

NON-BOND INTERACTIONS BETWEEN CHLORHEXIDINE DIACETATE AND KAOLINITE: A MOLECULAR MODELING STUDY

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Abstract

Chlorhexidine/kaolinite nanocomposite is a phyllosilicate-based material exhibiting antibacterial properties. For further optimization of preparation process and improvement of resulting nanocomposite the structure at an atomistic level should be known more precisely. Present work is focused on molecular modeling using empirical force field of complex systems containing kaolinite matrix (tetrahedral surface, octahedral surface, and edge of kaolinite are taken into account) and chlorhexidine diacetate, urea and water molecules. Accelrys Materials Studio modeling environment was used. The potential energies and non-bond energies of these systems were determined and the mutual chlorhexidine – kaolinite interaction was analyzed. Differences between the surfaces and edge of kaolinite with respect to the adsorption of molecules were found and the results revealed that surfaces of kaolinite are preferred by the molecules. Results also show the influence of water and urea molecules on the chlorhexidine – kaolinite interactions.

Keywords:

adsorption, chlorhexidine, clays, molecular modeling, nanostructures

1. INTRODUCTION

Chlorhexidine and its organic salts showing antibacterial and antiseptic effects [1] became useful primarily in dental medicine [2] and orthopedics [3]. The mechanism of their action is not precisely known, but in the first stage mainly electrical attraction between positively charged chlorhexidine and negatively charged outer surface of bacteria plays a role [4,5]. Molecular structure is shown in Fig. 1. In order to achieve a long-term antibacterial effect, anchoring of CH on the suitable matrix via non-bond interactions seems to be useful. Clay minerals are the natural host structures suitable for anchoring or intercalation of ionic molecules because of their unique crystallochemical properties and large surface area. Testing of long-term antibacterial activity of chlorhexidine showed significant improvement after its intercalation and adhesion on the surface of vermiculite [6,7]. Moreover, it was found that use of vermiculite is more advantageous than use of montmorillonite [8] because CH/montmorillonite composite exhibits lower effectivity [6].

This success prompted us to further study of clays as potential matrices for chlorhexidine. Kaolinite (KLT) was chosen and an extensive study has been realized by Holešová et al. [9] in which the chlorhexidine/KLT composite was characterized using various experimental methods. Molecular modeling was included only marginally in [9] as a supporting tool for X-ray diffraction analysis.

Present work summarizes the results of complete molecular modeling study of chlorhexidine on KLT. The study is focused on the adhesion capabilities of KLT and mutual chlorhexidine - KLT non-bond interactions. Differences between the surface and edge of KLT together with the influence of water and urea molecules (commonly used as a layer separating agent [10,11] on the chlorhexidine – KLT mutual interactions are discussed.

2. SIMULATION DETAILS

Chlorhexidine diacetate antibacterial specie was modeled as its salt (denoted as CA) and as set of non-protonated chlorhexidine and two molecules of acetic acid (denoted as CH+2AA). Therefore, while in the case of CA one chlorhexidine with charge 2+ and two CH₃COO⁻ were introduced into the model, in the case of CH+2AA electrically neutral molecules were handled. Other parts of initial models were urea (U) and water (W) molecules. Basic model of KLT with crystallochemical formula (Si₄)(Al₄)O₁₀(OH)₈ was prepared according to [12]. KLT supercell 8a × 4b × 1c (total charge was set to x = 0 el.) was used for the preparation of tetrahedral and octahedral KLT surfaces. Tetrahedral (denoted as KLTT) and octahedral (denoted as KLTO) surfaces were studied separately in three-dimensional periodic boundary conditions with length of c = 200 Å. In analogous manner, model of KLT edge (denoted as eKLT) was prepared. Prepared structures of KLT can be seen in Fig. 1. In order to simplify the calculations two modeling approaches of eKLT were chosen: 1) KLT layers (d₀₀₁ = 0.6458 nm) were fixed in their cartesian coordinates as in the non-intercalated KLT (denoted as eKLTa) while in 2) KLT layers were separated with constant distance corresponding to thickness of one layer (d₀₀₁ = 1.2916 nm) (denoted as eKLTb).

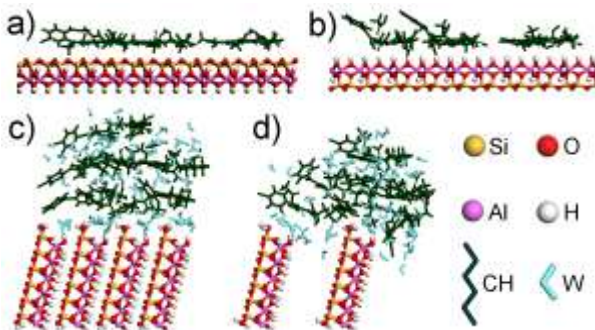


Fig. 1 Side view of optimized representative models of a) KLTT (KLTT9), b) KLTO (KLTO9), c) eKLTa (eKLTa2) and d) eKLTb (eKLTb2).

