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STRUCTURE OF SMALL ASSOCIATES OF GLYCYRRHIZIC ACID WITH CHOLESTEROL IN AQUEOUS SOLUTION: MOLECULAR DYNAMICS SIMULATION

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In a recent work by *Zelikman et al.(J. Struct. Chem., 2015, **56**(1))*, the molecular dynamics simulation of dimers of glycyrrhizic acid (GA) arising from the spontaneous collision of two GA molecules in water is performed. Several relatively stable dimer structures are found, and when a cholesterol molecule is inserted, associates are observed constituting a GA dimer with a cholesterol molecule "stuck" to it. Here, we simulate the associates consisting of three and four GA molecules and a cholesterol molecule. It appears that the cholesterol molecule, as a rule, also locates at the surface of the GA associate. Therewith, the trimers do not form any clear characteristic structures, as dimers do, and the tetramers can be two stuck dimers.

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INTRODUCTION

Glycyrrhizic acid (GA) exhibits a wide spectrum of biological activity and is used to treat and prevent different diseases [1, 2]. It has also been established that, apart from its own biological activity, GA is able to enhance the action of other drugs and improve the solubility of many hydrophobic medicinal compounds, however, the molecular mechanism of this GA action is yet not understood [2-4]. It is found experimentally that GA molecules can form self-associates in aqueous and water-alcohol solutions and complexes with many organic molecules [4-15]. The structure of these aggregates and the mechanism of their formation are also not known, although there are indications that the stoichiometry of GA complexes with an organic molecule at low concentrations is usually 2:1 or 4:1 (GA:guest molecule) [16].

The mechanism of GA promotion of the transport of slightly soluble molecules in water is a point of issue; see, for example, [4, 15]. We do not know the works on molecular dynamics simulation of GA associates and a hydrophobic molecule in water, except [17]. The attempts to simulate GA complexes using quantum chemical methods in vacuum [18] are not directly related to this problem because a solvent has a determining influence here.

In the previous work [17], the molecular dynamics simulation was used to study the spontaneous formation of GA dimers in water and its associates with cholesterol of the composition 2:1. We carried out 30 independent runs with a duration

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of 30 ns for each composition. In the first case, into a model box with 7000 water molecules two GA molecules were randomly placed as far as possible from each other; in the second case, these were two GA molecules and one cholesterol molecule. Then, the molecules freely diffused in the solution at room temperature and normal pressure. It was shown that the molecules stuck together did not decouple any more, but yet continued to move as a part of an aggregate. We identified several relatively stable dimer structures with different mutual orientations of terpene skeletons and sugar ends; with time, these structures turn into one another. In the presence of cholesterol, an associate arises in different ways: at first, a GA dimer can form, to which cholesterol then arrives, or cholesterol meets one GA molecule and then another GA molecule attaches to it. However, in the vast majority of cases, the resulting aggregate was a GA dimer with the known structure and a cholesterol molecule arranged on its surface, with the associate molecules remaining mobile.

Here, we simulate the spontaneous formation of aggregates from three of four GA molecules in the presence of a cholesterol molecule, i.e., we examine the associates of the compositions 3:1 and 4:1. As in [17], we are interested in the natural appearance of associates in solution. For that end, first we prepared a configuration in which GA and cholesterol molecules are located separately. Then, we calculated the molecular dynamics path and analyzed the structure of the aggregates formed. For the purpose of representativeness, we carried out 30 independent runs with a duration of 30 ns each.

MODELS

Fig. 1 depicts the initial configurations of our models to obtain 3:1 and 4:1 associates. The GA and cholesterol molecules were randomly placed in a model box as far as possible from each other (given the periodic boundary conditions). About 14000 water molecules were used, which is twice as large as in [17], to provide a sufficient initial separation of the molecules.

For each composition, 30 independent runs were carried out; each started from its own initial configuration. As in [17], the Gromacs software was used for the simulation [19]. Relaxation of the initial configuration was performed in two steps: at first, during 100 ps in the *NVT* ensemble using a Berendsen thermostat, then, also during 100 ps, in the *NPT* ensemble using a Nose–Hoover thermostat [20] in combination with a Parrinello–Rahman barostat [21], with equilibrium simulation parameters. The electrostatic interaction was calculated using the fast Ewald summation technique [22] with fourth-order interpolation. The force fields and parametrization of the GA and cholesterol molecules were the same as in [17]; for water, the Tip4p-Ew model was used. The whole equilibrium simulation was carried out at a pressure of 1 bar and a temperature of 300 K.

