Colloids and Surfaces A: Physicochem. Eng. Aspects 480 (2015) 328–335

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

Surface properties of Vancomycin after interaction with laser beams

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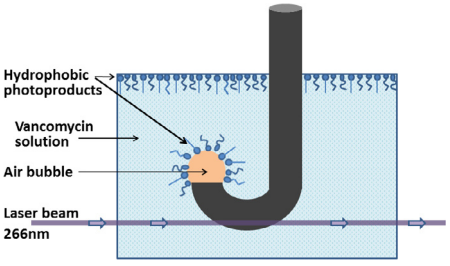
HIGHLIGHTS

- A novel method to evidence surface active compounds in water-VCM solutions produced by exposure to UV laser, measuring DIT at air/solution interface is reported.
- Production and presence of amphiphilic molecules without separating them from solution are reported.
- Four hydrophobic compounds are identified and their chemical structures are proposed.
- Surface activity modifications may lead to variations of VCM solutions wetting properties.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 June 2014
Received in revised form 25 August 2014
Accepted 26 August 2014
Available online 21 September 2014

Keywords:
Vancomycin
Dynamic surface tension
DIT
LC–MS
Molecular fragments
FTIR

ABSTRACT

This study presents results about UV laser beam interaction with Vancomycin (VCM) solutions in ultrapure water performed on bulk samples (5 mL). Photoproducts and molecular fragments resulting from the parent VCM and generated by exposure to 266 nm are evidenced by UV–vis absorption and FTIR spectroscopy and liquid chromatography electro spray ionization time-of-flight mass spectrometry (LC/ESI-TOF-MS) measurements. A novel method is reported to characterize surface active compounds produced in VCM solutions during exposure to UV laser radiation, by measuring in real time the dynamic interfacial tension of the irradiated solutions in emerging, constant volume bubble configuration. This shows that amphiphilic photoproducts are generated after the interaction of VCM molecules with laser beam that migrate at the interface air bubble/VCM solution. They accumulate at the interface leading at transition surface effects. In the paper four such amphiphilic photoproducts were identified and their chemical structures were proposed.

1. Introduction

Vancomycin (VCM) is an antibiotic used in some cases as ultimate solution in fighting multiple drug resistance (MDR) acquired by bacteria [1]. The glycopeptide VCM is active against most

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http://dx.doi.org/10.1016/j.colsurfa.2014.08.023
0927-7757/

Gram-positive bacteria and some anaerobic organisms (e.g., *Clostridium difficile*). Because of its severe side effects, the use of VCM is restricted mainly to the treatment of methicillin or penicillin-resistant staphylococcal infections [2–5].

Studies devoted to VCM action have shown that it forms homodimers and that dimerization enhances its antibacterial activity [4,5]. An important role in this has the asparagine side chain that bends in order to hold the binding pocket in a conformation convenient for peptide docking [6]. The loss of ammonia transforms VCM molecules in the degradation product CDP-I, which crystallizes as a monomer and has no antibacterial activity. As for the chlorine atom of CDP-I, it has a different orientation than in VCM [3,6–8]. It is known that the disaccharide increases the aqueous solubility of the VCM [6]. On the other hand, no data about molecules with surfactant properties, originating from VCM molecules are available.

VCM has a high toxicity for the human organism [2,3] and consequently studies are required to better know its properties and behavior in view of developing application procedures which use a minimum amount of medicine for treatments. This study aims to identify methods and means to obtain solutions samples containing VCM as parent compound and photoproducts obtained out of it by exposure to UV laser radiation, so that the samples contain small amounts of photoproducts with efficient antibacterial properties and lower overall toxicity. In this direction, photoproducts with enhanced antibacterial activity with respect to the parental compound were reported when Chlorpromazine is exposed to laser radiation [9]. This paper shows results about the laser beams interaction with VCM solutions in ultrapure water performed in bulk samples (5 mL), based on earlier reports introduced in [10]. Photoproducts generated in solutions by exposure to UV laser beams are evidenced by measuring surface properties of gas bubbles pendant in solutions [11,12]. These measurements are correlated with UV–vis and FTIR absorption data and with liquid chromatography electro spray ionization time-of-flight mass spectrometry (LC/ESI-TOF-MS) and allow the identification of VCM molecular fragments. The knowledge of surface tension evolution at the interface gas bubble–VCM solution after its interaction with the laser beam, may give information about the wetting properties of these molecular fragments in view of applications on biological targets. The use of measurements of surface properties of emerging gas bubbles in VCM (more general, antibiotics) solutions to detect molecular fragments produced by irradiation with laser beams constitutes a novel approach. The "accumulation" of photoproducts at the interface medicine solution/gas bubble and their surface activity is important in biological applications. Surface activity modifications may lead to variations of the wetting properties of solution as well as of the speed with which modified VCM and resulting photoproducts are delivered and penetrate biological targets. This kind of effect was reported recently for phenothiazines which were subject of exposure to laser beams [13].