Charges of KLT atoms were assigned using QEq method [13] while charges of molecules were calculated using Gasteiger method [14]. Energy minimization was performed in Materials Studio/*Forcite* module using Universal Force Field [15] with following convergence criteria: $\Delta E = 1 \cdot 10^{-4}$ kcal/mol, $\Delta l = 5 \cdot 10^{-5}$ Å. Lattice parameters *a*, *b*, *c*, γ were kept fixed during optimization of KLTT and KLTO, Si and O atoms (except O in OH groups) were fixed during optimization of eKLT. Optimized models were characterized using total potential energy (E_{tot}) computed during geometry optimization and cohesion energy (E_{nb}) calculated according to eq. (1).

$$E_{nb} = E_{complex} - E_{KLT} - \sum_{i=1}^{N_{mol}} E_i \quad (1)$$

Individual terms in above mentioned expression for E_{nb} correspond to non-bond energy of whole model system ($E_{complex}$), non-bond energy of KLT in model system (E_{KLT}) and non-bond energies of individual adhered molecules (E_i). The above limit of the sum (N_{mol}) corresponds to number of adhered molecules. Determination of the structure stability is straightforward as the more negative values of calculated energy indicate more stable structures. In order to estimate the dominant part of intermolecular interaction, E_{nb} was decomposed on van der Waals (E_{vdW}) and Coulombic (E_C) terms.

3. RESULTS AND DISCUSSION

3.1 KLT surface with urea and water molecules

Results of modeling of KLT surface with U molecules are summarized in Table 1. The lowest E_{tot} was obtained for the model KLTO3 containing the smallest number of atoms (*N*). In opposite, lowest E_{nb} was obtained in the case of model KLTT1 having the largest *N*. Based on these two opposite trends, dependence

of E_{tot} and E_{nb} on N may be expected. Small N facilitates the capturing of more favorable conformation due to larger free volume and, therefore, E_{tot} is decreasing. On the contrary, larger N accompanied with more interaction partners causes the decrease of E_{nb} . Although the interpretation of observed values seems to be very simple, the above mentioned assumptions do not apply in every case. Based on the comparison of E_{nb} values of models KLTT2 and KLTT5 or KLTO6 and KLTO1, one can see that lower E_{nb} values may be achieved also in case of models with smaller N . Evidence of this phenomenon is strongly coupled with presence of W in the structure, which stabilizes system through hydrogen bonds and van der Waals interactions (Table 1, E_{vdW} vs E_C). Lower values of E_{nb} for models containing a mixture of U and W molecules were observed on KLTO surface and correspond to better compatibility of KLTO to W molecules [16].

Table 1. Energies in [kcal/N] and composition of KLTT and KLTO models. N - number of all atoms, U - number of urea molecules, W - number of water molecules.

N	Model	U	W	E_{tot}	E_{nb}	E_{vdW}	E_C
1744	KLTT1	82	0	-55.20	-0.52	-0.48	-0.04
1416	KLTT2	41	0	-66.74	-0.34	-0.31	-0.03
1252	KLTT3	21	0	-74.75	-0.18	-0.17	-0.01
1319	KLTT4	21	21	-71.07	-0.29	-0.22	-0.07
1382	KLTT5	21	42	-67.94	-0.38	-0.26	-0.12
1445	KLTT6	21	63	-65.09	-0.48	-0.29	-0.19
1704	KLTO1	77	0	-56.39	-0.52	-0.44	-0.07
1396	KLTO2	39	0	-67.62	-0.32	-0.27	-0.05
1242	KLTO3	19	0	-75.27	-0.15	-0.13	-0.02
1319	KLTO4	21	21	-71.08	-0.30	-0.21	-0.09
1382	KLTO5	21	42	-67.97	-0.42	-0.26	-0.16
1445	KLTO6	21	63	-65.13	-0.52	-0.30	-0.23

3.2 KLT surface with chlorhexidine and water

Calculated energies from optimized models of KLT surface with CH are listed in Table 2.

Table 2. Energies in [kcal/N] and composition of KLTT and KLTO models. N - number of all atoms in the model, CH+2AA - number of “chlorhexidine + 2×acetic acid” sets, CA - number of chlorhexidine salt molecules, W - number of water molecules.

