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A Novel Impedimetric Sensor Based on Cyanobacterial Extracellular Polymeric Substances for Microplastics Detection

Wejdene Gongi^{1,2} · Hassen Touzi³ · Idris Sadly² · Hafedh Ben ouada³ · Ollivier Tamarin^{2,4} · Hatem Ben ouada¹

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Abstract

Cyanobacterial extracellular polymeric substances “EPS” have attracted intensive concern in biomedicine and food. Nevertheless, the use of those polymers as a sensor coating material has not yet been investigated mainly for microplastic detection. This study focuses on the application of EPS as a sensitive membrane deposited on a gold electrode and investigated with electrochemical impedance spectroscopy to detect four types of microplastics with a size range of 0.1 μm to 1 mm. The surface properties of this impedimetric sensor were investigated by Scanning electron microscopy, Fourier transforms infrared spectroscopy, and X-ray spectroscopy and, showed a high homogenous structure with the presence of several functional groups. The EPS-based sensor could detect the four tested microplastics with a low limit of detection of 10^{-11} M. It is the first report focusing on EPS extracted from cyanobacteria that could be a new quantification method for low concentrations of microplastics.

Keywords Extracellular polymeric substances · Impedimetric biosensor · Electrochemical impedance spectroscopy · Microplastics detection

Introduction

Over the past century, the production and demand for plastic have grown exponentially, especially during the global coronavirus outbreak. Plastic waste has thus become a worrying source of contamination [1]. Plastics stay in ecosystems for centuries because they are not biodegradable [2]. They turn into small particles with a diameter between 5 nm and 100 μm , defined as microplastics. Microplastics were quantified in freshwater environments, including lakes, rivers, surface waters, reservoirs, and river sediments [3–5].

These particles present severe and widespread ecological impacts [5]. They are transmitted through the trophic chain, altering the expression of genes in many aquatic organisms, causing toxic effects and endocrine disruption and adverse effects on the reproductive, neurological and immunological systems in humans and animals [6, 7]. Thus, it is crucial to identify plastic particles and determine their size and concentration both in the laboratory and in the natural environment.

Conventional measurement techniques for identifying and measuring microplastics (MP) in the field or the laboratory have been the subject of several reviews [8–11]. They can be summarized into three types of methods (Supplementary Table 3). (i) Visual methods using the naked eye or stereomicroscope or scanning electron microscopy (SEM) have often been applied for the identification of plastic in general [12]. They can be combined with staining using Nile red [13] or with energy dispersive spectroscopy using X-rays to identify elemental composition in addition to particle size [14]. (ii) More sophisticated methods used included microplastic degradation by pyrolysis [15] or thermal degradation, followed by gas chromatography–mass spectrometry analysis [16]. These techniques often overlap with visual methods for the identification and quantification of microplastic types

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[10]. (iii) The most used methods that showed promising results [17, 18] are based on the spectral signature of plastic polymers. They included Fourier-transform infrared (FTIR) methods as attenuated total reflection-FTIR (ATR-FTIR) [19, 20] or FTIR imaging [19, 21], and Raman spectroscopy analysis as Raman microspectroscopy [22, 23] or Raman imaging [24].

Overall, conventional methods for the identification and/or quantification of microplastics are subject to several limitations in terms of accuracy, time and cost of analysis [10, 11]. Samples often require heavy pre-treatment in the laboratory to avoid artefacts generated by organic or inorganic particles other than microplastics [25, 26]. Additionally, microplastics are usually highly dispersed in water bodies, requiring additional concentration processes to reach the detection limit of the analytical device. This preprocessing is time-consuming and labor-intensive.

Simpler and cheaper analytical methods are needed and, in this sense, biosensors have received great attention [27–29]. Biosensors belong to a category of analytical devices capable of providing a measurable output signal using the recognition of a substance of interest via a biological recognition unit. The main advantages of biosensors are low cost, low detection limit and fast sensitivity, allowing real-time and in situ monitoring [30]. So far, the detection of microplastics by biosensors and by sensors is at the stage of investigation despite the potential offered by these systems. In [31], the authors combined two optical methods (interference and specular reflection of light) to design a prototype optical sensor to detect translucent microplastics in fresh water. Malyskin [32] used a microwave resonance spectroscopy sensor to detect and quantify microplastics in soil and water at parts per thousand concentration levels.

Several biosensors based on enzymes, antibodies, nucleic acids and algal cells have been used as biorecognition elements [33–36]. They have demonstrated their effectiveness in the detection of different pollutants, including heavy metals, pharmaceutical pollutants, pesticides and biological contaminants. Recently, the efficiency of peptide biosensors for the detection of polystyrene and polypropylene microparticles in their unoxidized and oxidized forms was demonstrated [37]. Enzyme-based biosensors have also been shown to be effective in detecting nano and microplastics [38]. Huang et al. [39] detected low levels of microplastics by developing a surface plasmon resonance biosensor, using immobilized estrogens as bioreceptors. However, further progress is needed for a better and more acceptable detection process. In particular, the search for bioreceptors with a strong affinity for different types of microplastics while inducing a reliable and reproducible signal remains relevant.

