

Study of protein adsorption onto porous polymer synthesised in DBD plasma at atmospheric pressure

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This study focused on producing porous polymer under plasma conditions using two different monomers: ethylene glycol and styrene. A plasma discharge at atmospheric pressure was used to induce polymerisation reactions in order to obtain copolymer films based on the two monomers. The plasma source consists of a dielectric barrier discharge which operates in an argon gas flow with glass as dielectric. The porous polymer was obtained by dissolution and sonication of the hydrophilic groups from the material. The new porous material was tested for protein adsorption. The films were characterized by Fourier Transform infrared spectroscopy, X-ray Photoelectron Spectroscopy and Scanning Electron Microscopy techniques.

1. Introduction

Absorption is a very important phenomenon and highly dependent on the surface morphology and also on the chemistry [1-5]. Because of its very high active surface, porous materials are often used in drug absorption and controlled drug release, chromatography, biological separation molecules etc. [6-10]. However, the synthesis of such materials is difficult to control due to their repetitive chemical and topographic structure. In this way, the present study is focused on the synthesis of new polymeric porous materials using the technique of plasma polymerization at atmospheric pressure of two monomers with different wettability: ethylene glycol with a high hydrophilic character and styrene with high hydrophobic character. After synthesizing, the new polymer film was maintained in a water bath and exposed at ultrasonication process for 30 min at room temperature in order to obtain porous structure.

These new materials were tested for the adsorption of biomolecules. For this analysis we used bovine serum albumin (BSA). The adsorption process of biological molecules on the surfaces were analysed by Scanning Electron Microscopy (SEM) technique, X-ray Photoelectron Spectroscopy (XPS) and Fourier Transform InfraRed Spectroscopy (FTIR).

2. Experimental

The plasma chemical reactor consists of a discharge at atmospheric pressure, working in a plane parallel geometry with glass as dielectric barrier.

(Figure 1) The electrodes were circular with 30 mm diameter and the gap distance between them were 5 mm. Experiments were made in argon with a flow rate of 2 l/min for the plasma working gas (Flowmeter 1) and 0.9 l/min for ethylene glycol monomer bubbling (Flowmeter 2) and 0.1 l/min for styrene (Flowmeter 3), respectively. The spectral purity of argon was 99.999%.

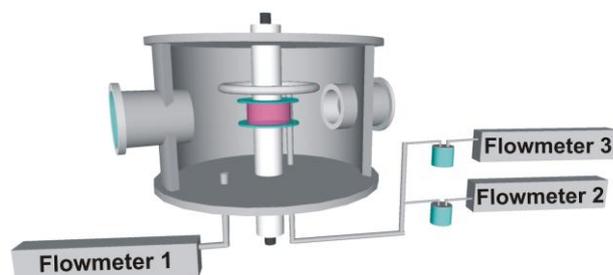


Figure 1. Plane parallel geometry of plasma reactor at atmospheric pressure

High voltage monopolar pulses were delivered using a voltage amplifier (Trek Inc.) connected with a waveform generator (Tabor Electronics Inc.). During the polymerisation process, the electrical parameters were maintained constant, respectively high voltage $V = 2$ kV, repetition rate $f = 2$ kHz and duration of polymerization was 10 minutes. The discharge mode is glow characterized by two current peaks at each applied voltage pulse, where the first current peak is called the primary discharge, and the second peak the secondary discharge. [11]

The chemical structure of the films was investigated with FTIR spectroscopy and the spectra were registered by a Bomem MB-Series 104 spectrometer

in the range of 4000-400 cm^{-1} , 4 cm^{-1} resolution and also by XPS (5000 VersaProbe spectrometer, equipped with a monochromatic Al $K\alpha$ X-ray source ($h\nu = 1486.7 \text{ eV}$).

The polymer films topography was investigated with Scanning Electron Microscopy (SEM) technique, using a Hitachi S-3400N microscope working at an acceleration voltage of 20 kV. The polymer sample was metalized with gold in a magnetron discharge for good conductivity.

3. Results and Discussion

Concerning morphological proprieties of the polymer surface, the SEM image of the Co PS-EG film shows a smooth surface without any defects (Figure 2 (a)). After the dissolution and sonication

process, the surface topography becomes porous, with a pore diameter around 50 nm (calculated with ImageJ program) (Figure 2 (b)). The SEM images prove that the adsorbed BSA proteins covering the entire surface of the polymer, the pores are not visible (Figure 2 (c)).

