ISSN 1990-7931, Russian Journal of Physical Chemistry B, 2016, Vol. 10, No. 7, pp. 1072–1077. © Pleiades Publishing, Ltd., 2016.
Original Russian Text © A.M. Vorobei, O.I. Pokrovskiy, K.B. Ustinovich, L.I. Krotova, O.O. Parenago, V.V. Lunin, 2015, published in Sverkhkriticheskie Flyuidy. Teoriya i Praktika, 2015, Vol. 10, No. 2, pp. 51–59.

Effect of Solvent Type and Concentration on Size and Morphology of Arbidol Microparticles Obtained by Supercritical Antisolvent Precipitation

A. M. Vorobei[a, b, *], O. I. Pokrovskiy[a], K. B. Ustinovich[a], L. I. Krotova[c], O. O. Parenago[a, b], and V. V. Lunin[a, b]

[a] *Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*
[b] *Department of Chemistry, Moscow State University, Moscow, Russia*
[c] *Institute of Laser and Information Technologies, Russian Academy of Sciences, Moscow, Russia*
**e-mail: vorobei@supercritical.ru*

Received April 30, 2015

Abstract—The capability of arbidol microparticle preparation by supercritical antisolvent (SAS) precipitation was demonstrated. A nonmonotonic dependence of the average particle size on the concentration was found, while the position of the minimum is dependent on the type of solvent used. It is possible to prepare Arbidol particles of various morphology and size from several microns to several hundred microns depending on the conditions.

Keywords: supercritical carbon dioxide, SCF micronization, supercritical antisolvent precipitation, Arbidol, bioavailability

DOI: 10.1134/S1990793116070198

INTRODUCTION

Arbidol (a pharmaceutical form of 6-bromo-4-((dimethylamino)methyl)-5-hydroxy-1-methyl-2-((phenylthio)methyl)-1H-indole-3-carboxylic acid ethyl ester hydrochloride) is an antiviral drug for the prevention and treatment of type A and B influenza and some other kinds of respiratory diseases such as severe acute respiratory syndrome (SARS) [1–4]. Arbidol activity is determined by the ability to inhibit the fusion of the virus lipid envelope with endosome membranes located within the cell. To date, arbidol is widely distributed in the domestic pharmaceutical market and is one of the main etiotropic anti-influenza drugs [5].

The main problem of using arbidol for viral disease treatment is the need for frequent administration. Arbidol is almost insoluble in water [6] and, therefore, has a low oral bioavailability (~40%). During treatment with arbidol, the drug should be taken every 3–4 h for several days, including at night to maintain the active substance concentration in blood above the therapeutic threshold (Pharmstandard, personal communication); it makes effective treatment inconvenient.

The most common approach to solve this problem is to develop a pharmaceutical form of arbidol with higher solubility in water. The methods for synthesis of arbidol complexes with acrylamide derivatives [5, 7], arabinogalactan [8], and phospholipid nanoparticles [9] were developed. A higher solubility in water is achieved for these products, but the duration for maintaining the required concentration remains unchanged. In addition, there is a risk of achieving the toxic threshold in the initial moments.

Designing the pharmaceutical form with prolonged action would be an alternative approach for this challenge. According to some data, encapsulation of the active pharmaceutical ingredient (API) in a bioresorbable polymer should be the best way to design such a form of Arbidol. The method of Supercritical AntiSolvent (SAS) precipitation has been successfully used to obtain such polymer complexes [10]. It has a number of technological advantages over the other methods of preparation of such substances; for example, it has high productivity and the absence of contamination with organic solvents.

Before the SAS coprecipitation of API and the polymer is performed, it is necessary to study this process for pure API and examine the influence of its parameters on the morphology of the particles; that is the aim of this work.

EXPERIMENTAL

The arbidol sample in the form of hydrochloride monohydrate was provided by O.I. Kiselev (Influenza

Research Institute) and was used without further purification and structure confirmation.

Dimethyl sulfoxide *puriss.* (Himmed, Russia) and methanol of HPLC grade (99.9%) (Lab-Scan, Poland) were used as solvents. Food grade carbon dioxide, used in this work, corresponded to *GOST 8085-50* (Linde Gas, Russia).

To obtain the solution of desired concentration (6.25–200 g/L), a sample of arbidol was added to a solvent, and then the mixture was treated in an ultrasonic bath until complete dissolution.