Cyanobacterial extracellular polymeric substances (EPS) have attracted great attention and have been extensively studied for several applications due to their unique and diverse

properties [40]. EPS are relatively easy to obtain, do not pose any ethical problems and could be used in bioremediation and bioleaching due to their great structural, physical and chemical diversity [41]. They are also potential candidates for the production of biofilms and thus replace the use of hazardous plastic materials [42]. Additionally, EPS have been used as a metal-binding material due to the abundance of negatively charged amino acids like aspartic acid and glutamic acid in their structure [43, 44]. These complex molecules can establish London forces, electrostatic interactions, hetero-aggregates and hydrogen bonds in the adhesion and cohesion of microplastic particles [45, 46].

These properties represent advantages for the use of cyanobacterial EPS as bioreceptors in the development of biosensors intended for the detection of microplastics.

In this context, this article presents a first investigation aimed at the development of an EPS-based biosensor for the detection of microplastics. It focuses on the microplastics most frequently found in marine wastewater effluent, including PS (polystyrene), PA (polyamide), PMA (polymethyl acrylate) in the form of nylon, and PE (polyethylene low density) in the form of polyester. Electrochemical impedance spectroscopy (EIS) was used to analyze the interaction between microplastics and EPS. EIS has been frequently used to characterize the stepwise modification of several biosensors [33, 47].

The main objectives of the current study are: (1) elaborate and characterize an EPS film solution; (2) immobilize and validate the EPS adhesion to a gold wafer electrode; (3) characterize the surface sensor using several techniques. Namely, Fourier transforms infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), and X-ray spectroscopy (XRD) characterize the elaborated device by EIS measurements; and finally (5) investigate its analytical performances.

Materials and Methods

Extraction, Elaboration and Characterization of the EPS Film Forming Solution

Extracellular Polymeric Substances (EPS) were extracted from the culture cultivation of the cyanobacteria *Gloecapsa gelationosa*. The EPS was concentrated using a tangential ultra-filtration cell (Vivaflow 50) in Millipore membranes with 5 kDa pore size and then washed with deionized water until constant conductivity to eliminate low molecular weight substances [48, 49]. Lastly, the recovery filtrate was freeze-dried, lyophilized, and weighed for EPS content determination. The film-forming solution was prepared by dissolving 1 mg of *Gloecapsa* EPS in 1 mL ethanol solution (99% of purity, purchased from Sigma Aldrich). In order to ensure the complete

dissolution of the EPS in the ethanolic solution, the pH was regulated at a value of 12.

The biochemical composition of the EPS film-forming solution was characterized using colorimetric and gravimetric methods. Total carbohydrates content was determined by the phenol sulphuric acid method [50] using d-glucose (Sigma, 50-99-7) as standard. Proteins content was determined according to Lowry et al. [51] using bovine serum albumin (Sigma, 10711454001) as standard. The sulphate groups were calorimetrically calculated using potassium sulphate as standard [52].

The zeta potential of the EPS film forming solution was measured on Zeta size 3000 HS. (Malvern, UK). Each sample was repeatedly measured 3 times and the values reported are the mean value \pm standard deviation for two replicate samples. The measurements were performed in triplicate at 25 ± 1 °C by measuring the electrophoretic mobility of the dispersed particles in a charged field. The software to convert the electrophoretic mobility measurements into zeta potential values used the Smoluchowsky mathematical model [53].

Sensor's Elaboration

The sensor was elaborated according to the method described by Touzi et al. [54]. In this study, we evaluated the applicability of the EPS as a sensitive membrane of gold wafers transducer. The gold wafers were cleaned with acetone and treated with heated piranha solution (H₂O₂/H₂SO₄ mixture 3:7 v/v) before being rinsed in ultrapure water and then dried under nitrogen flow.

The immobilization of sensitive EPS film on the cleaned gold surface was performed by means of spin-coating. A drop (10 μ L) of EPS film solution was deposited on a rotating gold wafer at a speed of 2000 rpm for 20 s. The resulting thin film on gold wafers was stabilized before use by drying (60 °C for 1 h) and soaking overnight in a 0.01 M acetate buffer solution.

Surface Characterization of the Sensitive Membrane

The surface of the EPS sensitive membrane was observed by Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray spectroscopy (XRD).

SEM analyses were performed using an HR-FESEM SU-70 Hitachi microscope, operating at 4 kV in the field emission mode and an angle of 90° with the surface, using different magnifications. Before imaging, samples were cryo-fractured by immersion in liquid nitrogen and fixed on the SEM support using double-sided adhesive tape, and observed under an accelerating voltage of 5.0 kV and

absolute pressure of 60 Pa, after sputter coating with a 5 nm thick gold.

FTIR analyses were performed using a Perkin-Elmer system (Perkin-Elmer) equipped with an attenuated total reflectance (ATR) cell. The spectra were recorded at room temperature, in the range of 500–4000 cm⁻¹ with a resolution of 2 cm⁻¹ and, evaluated by Agilent software.

