure. This shift of resonance wavelength indicates that the refractive index in contact with the gold surface is increased, showing that the antibodies were immobilized on the gold surface. The shift due to the antibody immobilization is about 25 nm.

3.2. PFOA detection

Fig. 5A shows the transmission spectra of the SPR biosensor, normalized to the reference spectrum, obtained by incubating solutions at increasing concentrations of PFOA in the range 0–100 ppb, dissolved in PBS buffer. The resonance wavelength is shifted to smaller values by increasing the concentration of PFOA in buffer solution, which demonstrates that the analyte really interacts with the functionalized gold surface, giving rise to a decrease of the refractive index value of the receptor layer. This effect is related to the chemical composition of the per-fluorinated compounds. In fact, these compounds are used, for example, to decrease the refractive index of PMMA, to realize the cladding in the manufacturing procedures of PMMA POFs.

On the other hand, in Fig. 5B are shown the SPR spectra obtained at different concentrations of PFOA dissolved in real matrices (460 mM NaCl). In Fig. 6 is shown the resonance wavelength shift (calculated with respect to the resonance wavelength at 0 ppb) versus the PFOA

Table 1
Analytical parameters of the Hill equation relative to PFOA detection in buffer and real matrices.

PFOA detection	Hill equation $\lambda_c = \lambda_0 + \Delta\lambda_{max} \cdot (c^n / (K_{Hill}^n + c^n))$							
	λ_0 [nm]		$\Delta\lambda_{max}$ [nm]		K_{Hill} [ppb]		n	
	Value	Std. dev	Value	Std. dev	Value	Std. dev	Value	Std. dev
Buffer	0.24	2.34	8.05	0.19	0.27	0.38	1.32	0.75
Real	0.88	2.42	6.10	0.64	0.27	0.99	1.24	3.50

Table 2
Analytical parameters at low concentration of analyte.

Matrices	Low concentration Hypothesis: ($c \ll K_{Hill}$)		Hill equation at low concentration (with $n \approx 1$) $\lambda_c - \lambda_0 \approx (\Delta\lambda_{max}/K_{Hill}) \cdot c$	
	Sensitivity at low c ($\Delta\lambda_{max}/K_{Hill}$) [nm/ppb]	Limit of Detection (LOD) [ppb] ($2 \cdot$ standard deviation of blank/sensitivity at low c)	Value	Value
	Value	Value		
Buffer	29.82	0.16		
Real	22.59	0.21		

concentration, in semi-logarithmic axes. The data show a different binding effect for PFOA in buffer solution and in real matrices respectively (dips in Fig. 5A and 5B). Each experimental value is the average of 5 subsequent measurements and the respective standard deviations (error bars) are shown. The experimental data, reported in Fig. 6, were fitted by Hill's equation, in order to determine the kinetic parameters of the interaction.

In Table 1 are listed the values obtained by OriginPro8.5, Origin Lab. Corp. (Northampton, MA, USA), while the Hill's equation is reported in the following:

$$\Delta \lambda = \lambda_c - \lambda_0 = \Delta \lambda \max \cdot (c^n / (K_{Hill}^n + c^n))$$

where c is the analyte concentration, λ_c is the resonance wavelength at the concentration c , λ_0 is the resonance wavelength at zero concentration (blank), $\Delta\lambda_{max}$ is the maximum value of $\Delta\lambda$ (calculated by the saturation value minus the blank value), whereas n and K_{Hill} are the Hill constants.

As shown in Table 2, from the Hill equation [26], at low concentration of the analyte (c), i.e. much lower than K_{Hill} , the Hill equation is linear, when $n \approx 1$, with sensitivity (slope) $\Delta\lambda_{max}/K_{Hill}$, defined as the "sensitivity at low concentration". The Limit of Detection (LOD) can be calculated by the ratio of twice the standard deviation of the blank (stand. dev. of λ_0 , reported in Table 1) and the sensitivity at low

concentration ($\Delta\lambda_{max}/K_{Hill}$, values reported in Table 1). For our system, the LOD has been evaluated (see Tables 1 and 2 values) and it is considerably lower than the maximum residue limit of PFOA, fixed at 0.5 ppb by European Union regulations.

3.3. PFOS detection

The same procedure was used to study the interaction between the produced antibodies (msAb-PFOA) and PFOS. Fig. 7 shows the transmission spectra, normalized to the reference spectrum, obtained when the functionalized surface was incubated with increasing concentrations of PFOS compounds (0–100 ppb) dissolved in PBS buffer. When the concentration of the PFOS increases the resonance wavelength decreases. In this case the SPR biosensor presents the same response obtained with PFOA, as shown in the inset of Fig. 7 (dose-response curve with Hill fitting of values). This effect should be due to the similar structure of both molecules exposed on the protein carrier during the immunization procedure (Fig. 1B). In other words, the produced antibody can be used, with the similar performances, to monitor both PFOA and PFOS molecules. This aspect is very significant because both analytes are interesting for several applications.

