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Molecular dynamics simulations of organic crystal dissolution: The lifetime and stability of the polymorphic forms of para-amino benzoic acid in aqueous environment.

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Abstract

Para-amino benzoic acid (PABA) manifests crystalline solid-state properties that are typical of a class of chemical compounds with important industrial applications. Hence, it is particularly worthwhile to investigate the lifetime and stability of representative molecular-clusters of two polymorphic forms of PABA in aqueous solution using molecular dynamics simulations. Simulations of five nano-seconds duration in the isothermal-isobaric ensemble (constant particle number, pressure and temperature (NPT) ensemble) were performed for the two polymorphic forms at three different temperatures 0 °C, 50 °C and 100 °C. The simulations revealed that at 0 °C the representative molecular-clusters of the two polymorphic forms remain ordered while at 50 °C the molecular packing within the clusters becomes partially disordered for both polymorphic forms and at 100 °C the clusters lose long-range order rapidly and come to resemble liquid drops. Care should be taken when assessing the relative stability of polymorphic forms, as a function of temperature, from such computational experiments which explore the dissolution of nano-scale crystals. The long range order of the clusters of the α-form at 50°C and 100°C was, respectively, partially and completely lost after 5ns which merits further investigation given the α-form is the high-temperature stable polymorph. Importantly, the initial shape of clusters, as well as the number of solute molecules they contained, affected the extent to which order was lost and how rapidly the loss occurred. Given that classical nucleation theory predicts a finite probability that clusters significantly larger than the critical size, in terms of number of molecules, may dissolve, building clusters containing a greater number of molecules could improve the simulated stability of the alpha polymorph at 50°C and 100°C. Furthermore, the simulations revealed that the selection of a suitable electrostatic potential is very important for the description of the dissolution process of the nano-sized crystals.

1. Introduction

The dissolution of crystals is a fundamental physical-process which has a remarkable impact on various applications in a broad range of scientific areas[1-4]. The dissolution of organic crystals is of particular interest to the pharmaceutical sector as it is a controlling factor in the development of formulations for health-care products.[5-8]. The mechanism by which molecules detach from the solid, crystalline phase in the presence of solution is not yet fully revealed by the available experimental techniques. Molecular dynamics approaches have

been used extensively as a powerful tool to reveal important information about the process of the dissolution at the microscopic level thus complementing experimental findings.[9-10]

Molecular dynamics simulations have been extremely useful for exploring biological function (protein-folding thermodynamics and kinetics, self-assembly in complex fluids and biological systems, lipid bilayer pressure profiles) for pharmaceutical applications in relation to proteins, lipids and nucleic acids[11-15]. One representative study of crystal dissolution has been reported by Gao and Olsen[16]. They simulated the drug-crystal dissolution of Acetaminophen (Aspirin) Form I in water. Their findings demonstrate that the dissolution process is influenced strongly by both the electrostatic and van der Waals solute-solute and solute-solvent inter-molecular interactions with non-negligible contributions from the latter. Hayakawa and co-authors reported the dissolution process of a cellulose triacetate-II nano-sized crystal in DMSO[17]. The crystal packing of cellulose triacetate-II is characterised by three, main intermolecular CH···O interactions where the H---O 'bond' is in line with the C-H bond forming an angle of 180 degrees. The strongest interaction is between three hydrogens of the glucopyranose ring and a carbonyl oxygen. The other two interactions are found between two acetyl carbonyl oxygens and two acetyl methyl hydrogens. Hayakawa reported that the three types of interaction occurring in the crystal structure were fully compensated in solution through solute interactions with DMSO solvent molecules and that the atoms involved in the main interactions were surrounded by the DMSO solvent. The energetic stability, in vacuum, of molecular clusters representing organic, molecular crystals, as a function of cluster size, has already been investigated extensively for L-glutamic acid[18], and D-mannitol[19] and in the case of benzophenone[20] particularly, cluster stability with respect to molecular, conformational flexibility has been addressed.

