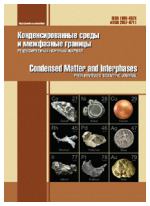


ISSN 1606-867X (Print)
ISSN 2687-0711 (Online)
Condensed Matter and Interphases
Kondensirovannye Sredy i Mezhfaznye Granitsy
https://journals.vsu.ru/kcmf/

Original articles

Research article
https://doi.org/10.17308/kcmf.2023.25/10982

Simulation of the molecular dynamics of the passage of liposome with cinnarizine through the blood-brain barrier

Yu. A. Polkovnikova✉

Voronezh State University,
1 Universitetskaya pl., Voronezh 394018, Russian Federation

Abstract

Liposomal preparations have a number of advantages: they protect the cells of the body from the toxic effects of drugs; prolong the action of the drug introduced into the body; protect medicinal substances from degradation; promote the manifestation of targeted specificity due to selective penetration from blood into tissues; change the pharmacokinetics of drugs, increasing their pharmacological effectiveness; make it possible to create a water-soluble form of a number of medicinal substances, thereby increasing their bioavailability. In this work, studies were carried out for the development of the method for determining the degree of inclusion of cinnarizine used as a corrector of cerebrovascular accidents into liposomes from soy lecithin. The aim of this study was to determine the distance between the membranes of endotheliocytes, which is critical for the passage of a liposome through the blood-brain barrier.

A simulation of changes in the structure of a liposome with cinnarizine located between two cell membranes was carried out using the molecular dynamics method at various distances between the membranes. A square planar fragment of a bilayer phospholipid membrane was assembled using the Internet service Charmm-GUI->Input Generator->Martini Maker->BilayerBuilder (http://www.charmm-gui.org/?doc=input/mbilayer). Geometry optimization and molecular dynamics simulation were performed in Gromacs 2019 using Martini 2.2 force field. According to the results of the simulation of coarse-grained molecular dynamics, a liposome from purified soy lecithin with cinnarizine adsorbed on its inner and outer surface is able to maintain integrity, being between the membranes of endotheliocytes at a distance between membranes of more than 8 nm. When the distance between the membranes of endothelial cells is less than 8 nm, the liposome with cinnarizine located between the endotheliocytes can lose its structural integrity due to fusion with the endothelial cell membrane.

As a result of the studies, the distance between the membranes of endotheliocytes was established, at which point the liposome with cinnarizine, located between endotheliocytes, can lose its structural integrity due to fusion with the endothelial cell membrane.

Keywords: Molecular dynamics, Liposomes, Cinnarizine, Blood-brain barrier

For citation: Polkovnikov Yu. A. Simulation of the molecular dynamics of the passage of liposome with cinnarizine through the blood-brain barrier. *Condensed Matter and Interphases* 2023; 25(1): 95–102. https://doi.org/10.17308/kcmf.2023.25/10982

Для цитирования: Полковникова Ю. А. Моделирование методом молекулярной динамики процесса прохождения липосомы с циннаризином через гематоэнцефалический барьер. *Конденсированные среды и межфазные границы*. 2023; 25(1): 95–102. https://doi.org/10.17308/kcmf.2023.25/10982

✉ Yulia A. Polkovnikova, email: juli-polk@mail.ru

1. Introduction

The tasks of targeted delivery of drugs, as well as increasing their bioavailability, are among the priorities in pharmacology. Currently, the development of a new generation of drugs based on liposomes is of strategic importance, since it will solve many problems associated with the targeted delivery of drugs [1]. Liposomes attract researchers as model systems for studying the mechanisms of functioning of biomembranes, as promising delivery vehicles for bioactive molecules and drugs, and also due to the possibility of wide use of liposomal forms of drugs for the treatment of various diseases [2]. Thus, the development of liposomal forms for the treatment of cerebrovascular diseases is relevant [3,4]. Currently, various groups of drugs are used for this purpose, including calcium channel blockers, one of the most widely used and economically available drug is cinnarizine [5–11]. Numerous studies of the drug have confirmed its effectiveness in diseases such as cerebral atherosclerosis without severe focal symptoms and an ischemic stroke. It is used after a haemorrhagic stroke and traumatic brain injury, with dyscirculatory encephalopathy [12].

