STRUCTURE ANALYSIS OF THE INTERLAYER SPACE OF VERMICULITE/CHLORHEXIDINE NANOCOMPOSITE USING MOLECULAR MODELING

Dominik HLAVÁČ, Jonáš TOKARSKÝ

IT4INNOVATIONS CENTRE OF EXCELLENCE, VŠB-TECHNICAL UNIVERSITY OF OSTRAVA, 17. listopadu 15, 708 33 Ostrava-Poruba, Czech Republic, dominik.hlavac@vsb.cz

Abstract
The aim of this work is to give closer information about structure of Na-vermiculite/chlorhexidine diacetate antibacterial nanocomposite. Special attention has been paid to the orientation of the molecules of chlorhexidine in the vermiculite interlayer space. Molecular modeling using empirical force field in Accelrys Materials Studio modeling environment was performed in this study and obtained results were confronted with available experimental data of real samples. Molecular modeling revealed that increasing interlayer distance of vermiculite in dependence on increasing number of chlorhexidine cations in the vermiculite interlayer space is not caused by simply higher amount of chlorhexidine cations but by the different space arrangement. Molecular modeling results also led to the conclusion that it is the vermiculite surface which is primarily responsible for the modulation of antibacterial properties of vermiculite/chlorhexidine diacetate nanocomposite.

Keywords:
Molecular modeling, structure, chlorhexidine, vermiculite

1. INTRODUCTION
Chlorhexidine (CH) is an organic compound belonging to the bis(biguanide) family. Due to the basic character it forms stable salts with several acids, i.e. in presence of acetic acid it forms a chlorhexidine diacetate (CA) [1,2]. The CH is nowadays one of the most widely used biocides in dental and hand washing antiseptic products [3]. The mechanism of CH action involves destabilization of the outer bacterial membrane which is penetrated by two chlorophenyl guanide groups [4]. Because the question of CH toxicity in higher concentrations remains open [5], the possible risks must be restrained. A simple method how to minimize these risks is a stabilization of CH with appropriate matrix. Montmorillonite [6] or silica [7] was used in some previous works. Holešová and her co-workers [8] decided to use vermiculite (VMT) matrix because the VMT is layered phyllosilicate with large specific surface area and high negative layer charge which causes the presence of greater amount of interlayer cations than in other clay minerals [9]. This novel synthesized vermiculite/chlorhexidine (VMT/CH) nanocomposite has a significant antibacterial activity at low concentrations against Gram-negative Escherichia coli and Gram-positive Enterococcus faecalis. In addition, the VMT/CH nanocomposite with higher CH concentrations successfully inhibited the growth of a very resistant Pseudomonas aeruginosa. Although the work of Holešová et al. [8] confirmed the antibacterial activity of VMT/CH nanocomposite, the question of structural arrangement of chlorhexidine on VMT matrix remained unanswered. Because only the combination of instrumental analytical methods and computer molecular modeling can provide the clear answer, the molecular modeling study based on the experimental data (elementary analysis, X-ray powder diffraction, thermogravimetry and IR spectroscopy) published in [8] was carried out. The main goal of present work was to describe the interlayer structure of VMT/CA nanocomposite, especially the space arrangement of the CH molecules in dependence on their number. Attention has also been paid to the influence of substitutions in the VMT structure on this arrangement.
Materials Studio (MS) modeling environment was used for the preparation and geometry optimization of the models.

2. STRATEGY OF MOLECULAR MODELING

2.1 Preparation of initial models

Structural data and unit cell parameters published by Shirozu and Bailey [10] were used to prepare the initial model of VMT under the periodic boundary conditions. According to the crystallochemical formula of the real VMT sample used by Holešová et al. [8] (i.e. (Si_{13.13} Al_{0.86} Ti_{0.02}) (Mg_{2.53} Fe^{2+}_{0.45} Al_{0.02}) O_{10} (OH)_2 (Na_{0.8}) the supercell (a = 3.744 nm, b = 2.776 nm, c = 4.5 nm) with the composition (Si_{13.1} Al_{36} Ti_{1}) (Mg_{106} Fe_{19} Al_{1}) O_{420} (OH)_{84} (Na)_{35} and layer charge x = - 35 el. was built. The layer charge was compensated by CH in the form of divalent (CH^{2+}) and monovalent (CH^{+}) cations, but because of steric hindrance the maximum number of cations (17 CH^{2+}+1 CH^{+}) cannot be reached in the interlayer space of VMT. In fact, 13 CH^{2+} and 1 CH^{+} represent the maximum amount of CH cations in the VMT interlayer space. These CH cations together with Na^{+} cations compensated the layer charge of our model. Set of initial models with various content of CH and Na^{+} cations and water molecules in the VMT interlayer space was prepared. The assumption of the rigid VMT layer requires the fixed cell parameters a, b, γ and variable parameters c, α, β [11].

