STRUCTURE CHARACTERIZATION OF THE ANTIBACTERIAL CHLORHEXIDINE / KAOLINITE COMPOSITE

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Abstract

Chlorhexidine/kaolinite (CH/KLT) composite is the nanostructured material exhibiting antibacterial properties. Distribution of the CH molecules in the structure of composite, i.e. the proportion of CH molecules on the KLT surface and in the KLT interlayer space, is one of the crucial factors affecting the antibacterial properties. In present work, thermogravimetric analysis, X-ray diffraction analysis and molecular modeling using empirical force field were involved to study the structure of CH/KLT composite. Since the X-ray diffraction analysis of real samples revealed the presence of more than one distinct KLT basal spacing suggesting the non-uniform intercalation, various models of KLT interlayer space and simulated diffractograms of these models were used in order to estimate the most probable composition and space arrangement of the KLT interlayer.

Keywords: kaolinite, chlorhexidine, structure, molecular modeling

1. INTRODUCTION

Chlorhexidine (CH) is an organic compound with basic character that forms stable salts with several acids [1,2]. For example in presence of acetic acid it forms a chlorhexidine diacetate (CA). CH is one of the most widely used biocides in dental and hand washing antiseptic products [3]. Mechanism of CH action is not completely understood but involves destabilization of the outer bacterial membrane which is penetrated by two chlorophenyl guanide groups [4]. Although CH has number of useful properties, there still remains an open question of CH toxicity in higher concentrations [5]. Very promising for exclusion of side effects seems to be its stabilization with appropriate matrix. This work follows up on some previous studies that investigated possibilities of CH stabilization on silica [6] or layered silicates montmorillonite [7] and vermiculite [8]. This study is focused on investigation the possibilities of fixing CH on another naturally occurring layered silicate – clay mineral kaolinite – having (in contrast to montmorillonite and vermiculite) smaller specific surface area and layer charge close to zero.

Prepared chlorhexidine/kaolinite (CH/KLT) composite is the nanostructured material exhibiting antibacterial properties. One of the crucial factors affectig the antibacterial properties is distribution of the CH molecules in the structure of composite, i.e. the proportion of CH molecules on the KLT surface and in the KLT interlayer space. In present work, thermogravimetric (TG) analysis, X-ray diffraction (XRD) analysis and molecular modeling using empirical force field in Materials Studio modeling environment were involved to study the structure of CH/KLT composite. Since the XRD analysis of real samples revealed the presence of more than one distinct KLT basal spacing suggesting the non-uniform intercalation, various models of KLT interlayer space and simulated diffractograms of these models were used in order to estimate the most probable composition and space arrangement of the KLT interlayer. An arrangement of the molecules on KLT tetrahedral and octahedral surfaces was also studied and special attention has been paid to CH – KLT adhesion forces. Amount of CH, urea and water molecules in models was compared with results of TG.
analysis of real samples and since the agreement is very good it can be concluded that the combination of molecular modeling with XRD and thermogravimetric analyses is very efficient tool for the characterization of such type of composites.

2. EXPERIMENT AND ANALYSES

2.1 Preparation of samples

Kaolinite (KLT) from Sedlec, Czech Republic served as a starting material (size fraction < 40 μm). Urea (CO(NH)₂, Vitrum, Czech Republic), Chlorhexidine diacetate (C₂₂H₃₀N₁₁Cl₂·2C₂H₄O₂, Sigma Aldrich, Czech Republic) and ethanol as a solvent were used for modification of the KLT matrix. Two-step process was used for the preparation of CH/KLT: (1) modification of KLT structure by urea and (2) mixing of KLT/urea sample with chlorhexidine.

2.2 Preparation of initial models

All computations were performed in Materials Studio (MS) modeling environment. KLT unit cell with crystallochemical formula Al₄Si₄O₁₀(OH)₈ has been built under the periodic boundary conditions using the data published by Neder et al. [9]. The unit cell with parameters: a = 5.14899 Å, b = 8.934 Å, c = 7.384 Å, α = 91.930°, β = 105.042°, γ = 89.791° was used to prepare a supercell structure Al₁₂₈Si₁₂₈O₃₂₀(OH)₂₅₆. Parameters for the KLT interlayer (KLTᵢₑ) model were a = 41.2 Å, b = 35.7 Å, c = 14.8 Å, α = 91.930°, β = 105.042°, γ = 89.791°. Parameters a, b, and γ were fixed during geometry optimization procedure. Total layer charge of KLT was 0 eL., partial atomic charges were assigned by QEq method [10]. Chlorhexidine (CH) molecules prepared in neutral (CH) and dissociated (CH²⁺) form were accompanied by two acetic acid molecules and acetate molecules (to maintain the zero charge), respectively. All molecules (i.e. CH²⁺, CH, urea (U), water (H₂O), acetic acid and acetate) were built in MS Visualizer module. Atomic charges of H₂O and organic molecules were assigned by QEq and Gasteiger method [11], respectively. Number and types of molecules in the KLTᵢₑ are summarized in Table 1. For each of this compositions five initial models with different space arrangement were built. All these models were optimized in MS Forcite module using Smart algorithm. Atoms were parameterized by Universal force field [12]. Two-step optimization procedure was used: (a) initial geometry optimization (convergence criterion: ΔE = 1·10⁻³ kcal/mol; external pressure: 101.325 kPa; Ewald accuracy: 1·10⁻² kcal/mol) for the pre-treatment of initial models, and (b) final geometry optimization (convergence criterion: ΔE = 1·10⁻⁴ kcal/mol, external pressure: 101.325 kPa; Ewald accuracy: 1·10⁻⁴ kcal/mol) leading to the final models from which the energy and structural parameters were obtained. Only models with the most negative value of total energy (Eₕₑ) from each group were further analyzed. Adhesion energy (Eₕₑ) of CH and CH²⁺ molecules on KLT was computed according to eq. (1)