One of the scenarios for the penetration of liposomes through the blood-brain barrier is their passage between endotheliocytes [13, 14]. Such a scenario becomes possible under pathological conditions, when the permeability of the tight junction between endotheliocytes of the blood-brain barrier increases due to an increase in the width of the gap between the membranes of adjacent cells [15].

The goal of the study was to determine the distance between endotheliocyte membranes, which is critical for the passage of a liposome through the blood-brain barrier.

2. Experimental

For the study of the process of passage of liposomes through the blood-brain barrier between endothelial cells, we simulated changes in the structure of a liposome with cinnarizine located between two cell membranes using the molecular dynamics method at different distances between the membranes. A spherical fragment of the system obtained by simulation of the molecular dynamics of liposome formation in the presence of cinnarizine was used as the initial structure. This fragment contained a liposome with a diameter of 14.7 nm with cinnarizine (125) molecules adsorbed on the inner and outer surfaces, as well as water molecules (Fig. 1).

Next, using the internet service Charmm-GUI-> Input Generator->Martini Maker->BilayerBuilder (http://www.charmm-gui.org/?doc=input/mbilayer) [16–19], a square flat fragment of a bilayer phospholipid membrane was assembled. The composition of this fragment was set based on the literature data on the composition of the cytoplasmic membrane of the endotheliocyte [20]. Also, on both sides of the membrane, layers of water molecules with the amount of Na^+ ions necessary to neutralize the negative charge of anionic phospholipids were placed (Table 1).

The liposome was placed in an aqueous environment and aligned with the endotheliocyte cell membrane model on both sides of the liposome. The initial distance between the membranes was 15.2 nm (151.81 Å) (Table 2).

Next, geometry optimization and molecular dynamics simulation were carried out in the Gromacs 2019 program using the Martini 2.2 force field [21]. During the simulation, periodic boundary conditions were used along all coordinate axes. The geometry of the system was preliminarily optimized by the gradient method. Next, thermodynamic balancing was carried out using thermodynamic balancing (310 K) and barostatting (Berendsen barostat, 1 atm) [22, 23]. The simulation step in the process of thermodynamic balancing varied from a lower (0.5 fs) to the higher (4 fs). Next, molecular dynamics simulation was carried out for 700 ns with a step of 5 fs (Table 3).

The molecular dynamics of this system was simulated in the same way with a reduced distance between the membranes. For the reduction of the distance, some of the water molecules were removed from the space between the membranes.

3. Results and discussion

The simulation results with indication of the distance between the membranes are shown in Table 4.

The spatial structures of the states of the studied system during 700 nanoseconds of molecular dynamics simulation after

