# STRUCTURAL DESCRIPTION AND PROPERTIES OF $Mg_2Al$-LAYERED DOUBLE HYDROXIDES INTERCALATED WITH THE FLUVASTATIN ANIONS SOLVED BY MOLECULAR SIMULATION METHODS

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## Abstract

We present the structure analysis of $Mg_2Al$ layered double hydroxides intercalated by fluvastatin anions based on published experimental data and show the advantages of molecular simulation methods for description of complex organo-clay hybrid materials. Models with different arrangements of intercalated fluvastatin anions, which fully compensate the positive charge of the LDH layers, were calculated. All presented models were analysed by calculated XRD patterns, basal spacing, and free volumes calculations to describe different behaviour and properties between individual models with different arrangement of the fluvastatin anions. Based on total energy values bilayer and pseudobilayer models are preferred with respect to monolayer models. Calculated results were thoroughly compared to published experimental data and a detail comparison between experimental and calculation X-ray data is presented. It was shown that calculated patterns for monolayer and bilayer arrangement do not reach a good agreement with experimental diffraction data so as the basal spacings. In opposite of this calculated XRD patterns proved a pseudobilayer arrangement with mixture of flat and tilted positions of fluvastatin anions in the interlayer space as well as equal distribution of water molecules in the interlayer among fluvastatin anions.

## Introduction

Layered double hydroxides (LDHs), known also as anionic clays, recently attract a great attention due to their unique properties allowing their modifications in various branches of industrial or pharmaceutical applications. This can be very well illustrated by several recently published reviews [1-5]. LDHs are layered materials with general chemical formula $[M_{1-x}^{II}M_x^{III}(OH)_2]^{x+}[A^{-n}]_{x/n}*mH_2O$, where $M^{II}$ represents a divalent metal cation (such as Zn, Ca, Mg, Ni, Fe), $M^{III}$ represents a trivalent metal cation (such as Al, Cr, Fe, Ga), $A^{-n}$ represents an exchangeable interlayer anion, which compensate the charge of the layers, *m* is number of moles of co-intercalated water molecules per formula weight of compounds and *x* is the number of moles of trivalent metal cation per formula weight of compounds. The LDH crystal structure is based on $M(OH)_6$ octahedral units which share edges in order to build $M(OH)_2$ brucite-like layers. The interlayer space contains anionic species compensating positive charge of LDH structure and water molecules.

Because of LDHs are biocompatible materials with human body it can be used as functional carrier or delivery system for different drugs. LDHs demonstrate perfect stability and capacity to intercalate drug species and preserve their durability, stability, decrease aging, and protect some parts of human bodies against undesirable influence of drugs in case of direct pharmacological action [6-8].

A lot of different organic species have been intercalated up to now into the interlayer space of LDHs, for example amino acids [9], DNA [10], antibiotics [11], anticardiovascular drugs (statins) [12,13], etc. All these results show the suitability of the drug-LDH systems for human body in comparison with direct application of pure drugs.

In this paper, we present a structural description and properties of $Mg_2Al$-LDHs intercalated with fluvastatin anions determined by classical molecular simulation methods based on previously published experimental data (XRD, chemical analysis, thermogravimetry, FTIR, TEM, AFM) [13]. Fluvastatin drug, belongs to class of statins, is used to reduce undesirable cholesterol in the blood stream. This drug inhibits 3-hydroxymethylglututaryl coenzyme A reductase, which decreases cholesterol biosynthesis [14]. Several studies presented cardiovascular benefits of statins, which are associated with their anti-inflammatory effects, for example [15].

Opposite of this, statins (included fluvastatin) can cause toxicity and adverse effects on human bodies [16]. The conformational changes significantly affect their solubility in aqueous solution and time of releasing. Hence, the use of LDHs as carriers of fluvastatin drug is possible way how to optimize and increase its chemical stability, durability, aging, and bioavailability during medication [17]. Moreover, pharmaceutically acceptable statins should be used as novel antimicrobials [18].

In this study, we focus on methods allowing us to describe structural properties like guest arrangement, position, mutual orientation, interaction of guests, and interactions with layers [19]. The presented calculated structural results bring more insight on experimental data [13] and also consider results from [12] where XRD patterns and basal spacings are directly compared for similar statin drug (pravastatin). Moreover, simulations allow us to obtain new additional detailed information about structural arrangement of all species in the interlayer space. This confirms potential usefulness and applicability of the calculation methods for solving of complex materials as LDHs intercalated with organic molecules.

