ISSN 1070-3632, Russian Journal of General Chemistry, 2014, Vol. 84, No. 5, pp. 1010–1017. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © Yu.N. Morozov, A.Yu. Utekhina, V.P. Shabatin, V.V. Chernyshev, G.B. Sergeev, 2012, published in Rossiiskii Khimicheskii Zhurnal, 2012, Vol. 56, Nos. 5–6, pp. 43–51.

Cryosynthesis of Nanosized Drug Substances

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Received November 1, 2012

Abstract—The specific features of cryochemical technology for producton of new nanosized modifications of drug substances are observed. The method is based on transformation of the initial substance in gas phase and followed by the organization of molecular flow to the cooled surfaces. The results of study of physico-chemical properties of nanosized drug substances obtained for different applications are presented: cardiological carvedilol, cytostatic imatinibe mesilate, steroid hormone Δ^5 androstendiol-3β,17β, psychoactive phenasepam. On example of the last compound the results of biological tests are presented.

DOI: 10.1134/S1070363214050387

Widespread attention received by nanoscale objects at the turn of the 21 century has led to emergence of new directions in physics, chemistry, materials science, and medicine. It was found that nanoparticles possess novel, previously unknown properties, which underlie creation of unconventional devices and materials and development of nanotechnologies. Particles measuring 1–10 nm display unusual chemical activity and can enter into interactions not found for large aggregates. For chemical application of nanoparticles, see monographs [1, 2].

In recent years, the focus in development of nanotechnology has shifted from materials science and electronics toward biology and medicine, so scientists have good reasons to believe that this will facilitate creation of new medicines.

One medicinal application of nanoparticles is early diagnosis of diseases, in particular, through the use of high-sensitivity semiconductor sensors and sensing devices able to monitor the human body condition [3].

Nanoparticles-mediated drug delivery to diseased organs is another medicinal application which is undergoing rapid development today. Specifically, drug substances are transported via preferential release of drug nanoparticles into the damaged tissue due to locally enhanced, because of pathology, microvascular permeability (passive transport). For active drug transport, it is suggested that the nanoparticle surface be labeled with antibodies or other recognizing elements to provide highly selective binding of nanoparticles to antibodies expressed on the damaged cell surface [4].

The third application, in our opinion, is regenerative medicine, which seeks to introduce a nanosized drug particle into the body. Such particles possess enhanced thermodynamic and kinetic activities compared to the initial agent and can be administered in smaller doses. In turn, fewer side effects will occur, and the own capacity of the body to fight diseases will be improved.

Synthetic routes to nanoscale organic compounds intended for development of new drug substances differ from those used for preparation of metal and semiconductor nanoparticles. The chemical reduction processes in solution, widely applied in the latter case, have virtually no practical application in synthesis of organic nanoparticles.

Today, nanoparticles of organic compounds are typically obtained by mechanical grinding. Brought into commercial implementation, this technique has widespread application in preparation of drug suspensions by pharmaceutical firms. For laser ablation systems, water-in-oil emulsions, and supercritical fluids as applied to preparation of nanoforms of drug substances and other organic nanoparticles, see review [5].

Since recently, much attention in pharmacy has been paid to the multiplicity structural forms of drug substances (polymorphism). Synthesis of new

polymorphs of molecular crystals underlies a possible approach to imparting new physicochemical and therapeutic properties to existing drugs [6]. Polymorphic transformations may change properties of compounds, e.g., their dissolution rate, solubility, morphology, and surface energy. In the case of drug substances, therapeutic properties may be affected. Particularly profound changes can be expected from synthesis of nanosized polymorphic modifications.

Here, we consider low-temperature methods used for preparation of nanosized modifications of drug compounds.

Cryochemical synthesis of organic nanoparticles is based on transferring the original substance to the gas phase by vaporization or sublimation and arranging a directed flow of molecules towards the cooled surface. The molecular flow interacts with the cooled surface, the substance from the gas phase is condensed, and solid nanoparticles are formed.