All supercritical antisolvent precipitation experiments were performed using a RESS/SAS apparatus produced by Waters (Milford, United States). The apparatus scheme and detailed description of micronization procedures were described earlier [11]. In brief, the process is as follows: the system was equilibrated under operating parameters and then the pure solvent was sprayed in order to saturate with them the supercritical (SC) CO_2 flow. Immediately after the solvent, the system was supplied with the arbidol solution. After spraying the necessary amount of arbidol, the spray nozzle was purged for some time with the pure solvent to wash the supply line and complete the arbidol addition to the system. Thereafter, the SC CO_2 flow was maintained in the system under the operating conditions to elute the organic solvent residue from the system. The washing time was defined as the time required for pumping 1–2 volumes of the precipitation vessel at the given mass flow rate and density of the fluid. The arbidol precipitation was performed at 40°C, a pressure of 15 MPa, a solution flow rate of 1 mL/min, and a CO_2 flow rate of 50 g/min. The diameter of the hole of the spray nozzle was 100 μm.

Analysis of the original arbidol sample and its micronized samples was performed by scanning electron microscopy (SEM) on a LEO 1450 microscope (Carl Zeiss, Germany) according to the standard procedure.

RESULTS AND DISCUSSION

The data on the effect of solvent type and concentration of arbidol on its form and the average size of the particles were of greatest interest. The original arbidol particles were irregularly shaped fragments, often elongated, with sizes from 0.5 to 30 μm (the average size was 14 μm).

At the first stage, a series of experiments on the arbidol micronization using methanol as solvent was performed. The anisotropic particles with either acicular or irregular structure were obtained in all studied conditions with methanol. In this paper, speaking about the size of these particles, we have in mind the length of the particle in the most extended direction. Figure 1 shows the dependence of the average particle length in the solution on the arbidol concentration. Three areas can be distinguished on this graph: (1) the region of a sharp fall of the particle length with increasing concentration (the range from 6.25 to 25 g/L); (2) the region of a soft fall of the length (from 25 to 75 g/L); (3) the region of the particle size growth with increasing concentration (from 75 to 200 g/L). In the first region there is rather sharp decrease in the average length of the arbidol particles with increasing concentration. When the concentration changes from 6.25 to 25 g/L the average particle size falls about 65 times: from 450 to 40 μm. When the concentrations of 6.25 and 12.5 g/L are used, the particles of a larger size than the Arbidol crystals before the SAS precipitation are formed. In the second region (25–75 g/L) the particle size also decreases with increasing concentration, but much more gradually: from 40 to 7 μm. The minimum of this dependence under the studied conditions is observed at the concentration of 75 g/L. The average size begins to increase with further increasing concentration. The average size of particles was equal to 35 μm at the maximum concentration used (200 g/L).

The tendency to a decrease in the particle size obtained by the SAS method with increasing concentration of the drug in the spraying solution is slightly unusual; however, this was detected not for the first time. Similar observations were made, for example, in the paper [12], wherein the micronization of the pharmaceutical substance risperidone was performed using the method of supercritical antisolvent precipitation. We hypothesize that this dependence is due to the fact that in the case of a more dilute solution, when mixing the solution and SC CO_2, the degree of supersaturation is very low, and therefore the nucleation rate is also small, and the growth process of already formed nuclei dominates. As the arbidol concentration rises in the solution, the instantaneous degree of supersaturation increases when mixing the methanol solution with supercritical CO_2, and the rate of nucleation increases proportionally. The crystal growth occurs on a greater number of crystallization nuclei, which defines a smaller average particle size.

Upon reaching a certain threshold, there is a qualitative change in the process of crystal growth with a further increase in the arbidol concentration. We hypothesize that the third region of the dependence corresponds to the situation when the volume content of crystal nuclei becomes so large that the growth of these nuclei does not takes place in isolation from each other, and proceeds in such a way that the bridges between the initially discrete nuclei grow and, as a result, connect them together. This assumption is evidenced by either the fact that the dependence of the particle size on the concentration changes its character or a qualitative change in the morphology of the particles.