## 1. Simulation methods

All simulations were performed using Forcite module in the Materials Studio software package [20]. This module is used to predict the structure, interactions, and energetic properties of LDH-fluvastatin models.

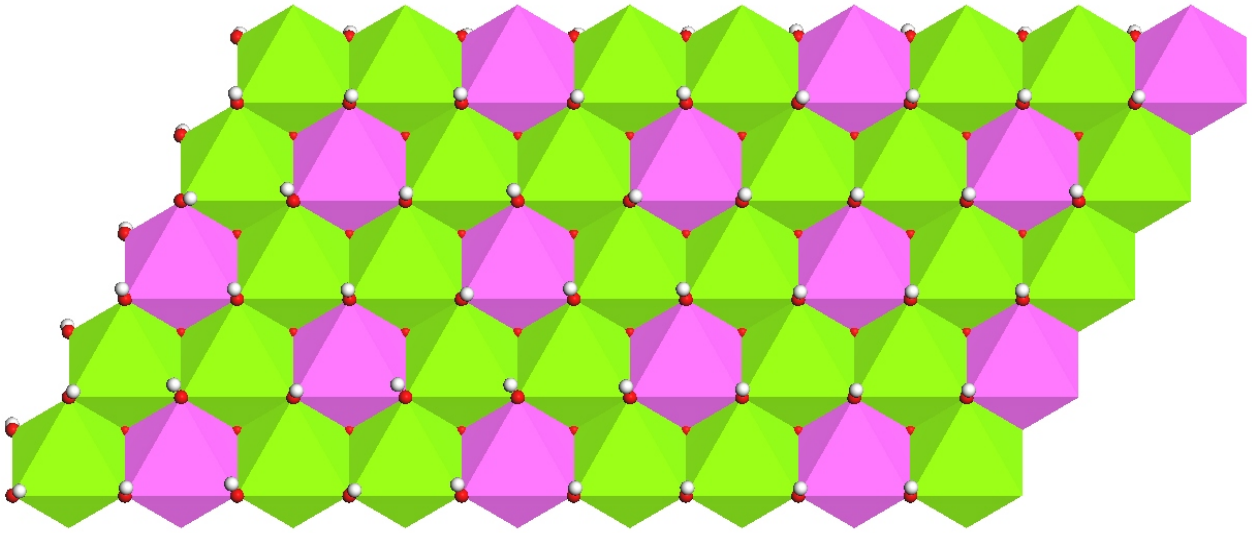

**Figure 1.** Polyhedron display style of honeycomb arrangement of atoms in $Mg_2Al$-LDH layer, Mg atom is green, Al is pink, O is red and H is white.

### 1.1 Initial model construction

Primitive unit cell of $Mg_2Al$-LDH structure was created with following cell parameters for $Mg_2Al$ structure: $a = b =$ 3.0460 Å, $c = 22.7720$ Å, $= = 90°$, $= 120°$, R$\bar{3}m$ space group [21]. A $5a$ $9b$ $1c$ supercell of $Mg_2Al$ LDH was built for subsequent calculations. The ratio between Mg:Al in the framework was 2:1. Mg atoms were not randomly distributed in the layer, but based on [22] in honeycomb arrangement, see Fig.1. Each layer consisted of 15 Al and 30 Mg atoms. Charges were calculated by QEq method [23] for each LDH layer and its charge compensating drug anions separately in order to keep the whole structure neutral. For computational purposes space group of super cell was changed to P1 but changes of crystallographic axes related to symmetry were constrained.

The model of fluvastatin molecule was build according to formula $C_{24}H_{26}FNO_4$ and its systematic formula (E,3R,5S)-7-[3-(4-fluorophenyl)-1-propan-2-ylindol-2-yl] -3,5-dihydroxyhept-6-enoic acid. Subsequently, the fluvastatin anion was created by removing hydrogen atom from carboxyl group, various conformations of fluvastatin were optimized and the selected model with the minimum energy was used as suitable intercalate, see Fig. 2. Charges of fluvastatin anion were calculated by the QEq method.

