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Conception and characterization of moleculary imprinted polymers nanofibers of poly(ethylene-co-vinyl alcohol) and their use as membrane in electrochemical sensor for creatinine detection

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Abstract

We report on the deposition of molecularly imprinted polymer (MIP) nanofibers (NFs) by electrospinning and on their potentialities in electrochemical biosensors. Electrospun polymer was the copolymer poly(ethylene-co-vinyl alcohol) denoted EVOH and model imprinted molecule was creatinine. The optimum conditions leading to uniform non-beaded fibers were reported. Infrared spectroscopy was used to establish actual molecular sites creation on the fibers. NF mats deposited on gold electrode were *characterized by electrochemical impedance spectroscopy (EIS). The EIS sensor based on creatinine MIP-EVOH NFs (25% of EVOH in DMSO) gave a large linear range for creatinine detection between 1fg/L and 2 ng/L with a low detection limit of 0.015 fg/L. Reproducibility of 1% to 9% on the same sensor, and 1% to 15% from one sensor to another with a very good selectivity for creatinine.*

Keywords: electrospinning; nanofibers; poly(ethylene co-vinyl alcohol); moleculary imprinted polymer; creatinine;electrochemical sensor

1. Introduction

The phenomenon of electrospining was first discovered in 1902 [1], where a high voltage supply was used to stretch a polymer solution outside very fine needle. The Taylor cone phenomenon was discovered in 1969 by Sir Geoffrey Taylor. This phenomenon was theoretically analyzed, and it was demonstrated that a conducting fluid can exist in equilibrium as a cone, under the action of an electric field and a jet can be formed in the cone if a continuous supply of liquid is provided [2]. Electrospinning is one of the most versatile methods for synthesizing 3D nanomaterials. In particular, it exhibits outstanding capabilities of producing polymer or oxide nanofibers assemblies of various shapes and sizes [4]. In recent years, scientific progress has been made in this area: the most notable in the manufacturing process of electrospun nanofibers, the electrospinning process of bioceramic fibers, and the co-axial electrospinning technology and production of nanotube fibers [3]. The production of nanofibers mats with remarkably high specific area and porosity enables the realization of high sensitivity chemical sensors and biosensors [4] as well as bioactive polymers [5]. In this work we went to concept and characterize nanofibers of Moleculary imprinted polymer of EVOH and test their use like membrane of an electrochemical sensor for creatinine detection. Creatinine is a product of waste of nitrogenated metabolism and is a biomarker of kidney dysfunction. We then decided to replace natural receptors used in biosensors that are very expensive, fragile and are difficult to produce by moleculary imprinted polymers (MIPs), as it was reported in references [6-8] that Molecularly Imprinted Polymers(MIPs) were powerful materials for high sensitive and selective detection.

Molecularly imprinted polymer membranes were reported for detection of urea and creatinine [9-10]. Most efficient active sites are located close to the host polymer surface, thus improved sensitivity is expected upon increasing active area of sensitive membrane. This could be achieved by replacing thin film membranes by mats of polymer nanofibers (NFs) hosting molecularly imprinted specific recognition sites. Recent works were reported MIP nanoparticles encapsulated in polystyrene (PS) [11] and in poly(ethylene terephtalate) (PET) electrospun NFs [12,13]. Potentialities of fluorescent biosensors based on MIP nanoparticles (amino acid derivative dansyl-L-phenylanyline) embedded in poly(vinyl alcohol) electrospun NFs were recently demonstrated [14]. Some

papers reported MIP polymer NFs processed by electrospinning of a solution containing a HP/TM mixture consisting of host polymer (HP) and recognition template molecule (TM) dissolved in same or compatible solvents: PET and poly(allylamine)/2,4-Dichlorophenoyacetic acid [15], acrylonitrile-co-acrylic acid/Theophylline (THO) [16] and Polyimide/estrone [17].