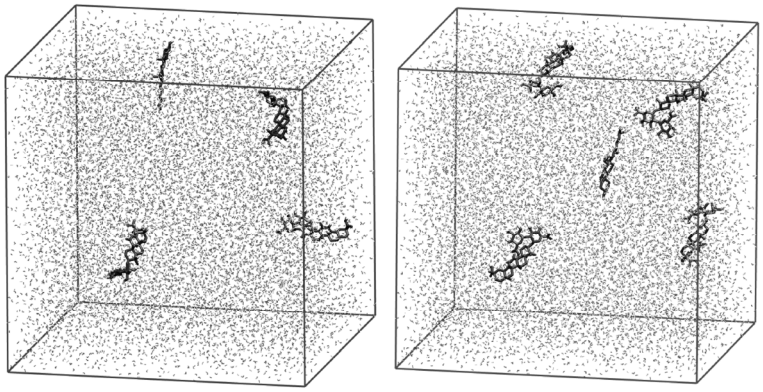

Fig. 1. Example of the initial configurations for the models of the compositions 3:1 and 4:1 (GA:cholesterol). The GA and cholesterol molecules are depicted by their skeletons, water molecules are shown by points.

IDENTIFICATION AND ANALYSIS OF THE ASSOCIATES

As shown in [17], the time when the GA and cholesterol molecules stick together can be simply and reliably determined by the value of the van der Waals interaction between these molecules. The Gromacs software provides the calculation of the interaction energy between the given groups of atoms. In this case, we propose to calculate the energy of Lennard–Jones interactions E_{LJ} between all atoms of chosen molecules not bound by covalent bonds. The E_{LJ} value experiences an abrupt jump when the molecules meet. Note that the total solvent-accessible surface area (SASA) changes in a similar way (in greater detail, see [17]).

The mutual orientation of a pair of GA molecules can be characterized by the angles between the vectors associated with these GA molecules. In [17], it was proposed to use two vectors characterizing a GA molecule (Fig. 2). The **A** vector determines the direction of the terpene skeleton of the molecule (connects the remote carbon atoms in the terminal rings of the skeleton). The **B** vector associated with a sugar end connects the remote carbon atoms in sugar rings.

For the analysis of the arrangement of a pair of GA molecules, it was proposed to calculate the angles (angle cosines) between these vectors in different molecules. For example, if the first GA molecule has the **A**1 and **B**1 vectors, and the second one, **A**2 and **B**2, then their mutual orientation is described by four angles between the respective vectors: (**A**1**A**2), (**B**1**B**2), (**A**1**B**2), and (**A**2**B**1). With these angles, we managed to identify the characteristic structures of the GA dimers [17].

For the analysis of an ensemble of dimers, it was proposed to calculate their density distribution diagram in the cos(**A**1,**A**2)–cos(**B**1,**B**2) coordinates. Concentrations (spots) on such a diagram are indicative of the characteristic dimer structures [17].

A cholesterol molecule can be characterized by one **C** vector associated with its sterane skeleton. The angles between the **C** vector and the vectors of a GA molecule (**A** and/or **B**) allow us to suggest a relative orientation of these molecules.

3:1 ASSOCIATES

Fig. 3 shows the time dependence of E_{LJ} for at once all thirty runs of the model of a solution containing three GA molecules and cholesterol. As noted above, $E_{LJ} \approx -170\ kT$ corresponds to the appearance of a GA–cholesterol pair (1:1 associate), whereas all the other molecules remain isolated; $E_{LJ} \approx -270\ kT$ corresponds to the appearance of a GA dimer (2:0); and $E_{LJ} \approx -400\ kT$ indicates the appearance of a 2:1 associate. In our figure, the level of $E_{LJ} \approx -500\ kT$ corresponds to three stuck GA molecules (3:0). Finally, the energy $E_{LJ} \approx -700\ kT$ suggests the appearance of a 3:1 associate. It is seen to form in

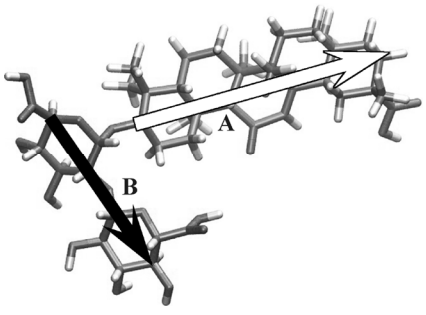

Fig. 2 Glycyrrhizic acid molecule and the associated vectors. The **A** vector characterizes the orientation of the terpene skeleton (connects carbon atoms in the terminal rings of the skeleton; the **B** vector characterizes the orientation of the sugar part (connects the remote carbon atoms in sugar rings).