\[
Eₕₑ = Eₚₛₛ - (Eₚₖₜ + Eₕₖ)
\]

where Eₚₛₛ corresponds to the potential energy of the whole CH/KLT system, Eₚₖₜ corresponds to the potential energy of KLT substrate and Eₕₖ is the potential energy of either CH or CH²⁺ molecule. In order to obtain directly the energy of mutual CH – KLT adhesion, H₂O and U molecules were omitted.

MS Reflex module was used to simulate the XRD patterns of optimized models under the same conditions as used in experimental XRD analysis of real samples, i.e. Bragg-Brentano geometry and CuKα irradiation (λ = 1.541078 Å).

2.3 Characterization methods

TG analysis was carried out with STA 409 EP (Netzsch, Germany) analyzer. Samples in α-Al₂O₃ crucibles were heated up to 1100°C (10°C/min) in dynamic atmosphere with air flow rate 0.1 dm³/min.

XRD diffraction patterns were obtained using the X-ray diffractometer INEL equipped with a curved position-sensitive detector CPSD 120 (Ge-monochromator, CuKα radiation). The measurements were taken in
ambient atmosphere (25°C, 43% of humidity) under constant conditions (35 kV, 20 mA). The samples were fixed in a flat rotation holder and measurement was carried out for 1500 s.

3. RESULTS AND DISCUSSION

XRD patterns of real samples, i.e. (a) pure KLT, (b) KLT intercalated by U and (c) CH/KLT composite, can be found in Fig. 1. It can be seen that basal reflection of pure KLT (Fig. 1a) remains at least partly present in diffraction patterns of sample after KLT treatment (Fig. 1b-c). Besides previously discussed basal reflection of pure KLT one new pronounced reflection appears after KLT treatment with urea indicating successful (although incompletely) intercalation (Fig. 1b). After mixing of KLT/urea with CH solution both previously discussed reflections are presented in diffraction pattern (Fig. 1c) but new reflection appears between them. This was recognized as hydrated KLT [13].

![XRD patterns](image)

**Fig. 1** XRD patterns of (a) pure KLT; (b) KLT intercalated by U; (c) CH/KLT composite

Various compositions of the KLT models and other parameters (M, E$_{ad}$, and d$_{001}$) are listed in Table 1. Models are ordered according to the increasing d$_{001}$ values. E$_{ad}$ values for models with more than one CH or CH$_2^+$ molecules are presented as an average of E$_{ad}$ calculated individually for each molecule. Visual observation of the models revealed that shapes of CH$_2^+$ molecules varies from bent shape to straight shape in dependence on growing number of them. U and H$_2$O molecules are oriented across the KLT surface tending to touch both tetrahedral and octahedral KLT surfaces (Fig. 2). Analysis of hydrogen bonds showed that donor – acceptor distance and donor-hydrogen-acceptor angle for CH$_2^+$ are in the range 2.61–3.69 Å and 116.7°–173°, respectively, while for CH varied from 2.49 to 3.61Å and from 114.4° to 173°. As expected, adhesion is stronger for CH$_2^+$ – KLT than for CH – KLT.
TG analysis showed that an amount of CH, U, and H2O molecules in real sample is 8.7 wt.% which is not in accordance with molecular modeling results in Table 1. However, it should be borne in mind that TG analysis shows the average amount of molecules in the whole sample but atomistic models represents only specific situations and XRD patterns are needed to prove the agreement of modeling and experiment. One can see that in the XRD pattern of CH/KLT sample (Fig. 1c) four distinct reflections (2θ = 8.318°, 8.780°, 10.566°, 12.351°) with intensities I = 204, 72, 163, 102 counts are clearly observable. These reflections correspond to various KLT interlayer contents and the intensities represent a quotient of these „phases“ in total volume of the real sample. Four models from Table 1 with corresponding d001 values can be assigned to these four reflections as follows: M9 – 1st reflection, M7 – 2nd reflection, M1 – 3rd reflection, M0 – 4th reflection. Subsequent recalculation of amounts of interlayer content according to the intensities (see Table 2) led to the value 10.22 wt.%. This value is close to the experimentally obtained value 8.7 wt.%. If it were possible to include also the less distinct reflections into the recalculation, the agreement with the experiment would be much better.