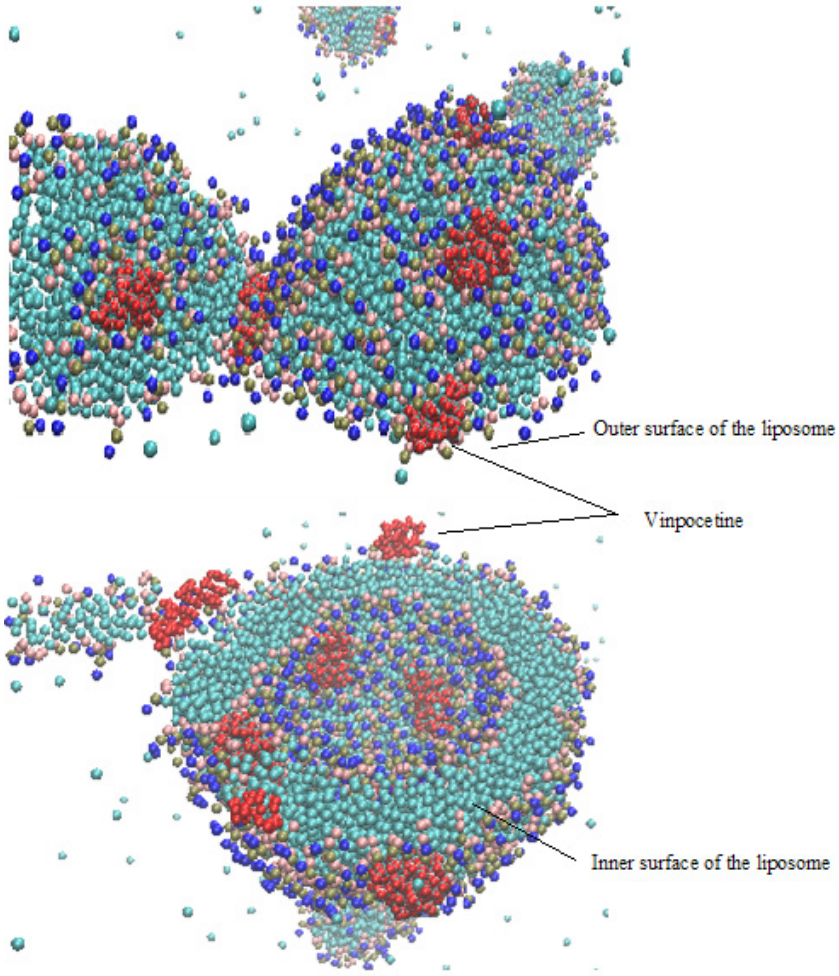


Fig. 1. Adsorption of cinnarizine molecules on the inner and outer surfaces of the liposome

Table 1. Composition of the endotheliocyte cell membrane model

Component	Abbreviation	Number of molecules
Dioleyl-phosphatidylcholine	DOPC	78
Dipalmitoyl-phosphatidylcholine	DPPC	368
Palmitoyl-oleyl-phosphatidylcholine	POPC	226
Dioleyl-phosphatidylethanolamine	DOPE	42
Dipalmitoylphosphatidylethanolamine	DPPE	72
Palmitoyl-oleyl-phosphatidylethanols	POPE	56
Dioleyl-phosphatidylserine	DOPS	14
Dipalmitoylphosphatidylserine	DPPS	12
Palmitoyl-oleyl-phosphatidylserine	POPS	14
Dipalmitoyl-phosphoinositol	DPPI	34
Palmitoyl-oleyl-phosphoinositol	POPI	52
Dipalmitoyl-sphingomyelin	DPSM	184
Palmitoyl-oleyl-sphingomyelin	POSM	98
Cholesterol	CHOL	534
Water	–	49972
Na^+ ion	–	126

Table 2. The composition of the simulated "liposome-cinnarizine-cell membranes" system

Component	Abbreviation	Number of molecules
Dilinoleoyl-phosphatidylcholine	DIPC	714
Dioleyl-phosphatidylcholine	DOPC	156
Dipalmitoyl-phosphatidylcholine	DPPC	736
Palmitoyl-oleyl-phosphatidylcholine	POPC	681
Dioleyl-phosphatidylethanolamine	DOPE	84
Dipalmitoylphosphatidylethanolamine	DPPE	144
Palmitoyl-oleyl-phosphatidylethanols	POPE	112
Dioleyl-phosphatidylserine	DOPS	28
Dipalmitoylphosphatidylserine	DPPS	24
Palmitoyl-oleyl-phosphatidylserine	POPS	28
Dipalmitoyl-phosphoinositol	DPPI	68
Palmitoyl-oleyl-phosphoinositol	POPI	104
Dipalmitoyl-sphingomyelin	DPSM	368
Palmitoyl-oleyl-sphingomyelin	POSM	196
Cholesterol	CHOL	1068
Water	-	213136
Na^+ ion	-	126
Cl^- ion		250
Cinnarizine-cation	-	125