Figures 2a and 2b shows photomicrographs of the Arbidol particles after micronization from the methanol solution (6.25 g/L). As can be seen, the arbidol morphology changes significantly after the antisolvent precipitation: the particles have a pronounced anisot-

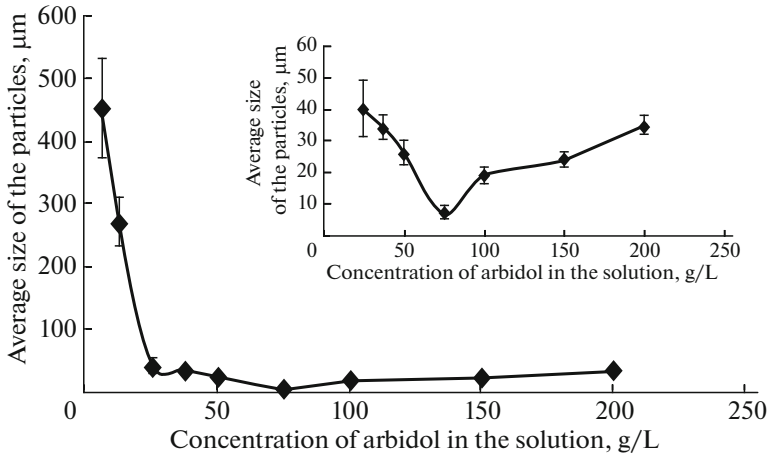

Fig. 1. Dependence of average length of the arbidol particle on concentration (solvent—methanol); sidebar—the same dependence zoomed along the axis of ordinates.

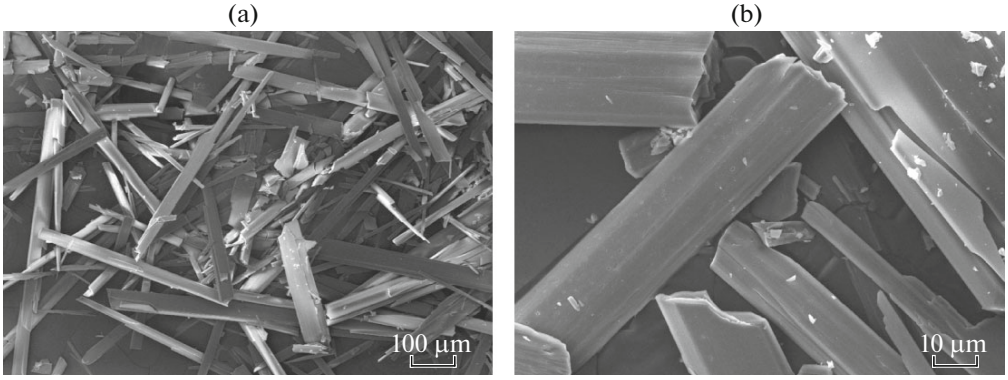

Fig. 2. Arbidol particles obtained from the methanol solution with the Arbidol concentration of 6.25 g/L; magnification: (a) 100; (b) 1000.

ropy. They represent needles with a length of 200–800 μm and a thickness of 30 μm.

When the solution concentration increases up to 25 g/L the form of the arbidol particles remains unchanged: the needles are formed, although of a significantly smaller size (length 20–110 μm, thickness 5 μm) than that at the concentration of 6.25 g/L (Fig. 3a). A gradual change in the particle morphology is observed at the concentration of 50 g/L (Fig. 3b): the fragments of irregular shape along with the needles are clearly seen on the photomicrographs (SEM); they have more pronounced structure irregularity compared with the needles. At the arbidol concentration of 75 g/L (Fig. 3c), which corresponds to the minimum particle size in the range of concentrations studied, the needles are almost absent, and the particles represent the fragments described above.

Such a change in morphology corresponds to the assumption mentioned above, which explains the reduction in the arbidol crystal size with increasing concentration. The growth process occurs on a small number of nuclei at sufficiently low concentrations. Growing crystals do not interfere with each other and grow along the most advantageous direction. The number of nuclei is much higher at a sufficient concentration and the fusion of growing nuclei becomes possible, resulting in the formation of less oriented, less ordered crystals, as well as an overall increase in the particle size. Such a qualitatively new picture is observed at a concentration of 75 g/L.

In the concentration range of 75–200 g/L the particle morphology has also undergone dramatic changes. The major part of arbidol forms needles with increasing concentration in the solution from 75 to 200 g/L. The number of less anisotropic fragments is reduced, and at the concentration of 200 g/L in the SEM photomicrographs, the needles with the average length of 35 μm constitute the bulk of the precipitated particles (Fig. 3d).