Two methods were developed for transferring a substance to the gas phase, which differ by the way in which the molecular flow towards the cooled surface is arranged and in the mechanism of solid particles formation. One method consists in vaporization or sublimation by local surface heating of the original substance layer in a high vacuum ($<5 \times 10^{-5}$ mmHg), whereby poorly volatile and thermally unstable organic compounds can be vaporized [7, 8]. Under vacuum condition, organic molecules leave the original substance surface with a low kinetic energy and reach the cooled surface after experiencing negligible number of collisions with other molecules. The contact with the cold surface may result in condensation, reflection, and migration. The accommodation of molecules on the surface is increased by lowering the temperature with the use of liquid nitrogen. When contacting the surface, the substance molecule loses the excess energy and gets stabilized by forming a solid-phase structure. High rates of losing the excess energy on the surface at liquid nitrogen temperature ensure formation of small-sized solid-phase nanoparticles and stabilization of possible metastable structures.

The other method consists in vaporization or sublimation in a flow of a heated carrier gas by which the compound vapor is entrained. When approaching the cold surface, the stream of the carrier gas with the substance vapor is rapidly cooled, which leads to multiple supersaturation of the gas phase with respect to the compound vapor pressure, whereby conditions conducive to rapid gas-phase nucleation are created in the system. High nucleation rates, in turn, are responsible for reduced compound vapor content in the gas phase, so further growth of crystallites is prevented. This method is unique in that, due to a temperature gradient, favorable conditions for a new nucleation stage are permanently reproduced in the system. The growth of the new phase nuclei is based on the molecules or clusters of the substance from the gas phase. The growing particles can collide in the gas phase and aggregate. The nanoparticles formed in the gas phase are subsequently captured by the cold surface and thus stabilized.

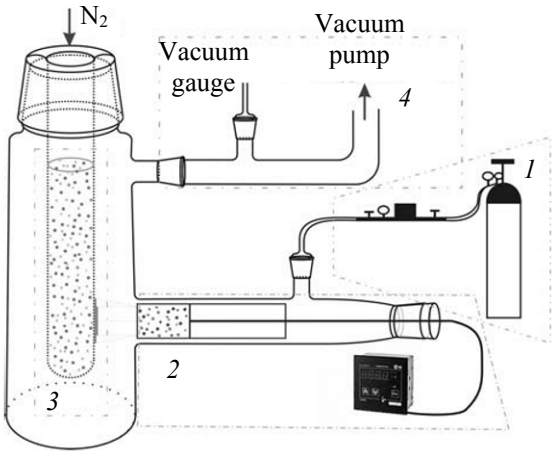


Fig. 1. Schematic of the setup for cryochemical modification of organic compounds by the sublimation with a carrier gas–low-temperature condensation route: (*1*) carrier gas stream generating unit, (*2*) vapor generating unit with temperature regime programmer, (*3*) low-temperature condenser unit, and (*4*) vacuum unit.

A setup for cryochemical modification includes several operating units (Fig. 1):

– a carrier gas stream generating unit which comprises a source of the carrier gas (typically, a metal container filled with the gas and a gas pipeline providing for the carrier gas supply to the system in the preset mode);

– a vapor generating unit represented by a container filled with the original organic compound particles that are uniformly distributed over its volume, through which the carrier gas stream passes; the container is provided with a heating system and a temperature control system; a special cap on the vapor generator nozzle provides for desired geometry of the gas jet enriched in the organic compound vapor;

– a low-temperature condenser unit constituted by a cylindrical finger into which a coolant, typically liquid nitrogen, is poured; and

– a vacuum unit for creating a directed flow of the carrier gas to the cold surface.

The above-described methods were used for preparation of a number of drug nanoforms without affecting the original drug substance composition.

To consider the specific features of cryosynthesis of drug nanosubstances and the methods proposed, we will take advantage of the conventional equations of thermodynamics and kinetics.