N	Model	CH+2AA	CA	W	E_{tot}	E_{nb}	E_{vdW}	E_C
2048	KLTT7	12	0	0	-46.19	-0.52	-0.49	-0.03
1568	KLTT8	6	0	0	-59.74	-0.36	-0.35	-0.01
1328	KLTT9	3	0	0	-70.17	-0.23	-0.21	-0.01
2048	KLTT10	0	12	0	-47.40	-2.13	-0.36	-1.78
1568	KLTT11	0	6	0	-60.52	-1.37	-0.25	-1.12
1328	KLTT12	0	3	0	-70.61	-0.85	-0.13	-0.72
1744	KLTT13	0	4	112	-54.43	-1.31	-0.28	-1.03
1584	KLTT14	0	2	112	-59.45	-0.95	-0.25	-0.70
1504	KLTT15	0	1	112	-62.38	-0.76	-0.23	-0.52
2048	KLTO7	12	0	0	-46.15	-0.48	-0.44	-0.04
1568	KLTO8	6	0	0	-59.72	-0.35	-0.33	-0.02
1328	KLTO9	3	0	0	-70.14	-0.20	-0.18	-0.01
2048	KLTO10	0	12	0	-47.36	-2.10	-0.33	-1.77
1568	KLTO11	0	6	0	-60.49	-1.36	-0.23	-1.12
1328	KLTO12	0	3	0	-70.60	-0.80	-0.14	-0.66
1744	KLTO13	0	4	112	-54.45	-1.33	-0.26	-1.06
1584	KLTO14	0	2	112	-59.47	-0.97	-0.23	-0.74
1504	KLTO15	0	1	112	-62.41	-0.80	-0.23	-0.57

Based on the comparison of E_{tot} as well as E_{nb} slightly stronger adsorption of CH is observed on KLTT for models without W . Because the values of E_{vdW} are the main fluctuating components of E_{nb} with respect to KLTO, hydrophobic nature of tetrahedral sheet seems to be responsible for preference of KLTT by CH.

Although E_C values are not changing rapidly between KLTT and KLTO, slightly lower values on KLTT correspond to the localization of negative charge mainly on the tetrahedral sheet [17]. Situation changes after the introduction of W into models. Because of hydrophilic character of KLTO, W molecules may better form hydrogen bonded net and, therefore, resulting structure is primarily stabilized through hydrogen bonds between W and KLTO [16] which are not achievable on KLTT. From this reason, models containing W show lower E_{nb} on KLTO. Also the simple relation between N and E_{nb} does not apply in the case of KLT surface with CH. Although the models KLTT13 and KLTT14 as well as their analogues KLTO13 and KLTO14 have higher N than KLTT11 and KLTO11, they exhibit weaker interaction according to E_{nb} . This finding is related to the presence of W molecules in models with higher N which (due to the large permittivity of water) attenuate the Coulombic interaction between CA and KLT. This idea is supported by E_{nb} values of KLTT8 and KLTO8 which do not reach the values of previously discussed models containing W molecules (i.e. KLTT13, KLTT14, KLTO13, and KLTO14) due to the zero charge on CH+2AA.

3.3 KLT surface with chlorhexidine, water, and urea

Similar trends discussed in the previous subsections 3.1 and 3.2 (correlation between N and E_{tot} or E_{nb}) were also observed in the case of CH, U and W (Table 3). However, in contrast to previously discussed models, no deviation from dependence of E_{nb} on N was recognized in this case. Taking into account the accentuating (KLT with U) and attenuating (KLT with CH+2AA/CA) role of W on E_{nb} , vanishing of those contradictory trends may be assumed when all molecules are present in the model. With respect to preferred type of KLT surface, KLTT is mostly preferred in opposite to KLTO in dehydrated models, while KLTO showed better E_{nb} values when W molecules are present.

Table 3. Energies [kcal/N] and composition of KLTT and KLTO models. N - number of all atoms in the model, CH+2AA - number of “chlorhexidine + 2×acetic acid” sets, CA - number of chlorhexidine salt molecules, W - number of water molecules.