To evaluate the effect of solvent type on the morphology of the arbidol particles, the experiments were carried out on its precipitation from the solution of dimethyl sulfoxide (DMSO). Figure 4 shows the

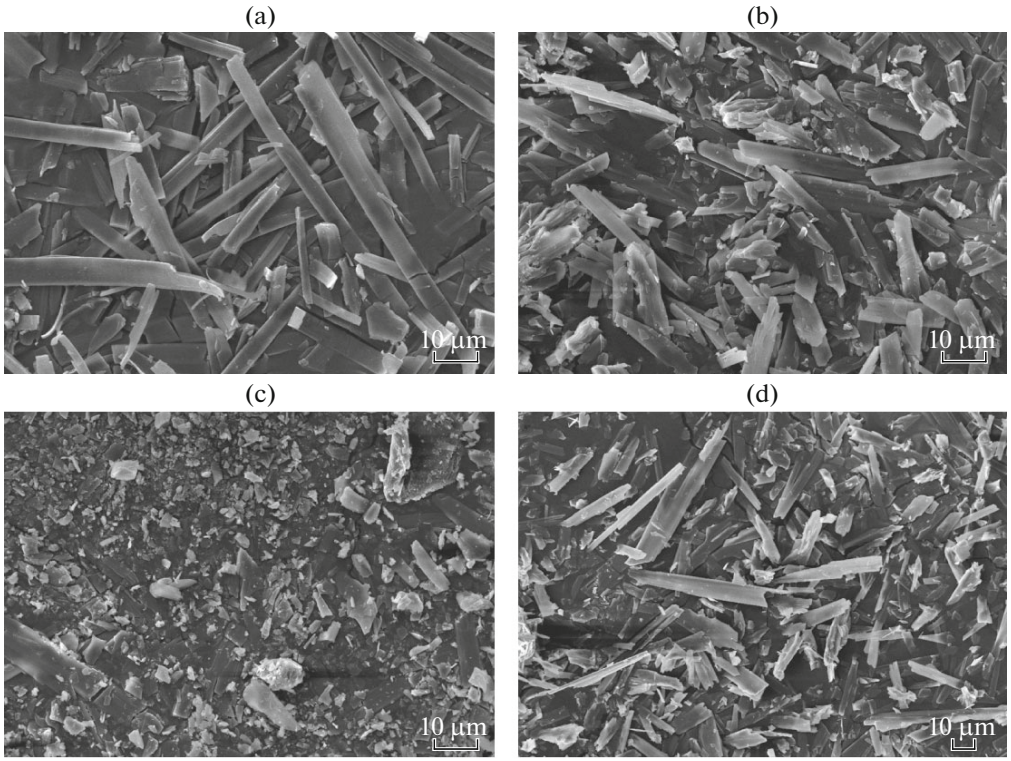

Fig. 3. Arbidol particles obtained from the methanol solution with the arbidol concentration of: (a) 25 g/L; (b) 50 g/L; (c) 75 g/L; (d) 200 g/L.

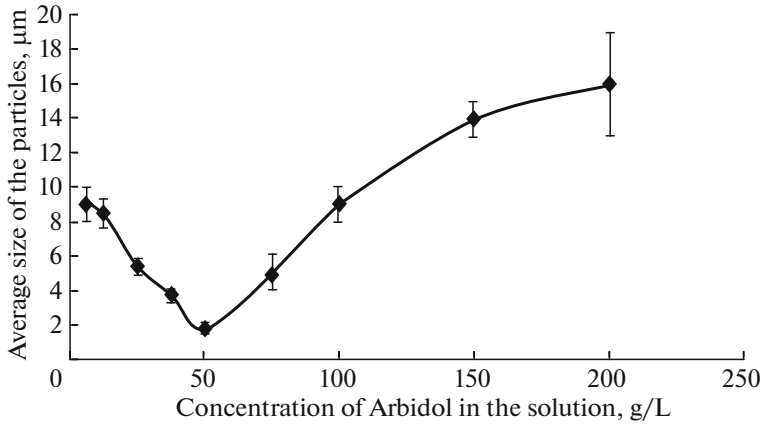

Fig. 4. Dependence of average arbidol particle size on its concentration in the solution (solvent – DMSO).

dependence of the average particle size on the arbidol concentration in the solution.