Table 3. Stages of simulation of the "liposome-cinnarizine-cell membrane" system

Stage	Trigger type	Number of steps	Step, fs	Duration, ns	Barostat/tensiostat	Thermostat
1	Geometry optimization	10000				
2	Molecular dynamics	2000000	0.5	1	Berendsen, semi-isotropic, 1 atm in z and 1 atm x and y, time constant 6 ps	Speed scaling, time constant 1 ps, temperature 310 K
3	Molecular dynamics	5000000	1	5	Berendsen, semi-isotropic, 1 atm in z and 1 atm x and y, time constant 6 ps	Speed scaling, time constant 1 ps, temperature 310 K
4	Molecular dynamics	200000000	4	800	Berendsen, semi-isotropic, 1 atm in z and 1 atm x and y, time constant 6 ps	Nose-Hoover, time constant 5 ps, temperature 310 K

thermodynamic balancing for the system with the smallest distance between membranes (3.8 nm) are shown in Table 5. At a distance between membranes of 3.8 nm, the liposome fuses with the cell membrane. Fusion begins with the adhesion of the liposome to the surface of the cell membrane (200 ns) and the formation of a bridge between the liposome and the cell membrane (300–400 ns). Next, the semi-fusion of the liposome with the cell membrane occurs: the outer layers of the membranes of the liposome and the cell (700 ns) are merged.

4. Conclusions

1. According to the results of simulation of coarse-grained molecular dynamics, a liposome from purified soy lecithin with cinnarizine adsorbed on its inner and outer surface is able to maintain integrity, being between the membranes of endotheliocytes

Table 4. Simulation of the molecular dynamics of the "liposome-cinnarizine-cell membranes" system at different distances between the membranes