In a previous work we reported MIP poly(ethylene-co-vinyl alcohol) (EVOH) membranes for the recognition of creatinine and urea [9,10]. The solvent used to dissolve EVOH was dimethylsulfoxyde (DMSO), which was already mentioned in the past for obtaining smooth EVOH membranes for neuronal culture [18]. In the present work, we explored potential improvement by replacing thin film membranes by EVOH NFs mats imprinted with creatinine. Most of reported works on electrospun EVOH NFs were carried out with either isopropanol (IPA)/water [19,20] or dimethyl acetamide (DMAc) [21] as solvent. The choice of solvent is known to be very important since a not convenient choice could result in the impossibility to electrospun the solution [22]. A comparison of DMAC with IPA/H2O [19,20] showed how the nature of the solvent influences the molecular chain in the liquid jet and, consequently the size of the deposited NFs [23]. In the following we describe electrospinning of EVOH NFs in DMSO solvent and of creatinine MIP-EVOH NFs. Highly sensitive electrochemical creatinine sensors were then produced.

2. Experimental

2.1. Products

Creatinine (anhydrous), dimethylsufoxide DMSO (99.8%), polyethylene(vinyl co-alcohol) EVOH (containing 27% of ethylene), tablets for the preparation of alkaline phosphate saline buffer (pH=7.5), bovine serum albumin (BSA), urea (>99%), potassium ferrocyanide and potassium ferricyanure $K_3Fe(CN)_6$ (>99%) and $K_4Fe(CN)_6.3H_2O$ (98.5-102%) were purchased from Sigma-Aldrich.

2.2. Preparation of gold electrodes

The gold substrates were provided by the laboratory for Analysis and Architecture of systems (LAAS, CNRS Toulouse). They were manufactured using standard silicon technologies. Oriented Silicon wafers of (100) P-type (3-5 Ω.cm) were thermally oxidized to grow an oxide layer of 800 nm thick followed by a gold layer of 300 nm thickness deposited by vacuum evaporation. A 30 nm-thick titanium under layer allowed for a better adhesion of gold on the silica surface. The wafer was cut into 1 × 1 cm2 square plates. Before use the gold electrode were washed with acetone for 15 min in an ultrasonic bath, dried with nitrogen, and then cleaned using a fresh mixture H_2O_2:H_2SO_4 3:7 v:v (Piranha) for 5 min rinsed with copious amounts of distilled water and ethanol and finally dried with nitrogen.

2.3. Influence of the viscosity of the polymer solution on the morphology EVOH NFs

Viscosity is a very important parameter influencing fibers shape and size. A too low viscosity leads to beaded fiber and even to electrospraying instead of electrospinning. If viscosity is too high, the coulombic force may not be able to stretch the fiber. For the optimization of the starting solution, four EVOH solutions in DMSO were prepared with 20, 22, 24 and 27 wt. % respectively. The EVOH pellets were mixed with DMSO and the solution was stirred at 80°C for two hours before obtaining full EVOH dissolution. Concentration beyond 27 % was not tested because of the difficulty to get full EVOH dissolution. After cooling down to the room temperature, the solution was filled into a glass syringe and electrospinning was processed. MIP EVOH solutions were obtained in a similar way by only adding 1 wt% of creatinine to a solution of 25% EVOH in DMSO.

2.4. Description of electrospining set-up

The electrospinning setup was consisting in a high voltage DC power supply (Spellman CZE1000R) delivering up to ±30kV, a syringe pump (Harvard Apparatus PHD2000), and a glass syringe with a blunt tip stainless-steel needle. The gauge of the needle was 20 corresponding to a 584 µm inner diameter. The electrically grounded collecting electrode was a 13 cm diameter aluminum disc onto which the substrates were maintained by vacuum. The distance between the tip of the needle and the collecting electrode (collection distance) can be varied from 0 cm to 38 cm. It is well known that fibers formation is much relying on relative humidity (RH) of the atmosphere. So the setup was kept into a Plexiglas box flushed with dry air and with continuous RH monitoring. All the processes reported in this work were carried at room temperature. Fiber morphology, diameters distribution and mat uniformity were observed with a FEG-SEM (TESCAN MIRA3) after metallization of the samples (10 nm Au).

