GLYCOPEPTIDE-CLAY NANOCOMPOSITE: CHEMICAL-PHYSICAL CHARACTERIZATION

NANOCOMPOSITO GLICOPÉPTIDO-ARCILLA: CARACTERIZACIÓN QUÍMICA-FÍSICA

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In the last two decade new applications has been found regarding the interaction clays-organic molecules [1–4]. In this sense, the use of Lithium-fluorohectorite (LiFh) -a synthetic clay from the smectite group- provides the right scenario to study a "pure" material, which allows a better understanding of the interactions host-guest. In previous work, the successful intercalation of pharmaceutical active ingredients into LiFh clay it has been demonstrated [5, 6]. In this paper we preliminary evaluate the interaction of the antibiotic Vancomicyn in hydrochloride form (VCM) with the LiFh clay. This clay has a monoclinic cell (space group C2/m) with unit-cell parameters $a = 0.52$ nm , $b = 0.91$ nm, $c = 0.11$ nm, $\beta = 99.21°$ [7]. VCM is a *special molecule* since it has a big variety of functional groups, which modulate its acid-base equilibrium. Also, in the study a comparison with another antibiotic -sulfamethoxazole, which has different structural characteristics -is carried out.

In order to obtain the best composite from the point of view of a high drug load per unit mass of clay, different parameters were assessed: pH (1, 3, 8, and 9), initial drug concentration (1, 3, 5 and 9 mg·ml^{-1}) and temperature (27, 35, 45 and 65°C). 0.1 g of clay were put in contact with 10 ml of a drug solution during 4 h, and agitation at 500 rpm. After that, the suspension was centrifuged, and the amount of VCM in dissolution was determined by UV-vis spectroscopy at 281 nm [8]. The LiFh-VCM composite obtained after the interaction was dried at 60°C, and characterized by X-ray diffraction (XRD). Diffraction patterns of samples (LiFh and LiFh-VCM) were obtained by means of a Philips Xpert diffractometer, using Cu Kα radiation ($\lambda = 0.154$ nm) in the angular range $2° \leq 2\theta \leq 10°$ at 0.6 °/*min*.

It was demonstrated that the VCM incorporation into clay increases linearly with the increment of the drug initial concentration, which suggests that the drug concentration provides a powerful driving force to overcome the mass transfer resistance between both phases. The relationship drug load vs. incorporation process efficiency indicates that the best incorporation of VCM into the clay is obtained at 3 mg· ml^{-1} of drug initial concentration.

Now we discuss the effects of pH. Six different pKa values (defined as –log of the acid dissociation constant) for the VCM are reported, based on its acid-base equilibrium. As a function of pH, the molecule could be in cationic (positive charge), anionic (negative charge) or zwitterionic form (like a dipole, with a positive and negative charge) [9]. Figure 1 illustrates the molecular structure of VCM and the functional groups responsible of its acid-base behavior, as well as its dimensions.

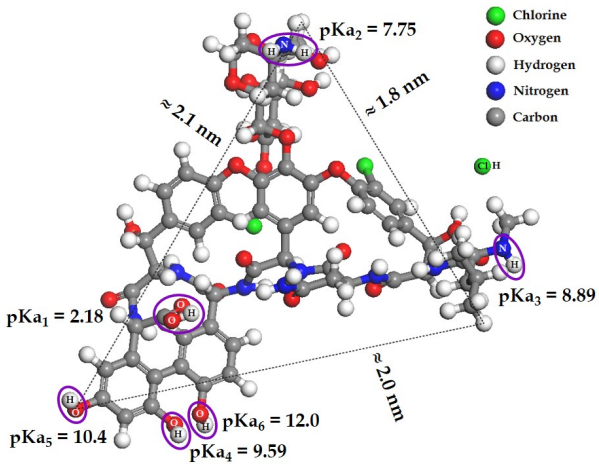

Figure 1. Molecular structure of the Vancomicyn (VCM), and estimated pKa values of the different functional groups.

