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Electrodeposition study of polypyrrole-heparin and polypyrrole-salicylate coatings on Nitinol

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HIGHLIGHTS

- PPy films containing Hep, Sa or both anions were formed onto NiTi alloy.
- The morphology of the formed film depends of the dopant species.
- The presence of Hep and Sa in the PPy film improve the anticoagulant properties.
- The PPy film containing Hep and Sa has the best pitting corrosion protection.

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ABSTRACT

Polypyrrole (PPy) films containing heparin (Hep), salicylate (Sa) or both anions were electrochemically deposited as single layers or bilayers onto Nitinol (NiTi) alloy. The PPy deposition onto the bare alloy was achieved through an anodic electropolymerization in the presence of the anions. The aim of this study was to determine the influence of the electrosynthesis parameters (electrolyte nature and monomer and electrolyte concentrations) on the electroforming mechanism and morphology of the PPy. The PPy coating containing Hep and Sa not only presents a very good anticoagulant activity but also presents excellent anticorrosive properties in Ringer's solution.

1. Introduction

The equiatomic alloy containing Ni and Ti elements, better known as Nitinol (NiTi), is widely used as a biomaterial in a great number of biomedical applications such as orthodontic wires, cardiovascular and nephrology stents, bone implants and as a surgical material [1–3]. Its high corrosion resistance and biocompatibility with the human body can be attributable to an oxide layer mainly composed of TiO_2 with a small amount of NiO on the alloy surface [4]. The main problem related to the use of Nitinol as implant material is the release of allergenic and toxic Ni^{2+} ions into the human body fluids [4–6]. The solution to this problem has stimulated researchers to develop different treatments to modify its surface.

Polypyrrole (PPy) is an attractive organic coating for a large number of biological and biomedical applications due to its biocompatibility with the human body tissues [7]. The electrochemical synthesis of hollow rectangular microtubes of PPy in salicylate (Sa) solutions on different metallic biomaterials, has been carried out successfully in our laboratory [8,9]. Sodium salicylate (NaSa) is a non-steroidal anti-inflammatory drug (NSAID) used in medicine as analgesic and anti-inflammatory agent. Moreover, Sa exhibits antiplatelet activity by reducing activation, adhesion and aggregation of platelets [10].

On the other hand, is widely known that heparin (Hep) has anticoagulant properties [11]. Hep is a linear polysaccharide

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composed of repeated units of uronic acid, containing an *o*-sulfate group at the C-2 position, and D-glucosamine, usually *N*-sulfated with an additional *o*-sulfate group at the C-6 position [12]. The general structure, although no always identical, is represented as shown below:

It produces its major anticoagulant effect by inactivating thrombin and activated factor X (factor X_a) through an antithrombin (AT)-dependent mechanism. Heparin binds to AT through a high-affinity pentasaccharide, which is present on about a third of heparin molecules. For inhibition of thrombin, heparin must bind to both the coagulation enzyme and AT, whereas binding to the enzyme is not required for inhibition of factor X_a [13]. The combination of NSAID with anticoagulant drugs as Hep enhances their anticoagulant properties.

Electropolymerization is an interesting method to develop a PPy coating, wherein the pyrrole (Py) monomer is dissolved in aqueous or organic solvent containing an anionic dopant [14]. The monomer is oxidized onto the metallic surface by a simple anodic oxidation to produce the polymer films. In this simple step the anionic dopant is incorporated into the polymeric matrix in order to assure the electrical neutrality of the coating. Moreover, PPy has the ability to incorporate bioactive molecules, such as Hep, to improve its compatibility with the human body [15]. The electrochemical synthesis of PPy in aqueous solutions containing Hep as a bulky anionic dopant has been studied by different authors over different metallic substrates in order to assess the anticoagulant ability of the composite coating [16–22].

The formation of PPy coatings onto NiTi alloy doped with different concentrations of Hep was studied in this work. Furthermore, the formation of a composite coating constituted by a first layer of hollow rectangular microtubes of PPy doped with Sa and a second layer of PPy that contains Hep was investigated. The electrosynthesis of a PPy layer in a solution containing both dopants (Hep and Sa) was also carried out. Finally, the anti-corrosive properties of the different PPy coatings in Ringer solution as well as their anticoagulant ability in blood plasma were determined.

