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Is the biocompatibility of copper with polymerized natural coating dependent on the potential selected for the electropolymerization process?

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ABSTRACT

With the intention of taking care of the environment and human health, the development of alternative eco-friendly methods to inhibit metal corrosion is intensively encouraged. In previous works we showed that some phytocompounds components of essential oils such as carvacrol (Carv) and thymol (TOH) are able to be electropolymerized on metals and they seem to be promissory for this purpose.

The aim this paper is to investigate if the biocompatibility of copper covered by coatings formed by electropolymerization of Carv and TOH (polyCarv and polyTOH) is related with the potential selected for the electropolymerization process. Potentiostatic perturbations at different potentials, AFM images, ATR-FTIR spectroscopy and measurements of copper ions release provided suitable information to make a detailed analysis of different stages of the electropolymerization process that leads to polyCarv and polyTOH layers on copper surface. The change of the characteristics of the coatings over time was evaluated after several polymerization periods and current transients were interpreted by using nucleation and growth models. Results showed interesting changes in the polymerization process with the electrochemical perturbation, nature of the isomer, and time of the treatment. The treatment that provides the most protective, transparent and homogeneous layer, that strongly increased the biocompatibility of Cu could be selected: electropolymerization of Carv at 0.4 V. Results highlight the importance of the detailed study of the evolution of the electropolymerization processes to select the best ecofriendly condition due the high impact of potential perturbation and polarization time on the biocompatibility of the resulting polymeric layer-copper system.

1. Introduction

Copper and its alloys are extensively used for diverse applications owing to their advantageous mechanical, thermal and conductivity properties together with their simple fabrication and joining. Additionally they are resistant to corrosion and biofouling and very suitable for piping, tubing, condensers and heat exchangers [1,2]. From environmental point of view their high recyclability and prolonged service life are also advantages [3–5]. On the other hand, due to their high conductivity and after accurately shielding, copper can be used to carry signals to small implants of diagnostic devices into the body.

However, copper-based metals are susceptible to corrosion in chloride-containing media [6–8] that are frequent in industrial and medical environments. Corrosion is detrimental both, owing to the economical costs involved in substitution or repair of the copper-containing devices and to the release of copper ions associated to the corrosion process that contaminates aqueous environments [9] and represents a potential risk for biological systems [10].

Coatings have been proposed in order to act as insulators to mitigate current transfer between anodic and cathodic areas and to obstruct the diffusion of oxygen and chloride towards copper surface. Several organic inhibitors have been suggested to hinder copper dissolution by adsorbing on copper surface. Some of them form self assembled monolayers (SAMs) on the metal sur-

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face [11,12]. Besides, hetero-cycles that are added to the corrosive solution can adsorb on copper and inhibit metal corrosion [13–19]. However, their impact on the environment is rarely evaluated notwithstanding the possible emergent risks.

With the aim of caring the environment, alternative eco-friendly methods, i.e. those that are not harmful to the environment, have been recently developed [20]. Natural compounds such as essential oils obtained from oregano and thyme show several advantages because they are low-cost, easily available and renewable. Importantly, they can be extracted inexpensively by simple procedures. On this respect, in a recent report [21] the fundamentals and recommended actions in decision-making to select eco-friendly procedures considering product design, manufacturing and usage phases for the succeeding product generation are discussed. In the case of oregano, among the several ways to produce the essential oil (supercritical fluid, solvent, water distillation), water distillation with full energy integration was selected as the better technology. The cost of oregano production by this procedure is 8.64 U$S/kg, close to the price of other synthetic corrosion inhibitors, but with a distinctive advantage: a low environmental impact is associated with this production (Potential environmental impact (PEI) 0.018 PEI/kg) and carbon footprint (0.80 kg CO_2-e/kg oil) for water distillation technology [22].

In a previous paper [23] we informed that the use of some phytocompounds seems to be suitable for corrosion inhibition. Thus, two of the components of *Origanum vulgare* and *Thymus vulgaris* essential oils, carvacrol (Carv) and thymol (TOH), were proposed as appropriate for the development of an eco-friendly treatment. They are also well known as antibacterial and antioxidant compounds [24]. The electrochemical results obtained by cyclic voltammetry showed that these phenolic isomers are able to electropolymerize on the metal surface. However, the characteristics of the layers should be optimized in order to improve biocompatibility and corrosion inhibition. A meticulous study of the process is imperative to select the best conditions.