At acid pHs, the VCM molecule is positively charged -pH=1, the VCM has charge 2^+ (divalent form) and at pH=3, it has charge 1^+ (monovalent form)-, which favors the interaction with the clay negatively charged through electrostatic interactions. The VCM amount per gram of clay, and the efficiency of the process, is very similar for both pHs. Thus, from the practical point of view it is convenient to work at pH=3. This pH value also contributes to the stability of the clay-drug resultingcomposite: i.e.,

the interaction of VCM with the clay -with negative surface charge density distributed heterogeneously-, is more favorable for monovalent (VCM^+) than for divalent species. However, at pH values $\approx$ 8 and higher, the efficiency of the process decreases progressively. It may be attributed to the low affinity between clay and drug, considering that at such pHs it is possible to find VCM species in its neutral form or negatively charged. From previous work it is known that LiFh shows an increase in the interlamellar space due to the temperature effect [10], which when it was put in contact with a drug solution at 70 ± 5°C [5]. However, for the VCM the results indicated a higher incorporation at room temperature ($\sim$ 27°C). Although the increase of temperature produces a raise in the kinetic energy of the VCM molecules, it should be kept in mind the size of the drug and its apparent structural rigidity, which difficult the best "accommodation" of the VCM molecules in the clay structure, which results in a decrease in the amount of drug incorporated.

Hence, the "optimal" composite is obtained at pH=3, VCM initial concentration of 3 $mg{\cdot}ml^{-1}$ and agitation at room temperature. In such case, the drug load is approximately 0.27 g of VCM per gram of LiFh.

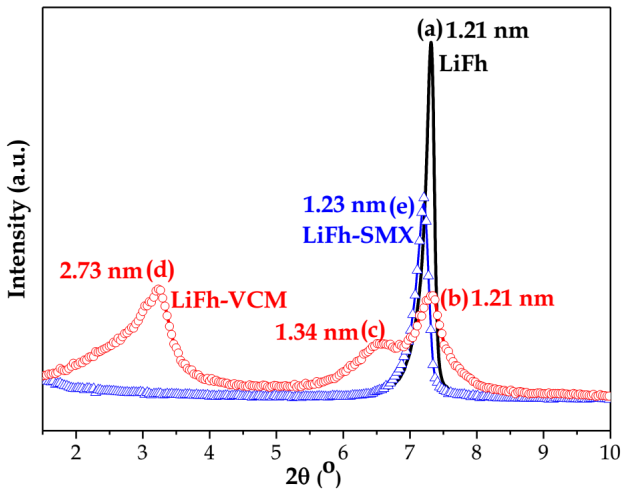

Figure 2. Diffraction patterns for LiFh, LiFh-VCM and LiFh-sulfametoxazole (LiFh-SMX) composites. The interplanar distances (d) for each peak are labeled.

Figure 2 shows the diffraction patterns for the LiFh and LiFh-drug composites. For the LiFh, the basal reflection (001), marked as (a) in the figure, corresponds to one layer of water intercalated between the stacks in ambient conditions [11].

In the LiFh-VCM sample three reflections were identified, marked as b-d in the figure. Reflections (c) and (d) could be interpreted as a fingerprintof clay stacks with VCM adsorbed: as illustrated in Figure 1, VCM is a big molecule with dimensions higher than water and produces a much largerd-value (interplanar spacing). Thus, the d-value after the interaction with the VCM is 1.34 nm, which suggests the existence of an intermediate regime (1.5 water layer). It is in good agreement with those reported for LiFh [11]. Reflection (d) may correspond to a different conformation of VCM molecules in the interlayer space of LiFh plus water layers also present. In such case, the d-value is 2.73 nm, i.e., VCM seems to be partially intercalated in the interlayer space (notice that an increment up to 3.99 nm has been reported in the literature for a sodium fluorohectorite [12]). However, when the LiFh is put in contact with the sulfamethoxazole drug (SMX), which has smaller dimensions than VCM ($0.9 \times 0.8 \times 0.7$ nm^3), the d-value does not seem to change significantly (see reflection marked as (e) in Figure 2). It clearly indicates that the intercalation process -which does not mean drug load per gram of clay- is governed basically by the affinity between the drug functional groups and the active sites of the clay. For example, in the SMX case, preliminary studies indicated a load of $\approx$ 0.25 g of SMX per gram of LiFh [13]. In such case, no intercalation took place. It suggests a weak interaction between the drug and the clay. Therefore, for the LiFh-SMX composite, in comparison with that LiFh-VCM, it is to expect a fast release of the drug from the support. This is relevant for future applications of the composite for controlled release of the drug in the gastrointestinal tract.

In summary, we have preliminarily characterized the adsorption and/or partial intercalation of an antibiotic (Vancomicyn, a big molecule) in a Li-fluorohectorite clay with potential applications in medicine (like slow release systems). On the other hand, the study revealed the actual difference between drug load -with and without intercalation- in the clay stacks: the comparison between two model antibiotics (VCM and SMX), with marked difference in the size and the functional groups, offers information related with the affinity host-guest, which will have implication in its future application.

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