FTIR spectra of Co PS-EG film show the sharp vibrations corresponding to the specific following groups of the polystyrene (ppPS) and polyethylene glycol (ppEG). Thus, we identified the aromatic C-H group vibrations at 700, 757 and 909 cm^{-1} , C-H in plane deformation bending at 1073 and 1029 cm^{-1} , aromatic C-H stretching vibrations at 3025 cm^{-1} and 3061 cm^{-1} and the aromatic C=C stretching at 1683, 1600 and 1493 cm^{-1} .

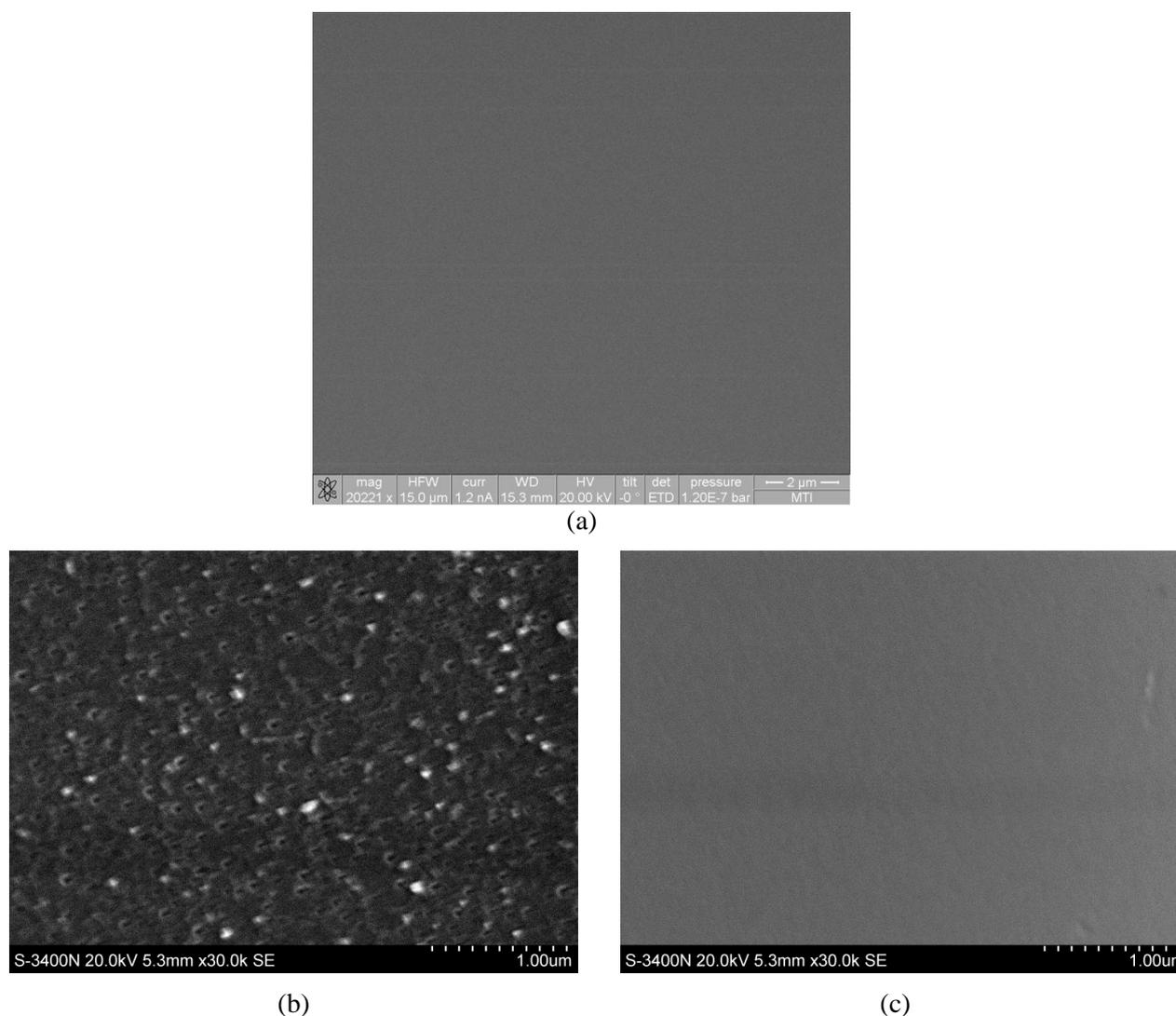


Figure 2. SEM images of Co PS-EG films (a), Co PS-EG films sonicated for 30 min (b), Co PS-EG films sonicated for 30 min and immersed in BSA (0.1 mg/ml) solution for 24 hours (c)