Series of initial models with various positions and orientations of fluvastatin anions in the interlayer of LDH with focus on monolayer, suggested in [13], bilayer suggested for similar statin drug pravastatin in [12], and later pseudobilayer arrangement were built and optimized see example in Fig. 3. For non-spherical anions, especially for the anions containing long chains, a lot of various arrangements in the interlayer space are available, namely for example, a monolayer/bilayer arrangement or their combination, parallel (flat) or tilted orientation of guest with respect to the LDH layers. Due to the size of fluvastatin anion and published experimental results we assumed, based on the set of optimized models, that the fluvastatin anions will be probably in tilted (almost perpendicular) bilayer arrangement into LDH galleries to reach agreement with experimental data. In opposite of this, basal spacing of

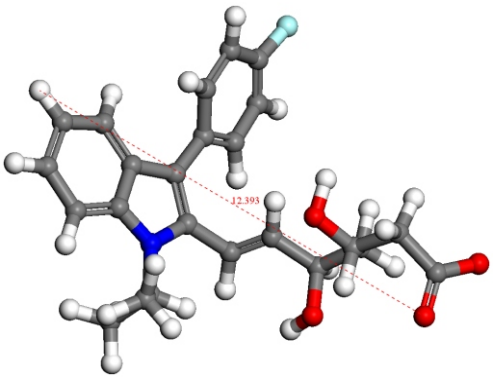


**Figure 2.** Optimized model of the fluvastatin anion with its dimension. O is red, H is white, C is grey, N is blue, F is light blue.

calculated results for bilayer arrangement was higher than experimental. Other tested and optimized models, especially monolayer arrangements of fluvastatin anions in the interlayer space, did not reach a sufficient agreement with published experimental data, which were used for verification of calculated results. So, we focused for the arrangement with combined positions of fluvastatin anions in the interlayer of LDH and set of initial models was created and calculated as pseudobilayer arrangement.

The amount of intercalated species was taken from experimental results published in [13]. Models with different arrangement of fluvastatin anions in the interlayer space of LDH as well as different amount of water molecules in the interlayer were created and tested to reach the best fit with experiments.

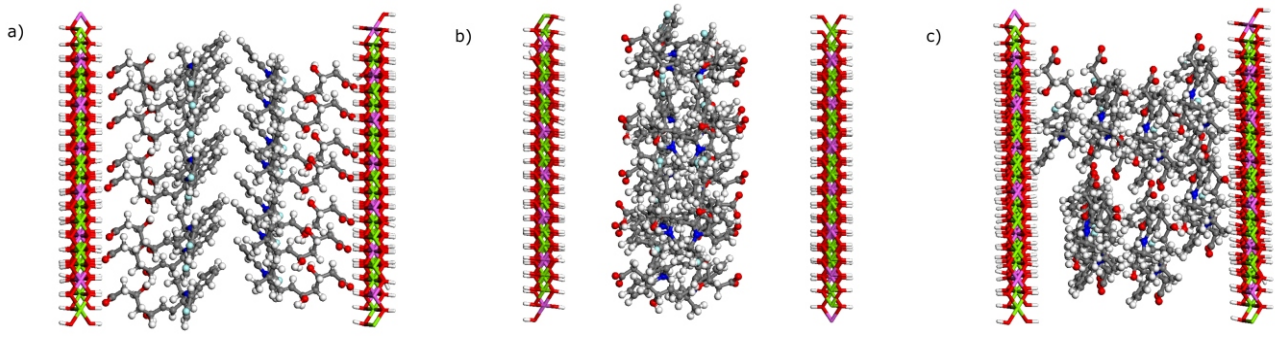


**Figure 3.** Initial models of $Mg_2Al$-LDH intercalated with fluvastatin anions a) bilayer arrangement, b) monolayer arrangement, c) pseudobilayer arrangement. View along *a* axis, Mg atom is green, Al is pink in LDH layer, O is red, H is white, C is grey, N is blue, F is light blue in the interlayer.

### 1.2 Parametrization and simulation strategy

The $Mg_2$-LDH model was built with the $d_{003}$ basal spacing based on experimentally obtained values [12] for $Mg_2Al$ $d_{003}$ = 31.3 Å for similar drug anion from statin group – pravastatin and also based on suggestion from [12] that in [13] was XRD measured from 5° 2-theta angle so there is a possibility of missing the first (003) diffraction peak. Fluvastatin were placed into the interlayer space of LDH in the amount derived from chemical elemental analysis in [13] and whole model was optimized. Number of water molecules placed into the LDH galleries of one supercell correspond with weight water loss (2 - 4 % for $Mg_2Al$) obtained experimentally from thermogravimetry measurements [13]. In our models that means 30 and 60 water molecules per model supercell (10 and 20 per one interlayer).