2.2 Modeling conditions

The molecular modeling procedure consisted of three following steps: (1) preliminary geometry optimization, (2) molecular dynamics run and (3) final geometry optimization. Geometry optimization of all prepared models was carried out in MS Forcite module using Universal force field [12]. This force field has previously been successfully used for the models containing organic compounds in the interlayer space of phyllosilicates [13,14,15]. Smart algorithm (cascade of steepest descent, conjugate gradient and quasi-Newton method) with 100 000 steps has been used for geometry optimization. Charges of atoms in CH molecules and VMT substrate have been assigned by Gasteiger method [16] and charge equilibration method (QEq) [17], respectively. During the geometry optimization of the initial models, positions of all atoms in the VMT layers and all the atomic coordinates of cations (CH^{2+}, CH^{+}, Na^{+}) and water molecules were optimized without any constraints. Molecular dynamics with NPT ensemble (i.e. constant number of atoms, constant pressure and constant temperature) was used with Berendsen barostat [18] and Nosé thermostat [19] (T = 298 K, p = 101.325 kPa). The sufficient length of dynamic trajectory which brought the system into the equilibrium state was found to be 500 ps. For each VMT/CH model, the last frame of the dynamics trajectory was optimized after the dynamics run. This final geometry optimization was realized under the same conditions as the preliminary geometry optimization. After this final step, the X-ray powder diffraction (XRPD) simulation was performed in MS Reflex module with parameters matching the experimental conditions: Bragg-Brentano geometry, X-ray lamp Cu Kα (λ = 0.154108 nm) and 2θ range = 5 – 40°. The experimental and calculated values of basal spacing (d_{001}) were compared in order to find the model most similar to real sample.

3. RESULTS AND DISCUSSION

Taking into account the number of exchanged Na^{+} interlayer cations, results of molecular modeling indicate that the Na^{+} cations remain in the interlayer space even after the VMT treatment with CH. This conclusion results from the comparison of calculated and experimental d_{001} values (2.006 - 2.146 nm, see [8]) and from the comparison of water content in models with thermogravimetric data (1 - 2 wt.% of water in real samples, see [8]). Calculated values are summarized in Tab. 1. The optimized model containing 10 CH^{2+} and 1 CH^{+} cations in the VMT interlayer space (i.e. model denoted as 10/1 and containing 0.0 wt.% of water; see Tab. 1 and Fig. 1) exhibits the best agreement with experimental values.
Tab. 1. The dependence of calculated \( d_{001} \) values for all models on number of CH\(^{2+}/\text{CH}^+ \) cations in the VMT interlayer space and calculated water content (wt.%) in the VMT interlayer space.

<table>
<thead>
<tr>
<th>water content [wt.%]</th>
<th>calculated basal spacing values ( d_{001} ) [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1.8194 2.0304 2.2073</td>
</tr>
<tr>
<td>1.5</td>
<td>-        -        2.3161</td>
</tr>
<tr>
<td>3.0</td>
<td>1.9710 -        2.0894</td>
</tr>
<tr>
<td>4.5</td>
<td>-        1.9632 -</td>
</tr>
</tbody>
</table>