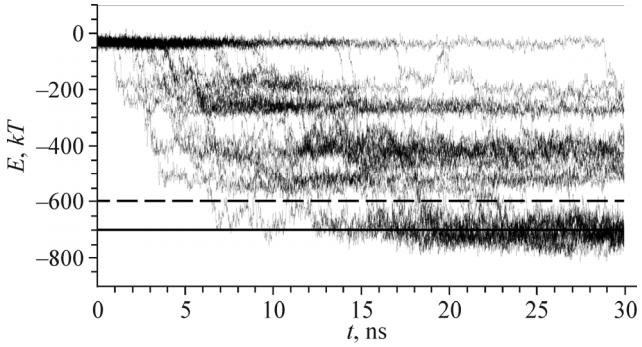

Fig. 3. Time dependence of the total Lennard–Jones interaction energy E_{LJ} between three GA molecules and cholesterol for 30 independent runs. The dashed line indicates the energy level of 600 kT, below which, we suppose, the associates have the composition 3:1. The solid line depicts the average energy level of these associates.

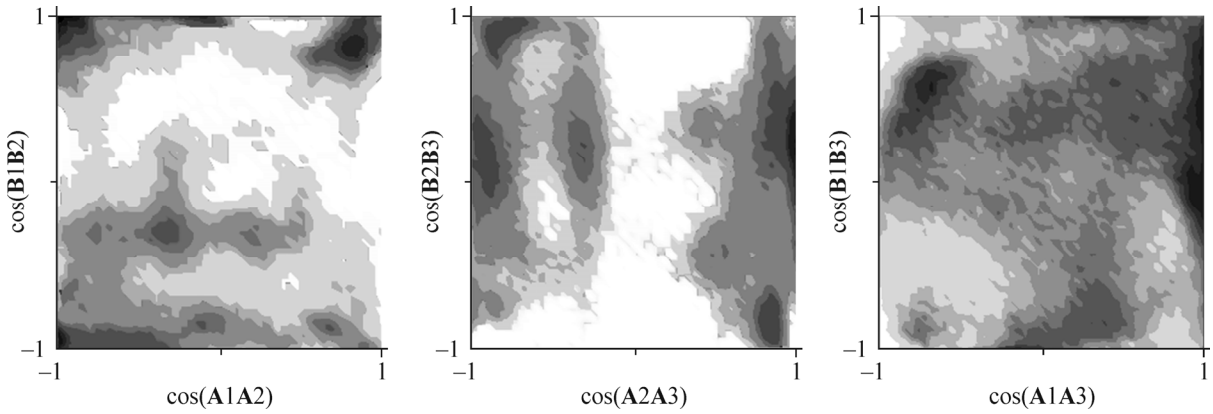

Fig. 4. Two-dimensional diagrams of occupancy in the cos(**A**1,**A**2)–cos(**B**1,**B**2) coordinates for each pair of GA molecules in the 3:1 associates.

most runs, but at different time and in different ways, and having arisen, it retains to the end of the simulation. To identify the configurations of molecular dynamics paths containing the 3:1 associate, we use the $E_{LJ} < 600\ kT$ condition (Fig. 3).

In the examination of the dimers in [17], one diagram showing the occupancy of the dimers in the cos(**A**1,**A**2)–cos(**B**1,**B**2) coordinates was calculated. Now, we have three GA molecules, i.e., there are three pairs of GA molecules in the associate. Hence, we construct three distribution diagrams rather than one (Fig. 4). Note that each 3:1 associate in these diagrams is represented by its own point with the coordinates equal to the angle cosines between the vectors of the respective pairs of molecules.

The diagrams were constructed using all 30 independent trajectories of the model with the discrimination $E_{LJ} < 600\ kT$. The approximate number of points in the diagram is 300 000. For each pair only fuzzy spots are observed. The absence of distinct areas on all three diagrams means that, for this ensemble of configurations, there are no characteristic structures similar to those observed for the dimers [17].

Considering the associates in the molecular dynamics model, we see that three GA molecules form a compact group, and a cholesterol molecule is located alongside the stuck GA molecules (Fig. 5).