Diffraction data of the EPS gold sensor membrane was collected with an X-ray diffractometer CICECO Empyrean (JDX 3532; Japan) equipped with CuK α radiation ($\lambda = 1.54184\text{\AA}$), a 4-circle kappa goniometer, and a CCD Detector. Data collection and processing were carried out using CrysAl-isPro Software System. The diffraction angle range of observation was 5° to 50°.

Electrochemical Impedance Spectroscopy « EIS »

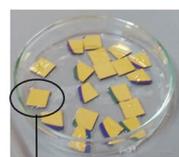
Impedance spectroscopy measurements were carried out at room temperature (20 ± 3 °C) in an electrochemical cell (Pyrex glass of 5 mL volume) connected to an impedance analyzer and controlled by a computer. Three electrodes electrochemical cells were used: the modified gold electrode as the working electrode (0.19 cm² active area defined by an O-ring seal), a platinum counter-electrode and a saturated calomel electrode (Ag/AgCl/KCl) used as a reference electrode (Fig. 1). Ammonium acetate (CH₃CO₂-NH₄⁺) aqueous solution (4 mL, 0.01 M at pH 6.8) was used as a background electrolyte. The excitation sinusoidal signal was 10 mV of amplitude to provide a linear response of the cell, which means a sinusoidal current response at the same frequency and shifted in phase. The frequency was scanned in a range of [10⁻³ Hz, 10⁶ Hz]. Many Factors induce destabilization of the response of the cell. A delay time is always needed to achieve a steady state. This stabilization was detected by the reproducibility of the response. In our case, a delay of 90 min was sufficient to minimize the drift in the system. The response of the EIS was investigated by Nyquist diagrams according to the microplastics. All our measurements were done at the negative bias of - 0.3 V which allows a great definition of the Nyquist plot and is not too high to induce the corrosion phenomenon. The equivalent circuit parameters for the electrolyte interface of the functionalized surface of EPS were directly deduced from the experimental data by applying the software Nova Autolab 1.5, after testing several models of the equivalent circuit.

Microplastics Solution

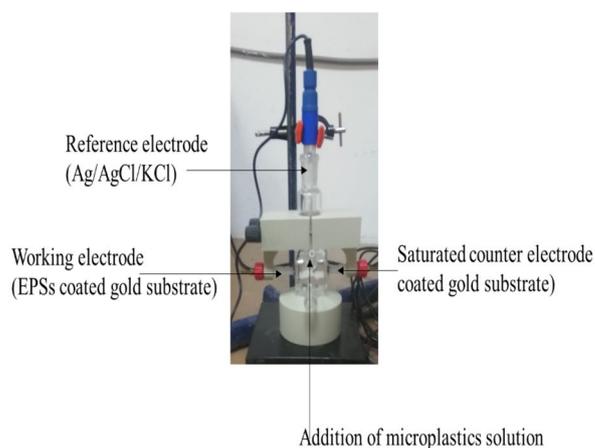
Four types of spherical-shaped microplastics, used in this study, including polystyrene (PSS) in size 0.1 μ m, polymethylacrylate (PMA) in size 10 μ m; Nylon polyamide (PA) in size 50 μ m and low-density polyethylene (PE) in size 1 mm were purchased from Sigma-Aldrich as aqueous suspensions

Fig.1 Scheme for the *Gleocapsa* EPS immobilization on the electrochemical sensor gold wafer (left) and the three electrochemical electrodes (right)

Gold electrode
electrochemical sensing



Gleocapsa EPSs



at 10% solid concentration. The microplastic solutions were prepared in water solution at different concentrations from 10^{-11} to 10^{-5} M.

Results and Discussion

Characterization of the EPS Film-Forming Solution

The chemical composition, particle size, and zeta-potential values of the film-forming solution are reported in Table 1. The chemical composition revealed that EPS solutions are principally composed of carbohydrates fraction. The total carbohydrate content was 70% of dry weight. The protein content was evaluated at 12%. The relatively high ratio of ester-sulfated groups (10%) proposes that the solution is of sulfated hetero-polysaccharides nature. The present results are in agreement with that found in the literature that revealed that several cyanobacterial EPS substances are sulfated polysaccharides [41, 55].

In general, polymers of polysaccharide nature are known for their ability to physical trapping or inclusion of several particles' material [56]. Furthermore, the presence of peptides confers on EPS the possibility of adsorbing MPs by hydrophobic binding [37].

From the results of the zeta potential listed in Table 1, it was found that the EPS film-forming solution had negative charges of -41 mV. The negative-charged cyanobacterial exopolysaccharides are commonly reported by other authors and attributed to the presence of anionic groups and uronic acids [44, 57]. However, according to our investigations, the

Zeta potential varied with the pH of the EPS film forming solution. At pHs below 10, the value of the Zeta potential was $+30$ mV (data not shown), but under these conditions the dissolution of the EPS is only partial. It is for this reason that we have maintained the pH of the EPS solution at 12 throughout the work, which guarantees the stability of the ionic charge and the homogeneity of the solution.