2. Material and methods

A cylindrical rod of NiTi alloy of 3.5 mm in diameter axially mounted in a Teflon holder was used as working electrode (WE) in the electrochemical experiments. The exposed area of the WE was 0.0962 cm^2 and its chemical composition (in wt. %) is: 55.8 Ni, 0.05 O, 0.02 C and Ti balance. In addition, NiTi square sheets of 1 cm^2 of exposed area were used as WE on the coagulation experiments. Before each experiment, the WE was abraded with SiC papers down to 1200 grit finish, then degreased with acetone and finally washed with triply distilled water. Then, the WE was immediately transferred to an electrochemical cell. A large Pt sheet was used as counter electrode and a saturated calomel electrode (SCE) was used as reference electrode. All potential values in this work are referred to SCE. Electrochemical experiments were performed in a Metrohm cell of 20 cm^3 employing a potentiostat-galvanostat PAR Model 273A. Electrochemical impedance spectroscopy (EIS) measurements were made using a potentiostat-galvanostat VoltaLab 40 Model PGZ301. The frequency was changed from 10 kHz to 100 mHz and a signal amplitude of 10 mV was used.

PPy films were obtained under potentiostatic conditions at 0.9, 1.0 and 1.5 V (SCE) during different polarization times (600 and 1800 s) from different solutions containing the monomer (pyrrole (Py, Sigma Aldrich)), previously distilled under vacuum before use.

The following solutions were used for the electrochemical synthesis:

1) x g L^{-1}Hep + 0.5 M Py, with x = 0.2, 0.3 y 0.4.
2) 0.5 M NaSa + 0.5 M Py
3) x g L^{-1}Hep + 0.5 M NaSa + 0.5 M Py, with x = 0.2 y 0.4.

Sodium salicylate and heparin sodium salt from porcine intestinal mucosa (Grade I-A with an activity of 180 USP units mg^{-1} and molecular weight between 17000 and 19000 Da) were purchased from Sigma-Aldrich and used as received.

The corrosion behavior of the coatings was evaluated by monitoring the open circuit potential (OCP) with time, linear sweep voltammetry (LSV), potentiostatic and EIS measurements in Ringer's solution (0.147 M NaCl, 0.00432 M $CaCl_2$ and 0.00404 M KCl), which is frequently used to simulate biological environment.

Tafel plots were used to determine the electrochemical parameters (anodic Tafel slope (βa), cathodic Tafel slope (β_c), corrosion potential (E_{corr}) and corrosion current density (i_{corr})). The i_{corr} was obtained by extrapolation of the linear part of the anodic and/or cathodic branches to E_{corr}.

Ni and Ti released concentrations in Ringer solution were analyzed using an inductively coupled plasma atomic emission spectrometer (ICP-AES) (ICPE 9000 - Shimadzu Corporation, Japan).

A scanning electron microscope (SEM) model LEO-EVO 40-XVP equipped with an X-ray energy dispersive system (EDX) model X-Max 50 (Oxford) was used for morphology characterization of the coatings. A fine carbon layer was used to improve the imaging of the samples.

The Fourier Transform Infrared (FT-IR) spectra of the different electrodeposited PPy films were recorded as KBr pellets in the 4000 to 400 cm^{-1} range on a Thermo Scientific Nicolet iS50 FTIR-NIR spectrometer.

The effect of PPy films doped with Hep, Sa or the combination of both anions on blood coagulation and platelet aggregation (PA) was evaluated. To that end, platelet poor plasma (PPP) was used in thrombin time (TT) and fibrinogen (F) measurements, and platelet rich plasma (PRP) for PA assays. PRP was obtained by centrifugation of citrated (0.38%) whole human blood at 240 × g for 20 min at room temperature, and PPP was obtained after centrifugation at 1200 × g for 15 min. Platelet concentration was adjusted to 3×10^{-8} cells mL^{-1}. Citrate was employed in order to remove calcium ions that are essential for blood coagulation. Slices were placed in a multiwell plate and incubated with PPP or PRP during 35 min at 37 °C with gentle movement. Immediately after, aliquots were taken for haemostatic assays performed as described below.

Coagulation tests (TT and F) were performed at 37 °C using Stago ST2 Coagulometer and commercial available kits (Wiener Lab, Argentina). TT test quantified the time taken for coagulation of an aliquot of PPP after thrombin addition. The results were expressed in seconds and represent the means of three independent experiments performed by duplicate.

F assay measured the plasmatic F content. To that end, thrombin was added to aliquots of PPP, immediately after the time taken for clot was determined, and the F concentration was quantified using a standardized preparation of F. Coagulation rate is inversely proportional to the F plasma concentration. The results were expressed in mg dL^{-1}, and represent the means of three independent experiments performed by duplicate.

PA was measured using a turbidimetric technique. Aliquots (285 μL) of each PRP were taken and set in an aggregometer cuvette (Chronolog 430) and prewarmed at 37 °C. Platelet aggregation was initiated by addition of 15 μL of 2×10^{-5} M ADP. Changes in light transmission were recorded for 5 min. Maximal PA (100%) was considered that induced by PRP alone. Results were expressed as percent of inhibition of PA (IPA).