Also, methylene C-H vibration bands at 2930 and 2866 cm^{-1} and C-H bending at 1450 cm^{-1} , specific for both polystyrene and polyethylene glycol (Figure 3) were identified. Evidence for carboxyl, COOH groups is confirmed by the broad band between 3600 – 3200 cm^{-1} , corresponding to OH vibration. This broad bands are decreasing after the sonication process, which means that the hydrophilic structures are removed from the polymeric surface (Figure 3b).

After the removing of ppEG structure from the copolymer matrix, the new material was immersed in a solution of BSA at 0.1 mg/ml concentration for 24 hours. The protein adsorption process is identify by the presence of C-N peaks between 1500-1650 cm^{-1} . Also, in the case of large wavenumbers, the

FTIR spectrum shows a peak at 3300 cm^{-1} , which is characteristic of the type NH bonds. All these peaks, which appear after BSA immobilisation on the polymeric surface, are in good correlations with BSA powder FTIR spectrum.

The protein adsorption process was demonstrated also by X-ray Photoelectron Spectroscopy (XPS) technique. In addition to the C1s peak at 284 eV and O1s peak presented at 534 eV, appears another peak at 399 eV, which can be attributed to N1s.

All data above prove a good affinity of the BSA onto the surface of porous polymeric material (Co PS-EG) synthesized by plasma polymerization process at atmospheric pressure.

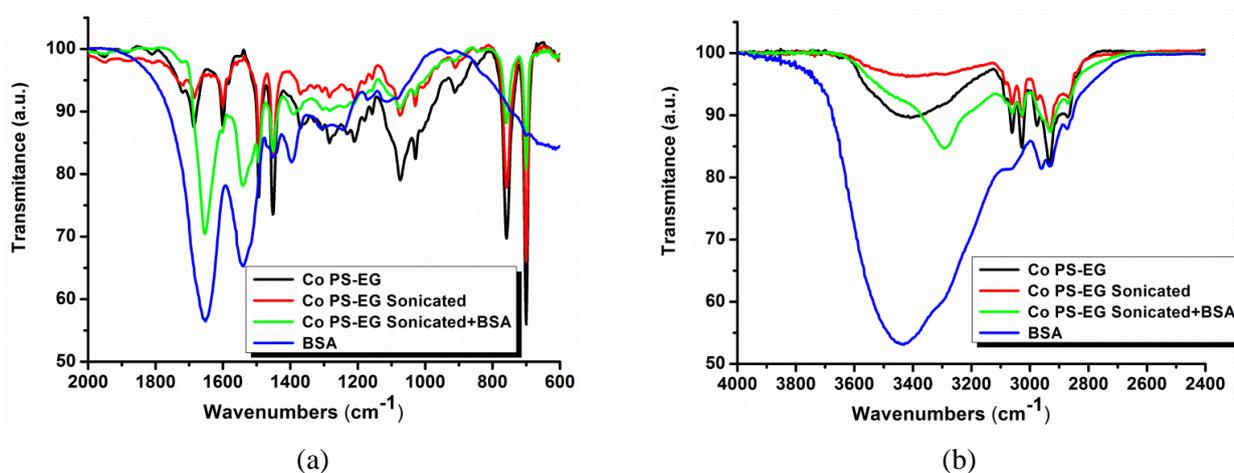


Figure 3. Comparative infrared spectra of Co PS-EG films, Co PS-EG films sonicated for 30 min, Co PS-EG films sonicated for 30 min and immersed in BSA (0,1 mg/ml) solution for 24 hours and BSA powder.

4. Conclusion

The plasma polymerization reaction in a mixture of styrene and ethylene glycol vapours is an efficient method to obtain copolymeric structure from two monomers having different wettability properties. These structures were modified by two processes (dissolution and sonication) providing a porous structure with a pore diameter around 50 nm.

These structures have been studied with respect to the BSA adsorption and present a good affinity of these proteins onto the polymeric surface. Thus, we demonstrate that bioactive porous structure may be easily prepared by plasma polymerization at atmospheric pressure and can be useful in biomedical applications.

5. Acknowledgement

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