Two polymorphs of PABA have been identified which constitute an enantiotropic pair. The α-form is the more thermodynamically stable above the transition temperature, independently reported to be 13.8 °C[21]and 16 °C[22], whilst the β-form is stable below this temperature. The α polymorph crystallises as a needle like shape with a cross section of a rhombus (slightly off a square shape).[23] The unit cell dimensions are a = 18.5712, b = 3.8431, c = 18.6321, β = 93.67°, space group is $P2_1/N$ and Z = 8[24]. This structure of the α polymorph is the structure used for this study and packing diagrams are shown in Figure 1b. The β polymorph crystallises as rhombic prisms with cell dimensions reported as a = 6.2782 Å, b = 8.5831 Å, c = 12.3649 Å and β = 100.13°; Z = 4 and space group $P2_1/N$[25]. The molecular packing is characterised by a closed, hydrogen bonded cycle of two identical OH…N and NH…O interactions that link four PABA molecules and is shown in Figure 1c. Of particular interest is the stability of carboxylic-acid dimers which are present in the α-form alone. The strength of the carboxylic-acid dimer interaction is of interest not only for single, isolated dimers but also in cases where the dimers are assembled into larger clusters, as the packing of these dimers is manifested in the solid-state structure of the α polymorph. Elucidating the energy landscape of solute clusters in supersaturated solutions is the key to understanding how particular polymorphs are selected via the solution crystallisation process according to factors such as solvent choice, de-supersaturation profile and temperature. In the present work, molecular dynamics simulations were used to investigate the stability of clusters of solute molecules for PABA as a function of solution supersaturation and temperature. The lifetime and stability of solute clusters were investigated with the objective of estimating an upper bound for the critical cluster-size, as a

function of the supersaturation, in a solvent environment based on observing a small probability for clusters to dissolve. A more accurate estimation of the critical cluster size, requiring many more simulations, was not undertaken however as this was not the main objective.

The stability of molecular clusters in solution was used to evaluate the performance and transferability of the potential employed in the simulations. In the case that sufficiently large molecular clusters of a particular shape, i.e. very much greater than the critical size, are employed, then for the specified temperature and supersaturation, the clusters are likely to remain stable in terms of retaining their original long-range order. It is recognised, however, that there is still a chance that such clusters can vanish by the process that starts 'uphill' with respect to the Gibbs free energy. From the molecular dynamics simulations the possible outcomes fall in to three different categories: (i) the clusters retain their order and cluster size, (ii) the clusters become liquid like but retain size, (iii) the clusters break-up and dissolve.

2. Computational details

The crystal structures of the α (AMBNAC06) and β (AMBNAC04) forms of PABA were retrieved from the Cambridge Crystallographic Data Centre. The asymmetric unit of the α-form comprises two molecules and the unit cell contains eight molecules. The asymmetric unit of the β form comprises one molecule and the unit cell contains four molecules. A series of α-form crystal lattices was constructed using the Material Studio package[26]. The crystal lattices were built and examined in two sets of computational experiments in which the size of the clusters was increased in order to estimate an upper bound to the critical-cluster size. The first clusters examined consisted of 192 molecules of the α-form and were constructed as a 2 x 6 x 2 array of unit cells (along the crystallographic a, b and c unit cell axes respectively) containing 24 unit cells in total. Similarly the cluster of β-form contained 256 molecules and was constructed as 4 x 4 x 4 array containing 64 unit cells. In the second set of simulations, the clusters examined consisted of a 504 molecule cluster of the α-form, in a 3 x 7 x 3 array containing 63 unit cells, and a 480 molecule cluster of the β-form in a 6 x 5 x 4 array containing 120 unit cells. Finally, as the crystal shape of the α polymorph is observed to be a needle-like another cluster was examined which consisted of 512 molecules of the α-form as a 2 x 16 x 2 array containing 64 unit cells. The shape of these molecular clusters, which represented nano-crystals of the respective polymorphs, was a parallelepiped for the β-form and rectangular parallelepiped for the α-form.