The calculated basal space value of optimized model 10/1 with 0.0 wt.% of water (2.0304 nm, see Tab. 1 and Fig. 1) is near to the basal space value of real sample that was prepared with solution of 0.2 x CEC (cation exchange capacity) CH cations concentration (2.006 nm, see [8]). Calculated basal spacing values of other optimized models (models with other number of CH cations in the VMT interlayer space) mismatch \( d_{001} \) of real samples (2.006 - 2.146 nm, see [8]). The comparison of \( d_{001} \) values, calculated from optimized models, with the experimental XRPD data suggests that the higher amount of CH cations in the solution will not cause the significant increase of these cations in the interlayer space of VMT. Visual analysis of the optimized 10/1 model can be seen in Fig. 1. There are two groups of CH cations marked in different colors according to their assembly in the VMT interlayer space after final geometry optimization. CH cations in the first group (denoted as CH 1, see Fig. 1) are situated along only single VMT layer. The second group (denoted as CH 2, see Fig. 1) contains CH cations oriented across the interlayer space so that each NH\(^2+\) function group is adjacent to another VMT layer. The ratio of CH 1 and CH 2 cations is 5:6.

**Fig. 1.** The model of VMT interlayer space after geometry optimization process. CH 1 cations (green) are situated along only one VMT layer while CH 2 cations (red) are adjacent to both VMT layers.

The less CH cations in the VMT interlayer space, the more CH cations situated along only single VMT layer and, consequently, the lower \( d_{001} \) values. On the other hand, the number of CH cations oriented perpendicular to the VMT sheet increases with increasing total amount of CH cations in the VMT interlayer space and this is accompanied with an increase of \( d_{001} \) values. It is evident that positively charged NH\(^2+\) groups determines the assembly of CH cations because of their interaction with negatively charged OH groups located in the ditrigonal cavities in VMT tetrahedral sheet. This is very similar to the behaviour of Na\(^+\) interlayer cations which also exhibit the tendency to settle into ditrigonal cavities (see Fig. 2) [20].
Fig. 2. Tendency of NH$_2^+$ functional groups to get close to the ditrigonal cavities of VMT tetrahedral sheet (similarly as Na$^+$ interlayer cations) can be seen in this detailed view on optimized model.

The more CH cations, the worse the access to these ditrigonal cavities because of steric hindrance. Therefore, CH cations have to take a different space arrangement in the VMT interlayer space. Instead of lying along only single VMT layer the CH cations begin to be oriented perpendicular to the VMT layer in order to reach the cavities in other VMT sheet. This change of arrangement leads to the increase of $d_{001}$ value (Tab. 1). Molecular modeling also revealed an important role of Al$^{3+}$ substitutions in VMT tetrahedral sheet in CH cations assembly. Fig. 3 shows the accumulation of CH cations at the Al-substituted sites of VMT tetrahedral sheet while unsubstituted sites are not occupied. This behaviour of CH cations (i.e. “looking for” Al-substitued sites) has been observed in all VMT/CH models.

Fig. 3. CH cations are accumulated close to Al$^{3+}$ substitutions in the VMT tetrahedral sheet while unsubstituted sites are empty. Double supercell is displayed in order to show the structure more clearly.

4. CONCLUSIONS
Molecular modeling of VMT interlayer space in the VMT/CH nanocomposite suggests that the initial solution used in laboratory preparation of composite contains sufficient amount of CH cations, even though it is low concentrated (see [8]). Higher concentration of initial solution does not lead to further intercalation of CH cations into the VMT interlayer space. Therefore, additional CH cations (i.e. when concentration higher than 0.2 x CEC is used, see [8]) have to be anchored on the VMT surface and, probably, it is the assembly on VMT surface which is primary responsible for the antibacterial behavior of the VMT/CH nanocomposite. The most important finding is that the increase of VMT interlayer distance in dependence on increasing number of CH cations is not the result of simply higher number of CH cations in the interlayer space. The molecular modeling revealed the effect of different space arrangement. Visual observation of optimized models showed
that NH$_2^+$ functional groups of CH cations exhibit a tendency to take a position very close to ditrigonal cavities in the tetrahedral sheet of VMT. The more CH cations in the VMT interlayer space, the more difficult access to these cavities. Therefore, CH cations have to be arranged perpendicular to both VMT sheets in order to reach the cavities in both of them and this is the reason for the increasing of $d_{001}$ value. From the molecular modeling results it can be also concluded that CH cations prefer to stay near the Al-substituted sites of VMT tetrahedral sheets.

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