The single exception to this rule was observed only in one run, where a cholesterol molecule occurred within a ring of three GA molecules (Fig. 6). This structure formed *per se* and retained to the end of the standard simulation (30 ns). To elucidate the stability of this associate, we continued the simulation with this structure. It turned out that it retained in the solution during 300 ns. However, after that, a spontaneous restructuring occurred: a ring disappeared and a usual compact trimer of GA molecules formed, while a cholesterol molecule appeared alongside. The subsequent simulation during 200 ns did not reveal any substantial changes in the structure of the associate; the ring did not form any more.

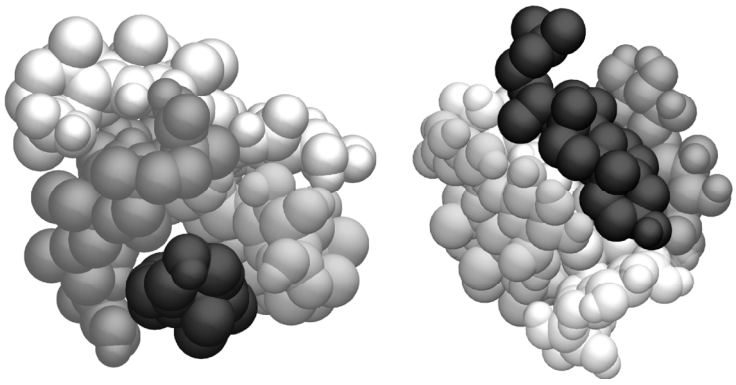

Fig. 5. Examples of the associates of the composition 3:1. Cholesterol molecule (shown in black) is located alongside the stuck GA molecules.

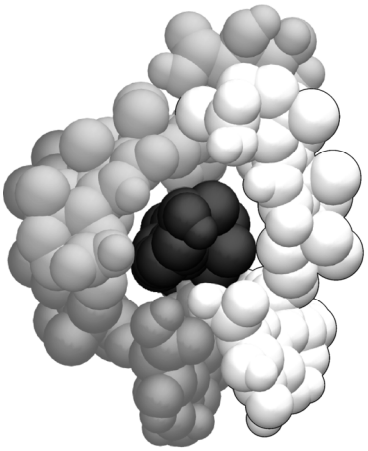

Fig. 6. Aggregate of the composition 3:1 where a cholesterol molecule (shown in black) is "captured" within a ring formed by GA molecules.

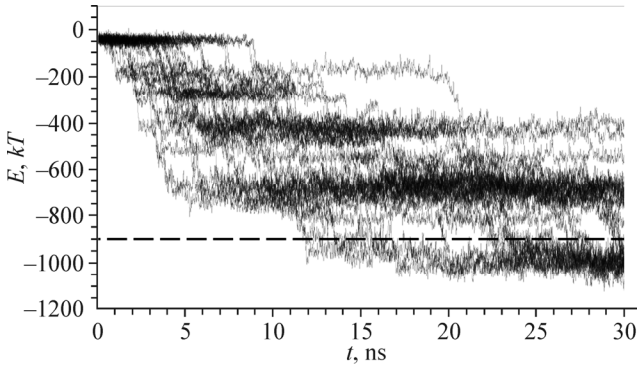

Fig. 7. Dependence of the total energy E_{LJ} for four GA molecules and one cholesterol molecule for 30 runs. The energy levels characteristic of the associates with different compositions are observed. To select the associates of the composition 4:1, the $E_{LJ} < -900\ kT$ condition is used (dashed line).

Tracking of the E_{LJ} value in this run showed that this energy is much the same both in the presence of the ring and after its breakdown. Interestingly that E_{LJ} fluctuates around –700 being the average value for isolated 3:1 associates (Fig. 3). Thus, the structure where cholesterol was within the ring formed by three GA molecules, is not characteristic. However, a quantitative estimation of the stability of these aggregates, for example, the calculation of their equilibrium constant, requires special approaches because the classical all-atom molecular dynamics does not give a direct solution to this problem.

4:1 ASSOCIATES

For the model with four GA molecules and a cholesterol molecule, we also carried out 30 runs of 30 ns. Here, the associates of all above mentioned compositions and, additionally, 4:0 and 4:1 can form. The calculation of the energy E_{LJ} makes it possible to see the formation of all these associates (Fig. 7). It is clear that the desired associates have time to appear in many runs and, as in Fig. 3, this occurs differently. The characteristic value of the energy E_{LJ} for the 4:1 associate is about –1000 *kT*. Given fluctuations, we refer to them all those for which $E_{LJ} < -900\ kT$.