The topology files for the clusters were created with antechamber[27] using Ambertools and the atom types defined from the GAFF[28]. All the clusters created were solvated with water and the TIP3P water potential was used. For the non-bonded interactions, parameters for the Lennard Jones potential derived from GAFF were used. For the electrostatic potential RESP charges based on the semi-empirical method AM1, derived from the Antechamber within Ambertools, were compared with RESP[29-30] charges created from the RED server based on a density functional theory method using the B3LYP functional. All MD simulations were performed using the GROMACS program version 4.5.5[31]. Periodic boundary conditions were used. The clusters were first energy minimized (2000 steps with the steepest descents technique). During these minimizations no significant changes were observed in the shape

or size of clusters and no molecules were observed to detach from the clusters. The Particle Mesh Ewald (PME) method was used to investigate the effect of long-range electrostatic interactions. The systems were equilibrated at the three temperatures at constant volume and temperature for 100 ps followed by a second phase equilibration at the three temperatures at constant pressure and temperature for 100 ps. Finally production simulations (5ns) were performed at constant pressure and temperature within the isobaric-isothermal (NPT) ensemble with a 2fs time step.

3. Results and discussion

The small molecular clusters employed in the first numerical experiment rapidly lost their long range order for all temperatures which implies that the clusters were much smaller than the critical cluster size required to maintain structural integrity. The same behaviour was observed for all the sets of atomic point charges employed (i.e. whether based on a semi-empirical or DFT level of theory). Therefore the use of larger clusters was indicated to further examine whether the failure of the molecular clusters to remain stable is size dependent or there is an issue of the atomic charges used to describe the electrostatic potential.

The simulation results obtained for the larger size clusters (3 x 7 x 3 array for the α-form and 6 x 5 x 4 array for the β-form) using partial charges derived from the semi-empirical AM1 method manifested clusters which also lost their long range order rapidly. This might imply that the clusters are smaller than the critical size. However, this inference is not supported by the results of simulations that employed DFT derived RESP charges which were significantly different. A possible implication is that the charges derived from the semi-empirical approach do not describe accurately the electrostatic interactions between molecules in the clusters. In particular, during these simulations the clusters appear to have transformed to configurations resembling liquid drops. On the other hand, when the DFT derived RESP charges were applied, the α-form cluster at 0°C was stable in water with only a small number of molecules becoming detached around the edges as is shown in Figure 2. This suggests that the lifetime and stability of the clusters depends not only on the size of the clusters chosen but on the partial charges selected as well. At 50°C the cluster representative of the α-form was found to retain its order for about 3ns whilst at 100C° the cluster transformed rapidly to a configuration resembling a liquid drop. As the α-form is observed to be the predominant form above the transition temperature experimentally, the simulations indicating that, at 100°C, the α-form clusters are less stable than the β-form clusters was an unexpected result. The degree of long range order in the simulated clusters was assessed by monitoring the total number of hydrogen bonding solute-solute interactions occurring during the simulations. For the 0°C simulations the cluster retains the number of hydrogen bonding interactions (figure 2e) whereas at 50°C it loses part of the long range order after 3 ns as the number of hydrogen bonding solute-solute interactions gradually reduces (figure 2f) and at 100 °C the cluster has lost part of the long range order from the equilibration simulations and the bonding solute-solute interactions are rapidly reduced. Similar results were obtained for the 6 x 5 x 4 cluster of the β-form the cluster largely retains its order at 0°C and also at 50°C losing a bit more of the edge of the cluster as can be seen from Figure 4. The results obtained for the β-form clusters are in better agreement with experimental observations as the β-form is found to be unstable above room temperature.

The next step was to examine a cluster that has an external morphology similar to that observed in experiments. Figure 3 shows the simulation results performed for the 2 x 16 x 2 cluster representing the α-form. The results obtained were significantly different compared to the 3 x 7 x 3 array for the α-form: the α-form cluster at 0°C remained ordered as with the more equant shaped cluster, with some detachment of PABA molecules at the edges. On the other hand, at 50°C the needle-shaped cluster retained long range order for a longer period (for about 4ns) although after 5ns the cluster has lost much of the original order. As for the 3 x 7 x 3 cluster, at 100°C the needle-shaped cluster transformed to a liquid drop rapidly although the cluster retained order for longer period. The relatively rapid reduction in long range order found for the α-form nano-crystal at 100°C, which one would envisaged should retain its order, suggests that, perhaps, a larger cluster should be used because of the implied greater solubility at the increased temperature. The results of monitoring the long-range order through time via counting the number of hydrogen bonding solute-solute interactions were similar to those found for the 3 x 7 x 3 cluster. In summary, it was found that the needle shaped cluster retained long range order at 50°C and at 100°C for longer than the 3 x 7 x 3 cluster.

