Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 136 (2015) 979–985

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Adsorption study of antibiotics on silver nanoparticle surfaces by surface-enhanced Raman scattering spectroscopy

Aline Luciano Filgueiras [a], Diego Paschoal [b], Hélio F. Dos Santos [b], Antonio C. Sant'Ana [a,*]

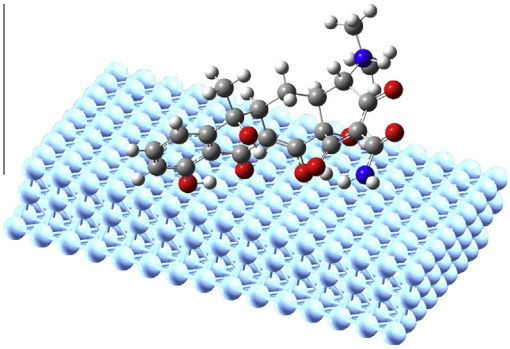
[a] Laboratório de Nanoestruturas Plasmônicas (LabNano), Departamento de Química, ICE, Universidade Federal de Juiz de Fora, 36036-900 Juiz de Fora, MG, Brazil
[b] Núcleo de Estudos em Química computacional (NEQC), Departamento de Química, ICE, Universidade Federal de Juiz de Fora, 36036-900 Juiz de Fora, MG, Brazil

HIGHLIGHTS

- Levofloxacin adsorbs strongly on silver surface by carboxylate moiety.
- Tetracycline adsorbs strongly on silver surface by carbonyl and hydroxyl moieties.
- Benzylpenicillin adsorbs weakly on silver surface by carbonyl from acyclic amide.
- SERS spectroscopy is outstanding technique for molecular adsorption analysis.

GRAPHICAL ABSTRACT

The adsorption geometries of the antibiotics tetracycline, levofloxacin and benzylpenicillin on the surfaces of silver nanoparticles were investigated by SERS spectroscopy.



ARTICLE INFO

Article history:
Received 24 July 2014
Received in revised form 23 September 2014
Accepted 25 September 2014
Available online 5 October 2014

Keywords:
Antimicrobial
Biomolecules
Synergy
Chemisorption
Resistant bacteria

ABSTRACT

In this work the adsorption of the antibiotics levofloxacin (LV), tetracycline (TC) and benzylpenicillin (BP) on the surface of silver nanoparticles (AgNP) have been investigated through both surface-enhanced Raman scattering (SERS) and UV–VIS–NIR spectroscopies. The SERS spectra were obtained using 1064 nm exciting radiation. Theoretical models for the antibiotic molecules were obtained from DFT calculations, and used in the vibrational assignment. The adsorption geometries were proposed based on the changes in the spectral patterns. The LV compound adsorbs through carboxylate group, TC compound interacts with silver atoms through carbonyl from intermediate ring, and BP compound adsorbs by carbonyl moieties from carboxylate and acyclic amide.

Introduction

The surface-enhanced Raman scattering (SERS) effect is responsible for the enhancement of Raman signal of molecules adsorbed on metallic nanostructures. The enhancing factor can attain 10^{11} times [1–4], allowing to obtain SERS spectrum from very diluted solutions. The signal enhancement is explained by two mechanisms: the electromagnetic one, by which the interaction of light with metallic nanoparticles produces large amplifications of the electric field on the surface through electronic excitations known as localized surface plasmon resonance [5–14], and the chemical one, which implies in a resonance involving a photon assisted charge transfer process [15–18]. The interpretation of SERS

* Corresponding author. Tel.: +55 32 2102 3310.
E-mail address: antonio.sant@ufjf.edu.br (A.C. Sant'Ana).

http://dx.doi.org/10.1016/j.saa.2014.09.120
1386-1425/

spectrum gives information about the orientation of molecules on the metallic surfaces in submonolayer regime, the molecular moieties involved in such interaction, and how strong is the adsorption process [19–23].

In the last decades antibiotics have been losing effectiveness due to the emergence of biological resistance [24–26]. Such problem leads to demand for the development of new strategies for the treatment of infections [25]. The use of silver nanoparticles (AgNP), which are known by the antibacterial properties [27–31] in association with antibiotic drugs can be an alternative for such treatments [32,33]. The knowledge of the molecular sites that are interacting with the metallic surface allows inferring if the mechanism of action of the drug is preserved or not. In another way the intensity of the interaction of the molecule with the silver surface could be useful for estimate the capability of AgNP to act as a vehicle for drug delivery as well as to facilitate the drug entrance through cell walls and membranes [34]. In suitable conditions, the antibiotic activity could be enhanced by silver nanoparticles in the biological medium [35]. In this way, SERS spectroscopy is an outstanding technique for investigations of the surface interactions of organic compounds on metallic surfaces. In this work SERS spectroscopy have been using aiming to obtain information about the adsorption of the antibiotics levofloxacin (LV), tetracycline (TC) and benzylpenicillin (BP) on silver surface for understanding some additive or synergic antibacterial effects observed *in vitro* in our previous results [36].