3.4. No binding specificity

In order to verify the non-specific binding between sensing surface and analyte, the response of SPR-POF sensor without the antibodies layer was checked. Fig. 8 shows the SPR curves at different concentrations of PFOA (0–100 ppb). When the PFOA concentration increases, from the analysis of data, it is clear that in absence of antibodies on the gold surface, a smaller shift of the resonance wavelength has been registered. This should be due to the PFOA/PFOS "bare surface" interaction. This effect does not influence the measurement because the smaller shift of the resonance wavelength, in the same direction of the binding shift, is around 0.8 nm for 100 ppb of PFOA in the bare sensor configuration, against 8 nm for the same concentration of PFOA in the functionalized surface sensor.

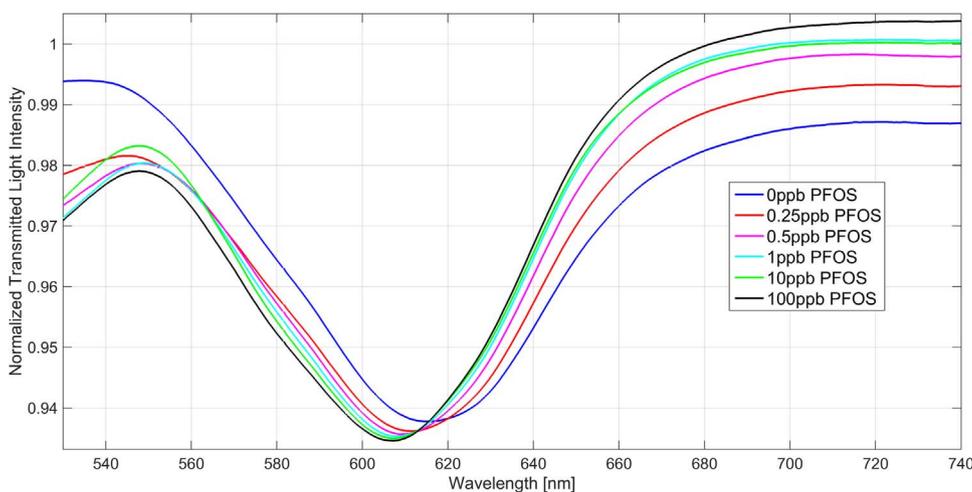


Fig. 7. SPR spectra obtained at different concentrations (0–100 ppb) of PFOS dissolved in buffer solution. The experiments were performed at room temperature, and each sample was incubated 10 min before acquiring the signal. Inset: Dose-response curve and Hill fitting of experimental values.

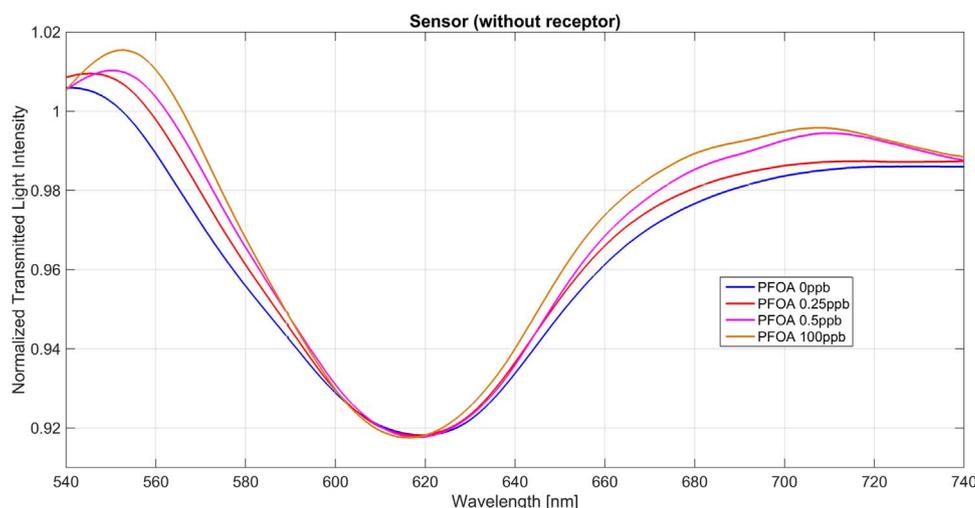


Fig. 8. SPR spectra acquired in the presence of different PFOA concentrations (0–100 ppb) on the "bare sensor" (gold film without bio-receptor layer). The experiments were performed at room temperature and each sample was incubated 10 min before acquiring the signal.

4. Conclusion

We designed, realized and tested a novel biosensor system, based on an optical fiber together with a novel bio-receptor, to monitor the presence of the PFOA/PFOS in seawater. This biosensor system could be also used for remote sensing, taking advantage of the capability of the optical fiber. The results showed that this analytical system is selective and able to sense very low concentrations (less than 0.21 ppb in seawater) of PFOA/PFOS.

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