2.5. Description of Infrared spectroscopy

Infrared spectra of NFs mats imprinted or not with creatinine were recorded at room temperature on a NICOLET by Continuµm microscope coupled with Nexus infrared spectroscopy in Attenuated Total Reflectance in monoreflexion with germanium crystal. Recordings were obtained with a resolution of 4 cm^{-1}, a spectral width between 650 and 4000 cm^{-1} and signal processing with HAPP-GENZEL apodization. (256 scans).

2.6. Impedance spectroscopy and electrical circuit model

The impedance measurements were performed using a conventional three electrode system, the working

electrode was the modified gold electrode (active surface area defined by an O-ring seal: 0.07 cm^2), the counter electrode was the platinum electrode (active surface area defined by an O-ring seal: 0.21 cm^2) and the reference electrode was an Ag/AgCl electrode. The impedance measurements were performed with the Voltalab 80 (PGZ 402), in a frequency range of 100 mHz to 100 kHz with applying potential of +200 mV to minimize impedance of the interface. All electrochemical measurements were carried out at room temperature in a Faraday cage.

3. Results

3.1. Manufacturing of EVOH nanofibers and EVOH nanofibers molecularly imprinted with creatinine

No useful results were obtained with EVOH concentration of 20 %. Only fibers including beads can be obtained whatever the collection distance or the polarization applied. Moreover, electrospraying is easily initiated instead of electrospinning if the polarization is somewhat too high. Figures 1 shows a set of significant NFs mats typically obtained in optimized spinning conditions without and with MIP.

For 22 % of EVOH in DMSO (fig. 1.a), a mixture of pure fibers (*i.e.* with no bead) and fibers with small beads can be produced within a narrow parameter range (collection distance between 28 cm and 32 cm and voltage between 12 kV and 14 kV). Diameter of pure fibers was mainly distributed around 250 nm with some occurrences up to 600 nm while beaded fibers are thinner with a diameter down to 150 nm. Diameter of the beads is about 1.5 µm.

For 24 % of EVOH in DMSO(fig. 1.b), pure fibers were obtained for a collection distance in the range 20-34 cm, and a polarization set around 11-12 kV. At 20 cm distance and below, the solvent had not enough time to evaporate during the fiber's time of flight and the fibers were not fully dry when reaching the collector, producing ribbon with 2.5µm width. At a distance of 34 cm and a polarization at 12 kV, very thin fibers (diameter around 150 nm) with beads were produced and also some extremely thin pure fibers with diameter as low as 26 nm. If the polarization was increased, more and more beads were produced and electrospraying started at 16 kV. At 38 cm distance, only fibers with beads were produced. Fibers with no beads were produced for 28 cm distance and 12 kV with diameters ranging from 290 nm to 1.15 µm. For 34 cm distance and 12 kV, a narrower diameter distribution was found, roughly between 300 nm and 400 nm.

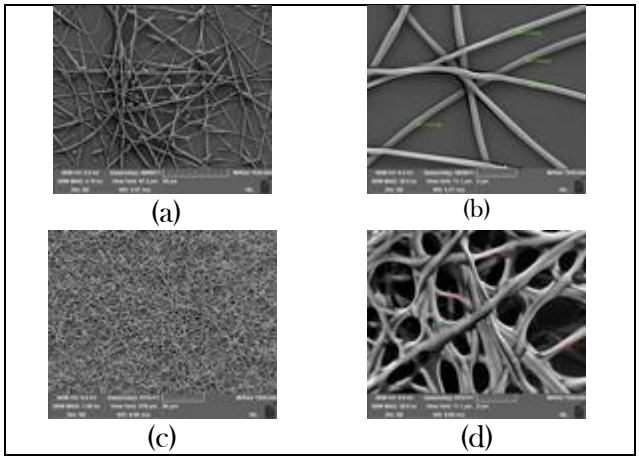

Figure 1. SEM micrographies of EVOH nanofibers a) EVOH/DMSO, 22 %, 32 cm, 12 kV; b) EVOH/DMSO, 24 %, 34 cm, 12 kV; c) and d) EVOH/DMSO (25 %) + creatinine (1 %), 28 cm, 12 kV.

