Surface-enhanced Raman and DFT study on zidovudine

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Abstract. This work presents a surface-enhanced Raman scattering (SERS) and density functional theory (DFT) study on neutral and deprotonated molecular forms of zidovudine. A pK_a value of 9.55 was found for this compound, the deprotonation occurring at the imide nitrogen of the pyrimidine ring. The characteristic bands of the SERS spectra were assigned considering both, the calculated vibrational wavenumbers and Raman intensity pattern. The adsorption geometry on the silver surface of zidovudine was derived based on the molecular electrostatic potential (MEP) and the selective Raman bands enhancement.

Keywords: SERS, DFT, pH-dependence, zidovudine

1. Introduction

Zidovudine, 3'-azido-3'-deoxythymidine (AZT), is an organic compound used to delay development of acquired immunodeficiency syndrome (AIDS) in patients infected with HIV, to reduce mother-to-child HIV transmission and as radiosensitiser in cancer radiotherapy, because it is a telomerase inhibitor with potential anticancer properties [14]. In order to avoid toxicity and also to achieve optimal drug concentration for viral suppression, it is now highly recommended a careful monitoring of drug levels [10].

In spite of its importance for pharmaceutical purposes, however, the reported spectroscopical studies on zidovudine are scarce. Cheng et al. [2] investigated different conformers of AZT by simulating their IR spectra. Improper hydrogen bonds caused blueshift in C–H bond stretch vibrations in three conformers of zidovudine that have been studied using DFT in gas phase and in aqueous solution. Recently, Peepliwal et al. [9] developed and then validated two analytical methods for the quantification of AZT containing solid dosage form by using FTIR and UV-Vis spectroscopies. AZT showed no interference from the common additives and excipients, demonstrating that the recommended procedure is well suited for the assay and evaluation of drugs in pharmaceutical preparations. Nayak et al. [7] studied the sustained release of AZT using chitosan as a polymer matrix by applying IR spectroscopy and differential scanning calorimetry (DSC). They have demonstrated the stability of the drug during the encapsulation process and have shown more than 95% drug release in 12 h. Rivas et al. [12,13] used normal Raman as well as SERS to carry out a structural study of AZT in solid state, aqueous solution and when adsorbed on silver nanoparticles prepared by reduction with citrate. Their study was focused

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on the effect of sugar substituents in the tautomerisation of thymine residue as well as on the conformational rearrangement of the molecule when adsorbed on the above metal surface. Raviolo et al. [11] investigated X-ray crystal and molecular structure, NMR, IR and Raman spectra, the thermal behavior of a novel carbonate of AZT and its lipophilic properties in order to enhance pharmacological effects. A study concerning the biodegradation of AZT in the environment was carried out by Kruszewska et al. [4] by using chromatographic methods, mass analyses, NMR and vibrational methods.

In this study, SERS and DFT calculations were used for a proper investigation of the structure and electronic properties of AZT, with the particular aim of evidencing the characteristic SERS bands of this compound in its neutral and deprotonated forms.

2. Experimental details

SERS spectra were recorded using an Advantage 532 spectrometer (DeltaNu, USA), equipped with a frequency doubled NdYAG laser emitting at 532 nm.

For SERS measurements, a silver colloidal SERS substrate was prepared by reducing Ag^+ with hydroxylamine hydrochloride [5]. Thus, 17 mg AgNO_3 were solved in 90 ml distilled water. 17 mg $\text{H}_2\text{NOH} \times \text{HCl}$ and 0.25 ml NaOH (2M) were solved in to 10 ml distilled water and then added rapidly to the silver nitrate solution under vigorous stirring. The final pH of the colloidal solution can be adjusted by varying the amount of NaOH added to the H_2NOH solution, or adding diluted HCl solutions to the prepared colloid.

3. Computational details

For the proper understanding of the spectra, DFT especially hybrid functional methods are used to determine the electronic structure of molecules. For the assignment of the marker bands from SERS spectra, DFT calculations were assessed using B3LYP hybrid exchange-correlation functional and the 6-31G(d) basis set. The gas-phase optimized geometry of the neutral form of AZT and the optimized one in water as solvent were obtained, according to the Polarizable Continuum Model (PCM) solvation model.