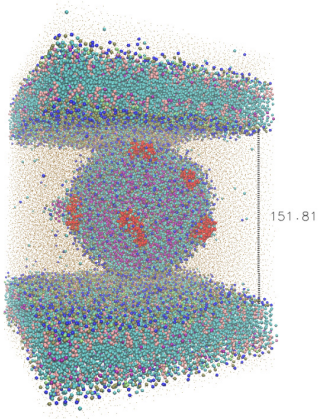
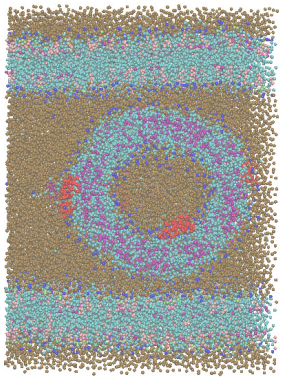
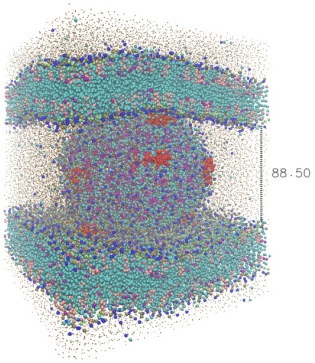
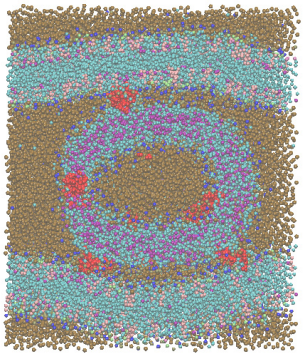
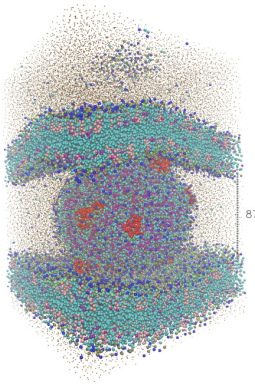
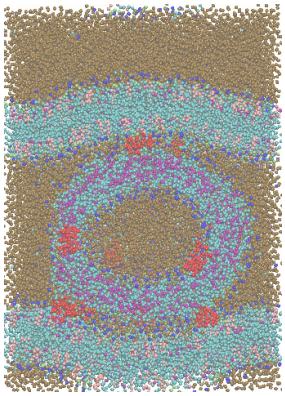
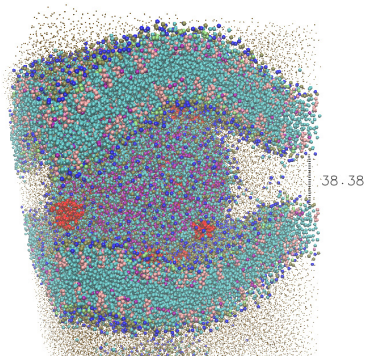
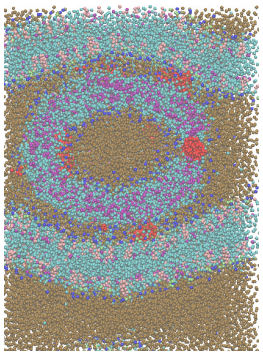
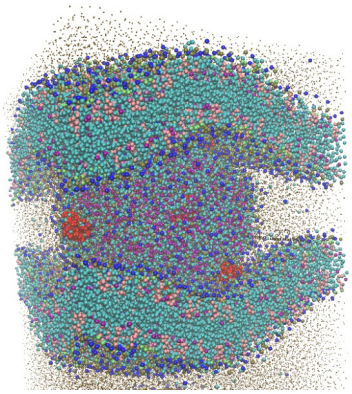
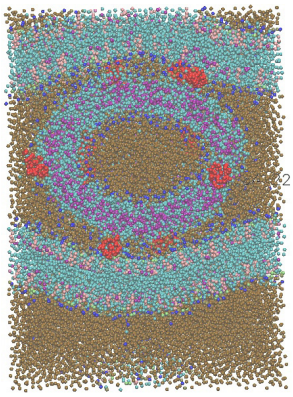
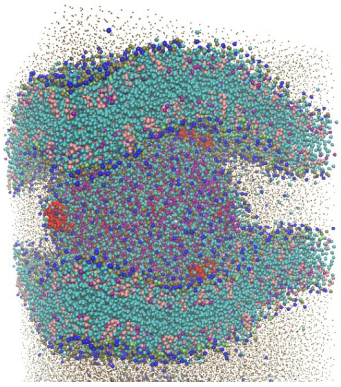
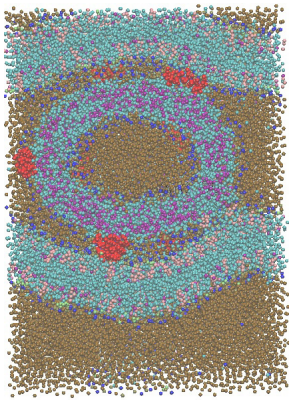
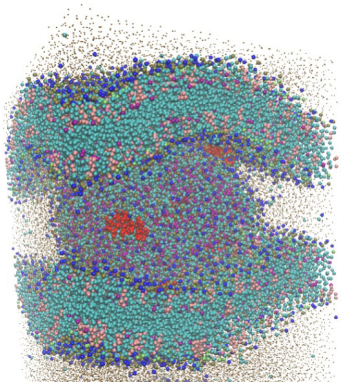
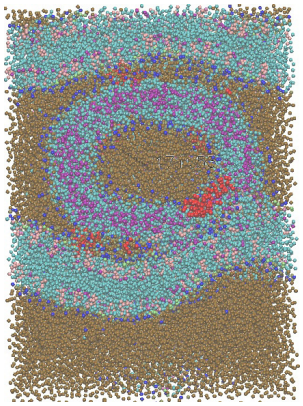
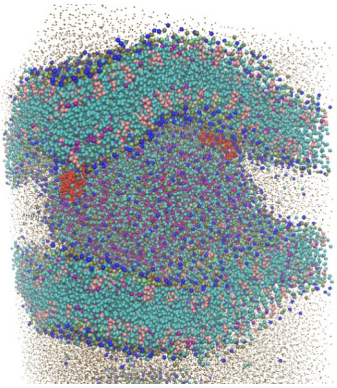
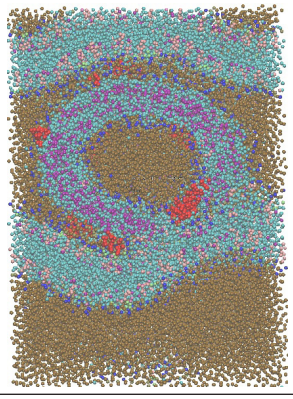
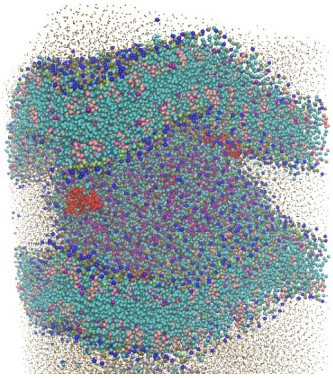
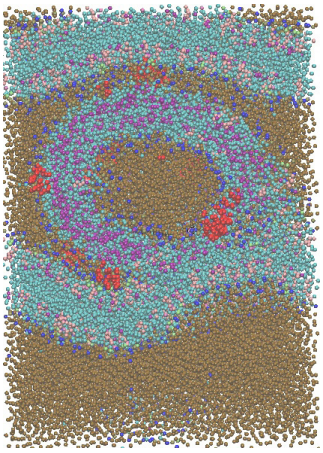
Distance between membranes, nm	System status	
15.2		
8.9		
8.7		
3.8		

