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Antimicrobial evaluation of new metallic complexes with xylitol active against *P. aeruginosa* and *C. albicans*: MIC determination, post-agent effect and Zn-uptake

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ABSTRACT

Xylitol ($xylH_5$) is metabolized via the pentose pathway in humans, but it is unsuitable as an energy source for many microorganisms where it produces a xylitol-induced growth inhibition and disturbance in protein synthesis. For this reason, xylitol is used in the prophylaxis of several infections. In the search of better antimicrobial agents, new copper and zinc complexes with xylitol were synthesized and characterized by analytical and spectroscopic methods: $Na_2[Cu_3(xylH_{-4})_2]\cdot NaCl\cdot 4.5H_2O$ (Cu-xyl) and $[Zn_4(xylH_{-4})_2(H_2O)_2]\cdot NaCl\cdot 3H_2O$ (Zn-xyl). Both copper and zinc complexes presented higher MIC against *Pseudomona aeruginosa* than the free xylitol while two different behaviors were found against *Candida albicans* depending on the complex. The growth curves showed that Cu-xyl presented lower activity than the free ligand during all the studied period. In the case of Zn-xyl the growth curves showed that the inhibition of the microorganism growth in the first stage was equivalent to that of xylitol but in the second stage (after 18 h) Zn-xyl inhibited more. Besides, the PAE (post agent effect) obtained for Zn-xyl and xyl showed that the recovery from the damage of microbial cells had a delay of 14 and 13 h respectively. This behavior could be useful in prophylaxis treatments for infectious diseases where it is important that the antimicrobial effect lasts longer.
With the aim to understand the microbiological activities the analysis of the particle size, lipophilicity and Zn uptake was performed.

1. Introduction

Xylitol (IUPAC name 2R, 3r, 4S)-Pentane-1,2,3,4,5-pentol, code $xylH_{-5}$) is a five-carbon sugar alcohol naturally found in plants, fungus and algae (Fig. 1). It is as sweet as sucrose but with only two-thirds of the food energy. Apart from that, it is an important intermediate product in the carbohydrate metabolism of mammalian cells. For instance, in human beings the concentration of xylitol in blood is up to 8×10^{-5} M and usually increases due to the use of this coadjuvant in food and medical products [1].

On the other hand, numerous clinical studies have suggested that xylitol presents antimicrobial effects following different mechanisms. Most sugars cannot be used as antimicrobial agents, as both microbes and host cells are able to utilize them. On the contrary, xylitol, which differs from most carbon sources due to its five-carbon polyol structure, is metabolized via the pentose pathway in humans, but is unsuitable as an energy source for many microorganisms [2]. Studies of mechanism of action of xylitol on *Streptococcus mutans* showed that xylitol-induced growth reduction is mediated through a fructose-dependent system. The xylitol is transported into the bacteria and phosphorylated through the constitutive fructose phosphotransferase system (PTS) that regulates many metabolic processes and the expression of various genes [3–4]. Due to the fact that these bacteria cannot utilize xylitol phosphate as an energy source the expulsion of xylitol from bacteria consumes energy. This effect and the harmful intracellular accumulation of xylitol phosphate can explain the xylitol-induced growth inhibition and the disturbance in protein synthesis [3].

Xylitol exposure may also disturb cellular metabolism in *Streptococcus pneumoniae* in addition to inhibiting its growth [3,5–6] and it may affect the cellular functions of *Haemophilus influenzae* leading to changes in morphology and virulence. Because these bacterial species along with *Moraxella catarrhalis* are the main etiologic agents of acute otitis media [7–8], xylitol is used in the prophylaxis of this infection. As an example, two-month regular use of xylitol chewing gum has been shown to decrease the occurrence of acute otitis media in children due to the effect of the growth reduction of *S. pneumoniae* [9–10].

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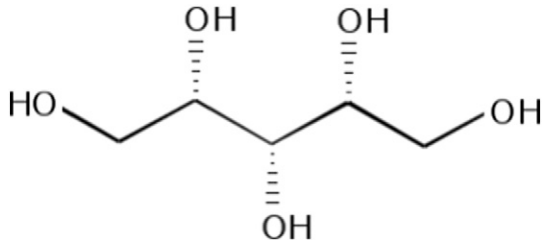

Fig. 1. Formula of xylitol ($xylH_5$).

Some studies have also suggested that xylitol reduces the adhesion ability of *S. mutans*, the main causal bacteria of caries, making its removal easier from plaques [11–13] There are several xylitol-containing chewing gums on the market recommended to safeguard the dental health [14–15].

Another important use of xylitol is on the treatment and prophylaxis of mucosal yeast infections in mammals, especially oral candidiasis which is the most common opportunistic infection in AIDS affecting up to 90% of the patients. Oral candidiasis interferes with the patient's nutrition and it is a risk factor for the development of *Pneumocystis carinii* pneumonia. Moreover, the presence of candidiasis is one of the most common reasons to discontinue chemotherapy in cancer patients [9].

Besides, xylitol is used in combined therapy in disrupting the structure of the *Pseudomona aeruginosa* biofilm and hence the persistence of this bacterium, for example in chronically infected wounds [16]. The use of xylitol was also studied in patients with cystic fibrosis [17] where the prevention of *P. aeruginosa* colonization is very important.