For 27 % of EVOH in DMSO, pure fibers were obtained for collection distance between 28 cm and 38 cm, and applied bias between 12 kV and 18 kV (depending on the selected distance). Diameters of the fibers are then randomly distributed between 550 nm and 1 µm with a regular cylindrical shape. At 20 cm distance, no fully dried fibers can be obtained and beyond 18 kV (tested for 34 cm distance), spraying starts instead of electrospinning.

These results show that EVOH in DMSO appears as a good solution to produce electrospun NFs since pure fibers without any beads could be obtained within a rather wide range of mass ratio (22-27%). Our results are in agreement with those of ref.22 where the importance of the use of a solvent with sufficient dipolar moment was pointed out (DMSO = 4.06 D). However, high boiling temperature of DMSO (T_b = 190.8 °C) would be a limiting factor to get dry fibers before they reach the collecting electrode unless working at sufficient collection distance to overcome this drawback. Depending on the solution viscosity, it is possible to get some coarse control on fibers mean diameter. At 22 %, fibers diameters around 250 nm are obtained, at 24 % and 34 cm distance one getfibers diameters distributed between 300 nm and 400 nm andwith 27 % concentration, diameters are distributed roughly between 500 nm and 1 µm.

A solution of EVOH/DMSO with creatinine as a template molecule was prepared and electrospun to obtain creatinine imprinted NFs mats. As expected the new solution remained processable by electrospinning with the same range of parameters as previously found for 24 % and 27 % concentrations. Figure 1.c shows a mat of randomly deposited fibers obtained after 1 hour electrospinning, with 28 cm collection distance and a 12 kV bias. A very dense mat is obtained with no bead and a fiber diameter in the range 200-500 nm. The mat density is very homogeneous and one can observe that the fibers are stuck together.

3.2. Characterization of nanofibers of MIPs EVOH by infrared spectroscopy

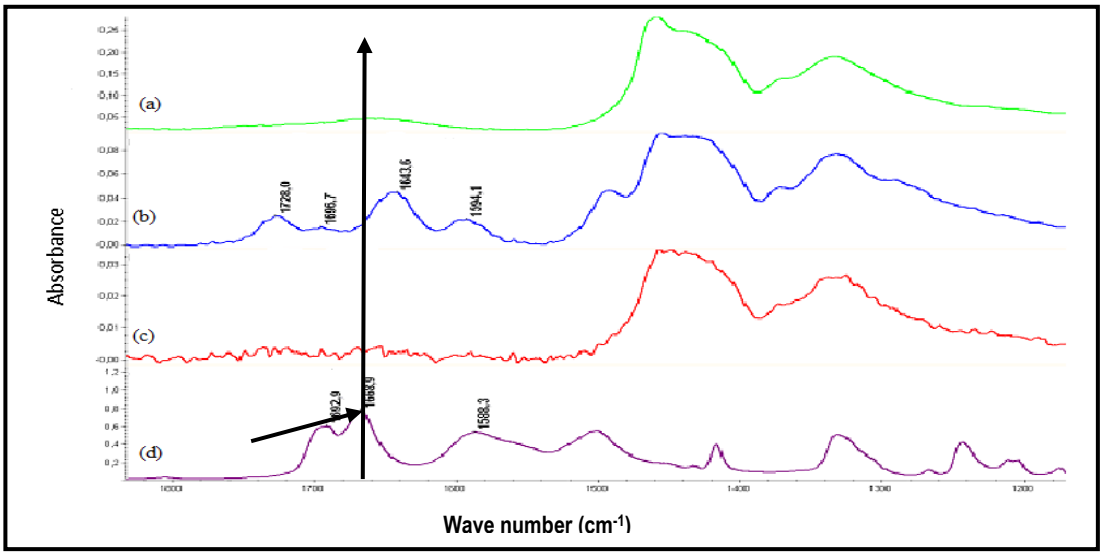

Figure 2. FTIR spectra (a) EVOH; (b) 1% creatinine in MIP-EVOH NF mat of before wash out; (c) MIP-EVOH NF mat after wash out; (d) creatinine