In particular, it is interesting to observe the molecules that detach first from the molecular cluster into the solvent. Figure 5 represents the superposition of the initial cluster generated for the α-form and the 2 x 16 x 2 cluster after 5 ns simulation. It can be seen that the first molecules of PABA to detach from the cluster are mainly from the edges and corners. This observation is in general agreement with the simulations performed for Acetaminophen crystal by Gao and Olsen[16]. Additionally, it is interesting to note that the results from the simulations are supported by the simple Kossel model[32].

4. Summary

The lifetime and stability of the two polymorphic forms of PABA at the nano-crystalline scale were investigated by means of molecular dynamics simulations. Simulations were performed at three different temperatures above and below the expected transition temperature to evaluate the performance of the selected force-field and to predict the critical-cluster size as a function of temperature and supersaturation ratio. The results demonstrate that the shape of the initial cluster of molecules selected i.e. being based on the experimentally observed morphology of the crystal, is important in terms of getting accurate predictions to which molecules will remain integral to the cluster and which will diffuse into the solution. The long range order of the clusters of the α-form at 50°C and 100°C were, respectively, partially and completely lost after 5ns which is not expected as the α-form is the high-temperature stable polymorph. Building clusters containing a greater number of molecules could improve the simulated stability of the alpha polymorph at these temperatures or alternatively we should consider changing aspects of the interatomic potential employed so as to improve the stability of α-form clusters in computational experiments. Additionally, the accurate description of the electrostatic potential is crucial for successfully modelling the dissolution process of nano-sized crystals via MD simulations. Overall, it has been demonstrated that molecular dynamics simulations can provide a better understanding of both the polymorph selection process and the dissolution process for organic crystals.

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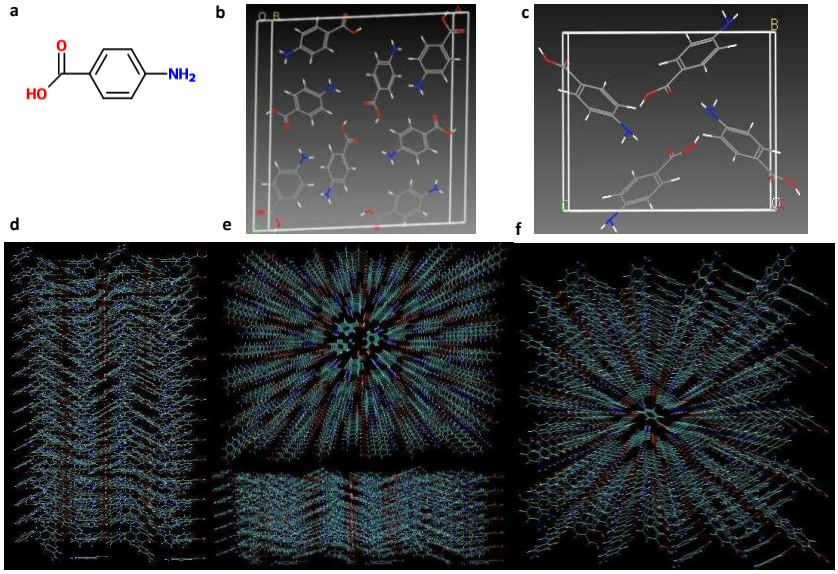

Figure 1. (a) Molecular structure of PABA. (b) Unit cell of anhydrous PABA α-form (c) Unit cell of anhydrous PABA β-form. (d) The 2 x 16 x 2 anhydrous crystal of PABA α-form (e) The 3 x 7 x 3 anhydrous crystal of PABA α-form (top and side view) (f) The 6 x 5 x 4 anhydrous crystal of PABA β-form