Table 1 Compositions of the KLT_{int} models and calculated adhesion energy (E_{ad}) and basal spacing (d_{001}) values. Models are denoted MX where X is the serial number of model. M0 is the model of pure KLT. M (wt.%) is the amount of molecules of given type in the model

<table>
<thead>
<tr>
<th>models of KLT_{int}</th>
<th>number of molecules</th>
<th>M [wt.%]</th>
<th>E_{ad} [kcal/mol]</th>
<th>d_{001} [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>– – – –</td>
<td>0.00</td>
<td>–</td>
<td>0.716</td>
</tr>
<tr>
<td>M1</td>
<td>– – – –</td>
<td>60</td>
<td>6.07</td>
<td>–</td>
</tr>
<tr>
<td>M2</td>
<td>– – – –</td>
<td>70</td>
<td>7.09</td>
<td>–</td>
</tr>
<tr>
<td>M3</td>
<td>9 – – –</td>
<td>11.56</td>
<td>–</td>
<td>0.964</td>
</tr>
<tr>
<td>M4</td>
<td>– 1 – –</td>
<td>5.63</td>
<td>-134.02</td>
<td>0.975</td>
</tr>
<tr>
<td>M5</td>
<td>10 1 –</td>
<td>9.66</td>
<td>-133.58</td>
<td>0.981</td>
</tr>
<tr>
<td>M6</td>
<td>5 – 1</td>
<td>5.79</td>
<td>-205.30</td>
<td>0.985</td>
</tr>
<tr>
<td>M7</td>
<td>10 – 1</td>
<td>6.91</td>
<td>-208.07</td>
<td>1.003</td>
</tr>
<tr>
<td>M8</td>
<td>– 6 –</td>
<td>18.51</td>
<td>-134.10</td>
<td>1.058</td>
</tr>
<tr>
<td>M9</td>
<td>17 – –</td>
<td>19.81</td>
<td>–</td>
<td>1.062</td>
</tr>
<tr>
<td>M10</td>
<td>10 – 4</td>
<td>18.07</td>
<td>-191.42</td>
<td>1.084</td>
</tr>
<tr>
<td>M11</td>
<td>30 4 –</td>
<td>20.67</td>
<td>-120.13</td>
<td>1.008</td>
</tr>
<tr>
<td>M12</td>
<td>20 4 –</td>
<td>20.44</td>
<td>-117.74</td>
<td>1.100</td>
</tr>
<tr>
<td>M13</td>
<td>30 – 4</td>
<td>20.67</td>
<td>-183.66</td>
<td>1.105</td>
</tr>
</tbody>
</table>

It must be noted that also the composition of interlayer contents of M0, M1, M7 and M9 models agree well with the experiment. One can see that the reflection at position 2θ = 8.318° is observable in the XRD patterns of both KLT intercalated by U (Fig. 1b) and CH/KLT composite (Fig. 1c). Therefore, it can be assumed that it is a result of U intercalation (see the model M9 in Table 1). Moreover, it is not very probable that U molecules were completely replaced by CH molecules (compare models M8 and M9 in Table 1) and it is rather expected that both U and CH molecules are present together in the KLT interlayer space. This situation can be found in the model M7 containing 1 CH2+ and 10 U molecules (Table 1, Fig. 2) which agrees with 2θ = 10.566° reflection from the XRD pattern in Fig. 1c. Reflection at position 2θ = 10.566° seems to be a result of presence of H2O molecules in the KLT interlayer space (see the model M1 in Table 1).
4. CONCLUSIONS

CH/KLT composite exhibiting antibacterial properties was characterized by a combination of experimental (TG and XRD analyses) and simulation (molecular modeling) techniques. Computer molecular modeling was used to study the space arrangement of chlorhexidine (both CH and CH$^{2+}$), urea and water molecules in the interlayer space of KLT and basal spacings of optimized models were calculated from simulated XRD patterns. Amounts of chlorhexidine (both CH and CH$^{2+}$), urea and water molecules in models were compared with results of TG analysis of real samples. Using recalculation based on XRD patterns of real samples and simulated XRD patterns of optimized models the very good agreement between simulation and experiment was reached. Taking into account that it is nearly impossible to directly observe and examine the interlayer content it can be concluded that the combination of molecular modeling with XRD and TG analyses is very efficient method for the characterization of such type of composites.

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