The typical average particle size distribution of the EPS film solution is plotted in Fig. 2. Particle size distributions showed monomodal shape dominated by small particles size of around 100 nm. The homogeneity of particle size distribution is confirmed by the low polydispersity index 0.036 (Table 1). According to Danaei et al. [58] a PDI lower than 0.05 indicate a highly monodisperse character. Small particle size polymers with low PDI have a great capacity to bind uniform micro-nanoparticles by encapsulation, electrostatic interaction or by covalent conjugation [58].

Characterization of the Sensor Surface

The surface of the EPS sensor membrane was observed by Scanning electron microscopy (SEM), Fourier transforms infrared spectroscopy (FTIR) and, X-ray spectroscopy (XRD).

The SEM top view and cross-section (Fig. 3) of the EPS membrane confirmed the homogeneous character and revealed a microstructural lamellar feature.

The presence of functionalities on the surface of the sensor was identified by FTIR measurements as shown in Fig. 4, revealing prominent functional reactive groups, such as sulfate, hydroxyl, or carboxylic. A broad band in

Table 1 Chemical composition: carbohydrates, protein, sulfate, mean particle size, and zeta potential of EPS film-forming solution

Proteins	Sulfate	Carbohydrates	Mean diameter (nm)	Polydispersity index	pH	Zeta potential (mV)
12.2 ± 0.02	10.1 ± 0.1	70.2 ± 0.1	99.7 ± 2.0	0.036 ± 0.001	12	-41.2 ± 3.5

Fig. 2 Size distribution of the EPSs film-forming solution

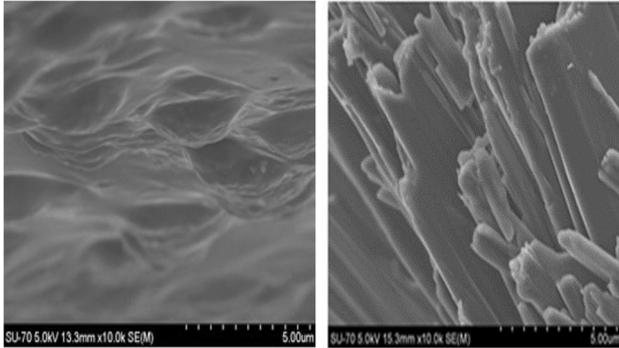
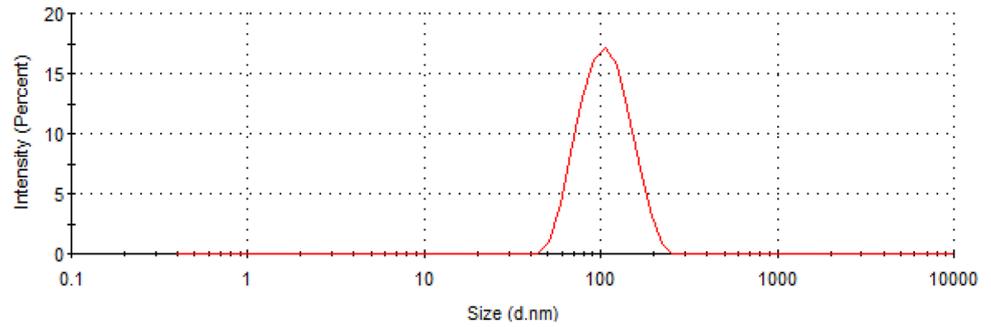


Fig. 3 Top-view SEM images of the EPS sensor membrane

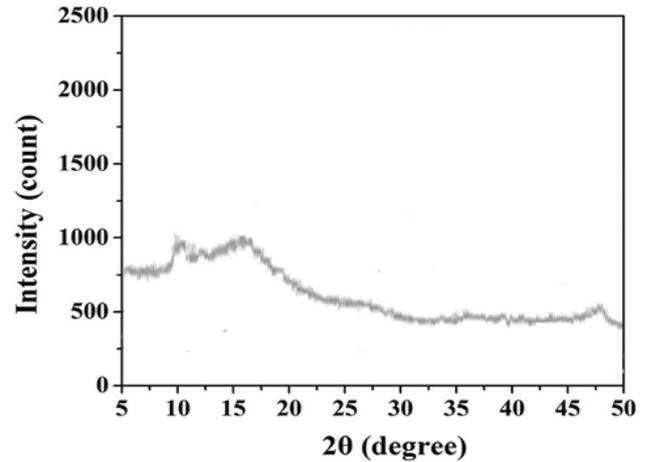


Fig. 5 Representative XRD profile of the *Gloecapsa gelatinosa* EPS sensor membrane

