Bioelectrochemistry 104 (2015) 35–43

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Antimicrobial ruthenium complex coating on the surface of titanium alloy. High efficiency anticorrosion protection of ruthenium complex

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ARTICLE INFO

Article history:
Received 5 October 2014
Received in revised form 13 February 2015
Accepted 15 February 2015
Available online 18 February 2015

Keywords:
Ruthenium complex
Electrochemical impedance spectroscopy
Cytotoxicity
Scanning electron microscope
Titanium alloy

ABSTRACT

A ruthenium complex was prepared and structurally characterized using various techniques. Antibacterial and antifungal activities of ruthenium complex were evaluated. High significant antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* was recorded. Minor cytotoxicity records were reported at the highest concentration level using MTT assay. The influence of Cu(II), Cr(III), Fe(III) and Ru(III) metal ions of salen Schiff base on the corrosion resistance of Ti-alloy in 0.5 M HCl was studied. In vitro corrosion resistance was investigated using electrochemical impedance spectroscopy (EIS) measurements and confirmed by surface examination via scanning electron microscope (SEM) technique. Both impedance and phase angle maximum (θ_{max}) values were at maximum in the case of the ruthenium complex with promising antibacterial and antifungal activities. The surface film created by the ruthenium complex was highly resistant against attack or deterioration by bacteria. The EIS study showed high impedance values for the ruthenium complex with increasing exposure time up to 8 days. SEM images showed uniform distribution and adsorption of Ru(III) ions on Ti-alloy surface. The ruthenium complex, as a model of organic–inorganic hybrid complex, offered new prospects with desired properties in industrial and medical applications.

1. Introduction

Coordination chemistry of ruthenium has served a vital role in the development of various fast emerging fields of science, technology and medicine [1–5]. The design and synthesis of the ruthenium complexes have received much interest up to date, due to the potential advantages over multi possible reactivities and a wide variety of applications in diverse types of research areas.

It is intriguing to envision the combination of organic–inorganic hybrid compounds with surface chemistry. The advantage offered by the use of this combination is directly explained by the protective action of organic–inorganic hybrid corrosion inhibitors on metal surface, therefore, the privilege of using inorganic-hybrid compounds promoted many researchers to undertake the fabrication and applicability of these compounds as chemical models for corrosion inhibition.

There are a few reports, in the literature, on the applications of the ruthenium metal ion as an effective corrosion inhibitor of stainless steel [6–9], for this reason the study was undertaken.

Ti–6Al–4V alloy has attracted great attention in the past decades due to its relatively low density and high specific strength [10,11]. It exhibited good corrosion resistance, due to the formation of a protective native stable oxide film of TiO_2 [12,13]. However, the industrial applications of Ti–6Al–4V still suffer from high processing cost, low ductility and relatively low creep strength.

Van der Lingen and Sandenberghon had incorporated ruthenium into American Society for Testing and Materials (ASTM) Grade 5 (Ti–6Al–4V) titanium alloy [14]. Binary titanium–ruthenium alloys would mainly be cost-effective replacements for the binary titanium–palladium alloys in many applications and would display the ability of the excellent corrosive resistance. In addition to the aforementioned effects, titanium alloys based on minimal Pd and/or Ru alloying additions have been proven to be among the most cost-effective commercial anticorrosion alloys to date [10,11].

In the last two decades, salen Schiff bases have been studied exhaustively as they may possess promising and potential applications in terms of catalytic aspects [15]. Considerable attention has been devoted to the chemistry of the Schiff bases and their metal complexes containing nitrogen, oxygen donor atoms, in particular Salen, tetradentate [OˆNˆNˆO] chelating bis-Schiff base, which derived from salicylaldehyde 1,2-diamines [16,17] (Scheme 1). Furthermore, the interaction of metal ions with salen Schiff base has recently been designed as an efficient target owing to the wide range of applications based on their numerous functionalities as antibactericide [18], antivirus [19,20] fungicide agents [21], heterogeneous and homogeneous catalysts for oxidation and polymerization of organic compounds [22–25].

We have previously prepared transition and lanthanides metal ions complexes [26,27]. The synthesis of the ruthenium complex was targeted with the goal of fostering chelation through coordinative O

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http://dx.doi.org/10.1016/j.bioelechem.2015.02.005
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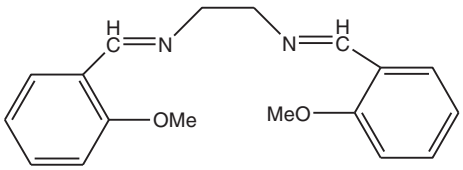

Scheme 1. The structure of the salen Schiff base.

and N atoms. The synthesis of the resulting ruthenium complex is described in this paper. The proposed structure of the complex is confirmed by the elemental analysis, vibrational spectroscopy, molar conductivity, UV–Vis, ^{1}H NMR and simultaneous TG-DTA analysis.

The antimicrobial activity of the complex was screened on four different microorganisms, Gram-positive and Gram-negative bacteria and two different fungi, under the same conditions. The ruthenium complex represented high inhibition record against Gram-positive and Gram-negative bacteria comparing with the other prepared compounds [26,27]. Cellular uptake was studied using two chosen cell lines, human embryonic kidney HEK 293 and L929 mouse fibroblast, as representative model to examine the cytotoxicity using MTT assay.

In addition, a comparative study between salen Schiff base, Cu(II), Cr(III), Fe(III) and Ru(III) complexes on the corrosion behavior of Ti-alloy in 0.5 M HCl has been studied. The aim of this study is to evaluate the protection efficiency of Ti alloy in an aggressive medium containing chloride ions (0.5 M HCl) in presence of different ions. The inhibition efficiency was recorded by the action of the ruthenium complex and compared to other reported complexes. Although the ruthenium complex was added in the solution and not incorporated in the alloy, it showed excellent protection efficiency for Ti–6Al–4V alloy.