On the other hand, it is well known that a good strategy for improving the antimicrobial activity is the complexation of active molecules with metals [18–19]. Xylitol is an efficient metal ion chelator. There are several studies that report the interaction between metal ions and xylitol in solution, focusing the study on speciation [20–21]. Norkus et al. determined the pKa values of 13.8 for terminal OH groups and 13.9 for the others. They also concluded that each xylitol could chelate up to two copper ions [21]. Besides, there are reports that present the synthesis and structural characterization in solid state of new metal–xylitol compounds, like $Li_5[Co_2(xylH_{-5})_2]Cl\cdot 8H_2O$ [22], $[Pd_2(en)_2(xylH_{-4})]$ (en: ethylendiamine) [23] and $[Si_4(xylH\text{-}2)_3]^{2-}$ [24]. In these cases xylitol forms complexes after the deprotonation of two or more OH-xylitol groups and the stability of all these complexes is related to the regioselectivity as indicated by the initial rules for alditol complexes, proposed by Angyal [23]. Similarly, complexes with erythritol, a four-carbon polyol, were also reported: $Na_2[Fe(AnErytH_{-2})_2(OH)]\cdot 0.5NaNO_3\cdot 3.5H_2O$ and $Na_4[Fe_3(AnErytH_{-2})_6(OMe)]\cdot 2.5\ NaNO_3$ ($AnErytH_{-2}$: anhydroerythritol) [25]. Hence, polyols like xylitol can form new interesting complexes with metals having the ability to change their activities.

As a part of our work on metal complexes with pharmacological or pharmaceutical interest [26–32], in this article the synthesis and characterization of new Cu(II) and Zn(II) complexes with xylitol are reported. Moreover, in the search for new and better antimicrobial agents, the effect of these new complexes on the growth of *Candida albicans*, Gram positive and Gram negative aerobic bacteria was determined and compared with the effect of free xylitol and metal ions taken as controls. Looking for information about plausible mechanisms of action, the post-agent effect and the Zn uptake in the cells were also determined. It is noteworthy that these tests are not usually performed in the evaluation of antimicrobial properties of metallic complexes but they provided interesting information.

The selection of Cu(II) and Zn(II) was based on the fact that both cations are related to antibacterial and/or antifungal activities [33]. Furthermore, there are studies showing that Zn supplementation is beneficial for the teeth since it inhibits dentine demineralization. This element is incorporated into enamel, for example through chewing-gum and probably the Zn substitution takes place in the calcium position in enamel hydroxyapatite [34]. For this reason a complex with Zn and xylitol could be interesting in the caries prophylaxis.

2. Experimental section

2.1. Materials

All metal salts and solvents were purchased in SIGMA. The methanol 99.6% was not previously dried. The xylitol used was from Shanghai Richem International Co.

2.2. Synthesis of the complexes

The complexes were synthesized mixing methanolic solutions of xylitol (0.2 mmol, 30.4 mg) and $Cu(Cl)_2\cdot 2H_2O$ (0.4 mmol, 68.2 mg) or $ZnCl_2$ (0.4 mmol, 54.5 mg) at 60 °C. To the resulting solution, 1 M methanolic NaOH was added until the formation of a green solution and subsequently a green precipitate for the case of copper complex, and a colorless solution and white precipitate for the zinc one. After stirring for 1 h, the precipitates were filtered, washed with methanol and dried at room temperature.

The elemental analysis of complexes was performed with a Carlo Erba EA1108 elemental analyzer. Absorption atomic measurements of Zn and Cu were performed with a Perkin Elmer 5000 equipment with a hollow-cathode lamp, single-element, Photron®, Cu (324.8 nm), Zn (213.9 nm).

2.3. Physical measurements

IR spectra, in the range of 4000 and 200 cm^{-1}, were recorded on a BOMEM M 102 FTIR spectrophotometer using the KBr pellet technique.

Electronic spectrum of copper-complex aqueous solution was registered on a Milton Roy Spectronic 3000 spectrophotometer. The Zn-xyl do not present electronic spectrum due to the d^{10} electronic configuration of Zn(II).

^{1}H-NMR spectrum of deuterated aqueous solution of zinc-complex was performed with a Bruker Avance DPX-400 instrument. NMR spectra for Cu-xyl were not performed due to the paramagnetism of Cu(II).

EPR spectrum of copper-complex in solid state determined at 4.3 K was performed using a JEOL JES-FA200 spectrometer and a cavity with 100 kHz field modulation.

Thermal analyses were obtained with a Shimadzu TGA 50 thermobalance, with a platinum cell, working under flowing air (50 mL min^{-1}) and at a heating rate program of 0.5 °C min^{-1} from 30 to 200 °C and 5.0 °C min^{-1} from 200 to 650 °C.

The X-ray powder diffraction data were obtained for all the samples using a Rigaku ULTIMA IV powder diffractometer, operating in Bragg Brentano geometry. The selected radiation corresponds to CuKα ($\lambda = 1.5418$ Å), monochromatized with a diffracted beam bent germanium crystal. The data was collected over the 2θ range 10–80°, in steps of 0.02°, using a scintillation detector. Fixed slits of 2/3° were used for data collection to prevent beam spillage outside the 2 cm long sample (along the beam-path) at low angles.

The particle size analysis was performed using a Zetasizer Nano ZS (Malvern, UK), equipped with a 633 nm He–Ne laser beam, using a back-scattering angle of 173°. The sample preparation involves the suspension of all the samples in ethanol. Each experiment was carried out with three replicates ($n = 3$) and the data are presented as mean ± standard deviation (SD).

2.4. Lipophilicity

Lipophilicity tests were performed determining the partition coefficient between n-octanol and water [35]. The concentration of