The chemical properties of MIPs nanofibers (NFs) of EVOH were characterized by FTIR spectrometry. This analytical technique allowed us to analyse the chemical composition of the deposited MIP-EVOH NFmaton gold electrode, the kind of interactions between creatinine and EVOH nanofibers and at last to see the removal of creatinine from the MIP-EVOH NFs after washing it out with ethanol. By comparing spectra of EVOH NFs (NIP) and EVOH including creatinine template in (fig. 1: b and c). The presence or absence of carbonyl functions (γ C=O 1696.7 cm^{-1}) and imides functions (γ C=N 1643.6 cm^{-1}) assigned to the creatinine confirmed capture and removal of template molecule after the washing step with ultrapure water and ethanol. Comparing the FTIR spectra of the pure components creatinine and EVOH with that of MIP (1% by weight of creatinine), all of EVOH characteristic peaks remain the same, even after adding of creatinine. In the case of creatinine there was a slight shift in the wavenumber for (γ C = N 1668.9 cm^{-1} to 1643.6 cm^{-1}) showing the existence of weak interactions between creatinine and EVOH skeleton. In Fig 2. (b) the characteristic peak of (γ C = N) moves towards lower wave numbers, demonstrating the existence of hydrogen bonding due to the electron donor properties of the nitrogen atom.

3.3. Detection of creatinine:

Fig. 3 shows the impedance change with the different creatinine concentrations. Creatinine was injected in PBS buffer 5 mM pH 7.5 containing 5 mM of ferri/ ferrocyanide with continuous stirring, the concentration varying from 1 $fg.l^{-1}$ to 2 $mg.l^{-1}$. The impedance measurements decrease with increasing the concentration of creatinine , as was observed in the work published in reference 10 with thin layer of MIP EVOH of creatinine.

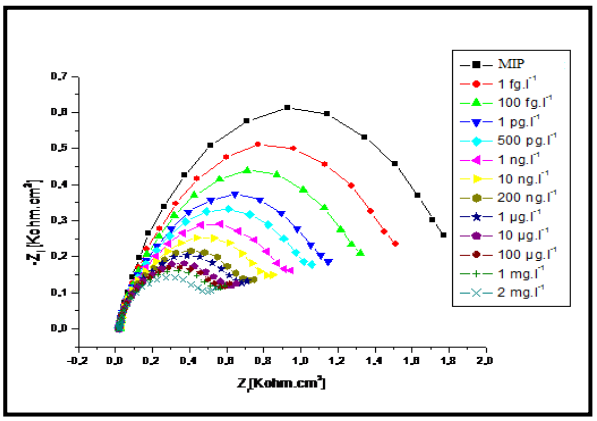

Figure 3. Complex impedance spectra (Nyquist plots) for the mat of MIP-EVOH NFs with different concentration of creatinine in a solution of PBS 5mM, containing 5 mM of ferri/ferrocyanure: from 1 fg/L to 2 mg/L. Under a potential of 200 mV.

The electrical properties at the electrode / electrolyte interface can be modeled by the electrical circuit shown in Figure 4. After modeling the impedance spectra in figure 3 with the electrical equivalent circuit in figure 4 using the Zview software, the main parameter that varies when the creatinine concentration varies is the charge transfer resistance. The calibration curve was plotted in figure 5 as the relative variation of charge transfer resistance versus logarithm of creatinine concentration.

In figure 5, we see that the response of MIP-EVOH NF based sensor is linear between 1 fg/L and 2 mg/L of creatinine concentration with a detection limit of 0.015 fg/L. The NIP nanofibers of EVOH was tested for the detection of creatinine and the calibration curve in figure 5 shows reduced linearity range and low sensitivity for creatinine.

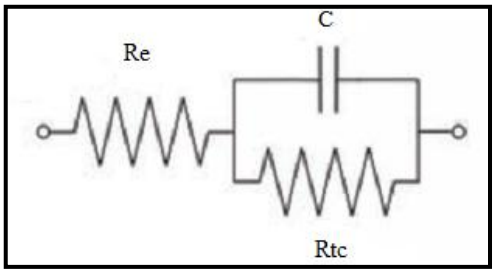

Figure 4. Equivalent electrical circuit used for impedance spectra modelling.