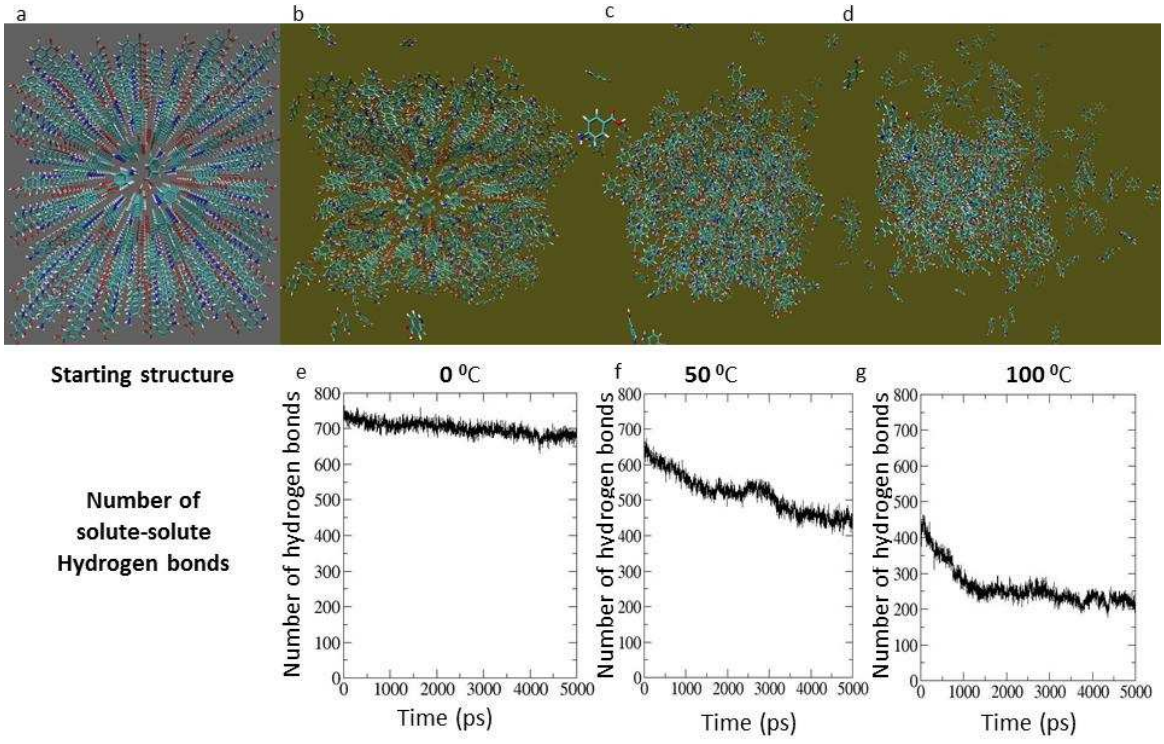

Figure 2. Results of the MD simulations of a 3 x 7 x 3 nano-crystal of PABA: Starting structure (Crystal lattice constructed in Materials Studio not the equilibrated structure) (a). Snapshot taken and the number of hydrogen bond of solute-solute after 5ns simulation respectively for 0C (b) and (e), 50°C (c) and (f) and 100°C (d) and (g).