To study the structure of these associates, we also calculated two-dimensional diagrams of the occupancy in the cos(**A**1,**A**2)–cos(**B**1,**B**2) coordinates. In this case, there are six diagrams according to the number of possible pairs of four

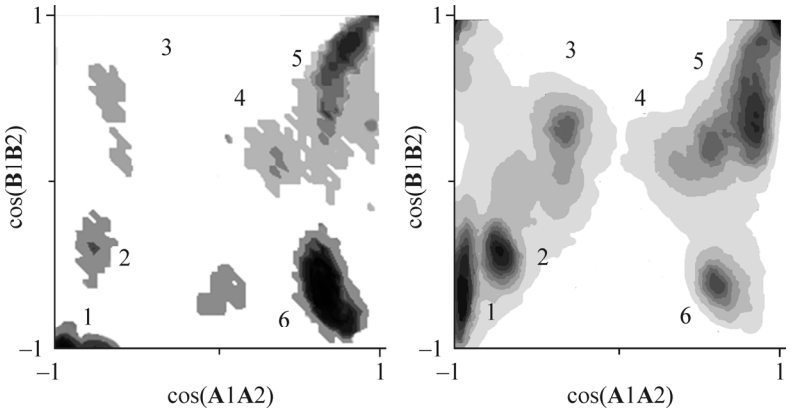

Fig. 8. Two-dimensional occupancy diagrams in the cos(**A**1,**A**2)–cos(**B**1,**B**2) coordinates for one of the pairs of GA molecules of the 4:1 associate (left) and for the dimer from [17] (right).

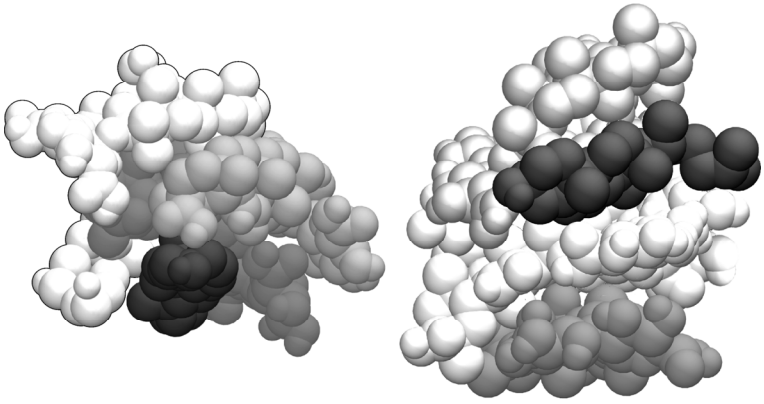

Fig. 9. Examples of the associates of the composition 4:1. Cholesterol molecule (shown in black) is located alongside the stuck GA molecules.

molecules. It turned out that in all these diagrams we can see spots that are much more isolated than those in the diagrams for the composition 3:1. Interestingly that for some pairs their location is similar to that observed for the dimers. Fig. 8 shows one of six diagrams for the 4:1 associate and the diagram for the dimers from [17]. It is evident that the similarity in the arrangement of spots cannot be accidental. The spots in this diagram for the 4:1 associate correspond to all characteristic structures of the dimer (spots in the diagram). The mutual arrangement of this pair of GA molecules in this associate is clearly the same as in the dimer.

The visualization of the 4:1 associates allows us to see that they constitute compact groups of molecules. By and large, it is difficult to judge about the structure of these clusters, especially as the molecules are in a constant relative motion. However, in many cases it can be perceived that a cluster formed by four GA molecules resembles two stuck pairs of GA dimers with the known structure [17].

Observing a cholesterol molecule in the 4:1 associates we did not find any preferred structures. As for the composition 3:1, cholesterol also tends to locate outside the cluster (Fig. 9).

Theoretically it seems possible that four GA molecules can form a ring around a cholesterol molecule. Yet, we did not observe such structures in our simulation, although sometimes a cholesterol molecule slightly penetrates into a ball cluster of GA. Nonetheless, generally it could be said that it to locates at the side of the GA cluster.

CONCLUSIONS

The molecular dynamics simulation of aqueous solutions consisting of three and four GA molecules with the insertion of a cholesterol molecule was performed. The associates of the composition 3:1 and 4:1 formed are sufficiently stable in water: during the simulation the molecules do not decouple, but remain mobile and can change their mutual orientation being a part of an associate.