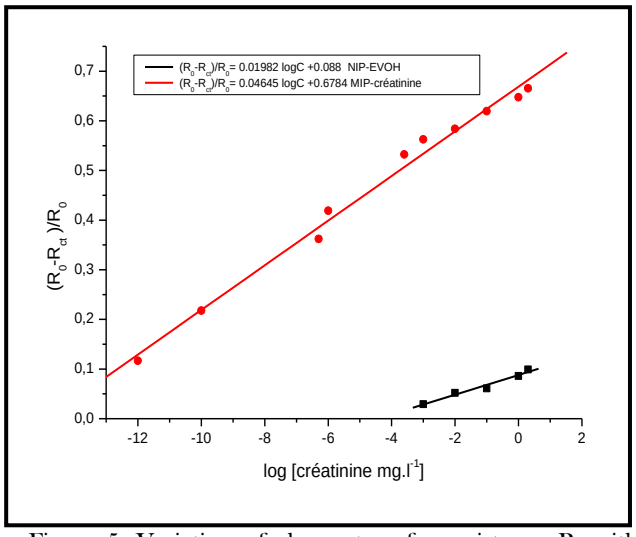

Figure 5. Variation of charge transfer resistance R_{th} with the increasing logarithm of creatinine concentration for matof MIP-EVOH NFs and of NIP-EVOH NFs.

3.4. Selectivity and reproducibility

Urea and BSA were used to test the selectivity of the sensor based on EVOH NPs imprinted with creatinine. The calibration curve of the response of the sensor to urea and BSA were plotted in figure 6 with that of creatinine. The relative sensitivities were respectively 1,0.442,0.24 for creatinine, urea and BSA. The sensor based on EVOH NFs imprinted with creatinine gave a very good reproducibility with an error factor which varies between 1% and 9% on the same sensor and from 1% to 15% from one sensor to another.

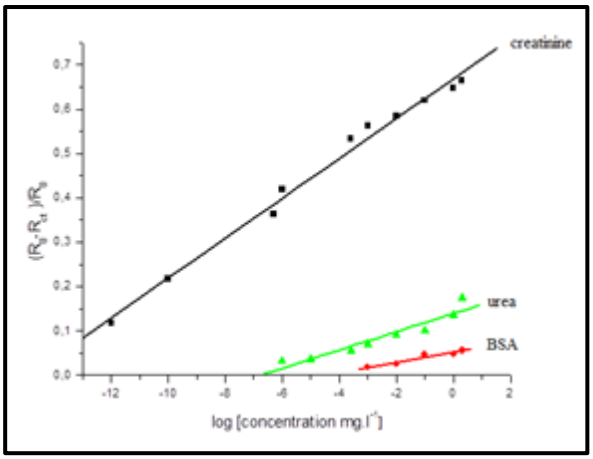

Figure 6. Selectivity of NFMIPs of EVOH toward creatinine.

4. Conclusion

The objective of this work is the manufacture and characterization of EVOH nanofibers imprinted with creatinine and their application to electrochemical sensor. The morphology and size of EVOH NFs were characterized using scanning electronic microscope (SEM),
It was found that the fibers without beads were obtained from 22% to 27 % of EVOH in DMSO. When percentage of EVOH in DMSO is less than 22%, the obtained NFs are filled with beads and when percentage is higher than 27% solutions not electrospinnable.

The electrochemical impedance spectroscopy (EIS) was the electrochemical method used to detect creatinine by the sensor based on MIP-EVOH NFs. The sensor gave a linear calibration curve between 1fg/L and 2 mg/L with a limit detection of 0.015 fg/L and a reproducibility of 1% to 9% for the same sensor, and 1% to 15% from one sensor to another with a very good selectivity for creatinine. The obtained low detection limit for creatinine and large detection range are due to the high affinity of creatinine for the EVOH and to the high specific surface area of MIP-EVOH NFs. This type of sensor is of high interest for the detection of small molecules in biological samples.

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