The geometry optimization of initial models was carried out using Universal Force Field (UFF) [24], the electrostatic energy was calculated by Ewald summation method [25], and van der Waals energy was calculated using cubic spline with cut-off distance of 15.0 Å. The charges were calculated by QEq method except charges of water molecules. Charges from SPC/E model [26] were used for water molecules. The host layers were kept as rigid units, all atomic positions in the interlayer space were variable, c parameter of the supercell was variable, and other supercell parameters were fixed.

The quench molecular dynamic allowing geometry optimization after defined number of steps was done for selection of new possible initial models promising the best agreement with experiments. LDH layers were kept fixed and all species in the interlayer space were without constraints. The MD was carried out in an NVE statistical ensemble (N - constant number of atoms, V - constant volume, E - constant energy) at 298 K to obtain a new series of optimized models. One dynamic step was set to 0.1 fs and dynamic calculations last in total 10 ps.

## 2. Results and discussion

Several series of initial models with various arrangements and orientations of fluvastatin anions were created and optimized as $Mg_2Al$-LDH intercalate and compared with available published experimental results in [13] and [12]. Based on experimental data, due to lower crystallinity of $Mg_2Al$-LDH samples and due to amount of fluvastatin anions which fully compensate layer charge we present only models with the highest number of fluvastatin anions mostly from monolayer to bilayer arrangement. Calculation results from all tested geometry optimizations and MD showed that fluvastatin anions formed a pseudobilayer arrangement in LDH galleries see Fig. 4. Monolayer arrangement was also calculated and the results showed that fluvastatin anions had tendency to be anchored by $COO^-$ group to one LDH layer and the opposite part of chain had tendency to be directed to the opposite LDH layer by F atom. Fluvastatin anions were mostly tilted in monolayer arrangements and moreover, basal spacing did not correspond with experimentally obtained basal spacing [13] even when we consider that (003) basal reflection in [13] is (006) according to [12]. Moreover, calculated total energy of monolayer arrangements was higher than for bilayer and pseudobilayer models. We can conclude that in all calculated models fluvastatin anions do not prefer monolayer arrangement in the interlayer space.

In bilayer and pseudobilayer arrangements several fluvastatin anions were anchored by their $COO^-$ groups directly to one LDH layer and several directly to second layer. The part of fluvastatin anion molecule represented by F atom had tendency to be anchored to the same LDH layer like $COO^-$ group. This arrangement limit connection of all fluvastatin anions close to LDH layer and several of them remain in the middle of interlayer with active edges oriented to LDH layers, see pseudobilayer arrangement in Fig. 4c. Despite of this small amount of fluvastatin anions remain trapped in the middle due to steric hindrances creates pseudobilayer arrangement. In our calculations models with bilayer arrangement do not fully agree with experimental X-ray data but this arrangement is only with about 5 % difference energetically preferred with respect to pseudobilayer arrangement.

Let's compare calculated X-ray diffraction pattern with available experimental data published in Panda [13] for $Mg_2Al$ LDH intercalated with fluvastatin. Samples preparation took 24 hours under 65 °C under inert atmosphere. Based on published data, although low molar concentration 0.017 mol/L was used high concentration of fluvastatin anions in the interlayer space of $Mg_2Al$ was reached. When we look at these experimental diffraction patterns we can see sharp and thin reflection peaks for these 24 hours prepared intercalates. Molecular simulations have an advantage that resultant models can be analysed by calculated X-ray diffraction patterns which can be directly compared