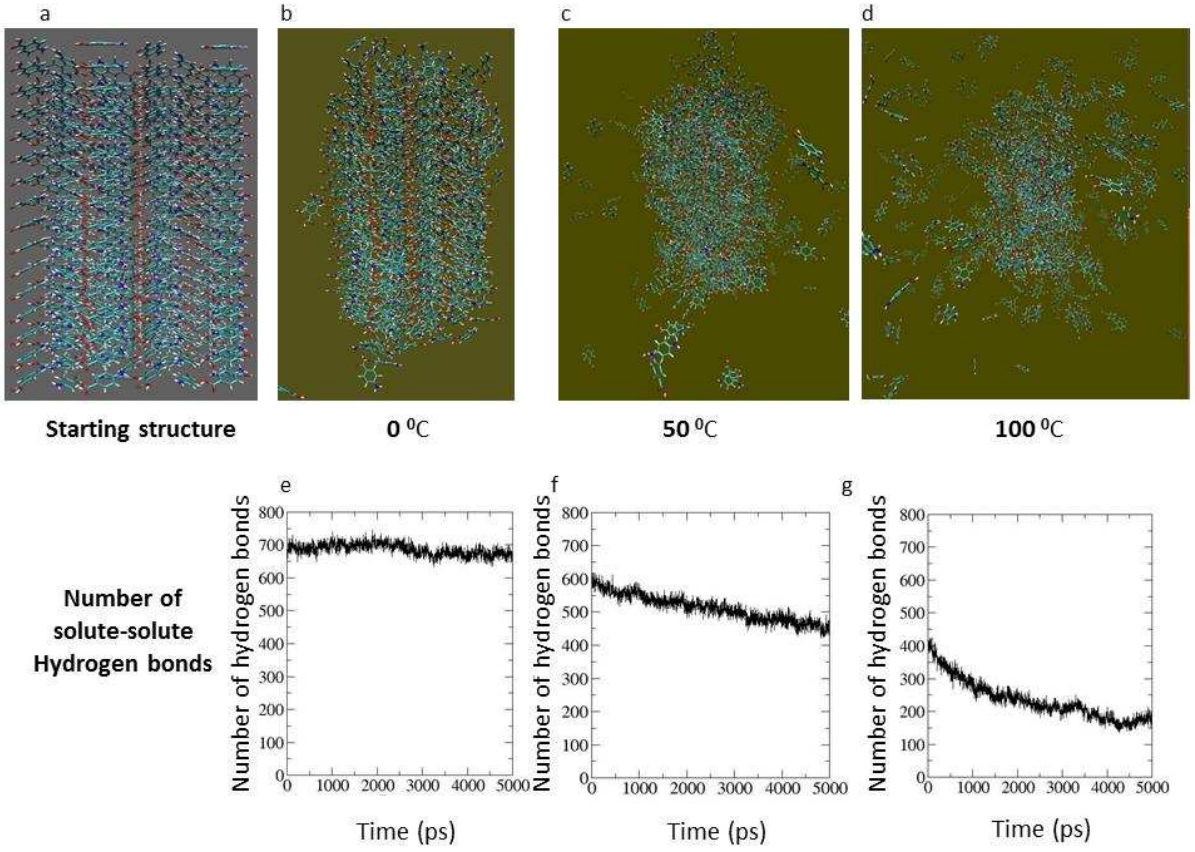

Figure 3. Results of the MD simulations of a 2 x 16 x 2 nano-crystal of PABA: Starting structure (Crystal lattice constructed in Materials Studio not the equilibrated structure) (a). Snapshot taken and the number of hydrogen bond of solute-solute after 5ns simulation respectively for 0°C (b) and (e), 50°C (c) and (f) and 100°C (d) and (g).