Table 5. Simulation of the molecular dynamics of the "liposome-cinnarizine-cell membrane" system for a system with a distance between membranes of 3.8 nm

Time, ns	System state	
1	2	
100		
200		
400		
600		

End of table 5

700		

at a distance between the membranes of more than 8 nm.

2. The results of simulation of coarse-grained molecular dynamics also demonstrate that if the distance between endotheliocyte membranes is less than 8 nm, a liposome with cinnarizine located between endotheliocytes can lose its structural integrity due to fusion with the endotheliocyte membrane.

Conflict of interests

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Hou G., Niu J., Song F., Liu Z., Liu S. Studies on the interactions between ginsenosides and liposome by equilibrium dialysis combined with ultrahigh performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*. 2013;923–924: 1–7. https://doi.org/10.1016/j.jchromb.2013.01.035

2. Sariev A. K., Abaimov D. A., Seyfulla R. D. Drug bioavailability improvement by means of nanopharmacology: pharmacokinetics of liposomal drugs. *Experimental and Clinical Pharmacology*. 2010;11: 34–38. (In Russ.). https://doi.org/10.30906/0869-2092-2010-73-11-34-38

3. Kamchatnov P. R., Salnikova G. S., Mikhailova N. A. Chronic disorders of bran blood circulation and possibilities of their pharmacological correction. *Zhurnal Nevrologii i Psikhiatrii imeni S.S. Korsakova*. 2012;112(6): 72–75. (In Russ.). Available at: https://www.elibrary.ru/item.asp?id=17912111

4. Dolgova I. N., Starodubtsev A. I. The chronic cerebrovascular pathology in young patients. *Medical Bulletin of the North Caucasus*. 2011;1:26–29. (In Russ., abstract in Eng.). Available at: https://med-click.ru/uploads/files/docs/hronicheskaya-tserebrovaskulyarnaya-patologiya-u-patsientov-molodogo-vozrasta.pdf

5. Glukhova O. E. Liposome Drug Delivery System across Endothelial Plasma Membrane: Role of Distance between Endothelial Cells and Blood Flow Rate. *Molecules*.2020;25:1875. DOI: https://doi.org/10.3390/molecules25081875

6. Jo S., Kim T., Iyer V.G., Im W. CHARMM-GUI: A Web-based Graphical User Interface for CHARMM. *Journal of Computational Chemistry*. 2008;29: 1859–1865. https://doi.org/10.1002/jcc.20945

7. Qi Y., Ingólfsson H. I., Cheng X., Lee J., Marrink S. J., Im W. CHARMM-GUI Martini Maker for Coarse-Grained Simulations with the Martini Force Field. *Journal of Chemical Theory and Computation*. 2015;1: 4486–4494. https://doi.org/10.1021/acs.jctc.5b00513

8. Tian Y., Shen S, Gu L., Zhou J., Li Y., Zheng X. Computer-aided design of glucoside brain-targeted molecules based on 4PYP. *Journal of Molecular Graphics and Modelling*. 2021;103: 107819. https://doi.org/10.1016/j.jmgm.2020.107819