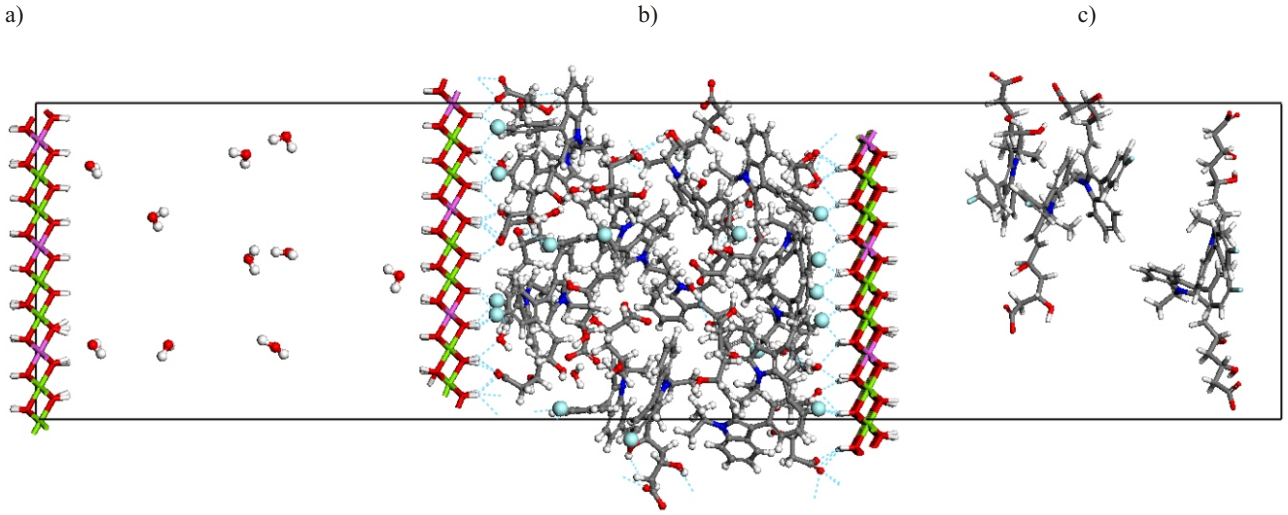


**Figure 4.** Optimized models of $Mg_2Al$-LDH intercalated with fluvastatin anions a) only water molecules are visible, b) all molecules in the interlayer are visible, F atoms highlighted, H-bridges displayed by blue-dashed lines c) only selected fluvastatin anions are visible. View along *a* axis, Mg atom is green, Al is pink in LDH layer, O is red, H is white, C is grey, N is blue, and F is light blue in the interlayer.

with experimental patterns. When we compared basal peaks of X-ray diffraction data published in [13], when we consider (003) basal reflection below 5° 2-theta angle as suggested in [12], with our calculated X-ray diffraction data we obtained very good agreement in basal peaks for the first seven basal peaks of the calculated pseudobilayer model presented in this paper. For this reason XRD patterns of intercalated LDH by fluvastatin anions were calculated for bilayer, pseudobilayer and monolayer arrangement in the interlayer space. Direct comparison of these three calculated patterns for $Mg_2Al$ LDH with fluvastatin anions is shown in Fig. 5. The comparison between calculated basal spacings for individual calculated arrangements and experimental $d_{006}$ values published in [13] is given in Table 1.