N	Model	U	CH+2AA	CA	W	E_{tot}	E_{nb}	E_{vdW}	E_C
1416	KLTT16	21	2	0	0	-66.31	-0.27	-0.25	-0.02
1736	KLTT17	21	6	0	0	-54.43	-0.40	-0.37	-0.03
1408	KLTT18	30	1	0	0	-66.87	-0.30	-0.28	-0.02
1416	KLTT19	21	0	2	0	-66.62	-0.68	-0.24	-0.43
1736	KLTT20	21	0	6	0	-55.20	-1.40	-0.33	-1.08
1408	KLTT21	30	0	1	0	-67.04	-0.51	-0.28	-0.23
1498	KLTT22	30	1	0	30	-62.98	-0.41	-0.31	-0.10
1588	KLTT23	30	1	0	60	-59.55	-0.53	-0.36	-0.17
1498	KLTT24	30	0	1	30	-63.14	-0.61	-0.31	-0.30
1588	KLTT25	30	0	1	60	-59.69	-0.71	-0.35	-0.37
1416	KLTO16	21	2	0	0	-66.32	-0.28	-0.25	-0.03
1736	KLTO17	21	6	0	0	-54.41	-0.38	-0.34	-0.04
1408	KLTO18	30	1	0	0	-66.85	-0.28	-0.24	-0.04
1416	KLTO19	21	0	2	0	-66.58	-0.64	-0.18	-0.46
1736	KLTO20	21	0	6	0	-55.19	-1.39	-0.30	-1.08
1408	KLTO21	30	0	1	0	-67.01	-0.69	-0.22	-0.47
1498	KLTO22	30	1	0	30	-63.00	-0.43	-0.30	-0.13
1588	KLTO23	30	1	0	60	-59.58	-0.56	-0.35	-0.21
1498	KLTO24	30	0	1	30	-59.60	-0.58	-0.36	-0.22
1588	KLTO25	30	0	1	60	-59.72	-0.75	-0.34	-0.40

3.4 Edge of KLT with chlorhexidine, water, and urea

Calculated energies of optimized models from serie eKLTa are summarized in Table 4. Model eKLTa2 having the lowest N in the eKLTa serie, exhibits the lowest E_{tot} . Although eKLTb models have more free space due to higher value of interlayer twice lower in the case of eKLTa in contrast to eKLTb. This observation is consistent with experimentally evidenced fact of difficult intercalation of highly ordered KLT [10,18]. Therefore, the eKLTa can be considered more probable than eKLTb. E_{nb} values are also contrary to the

previously discussed dependence of calculated energies on N because models from serie eKLTb (having lower N) exhibit lower E_{nb} . This anomaly may be attributed to higher surface area available to molecules adhered on KLTb which enter the interlayer space after the separation of layers. Comparison of KLTT, KLTO, and eKLTa based on the lowest E_{nb} (i.e models KLTT10, KLTO10, and eKLTa1) shows that KLT surfaces are more suitable for anchoring of CH than KLT edge.

Table 4. Energies in [kcal/N] and composition of eKLT models. containing edge of KLT () with CH. N - number of all atoms in the model, CA - number of chlorhexidine salt molecules, W - number of water molecules.

N	Model	U	CA	W	E_{tot}	E_{nb}	E_{vdW}	E_c
2528	eKLTa1	0	10	0	-550.13	-1.39	-0.15	-1.23
2428	eKLTa2	0	5	100	-572.36	-1.02	-0.14	-0.88
2292	eKLTa3	33	0	100	-606.16	-0.45	-0.20	-0.26
2572	eKLTa4	48	2	100	-540.69	-0.72	-0.24	-0.48
1664	eKLTb1	0	10	0	-209.90	-2.15	-0.26	-1.90
1564	eKLTb2	0	5	100	-222.59	-1.55	-0.23	-1.33
1428	eKLTb3	33	0	100	-243.49	-0.65	-0.28	-0.37
1708	eKLTb4	48	2	100	-204.38	-1.06	-0.36	-0.70

4. CONCLUSIONS

Interaction between chlorhexidine and kaolinite was investigated using molecular modeling approach. Dependence of adhesion on number of co-adsorbed urea and water molecules and on type of chlorhexidine (i.e., non-protonated chlorhexidine with two molecules of acetic acid, and chlorhexidine salt) as well as surface of kaolinite (tetrahedral surface, octahedral surface, edge) was studied. Analysis of results revealed that edges of kaolinite are not preferred as location of adsorption with respect to kaolinite surfaces. Comparison of adsorption behavior of non-protonated chlorhexidine with two acetic acids and chlorhexidine salt showed that chlorhexidine salt adheres more strongly to the kaolinite. This finding allows us to conclude that preparation in basic environment does not improve the docking of chlorhexidine on kaolinite due to the loss of chlorhexidine's charge and the usage of acidic environment is more suitable.

Moreover, preparation of chlorhexidine/kaolinite nanocomposite by wet chemical route is undoubtedly possible [9] but the prevention of co-adsorption of other molecules in order to avoid shielding of Coulombic attraction between chlorhexidine and kaolinite seems to be a better way. This can be probably achieved by mechanochemical intercalation similarly as in the case of kaolinite-urea intercalate reported by Makó et al. [10,19]. Co-grinding of kaolinite and chlorhexidine in the absence of urea and water will be included in the future plan of our research group.

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