9. Dar K. B., Bhat A. H., Amin S., ... Ganie S. A. Modern computational strategies for designing drugs to curb human diseases: a prospect. *Current Topics in Medicinal Chemistry*. 2018;18(31): 2702–2719. https://doi.org/10.2174/1568026619666190119150741

10. Scholtz A. W., Hahn A., Stefflova B., ... Weisshaar G. Efficacy and safety of a fixed combination of Cinnarizine 20 mg and Dimenhydrinate 40 mg vs Betahistine Dihydrochloride 16 mg in patients with peripheral vestibular vertigo: a prospective, multinational, multicenter, double-blind, randomized,

non-inferiority clinical trial. *Clinical Drug Investigation*. 2019;39(11): 1045–1056. https://doi.org/10.1007/s40261-019-00858-6

11. Ivanova L., Nikolov R., Tsikalova P., Nikolova M. Experimental rheoencephalographic studies on the effect of the cinnarizin analogue As2 on cerebral circulation. *Acta Physiol Pharmacol Bulg*. 1979;5(2):47–52.

12. Asadi P., Zia Ziabari S. M., Majdi A., Vatanparast K., Naseri Alavi S. A. Cinnarizine/betahistine combination vs. the respective monotherapies in acute peripheral vertigo: a randomized triple-blind placebo-controlled trial. *European Journal of Clinical Pharmacology*. 2019;75(11): 1513-1519. https://doi.org/10.1007/s00228-019-02741-x

13. Sethi S., Mangla B., Kamboj S., Rana V. A. QbD approach for the fabrication of immediate and prolong buoyant cinnarizine tablet using polyacrylamide-g-corn fibre gum. *International Journal of Biological Macromolecules* 2018;117: 350–361. https://doi.org/10.1016/j.ijbiomac.2018.05.178

14. Maghsoodi M., Nokhodchi A., Oskuei M. A., Heidari S. Formulation of Cinnarizine for stabilization of its physiologically generated supersaturation. *AAPS PharmSciTech*. 2019;20(3): 139. https://doi.org/10.1208/s12249-019-1338-7

15. Wang X., Liu W., Du K. Palaeontological evidence of membrane relationship in step-by-step membrane fusion. *Molecular Membrane Biology*. 2011;28: 115–122. https://doi.org/10.3109/09687688.2010.536169

16. Hsu P-C., Bruininks B. M. H., Jefferies D., ... Im W. CHARMM-GUI Martini Maker for modeling and simulation of complex bacterial membranes with lipopolysaccharides. *Journal of Computational Chemistry*. 2017;15: 38(27):2354–2363. https://doi.org/10.1002/jcc.24895

17. van Hoogevest P., Wendel P. A. The use of natural and synthetic phospholipids as pharmaceutical excipients. *European Journal of Lipid Science and Technology*. 2014;116: 1088–1110. https://doi.org/10.1002/ejlt.201400219

18. Marrink S. J., Risselada H. J., Yefimov S., Tieleman D. P., de Vries, A. H. The MARTINI force field: Coarse grained model for biomolecular simulations. *Journal of Physical Chemistry B*. 2007;111: 7812–7824. https://doi.org/10.1021/jp071097f

19. Berendsen H. J. C., Postma J. P. M., van Gunsteren W. F., Di Nola A., Haak J. R. Molecular dynamics with coupling to an external bath. *The Journal of Chemical Physics*. 1984;81(8): 3684–3690. https://doi.org/10.1063/1.448118

20. Cansella M., Gouygoub J.-P., Jozefonvicza J., Letourneura D. Lipid composition of cultured endothelial cells in relation to their growth. *Lipids*. 1997;32: 39–44. https://doi.org/10.1007/s11745-997-0006-3

Information about the authors

Yulia A. Polkovnikova, Cand Sci. (Pharmacy), Associate Professor at the Department of Pharmaceutical Technology and Pharmaceutical Chemistry, Faculty of Pharmacy, Voronezh State University (Voronezh, Russian Federation).

https://orcid.org/0000-0003-0123-9526

juli-polk@mail.ru

Received 12.09.2022; approved after reviewing 10.10.2022; accepted for publication 15.10.2022; published online 25.03.2023.