The associates of the composition 3:1 are three closely bonded GA molecules with a cholesterol molecule stuck to them. We failed to find any preferred arrangement between the molecules in the associate. Three GA molecules can “capture: a cholesterol molecule in a ring. However, we have seen this configuration only once. During the subsequent simulation the ring disappeared and a usual trimer with a cholesterol molecule outside formed.

The 4:1 associates are also a close group of GA molecules with a cholesterol molecule at its surface. However, here we can perceive that in many instances four GA molecules are two stuck GA dimers, which, as has been shown previously [17], have a set of characteristic structures.

The identified stable associates of GA molecules with cholesterol on their surface illustrate the mechanism of GA promotion of the solubility and transport of hydrophobic molecules in water.

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REFERENCES

1. G. A. Tolstikov, L. A. Boltina, R. M. Kondratenko, et al., *Glycyrrhiza: Biodiversity, Chemistry, and Application in Medicine* [in Russian], NP "Geo" Academic Publishing House, Novosibirsk (2007).
2. T. G. Tolstikova, M. V. Khvostov, and A. O. Bryzgalov, *Mini-Rev. Med. Chem*., **9**, 1317-1328 (2009).
3. T. G. Tolstikova, M. V. Khvostov, A. O. Bryzgalov, A. V. Dushkin, and E. S. Meteleva, *Biomed. Khim*., **56**, No. 2, 187-194 (2010).
4. N. E. Polyakov and T. V. Leshina, *The Open Conf. Proc. J*., **2**, 64-72 (2011).
5. V. A. Vavilin, N. F. Salakhutdinov, Yu. I. Ragino, N. E. Polyakov, M. B. Taraban, T. V. Leshina, E. M. Stakhneev, V. V. Lyakhovich, Yu. P. Nikitin, and G. A. Tolstikov, *Biomed. Chem*., **54**, 301-313 (2008).
6. Yu. I. Ragin, V. A. Vavilin, N. F. Salakhutdinov, S. I. Makarov, E. M. Stakhneva, O. G. Safronova, Yu. P. Nikitin, and G. A. Tolstikov, *Bull. Exp. Biol. Med*., **145**, 285-287 (2008).
7. N. E. Polyakov, T. V. Leshina, N. F. Salakhutdinov, et al., *J. Phys. Chem. B*, **110**, 6991-6998 (2006).
8. V. S. Kornievskaya, A. I. Kruppa, N. E. Polyakov, and T. V. Leshina, *J. Incl. Phenom. Macrocycl. Chem*., **60**, 123-130 (2007).
9. K. C. James and J. B. Stanford, *J. Pharm. Pharmacol*., **5**, 445-450 (1962).
10. H. Hibasami, H. Iwase, K. Yoshioka, and H. Takahashi, *Int. J. Mol. Med*., **17**, 215-219 (2006).
11. S. Nafisi, F. Manouchehri, and M. Bonsaii, *J. Photochem. Photobiol*., **111**, 27-34 (2012).
12. N. E. Polyakov, T. V. Leshina, N. F. Salakhutdinov, et al., *Free Radical Biol. Med*., **40**, 1804-1809 (2006).
13. N. E. Polyakov, V. K. Khan, M. B. Taraban, et al., *J. Phys. Chem. B*, **112**, 4435-4440 (2008).
14. K. C. James and J. B. Stanford, *J. Pharm. Pharmacol*., **5**, 445-450 (1962).
15. O. Yu. Gluschenko, N. E. Polyakov, and T. V. Leshina, *Appl. Magn. Reson*., **41**, 283-294 (2011).
16. M. V. Zelikman, A. V. Kim, N. N. Medvedev, O. Yu. Selyutina, and N. E. Polyakov, *J. Struct. Chem*., **56**, No. 1, 67-76 (2015).
17. L. V. Lekar', A. A. Milov, S. N. Borisenko, et al., *Vestn. Yuzhn. Nauch. Tsentra*, **8**, No. 2, 18-26 (2012).
18. D. Van der Spoel, E. Lindahl, B. Hess, G. Groenhof, A. E. Mark, and H. J. C. Berendsen, *J. Comput. Chem*., **26**, No. 16, 1701-1718 (2005).
19. W. G. Hoover, *Phys. Rev. A*, **31**, 1695-1697 (1985).
20. M. Parrinello and A. Rahman, *J. Appl. Phys*., **52**, 7180-7182 (1981).
21. U. Essmann, L. M. Perera, L. Berkowitz, T. A. Darden, H. Lee, and L. G. Pedersen, *J. Chem. Phys*., **103**, 8577-8593 (1995).