Two regions in the graph of the dependence of the average particle on the concentration can be distinguished for DMSO solutions. The first region, corresponding to the concentration from 6.25 to 50 g/L, is characterized by a decrease in the arbidol particle size with increasing concentration. In the second region (50 to 200 g/L), in contrast, the particle size increases with increasing concentration. However, in contrast to methanol, it is clearly seen that in the region of initial concentrations in the case of DMSO there is no sharp decrease in the particle size with increasing concentration. In the concentration range of 6.25–50 g/L, a relatively weak (compared with methanol) reduction in the particle size (from 9 to 1.8 μm) occurs. Apparently, this is due to a lower solubility of the CO_2–DMSO mixture towards arbidol compared with the CO_2–methanol mixture and, hence, a higher degree of supersaturation at low arbidol concentrations. Proba-

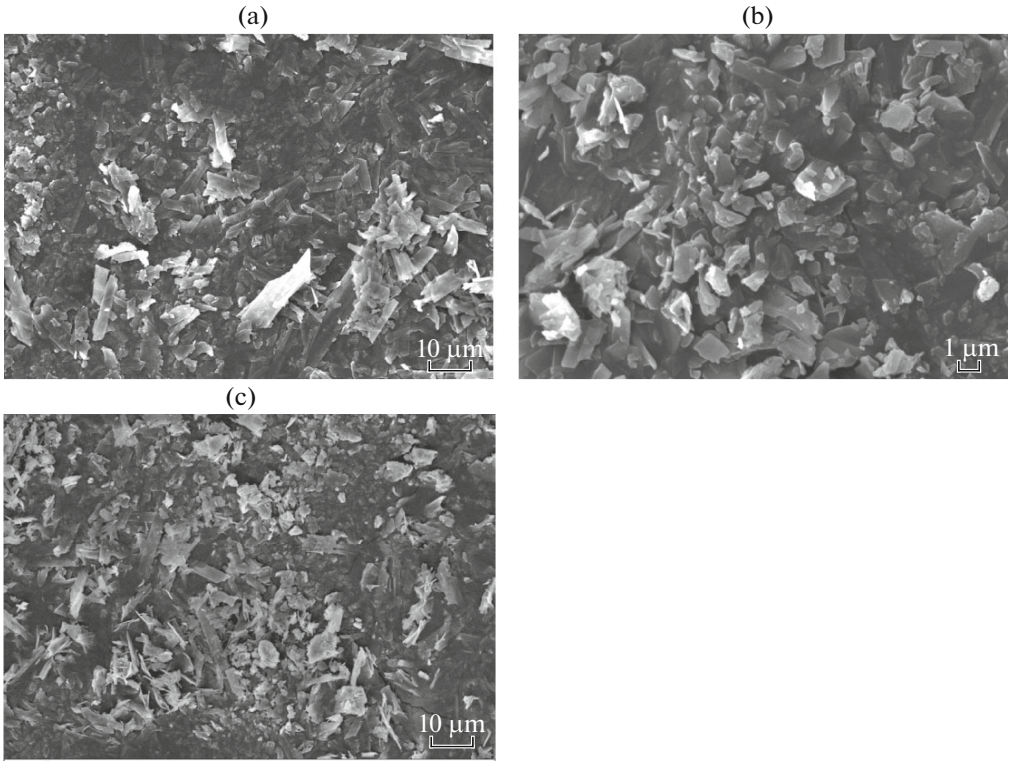

Fig. 5. Arbidol particles obtained from the DMSO solution with the concentration of: (a) 6.25 g/L; (b) 50 g/L; (c) 200 g/L.

bly, dependences similar to the first region of the concentration curve for the methanol solutions will be observed under further decrease in the arbidol concentration in DMSO or decrease in the ratio of DMSO and SC CO_2 mass flows.

In the concentration range of 50–200 g/L, the particle size increase is observed with increasing arbidol concentrations in DMSO. Most likely, the causes for this change in the character of dependence of the particle size on the concentration are similar to the causes described above for the case of spraying of the methanol solution.

The arbidol concentration, at which the particle size is minimal, is 50 g/L for DMSO and 75 g/L for methanol.

The SEM photomicrographs of the arbidol samples, obtained by antisolvent precipitation from the DMSO solutions with the concentration of 6, 25, 50, and 200 g/L, respectively, are given in Figs. 5a, 5b and 5c. From the presented data we can conclude that the type of the solvent has a determining influence on the morphology of the obtained arbidol particles. In contrast to the particles obtained by the precipitation from methanol solutions, for methanol, the typical needles are not formed in the studied concentration range when using DMSO. In the case of DMSO there are various fragments of an irregular shape, often having a high anisotropy. In general, a wide variation in the shape is typical for the particles precipitated from DMSO. The search for correlation between the morphology of the particles and the arbidol concentration in DMSO in severely hindered, i.e., explicit dependence between the form of the precipitated particles and the arbidol content in the solution was not established.