The aim this paper was to investigate if the biocompatibility of copper covered by coatings formed by electropolymerization of Carv and TOH is related with the potential selected for the electropolymerization process. A detailed analysis of different stages of the process was made using potentiostatic polarization at several potentials and polarization periods. The change of the characteristics of the coatings over time was evaluated by AFM images, by measurements of copper ions release and by ATR-FTIR spectroscopy of the polyCarv and polyTOH layers. Current transients were interpreted by using nucleation and growth models. Proliferation of cells in the vicinity of the coated metal samples was examined to find the relationships between biocompatibility and the selected potentials used for the electropolymerization process.

2. Experimental section

2.1. Materials

Carvacrol (Carv) (Sigma, St. Louis, MO, USA), and Thymol (TOH) (Sigma, St. Louis, MO, USA) were used in the experiments. All chemicals employed in the assays were of analytical grade and ultrapure water was employed to prepare the solutions.

Cylindrical copper bars (99.7% electrolytic metal copper, 9 mm diameter) (Merck, Darmstadt, Germany) were used for electrochemical experiments and copper sheets (99.7%, 0.1 mm thick, 6 mm diameter) were used for biological experiments. These discs and bars were washed with (5% v/v) H_2SO_4, vigorously rinsed with ultrapure water and then dried with nitrogen.

2.2. Cu samples and generation of electropolymerized layers

Cylindrical copper bars, whose lateral surfaces were covered with polyoxymethylene, leaving an exposed area of 0.626 cm^2 were used as working electrodes for electrochemical experiments. Each electrode was mechanically polished with emery papers of different grain sizes using water as lubricant and then washed with water and ethanol, and dried with nitrogen. The electrode surface was carefully observed under optical microscope (Olympus BX51, Olympus Corp., Tokyo, Japan), before and after the experiments, to evaluate possible changes in color and/or texture of copper surface.

Electrochemical assays were made in a conventional electrochemical cell. A platinum foil was used as counter electrode and a saturated calomel electrode (SCE) as reference electrode. The potential values in the text are referred to the SCE.

Electropolymerized films of Carv and TOH (polyCarv and polyTOH) on copper (sheets or cylinders) were attained by potentiostatic electrochemical methodology (Chronoamperometric measurements, CA) by modifying that reported by Guenbour et al. [25,26]. The potential range was selected according to the electrooxidation region (0.20 V-0.55 V potential region) characterized by cyclic voltammetry (Fig. S1, Supplementary information). Potentiostatic steps from the open circuit potential (OCP) to the selected potential were made. 0.1 M Carv or 0.1 M TOH water/ethanol (70:30) alkaline solution (0.3 M NaOH) [25–27] was used as electrolyte for this treatment. The presence of EtOH in Carv solutions is necessary to improve Carv solubility.

Each test was run in triplicate to verify the reproducibility of the data. In all cases a potentiostat-galvanostat TEQ03 was used.

2.3. Measurement of copper ions release

The copper ions released from the discs covered by polyCarv or polyTOH films after their immersion in 3 ml of 0.136 M KCl solution for 6 days at room temperature was measured by colorimetric analysis and by atomic absorption spectroscopy. Colorimetric method is based on the addition of 1-(2 pyridylazo)-2-naphthol (PAN) to the samples. This dye forms coloured complexes with a large number of metal ions, including Cu(II) which are suitable for spectrophotometric analysis. Briefly, an appropriate volume of H_2SO_4 was added to each sample to reach a final concentration of 0.25 M. An aliquot of 100 μl of these acidic samples was mixed with 100 μl of 4 mM PAN ethanolic dissolution and 800 μl of water. The absorbance was measured in a Shimadzu UV 1800 spectrometer at λ=560 nm, maximum of the absorption spectra of the Cu(II)–PAN complex. The copper content in an unknown sample was determined by comparing with a calibration curve [28].

The concentration of soluble copper in case of the bare copper was also determined by Flame atomic absorption spectrometer (Shimadzu AA-7000, Kyoto, Japan) after total dissolution with 1 ml 0.28 M nitric acid. Hollow cathode lamps were used as radiation sources (limit of detection = 0.02 μg/ml, obtained using internal quality control, according to standard procedures).

2.4. Surface analysis by ATR-FTIR and AFM

ATR-FTIR spectra were obtained in a Varian 660 spectrometer equipped with an attenuated total reflection (ATR) accessory (MIRacle ATR, Pike technologies) with a ZnSe prism. In all cases, each spectrum was the result of 256 scans taken with a resolution of 2 cm^{-1}.

Tapping® mode AFM (Nanoscope V; Bruker, Santa Barbara, CA) in topographic mode was used to characterize the substrates, using silicon tips (Arrow™ NCR; NanoWorld, Neuchâtel, Switzerland) (spring constant, 42 N/m; resonance frequency,