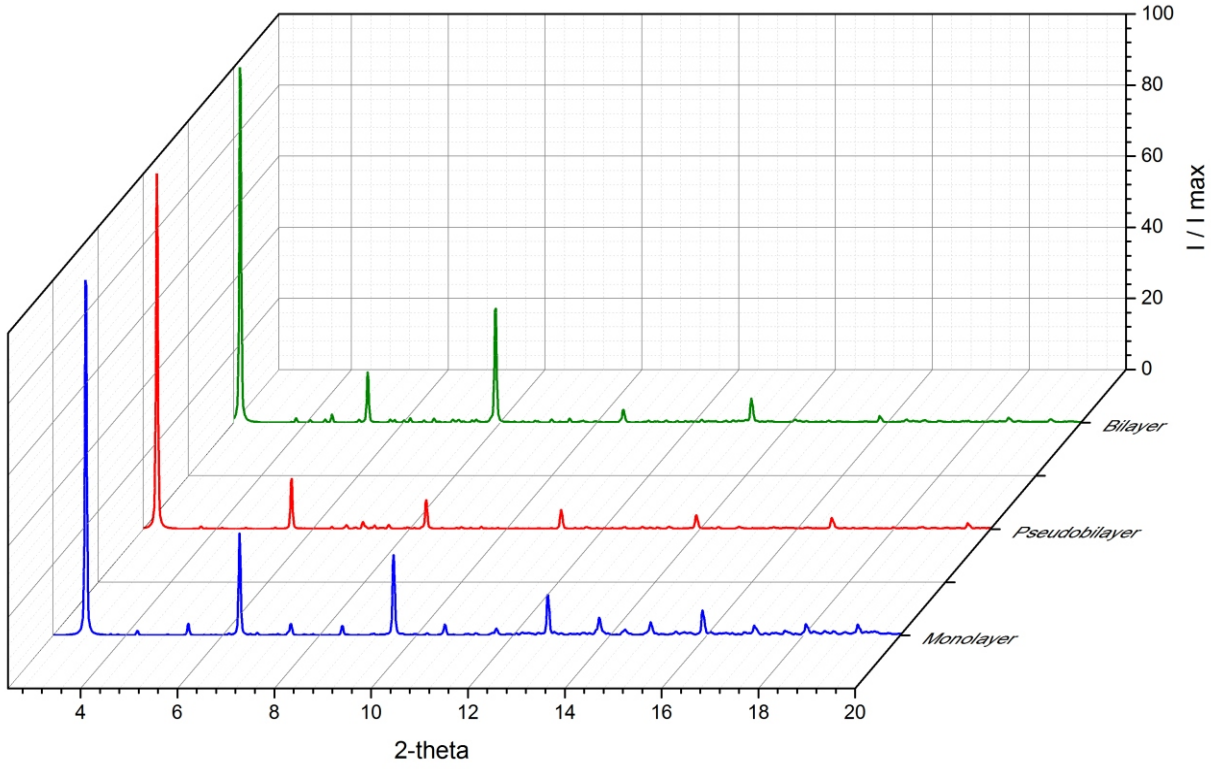


**Figure 5.** Calculated XRD patterns of $Mg_2AL$-LDH intercalated with fluvastatin anions for monolayer, pseudobilayer and bilayer arrangement.

**Table 1**. Comparison of calculated and experimental basal spacing for intercalated LDH with fluvastatin anions.

| | $Mg_2Al$-LDH | | | |
|---|---|---|---|---|
| Type of fluvastatin arrangement | $d_{003}$(simulation) [Å] | $d_{006}$(simulation) [Å] | $d_{006}$(experimental) [Å], [13]* | Total energy [kcal/mol] |
| Monolayer | 27.8 | 13.9 | 15.5 | -9284 |
| Bilayer | 33.5 | 16.8 | 15.5 | -11474 |
| Pseudobilayer | 31.8 | 15.9 | 15.5 | -10963 |
| Pseudobilayer with 30 water molecules | 31.8 | 15.9 | 15.5 | -9281 |
| Pseudobilayer with 60 water molecules | 31.8 | 15.9 | 15.5 | -9916 |

*experimental $d_{006}$-spacing for $Mg_2Al$-LDH is 15.5 Å, marked as $d_{003}$ in publication

As it is shown in Table 1 the best agreement between published and calculated basal spacing for $Mg_2Al$-LDH with fluvastatin anion is pseudobilayer arrangement (but the first peak there is in fact the second order of diffraction as suggested in [12]) despite a bit higher total energy. Calculated values of basal spacing of models with bilayer or monolayer arrangement are higher or lower and do not agree with experimentally determined basal spacings.

Based on TGA measurements we included related amount of water molecules into intercalate. We tested addition of 30 and 60 water molecules per one model, which corresponds to 2-4 % weight water loss in [13]. Based on calculation results we can say that there is no special arrangement of water molecules in the interlayer space of LDH but all water molecules are accidentally spread between LDH layers. We can see slight tendency for presence of water molecules in the middle of interlayer because of most of water is expelled from LDH layers due to presence of active sites of fluvastatin how it is seen in Fig. 4a. We can expect that water molecules prefer to occupy the free volume in intercalate, which also plays major role in ion exchange during drug release process. This behaviour can be characterized by calculation of free atom volume, which can be described by Connolly surfaces [27]. The Connolly surfaces are determined by moving a probe sphere over the van der Waals surfaces, and then the Connolly surface is at the boundary between this probe and the atoms represented by their van der Waals radii. In our case the radius of Connolly probe is 1 Å and Connolly surfaces are displayed by grey colour on outer sphere and blue colour represents inner surfaces, see Fig. 6. In fact, those surfaces are a part of the van der Waals surfaces of molecule that is accessible to a solvent. For LDH intercalates with monolayer, bilayer and pseudobilayer arrangement without water molecules, the free volume is 2923 $Å^3$, 9777 $Å^3$, and 7717 $Å^3$ respectively. As we can see, free volume for monolayer show very compressed arrangement of fluvastatin anions together with difference in basal spacing and high total energy we can conclude that this arrangement is not preferred. Bilayer arrangement has higher free volume allowing for example higher absorption of water molecules but basal spacing is not preferred like in the case of monolayer. Free volume of pseudobilayer arrangement allows absorption of water molecules and it seems as reasonable compromise for resultant model with the best agreement with experimental basal spacing and value of total energy from calculation point of view.