This work was conducted to represent the ruthenium complex as new promising organic–inorganic hybrid candidate of valuable antibacterial activity together with high biocompatibility profile, furthermore its amenability to use as an effective corrosion inhibitor for Ti-alloy has triggered the explosive growth of interest in this new material across the discovery of novel anticorrosion inhibitors applications.

2. Experimental

2.1. Reagents and physical measurement

All chemicals used were analytical grade reagents. They included 2-methoxybenzaldehyde, ethylenediamine, $Ru(Cl)_3 \cdot 3H_2O$ (Sigma), absolute ethyl alcohol, diethyl ether (Acros). Yeast extract and agar (Sigma) were also used.

The salen Schiff base, Cu(II), Fe(III) and Cr(III) complexes were prepared using the reported method [26,27]. The ruthenium complex was prepared by adding a hot water–ethanolic solution (60 °C) of the Ru(III) metal salts (25 mL, 0.1 mmol) to a hot ethanolic solution of salen Schiff base (25 mL, 0.1 mmol). The resulting mixture was stirred under reflux for 2 h and left to cool, whereby the complexes precipitated as fine dark green powders. The solid complex was filtered, washed with ethanol, then with diethyl ether, dried in a vacuum desiccator over P_4O_{10} and obtained as a solid powder yield greater than 96%. M.P. ≥ 300 °C.

2.2. Electrode preparation

Ti–6Al–4V alloy [28] supplied from Johnson and Matthey (England) with composition (wt%); 5.7 Al, 3.85 V, 0.18 Fe, 0.038 C, 0.106 O and 0.035 N and balance titanium was tested in the present study. The coupon was welded to an electrical wire and fixed with Araldite epoxy resin in a glass tube leaving cross-sectional area of the specimen 0.196 cm [6–9]. The surface of the test electrode was mechanically polished with emery papers with 400 up to 1000 grit to ensure the same surface roughness, degreased in acetone, rinsed with ethanol and dried in air.

2.3. Solutions preparation

Hydrochloric acid of 0.5 M concentration (Analar reagent) was prepared using triply distilled water.

2.4. Antimicrobial activity

Antimicrobial activity of the tested samples was determined using a modified Kirby–Bauer disk diffusion method [29,30]. In the disk diffusion method, 100 μL of the test bacteria/fungi was grown in 10 mL of fresh medium with a concentration of 108 cells/mL for bacteria or 105 cells/mL for fungi [31]. 100 μL of microbial suspension (20 mg/mL) was spread onto sterile melted agar and poured into plates. Holes of 9 mm diameter were cut in the agar and filled with 50 mL of a 50 ppm solution of the complexes or ligand at room temperature (dissolved in 1% dimethylsulfoxide). Fungi (as *Aspergillus flavus*, *Candida albicans*) were grown in nutrient agar medium and incubated at 25 °C for 48 h; both Gram-positive (as *Staphylococcus aureus*) and Gram-negative (as *Escherichia coli*) were grown in nutrient agar medium and incubated at 35–37 °C for 24–48 h, followed by frequent subculture to fresh medium and used as test fungi and bacteria, respectively. The diameters of the inhibition zones were measured in millimeters after incubation taking into account, the standard discs of Tetracycline (antibacterial agent), Amphotericin B (antifungal agent) served as positive controls, while filter discs impregnated with 10 μL of solvent (distilled water, chloroform, dimethyl sulfoxide) were used as a negative control.

2.5. In vitro cytotoxicity tests

2.5.1. Pretreatment of the complexes for cytotoxicity tests

The prepared complex was sterilized by UV–Vis (ultraviolet–visible) light for 6 h. To prepare the stock solutions, cell culture medium supplemented with FCS (fetal calf serum) was used; each suspension was sonicated for 15 min. before to exposure to the cells to avoid any agglomerates.

2.5.2. Cell culture and cytotoxicity assay by MTT

To examine in vitro cytotoxicity, L929 mouse fibroblast and HEK 293 cells derived from human embryonic kidney were used. The viability of these cells upon treatment with the title complex at different incubation periods was checked using MTT assay [32,33]. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is absorbed by mitochondria, where, by the action of enzyme succinic dehydrogenase, it is transformed into formazan (purple crystals). The activity of viable cells in a cell population was measured, after treatment with the prepared complex for a different time period, both of the sensitivity of the cells to the concentrations and incubations period are determined to assess the activity of the mitochondrial dehydrogenases. Growing HEK 293 and L929 cells are initially seeded at 150 μg/mL of cells/mL and were treated with 1 mg/mL of the ruthenium complex for 24 and 48 h at 37 °C in an atmosphere supplemented with 5% CO_2. MTT was added to the final concentration of 5 mg/mL and they were further incubated for 1 h at 37 °C 5% CO_2 in order to transfer MTT (yellow) into formazan crystals (purple) by the viable cells. Upon adding a solution containing dimethyl sulfoxide the formazan crystals were solubilized. The results from MTT assay are displayed as the means of the absorptions at 630 nm, the measured absorbance is correlated with the number of viable cells. Cells incubated with media alone were employed as control and this was considered as 100% cell viability. The IC_{50} values or the concentrations at which the cell growth inhibition was 50% compared to untreated controls.

2.6. Characterization techniques

FTIR spectra were obtained from dispersions in KBr using a Perkin–Elmer FT-IR type 1650 spectrophotometer. The spectra were collected