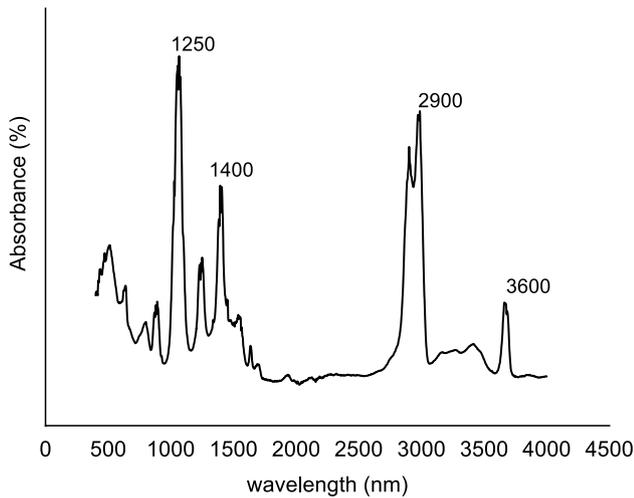


Fig. 4 Infrared spectra of the *Gloecapsa gelatinosa* EPS sensor membrane

the range of 2900–3600 cm^{-1} is attributed mainly to the stretching vibration (ν) of $\nu\text{O-H}$ or $\nu\text{C-H}$ groups, typical of the hydroxyl and alkyl functionality of carbohydrates [59]. The prominent absorption observed at the region 1400 cm^{-1}

could be attributed to the bending vibration (δ) of $\delta\text{N-H}$ and the $\nu\text{C-N}$ [60] indicating the existence of amino acids from peptide/proteins. The important peak observed at 1250 cm^{-1} corresponds to the stretching vibration of the ester sulfate groups (S=O) [60] and is further evidence of the hetero-sulfated polysaccharides nature [61, 62].

In this regard, the plastic binding capacity would be directly proportional to the availability of these functional groups at the surface [63]. Restrepo-Flórez et al. [64] reported that the interaction between polymers/organisms and plastic particles leads to changes in functional groups and physical properties of plastic. However, as far we know, no studies have analyzed these interactions in depth.

X-ray spectroscopy (XRD) is a rapid analytical technique most widely used for phase identification of crystalline material. The XRD profile of the EPS membrane surface (Fig. 5) did not exhibit well-differentiated peaks, suggesting an amorphous nature. Similar to previous profiles of native EPSs produced by several microorganisms [52, 65].

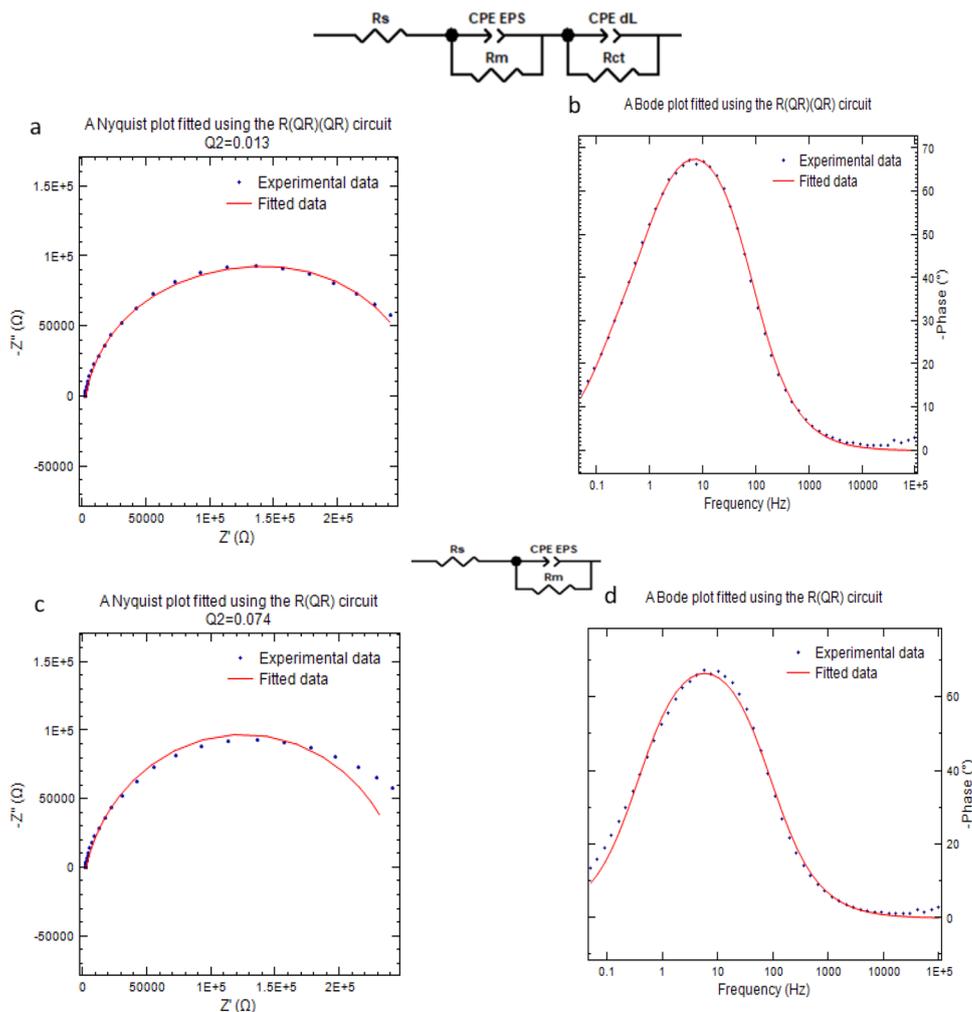
Impedance Measurements and Equivalent Circuit Modeling