The antibiotic LV is a fluoroquinolone compound, with broad action range against Gram-positive and Gram-negative bacteria, and such class of antibiotics acts by inhibition of DNA synthesis [37]. The mechanism is not completely known, but possibly involves interactions with fluorine and others lateral structures around aromatic nucleus [38,39]. Copper(II)–LV complex involving coordination by carboxylic moiety, presented enhanced antibacterial activity [40,41]. In another system, gold(III)–LV complex, formed by coordination through piperazine moiety, presents anticancer properties [42]. The antibiotic TC has a mechanism of antibacterial action involving the presence of dimethylamine moiety [43]. Such a compound forms complexes with different metals as platinum(II) [44], palladium [45] or copper(II) [32,46], changing its chemical properties, and being used in biological applications such as anticancer action [44,47], DNA interactions [32], and antimicrobial activity against resistant bacteria [48–50]. The antibiotic BP has the beta-lactam ring as the biological active site, acting through inhibiting of protein syntheses, which are necessary for the formation of bacterial cell wall [51]. Complexes involving beta-lactam ring have been synthesized and characterized by infrared spectroscopy, shown that such molecular moiety is an important interaction site with metals [52,53].

The SERS and resonance Raman spectra of some quinolones and the FTIR spectrum of LV, with vibrational assignments based on DFT calculations are reported [54–56]. The Raman, resonance Raman and FTIR spectra of TC, derivative species and metallic complexes, with the vibrational assignments have also been reported [44,48,57,58]. The Raman and FTIR spectra of beta-lactam ring compounds [52,59], as well as the SERS spectra of BP adsorbed on AgNP aqueous suspension or on silver electrode are reported [60–62], but the adsorption mechanism is yet no clear.

In the present work it was studied the interaction of LV, TC and BP antibiotics, with AgNP through SERS experiments, with excitation in the near infrared. The vibrational assignment of each SERS spectrum was proposed based on DFT calculations of the compounds, in appropriate protonation states, taking into account the pK_a values (Scheme 1). The results allowed inferring the molecular anchor sites, the adsorption geometries and the nature of the surface interaction forces. All assumptions on the interactions of the antibiotics with silver surface were also supported by UV–VIS–NIR spectroscopy studies based on changes in the localized surface plasmon resonance (LSPR) features.

Materials and methods

Materials

The chemicals sodium borohydride, trisodium citrate dihydrate, silver nitrate, BP, LV and TC were purchased from Sigma–Aldrich and used without additional purification. The deionized water with Milli-Q pattern (18.2 MΩ cm resistivity) was used in all aqueous solution preparation. All glass containers were cleaned using aqua regia ($HCl{:}3HNO_3$) and copiously rinsed with deionized water.

AgNP aqueous suspension preparation

AgNP aqueous suspension was prepared as the synthesis of Creighton et al. [63], modified as described: 20 mL of 2.0×10^{-3} mol L^{-1} of silver nitrate aqueous solution was added dropwise to 40 mL of 8.0×10^{-3} mol L^{-1} sodium borohydride aqueous solution in ice bath, with continuous stirring, and 600 μL of 3.4×10^{-2} mol L^{-1} trisodium citrate dihydrate aqueous solution was added after 30 min for stabilizing the AgNP suspension. The resultant colloidal suspension, with 9.8×10^{-4} mol L^{-1} Ag concentration, was yellowish gray, has been used in SERS experiments when recently prepared.

Conditions for obtaining spectroscopic results

The UV–VIS–NIR spectra of adsorbates and AgNP aqueous suspensions were obtained in a Shimadzu spectrophotometer, model UV-1800, using 0.1 cm path length quartz cuvette. The SERS and Raman spectra were obtained from a Bruker spectrometer, model RFS-100, using a Nd^{3+}/YAG laser line with wavelength at 1064 nm, coupled with a germanium detector, cooled with liquid nitrogen. The spectral resolution used in all Raman spectra was 4 cm^{-1}. The Raman spectra of the BP, TC, and LV in solid state were obtained with a 30 mW laser power, accumulated with typically 100 scans. The SERS spectra of antibiotics were obtained in silver aqueous suspension using 1.0×10^{-3} mol L^{-1} concentration for TC and BP and 1.0×10^{-4} mol L^{-1} for LV, and for all samples the pH remained around 6.0. In SERS experiments it was used a 500 mW laser power, accumulated with typically 200 scans. Several spectra were recorded in different points of the suspension to check the reproducibility. It was not observed any light–induced aggregation process at these accumulation conditions that was monitored by the stability of the SERS background [64,65]. Since the Raman signals from antibiotics solutions were rather weak, even in higher concentration, their contributions were not considered in the SERS spectra, for all analytes. Such discussion, together the Raman spectra of the analytes in aqueous solutions are presented in Supplementary Material.

Computation details

The geometries for the free ligands were fully optimized in gas phase and characterized as stationary points on the potential energy surface (PES) through harmonic frequency calculations at DFT level with the PBE1PBE functional [66] and LANL2DZ ECP basis set for Ag [67] atom and 6-31 + G(2d) [68] basis set for all other atoms. Raman spectra were calculated at the same level of theory and the frequencies and intensities [69] used as input to fit a Lorentzian function [70] in order to represent the band spectra. The main bands were assigned by visual inspection of the normal modes. All calculations were carried out using Gaussian 09 package, revision A.02 [71].