2. Materials and methods

The utilized VCM is a hydrochloride powder form (Sigma, Germany) and VCM solutions were prepared in ultrapure deionized water [10]. In Fig. 1 is shown the VCM chemical structure, indicating the disaccharide (glycan component) and the heptapeptide that contains 5 distinct amino acids.

The set-up used to irradiate VCM samples in ultrapure water at 2×10^{-3} M concentration and to identify the generated photoproducts is shown in Fig. 2.

The clinical use of VCM consists in the systemic delivery of the medicine at concentrations of 0.25–1 mg/L for susceptible strains and 8 mg/L (i.e. 5.4×10^{-6} M) for resistant strains of bacteria. These

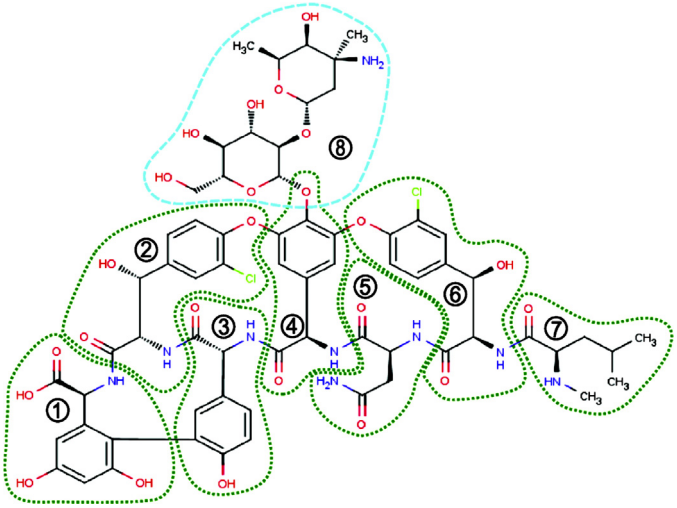

Fig. 1. The VCM chemical structure. (1) Dihydroxyphenylglycine (DPG); (2) β-hydroxy chlorotyrosine (βhTyr); (3) hydroxyphenylglycine (HPG); (4) HPG; (5) asparagine (Asn); (6) βhTyr; (7) N-methyl-leucine (NML); (8) glycan component.

concentrations are very low for studying the properties of VCM, in particular by optical/laser methods and this is the reason for which we studied samples at higher concentrations (2×10^{-3} M, i.e. 2.9×10^{3} mg/L). At this concentration one may obtain stronger optical signals which may be interpreted in terms of modifications of the samples at interaction with laser beams and more important quantities of photoproducts resulting from the VCM parent compound. The absence of dimers in the parent solutions are observed in the liquid chromatography electro spray ionization time-of-flight mass spectrometry (LC/ESI-TOF-MS) spectra which are measured at mass ranges that cover the area where the dimers could be found. A solution volume of 5 mL is irradiated in a cuvette of the PAT-1 system (Sinterface, Germany) in which a curved capillary is used to produce an air bubble in emerging position. PAT-1 allows measuring the dynamic interfacial tension (DIT) in a characteristic time ranging from few seconds to hours.

The laser beam is filtered spatially and spectrally as shown in Fig. 2 and its optical path in PAT-1 cuvette is 20 mm. The beam is parallel with the capillary passing laterally at 1 mm distance from the bubble. Once the VCM sample is irradiated, one may extract smaller volumes out of it and perform measurements to identify the modifications of the VCM molecules.

The first is the measurement of UV–vis absorption spectra to identify modifications of the VCM solution exposed to laser beam, with respect to the unexposed solutions, at the same concentration. These spectra were recorded with a UV-Vis-NIR spectrophotometer Lambda 950 (Perkin-Elmer, USA) in cuvettes with 1 mm optical path length.

The second is the measurement of FTIR spectra of solutions to identify modifications of the vibrational states specific to functional groups in VCM irradiated molecules with respect to the unirradiated ones; this gives an insight about the formation of new photoproducts by exposure to laser beam. To acquire the spectrum of a sample, 20 μL of solution were placed on a KRS-5 (Thallium Bromo-Iodide) optical crystal and absorbance was recorded with a FTIR spectrometer Nicolet iS50 (Thermo Scientific, USA). Samples were dried before recording the spectra to minimize the influence of water.

The third experiment consists in the analysis of unirradiated and irradiated solutions by LC/ESI-TOF-MS, to identify the particular compounds produced by VCM molecules dissociation that is induced via exposure to laser beams. The measurements were made using an Agilent 1260 Infinity series LC system equipped