The electrochemical measurement is well-suited to detect changes in the electrode-medium interface [66]. Thus, in the field of biosensors, monitoring the immobilization procedure of the recognition element is based on the measurement of the electrochemical impedance variation. EIS is a steady-state technique using low amplitudes signal analysis and detect signal relaxations over a very wide range of applied frequency. The Impedance measurement results are illustrated in the Nyquist plot using the imaginary part (Z'') and the real part (Z') of impedance (Z). To design the electrical model of the electrochemical cell, we consider the gold-EPS interface in series with the EPS membrane and in series with the electrolyte solution. The model of the electrolyte solution should be a resistor R_s , the interface gold- EPS is modeled by a real capacitor (i.e. a capacitance CdL in parallel with a resistor Rct) and the membrane EPS is assumed to another real capacitor with a capacitance Cm and a resistance Rm. The three dipoles are assembled in series. CdL is the double

layer capacitance and Rct is the charge transfer resistor. This model is denoted $R(C//R)(C//R)$. In the Nyquist plot, the dipole $C//R$ correspond to a semi-circle with a diameter R. In the case of a semi-circle whose center is shifted towards the negative Z'' , we replace the capacitance C with a constant phase element CPE. The constant phase element is considered as a “power-law-dependent specific layer capacitance”. The constant phase element reflects non-homogeneity and defecting area of the electrode surface. (Rct) corresponds to the electron transfer resistance between the bulk and the electrode’s surface [67]. In our specific case, to take into account our EPS chemical layer immobilized on the electrode surface, the model “ $R(CPE//R)(CPE//R)$ ” become “ $R_s(CPE_EPS//R_m)(CPE_dL//R_{ct})$ ”.

In this work, the equivalent electrical circuit models $R_s(CPE_EPS//R_m)$ (i.e. without the modelization of the interface between the gold substrate and the EPS film), and $R_s(CPE_EPS//R_m)(CPE_dL//R_{ct})$ have been tested. Based on the chi-square values of each model fitting, the Nyquist diagrams obtained for the gold electrode after the EPS deposition (Fig. 6) show that $R_s(CPE_EPS//R_m)(CPE_dL//R_{ct})$

Fig. 6 a A Nyquist plot fitted using the $R_s(CPE_EPS//R_m)(CPE_dL//R_{ct})$ circuit. b A Bode plot fitted using the $R_s(CPE_EPS//R_m)(CPE_dL//R_{ct})$ circuit. c A Nyquist plot was fitted using the $R_s(CPE_EPS//R_m)$ circuit. d A Bode plot fitted using the $R_s(CPE_EPS//R_m)$ circuit. Z'' : imaginary part of impedance and (Z') the real part of impedance R_s : solution resistance; R_m : resistance of the EPS film; CPE_EPS: constant phase element of the EPS film; CPE_dL: constant phase element of the interface; Rct: charge transfer equivalent resistance; χ^2 : chi-square.



circuit was more suitable (corresponding chi-square values $\chi^2=0.013$) for describing the EPSs gold sensor for microplastics detections. The observed non symmetric profile (Fig. 6 b) of the phase spectra confirms this choice. The good agreement between the measured data and the fitting curves indicates that this equivalent circuit is suitable and meaningful for this electrochemical system and is therefore used to fit the impedance spectroscopy data and extract R_s , R_m , CPE_{EPS} , R_{ct} , and CPE_{dL} values. These were obtained by plot fitting with a less than 5.0% error percentage (Table 2).

Influence of Microplastics on the Electrochemical Response

Nyquist diagrams resulting from electrochemical impedance spectroscopy were plotted (Fig. 7) at different concentrations ranging from 10^{-11} to 10^{-5} M of each studied microplastics (PSS, PMA, PA, and PE).

The system stability for application as a sensor was recorded after the EPSs film deposition. The Nyquist diagram corresponding to the blank representing EPS without microplastics (EPS acetate) showed a semicircle with a Z' value of 1 E^{+5} to $1.5 \text{ E}^{+5} \Omega$ suggesting a high stability of the EPS electrode. The shape of Nyquist plots is dependent mainly on the electrochemical responses taking place at the surface of the working EPS electrode. A difference in the

electrical signal was created due to the kinetic binding of each type of microplastics at the sensors surface. The electrochemical sensor was able to detect all tested microplastics at a very low concentration of 10^{-11} M.