$\text{p}K_a$ values of AZT were calculated using ACDLABS 12.0 (Advanced Chemistry Development, Canada) software. Both species, neutral and deprotonated, are present, with equal contributions, for the $\text{p}K_a$ value 9.55. In a previous study, Checa et al. [1] predicted a $\text{p}K_a$ value of 9.78.

4. Results and discussion

Raman and SERS spectroscopies provide important structural information making them a powerful molecular investigation tool. For SERS measurements, a colloidal substrate at $\text{pH} = 7$ was prepared, where the neutral molecular form of AZT is predominantly present (in the 5–9 pH values interval), and also an alkaline one at $\text{pH} = 12$, where the deprotonated molecular form is dominant.

As shown in Fig. 1, the most intense bands of zidovudine are shown at 1648 cm^{-1} for the deprotonated species (pH 12) and at 1643 cm^{-1} for the neutral zidovudine species (pH 7), assigned to the symmetric stretching vibration of the $\text{C}=\text{C}$ bond of the pyrimidine ring. Another marker band for zidovudine is that

Fig. 1. SERS spectra of 10^{-5} M zidovudine at pH = 7 (top) and pH = 12 (bottom).

corresponding to the breathing vibration of the pyrimidine ring, shown at 789 and at 793 cm^{-1} for the deprotonated species and for the neutral one, respectively.

Comparing the two SERS spectra, the most notable shifting occurs for the band at 1588 cm^{-1} in the case of the deprotonated form of zidovudine, to 1575 cm^{-1} in the spectrum of the neutral molecular species. This band has no correspondent in the FT-Raman spectrum. In our opinion, this band is due to the C=O stretching vibrations of the pyrimidine ring, strongly shifted to lower wavenumbers, due to the interaction of oxygen with the silver surface. Furthermore, a characteristic band of zidovudine is the N $\equiv$ N stretching vibration of the azide group present in the SERS spectrum at 2114 cm^{-1} .

4.1. Adsorption geometry of zidovudine

MEP is widely used as a reactivity map, displaying most probable regions for the electrophilic attack of charged point-like reagents on organic molecules [3]. Also, it is a useful tool in determining the adsorption geometry on the silver surface, considering also several marker bands. The MEP distribution of AZT molecule obtained from DFT calculations (Fig. 2) shows that the negative charge is located mainly on the oxygen atoms, thus, it is supposed that AZT adsorbs to the silver surface by the oxygen atoms of the pyrimidine ring. This supposition is sustained by the strong shifting of the C=O band of the pyrimidine ring. The presence of the C=O stretchings as well as of the breathing vibration of the pyrimidine ring as intense bands indicates a predominant perpendicular adsorption of the pyrimidine ring in respect to the silver surface, as can be deduced from the SERS selection rules [6,8]. Also, the enhancement of the =C–H stretching of the pyrimidine ring at 3073 cm^{-1} sustains the predominant

Fig. 2. B3LYP/6-31G(d) calculated 3D electrostatic potential of zidovudine (a.u) mapped onto the electronic density isosurface of 0.2 a.u. (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/SPE-2011-0544>.)

perpendicular orientation of the pyrimidine ring. A predominant parallel orientation of the oxalate ring, with respect to the silver surface is proposed, due to the weak SERS bands associated with this ring.

5. Conclusions

The calculated pK_a value for AZT is 9.55 and the deprotonation of the molecule occurs at the imide nitrogen of the pyrimidine ring.

Normal modes of vibration including Raman intensities were calculated at B3LYP/6-31G(d) level of theory on the optimized structures of neutral and deprotonated molecular forms of zidovudine.

The characteristic bands of the neutral and deprotonated molecular forms were evidenced in the SERS spectra recorded at pH 7 and pH 12, respectively.

The pyrimidine ring appeared to have a perpendicular orientation on the silver surface, while the oxalate ring seems to be adsorbed predominantly parallel to the surface.

Acknowledgements

This work was supported by CNCS-UEFISCDI, project number PN II-RU TE_323/2010. Financial support from the Sectoral Operational Programme for Human Resources Development 2007-2013, co-

financed by the European Social Fund, under the project number POSDRU/107/1.5/S/76841 is highly acknowledged by N.E.M.

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