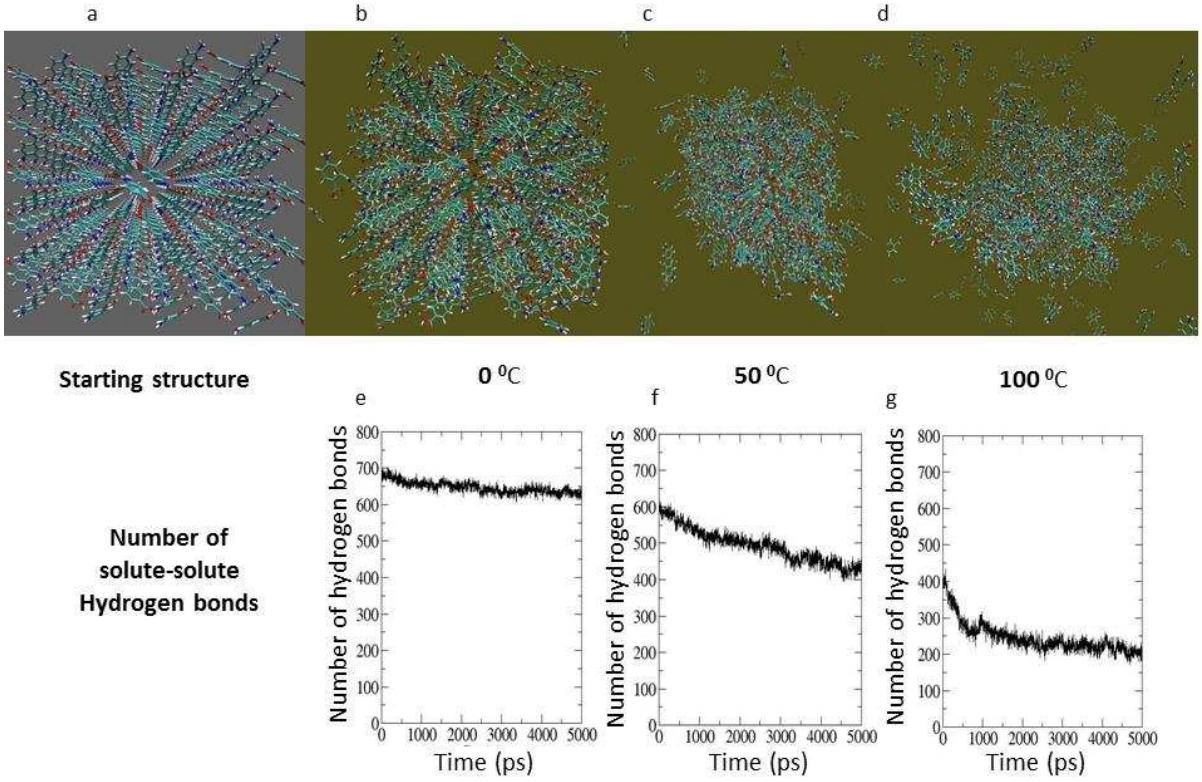

Figure 4. Results of the MD simulations of a 6 x 5 x 4 nano-crystal of PABA: Starting structure (Crystal lattice constructed in Materials Studio not the equilibrated structure) (a). Snapshot taken and the number of hydrogen bond of solute-solute after 5ns simulation respectively for 0°C (b) and (e), 50°C (c) and (f) and 100°C (d) and (g).

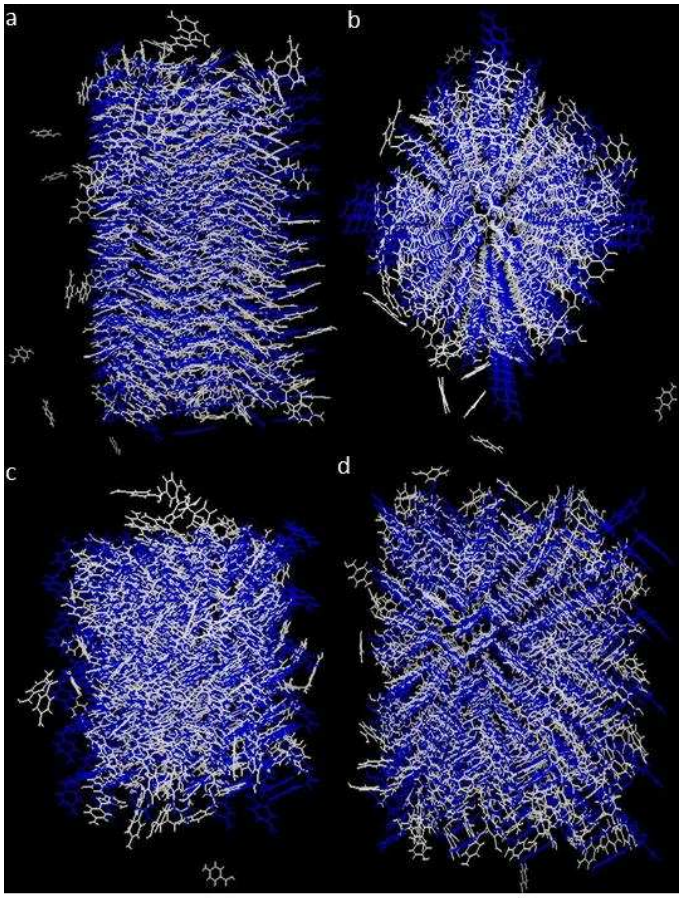

Figure 5. Overlap of the starting 2 x 16 x 2 crystal structure of the α-form (a) side view (b) top view and 6 x 5 x 4 crystal structure (c) side view (d) top view with the crystal structure image taken after 5ns simulation at 0°C.