Based on the calculation results we suppose that the positions of water molecules are equally spread in the interlayer space of LDH and are connected by hydrogen bonds with Fluvastatin anions see Fig. 4. Opposite of this hydrogen bond interactions play crucial role in description of mutual interactions among fluvastatin anions and LDH layers as we can see from Fig. 4b, where hydrogen bonds

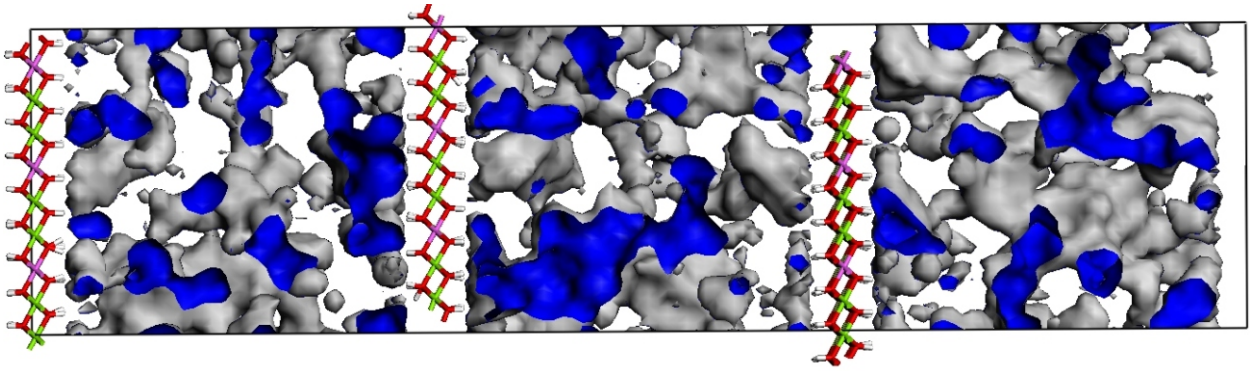

**Figure 6.** Connolly surfaces for pseudobilayer arrangement of $Mg_2Al$-LDH intercalated with fluvastatin anion and 30 water molecules per supercell.

are presented by dashed light blue lines. Various possible definitions and descriptions of hydrogen bond can be found in literature for example [28]. The following hydrogen bond definition was used based on [20, 29]: the hydrogen bond is considered to exist if the acceptor - hydrogen distance was lower than 2.5 Å ($r_{AH} < 2.5$ Å) and also the acceptor–hydrogen–donor angle was higher than 90° ( $_{AHD} > 90°$).

## 3. Conclusions

In summary, molecular simulations have been used to explore structure and properties of $Mg_2Al$-LDH intercalated with various arrangements of fluvastatin anions. We studied several different orientations of interlayer species and various amount of water molecules. Models, X-ray diffraction patterns, basal spacing, and Connolly surfaces were calculated from optimized models and compared with experimental data. The results of the molecular simulations showed that optimal arrangement of fluvastin anions is pseudobilayer arrangement with tilted and partially flat positions of the fluvastatin anions with respect to LDH layers and this model shows the best fit with experimental basal spacing and is energetically preferable with respect to monolayer arrangement. The tilted or nearly flat arrangement in the middle of interlayer is caused by mutual electrostatic repulsions between fluvastatin anions. We suppose that electrostatic repulsions and hydrogen bond interactions play important role in water-fluvastatin, fluvastatin-LDH and water-LDH interactions. Calculated results very well correspond with previously published experimental results and improve them by addition of new information about basal spacing related to lower 2 theta angles. Based on the presented results we can see the effectiveness and usefulness of molecular simulation methods for structural analysis and properties description of the intercalated materials.

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