The differences monitored between the four experimented types of plastics was mainly related to their sizes. By comparing the Nyquist diagrams, a fast saturation of the sensor membrane was detected in the case of PA at a concentration of 10^{-10} M and of PE at a concentration of 10^{-11} M. The real impedance Z' was evaluated in these cases respectively at 1.8 E^{+5} and $2.5 \text{ E}^{+5} \Omega$. This behavior could be mainly correlated to the high size of both PA (50 μm size) and PE (1 mm size) that blocks EPSs micropores. It was reported that the plastics size affects the ability of polymers to adsorb particles [68, 69].

On other hand, the diameter of the semicircle increased with increasing the concentration of both PMA (10 μm) and PSS (0.1 μm). A detection limit was observed at 10^{-6} M in the case of PMA and 10^{-5} M in the case of PSS. At all tested concentrations, PSS showed the highest Z' value, higher than that recorded with PMA, thereby indicating more affinity of EPS sensor to detect large range of PSS than the other types of microplastics.

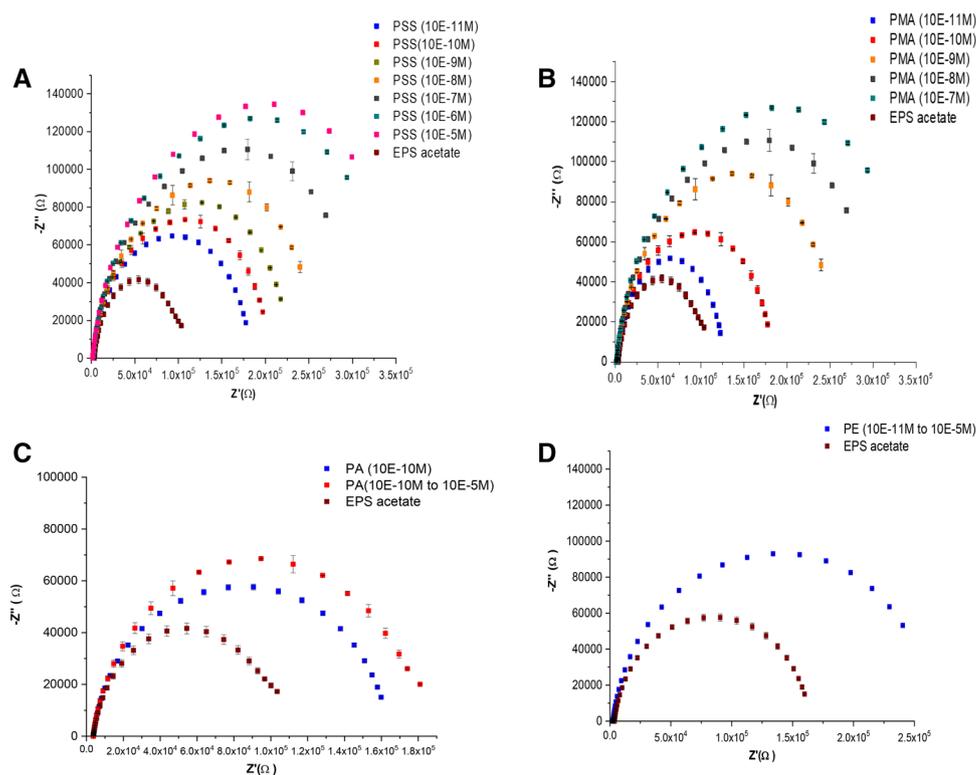
Table 2 shows the different results obtained after fitting the experimental data, revealing that the detection was influenced by the microplastic concentrations. Little variation was observed in constant phases elements of both the

Table 2 Parameters of the electrical circuit equivalent to the EPS electrode for different concentrations (10^{-11} to 10^{-5} M) of microplastics: PA, PMA, PSS, and PE (values are reported until saturation)