The development of methods for obtaining arbidol complexes with bioresorbable polymers in order to produce a pharmaceutical form of the drug with prolonged action will be a further direction of the present work. Currently, various candidates for the role of bioresorbable polymer support, such as copolymers of lactic and glycolic acids, poloxamers, polyanhydrides, alkyl derivatives of cellulose, etc., are considered. The search for the most effective combination of the polymer with arbidol is a complex multiparameter task; it is very labor-intensive. A high-performance rapid method for producing test batches of AFI complexes with polymers is required to carry out this study. Previously, it has repeatedly been demonstrated that the SAS method is well suited for rapid screening of crystallization conditions for different pharmaceutical substances, including the search for optimal conditions for preparing cocrystals [13] or various polymorphic forms of API [14]. We suggest that the SAS method may be used just as effectively for screening AFI complexes with bioresorbable polymers. The regularities of arbidol crystallization during the SAS process, disclosed in the present work, will be taken into

account in the future study. The found values of the concentrations, corresponding to the minimum in the concentration dependences, will serve as a starting point during the development of polymer/arbidol coprecipitation methods.

CONCLUSIONS

Thus, the possibility of obtaining Arbidol microparticles of different sizes and morphology was demonstrated by supercritical antisolvent precipitation. The effect of the solvent and arbidol concentration in the solution on the micronization results was considered. It was shown that the dependence of the average particle size on the substance concentration in the sprayed solution is nonmonotonic. An increase in the concentration results in a decrease in the particle size until a certain threshold value; the trend is reversed when this threshold is reached. This threshold value depends on the type of organic solvent. The values of the concentrations, at which the crystal size is minimal, were found. The dependence of the crystal morphology on the arbidol concentration in the sprayed solution is also nonmonotonic. The inflection of the curve on the concentration dependence graphs is probably due to particle fusion.

ACKNOWLEDGMENTS

The authors would like to thank O.I. Kiselev (Research Institute of Influenza) for providing samples of Arbidol. This work was financially supported by the Russian Foundation for Basic Research, project no. 12-03-12270 ofi-m (production of micron powders), and the Russian Science Foundation, project no. 14-33-00017 (studying of the products by scanning electron microscopy).

REFERENCES

1. Y. S. Boriskin, I. A. Leneva, E.-I. Pecheur, and S. J. Polyak, Curr. Med. Chem. **15**, 997 (2008).
2. M.-Y. Liu, S. Wang, W.-F. Yao, H. Wu, S.-N. Meng, and Min-Jie Wei, Clin. Ther. **31**, 784 (2009).
3. Y. S. Boriskin, E.-I. Pecheur, and S. J. Polyak, Virol. J. **3**, 56 (2006).
4. M. J. Brooks, E. I. Burtseva, P. J. Ellery, G. M. Marsh, A. M. Lew, A. N. Slepushkin, S. M. Crowe, and G. A. Tannock, J. Med. Virol. **84**, 170 (2012).
5. M. Yu. Eropkin, M. V. Solovskii, M. Yu. Smirnova, et al., Khim.-Farm. Zh. **43** (10), 27 (2009).
6. Arbidol. Pharmacopeia art. FS 42-0216-07 instead of art. GF-XII, Pt. 1.
7. RF Patent No. 2394618 (2008).
8. RF Patent No. 2475255 (2011).
9. RF Patent No. 2411942 (2009).
10. M. J. Cocero, A. Martin, F. Mattea, and S. J. Varona, Supercrit. Fluids **47**, 546 (2008).
11. A. M. Vorobei, K. B. Ustinovich, O. I. Pokrovskiy, O. O. Parenago, and V. V. Lunin, Russ. J. Phys. Chem. B **9**, 1103 (2015).
12. V. N. Bagratashvili, A. M. Egorov, L. I. Krotova, A. V. Mironov, V. Ya. Panchenko, O. O. Parenago, V. K. Popov, I. A. Revelsky, P. S. Timashev, and S. I. Tsypina, Russ. J. Phys. Chem. B **6**, 804 (2012).
13. L. Padrela, M. Rodrigues, S. P. Velaga, et al., Eur. J. Pharm. Sci. **38**, 9 (2009).
14. WO Patent No. 2005/105293 A1 (2005).

Translated by D. Novikova