	R_s (k Ω)	R_m (K Ω)	CPE_{EPS} (μF)	μ	R_{ct} (K Ω)	CPE_{dL} (μF)	μ
EPS-acetate	3.4 ± 0.5	127.6 ± 2.0	2.8 ± 0.0	0.9 ± 0.0	59.4 ± 1.3	1.8 ± 0.2	0.9 ± 0.0
PA 10E^{-11}	3.0 ± 0.3	121.0 ± 2.2	3.2 ± 0.5	0.9 ± 0.0	60.8 ± 1.2	1.8 ± 0.0	0.9 ± 0.0
PA (10E^{-10} – 10E^{-5})	2.1 ± 0.2	177.1 ± 1.9	3.3 ± 0.5	0.8 ± 0.0	60.8 ± 1.2	1.7 ± 1.0	0.9 ± 0.0
PSS 10E^{-11}	3.3 ± 0.1	118.5 ± 4.3	3.0 ± 0.6	0.9 ± 0.0	60.3 ± 2.2	1.8 ± 0.7	0.9 ± 0.0
PSS 10E^{-10}	2.5 ± 0.5	142.0 ± 3.2	3.2 ± 0.8	0.9 ± 0.0	60.0 ± 2.3	1.7 ± 0.9	0.9 ± 0.0
PSS 10E^{-9}	1.9 ± 0.2	168.2 ± 2.2	3.3 ± 0.2	0.9 ± 0.0	59.3 ± 2.0	1.8 ± 0.0	0.9 ± 0.0
PSS 10E^{-8}	1.8 ± 0.6	195.0 ± 2.0	3.5 ± 0.3	0.9 ± 0.0	58.3 ± 2.1	1.5 ± 0.1	0.9 ± 0.0
PSS 10E^{-7}	1.7 ± 0.5	223.1 ± 2.2	3.7 ± 0.8	0.9 ± 0.1	54.8 ± 1.2	1.5 ± 0.2	0.9 ± 0.0
PSS 10E^{-6}	1.6 ± 0.2	252.4 ± 2.0	3.9 ± 0.7	0.9 ± 0.1	52.4 ± 2.2	1.4 ± 0.3	0.9 ± 0.0
PSS 10E^{-5}	1.41 ± 0.54	267.76 ± 2.4	3.9 ± 0.5	0.9 ± 0.1	49.1 ± 1.8	1.4 ± 0.5	0.9 ± 0.1
PE (10E^{-11} – 10E^{-5})	2.4 ± 0.7	208.3 ± 2.5	4.0 ± 0.4	0.9 ± 0.0	59.4 ± 2.2	1.1 ± 0.2	0.9 ± 0.1
PMA 10E^{-11}	3.5 ± 0.9	171.9 ± 3.2	2.2 ± 0.3	0.9 ± 0.0	65.9 ± 2.0	1.7 ± 0.1	0.9 ± 0.0
PMA 10E^{-10}	3.2 ± 0.2	189.1 ± 3.5	2.7 ± 0.2	0.9 ± 0.0	64.9 ± 1.7	1.7 ± 0.0	0.9 ± 0.0
PMA 10E^{-9}	3.1 ± 0.9	245.3 ± 4.0	2.8 ± 0.6	0.9 ± 0.0	51.7 ± 2.2	1.6 ± 0.1	0.9 ± 0.0
PMA 10E^{-8}	2.0 ± 0.9	274.8 ± 4.6	2.8 ± 0.8	0.9 ± 0.1	54.8 ± 1.3	1.5 ± 0.3	0.9 ± 0.0
PMA 10E^{-7}	2.0 ± 0.5	292.3 ± 2.0	3.2 ± 0.3	0.9 ± 0.0	51.6 ± 3.2	1.5 ± 0.1	0.9 ± 0.2
PMA 10E^{-6}	1.9 ± 0.7	293.0 ± 2.3	3.4 ± 0.3	0.9 ± 0.0	50.3 ± 1.5	1.5 ± 0.2	0.9 ± 0.1
PMA 10E^{-5}	1.9 ± 0.1	299.5 ± 1.2	3.7 ± 0.2	0.9 ± 0.0	45.1 ± 2.2	1.5 ± 0.2	0.7 ± 0.0

R_s solution resistance, R_m resistance of the EPS film, CPE_{EPS} constant phase element of the EPS film, CPE_{dL} constant phase element of the interface, R_{ct} ion transfer equivalent resistance, μ ion mobility

Fig.7 Nyquist diagram Z' (real) vs Z'' (imaginary) of electrochemical impedance spectra for the microplastics detection [PSS (A), PMA (B), PA (C), and PE (D)] with the gold sensor coated with EPS film. Values are expressed as the mean six replicates ($n=6$) \pm standard deviation



EPS film (CPE_EPS) and the interface (CPE_dL). The ion transfers equivalent resistance (R_{ct}) and the resistance of the solution (R_s), showed a decrease with the increase of the concentration. These results clearly signify that the microplastics solution were incrustated on the EPSs electrode which provide easier access for intercalation of ions on EPS electrode surface and easy charge-transfer at electrode–electrolyte interface [70].

The EPS resistance (R_m) increased with increasing the concentration of microplastics. Indeed, according to the pore-impedance relationship, the R_m can be attributed to the impediment to the microplastics passing through the pores. Several studies showed that small particles tend to aggregate into the pores in polymers and then adsorb to the surface wholly or individually [46].

Conclusion

This work demonstrates the validity of using EPS as a bioreceptor for the development of biosensors allowing the detection of low concentrations of different types of microplastics. The detection limits are 10^{-11} to 10^{-5} M, but the larger the particle size, the faster the saturation of the EPS membrane and the smaller the detection interval in terms of concentration. Investigations using the same type of biosensor for the detection of nanoparticles can be envisaged in this direction. However, more investigations are also needed to understand

the behavior of the biosensor in the presence of a mixture of particles of different sizes.

EPS represent a natural substance, which is complex in its physical and chemical structure. The EPS film is monodisperse at the molecular scale with a homogeneous structure and adheres to the surface of the gold wafer without adding a specific ligand, which represents an advantage in the development of biosensors. However, its negative charge and the presence of different functional groups in its structure reduce its selectivity for plastic in a solution containing several types of pollutants. The use of the impedimetric biosensor thus designed in the field and the study of interactions with pollutants of different nature remains subject to additional investigations.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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