The vapor generator efficiency can be estimated using the Hertz–Knudsen equation describing the sublimation-desublimation processes [9]:

$$r_{\text{eff}} = \beta(P_0 - P)\sqrt{\frac{1}{2\pi\mu RT}}, \qquad (1)$$

where P_0 is the saturated vapor pressure of the substance at temperature T; P, real vapor pressure of the substance in the gas phase; m, molecular weight of the substance; and β, sublimation–condensation coefficient ($0 < \beta < 1$).

Equating the rate of ejection of the substance from the vapor generator nozzle

$$\frac{dN}{dt} = \frac{P}{RT}\frac{dV}{dt} \qquad (2)$$

to that of the substance flow to the gas phase during sublimation and considering the vapor generator temperature T as constant, we obtain:

$$\frac{P}{P_0} = \frac{1}{1 + (dV/dt)\,(1/\beta S_{\text{eff}})\sqrt{2\pi\mu/RT}}, \qquad (3)$$

where P is vapor pressure of the substance in the flow ejected from the generator nozzle; S_{eff}, effective total surface area of the substance that undergoes sublimation; and dV/dt, carrier gas flow rate.

The carrier gas flow rate is typically 900 mL h^{-1} (0.25 cm^3 s^{-1}) at atmospheric pressure and temperature of 298 K. Under real conditions, the pressure in the reactor is several millimeters of mercury. Taking the lowest pressure in the reactor as 1 mmHg, we obtain the carrier gas flow rate of 0.26×10^{-3} m^3 s^{-1}.

The nucleation in the gas phase can be described using the modified Volmer equation which, when applied to nucleation of cubic nuclei, appears as follows [10]:

$$I = \frac{6d_k^2 Pc}{\sqrt{2\pi mkT}}\sqrt{\frac{1}{2\pi kTN_k^2}}\exp\left(-\frac{A_z}{kT}\right), \qquad (4)$$

$$A_z = \frac{32\sigma^3\mu^2}{\rho^2(RT)^2[\ln\{P/P_0(T)\}]^2} = 32\sigma^3\left[\frac{V_m}{RT\ln[P/P_0(T)]}\right]^2, \qquad (5)$$

$$d_k = \frac{4\sigma\mu}{\rho RT\ln[P/P_0(T)]} = \frac{4\sigma V_m}{RT\ln[P/P_0(T)]}, \qquad (6)$$

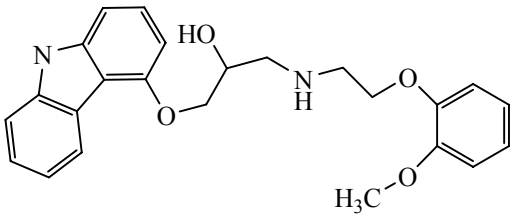
where I is the nucleation rate, particles m^{-2} s^{-1}; A_z, free energy of formation of critical nucleus; d_k and N_k, size of the cubic critical nucleus and number of molecules therein, respectively; σ, surface energy; ρ, density; V_m, molar volume; and $P/P_0(T)$, degree of supersaturation. This equation contains a Zel'dovich correction which takes into account possible loss of critical nucleus.

The Volmer equation allows estimating the maximum achievable nucleation rate. The degree of supersaturation can be determined by the Clapeyron-Clausius equation:

$$\frac{P_0(T)}{P_0(T_0)} = \exp\left[\frac{\Delta H_{\text{subl}}}{R}\left(\frac{T - T_0}{TT_0}\right)\right]. \qquad (7)$$

Detailed analysis of the crystal formation and growth processes occurring in a carrier gas jet requires that the system of Eqs. (4)–(7) be supplemented by the mass and heat balance equations.

Below we give several examples of how cryochemical methods were used for preparation of drug substance nanoparticles and present the results of analysis of carvedilol, imatinib mesylate, androstenediol, and phenazepam that were synthesized by the cryochemical techniques.



Carvedilol [11, 12]

Carvedilol is a drug belonging to the class of cardiologic α- and β-adrenoblockers. Cryochemical modification of carvedilol was carried out by the sublimation with a carrier gas–low-temperature

condensation route and yielded an amorphous powder. The nuclear magnetic resonance examinations (^{13}C NMR, ^{1}H NMR) revealed identity of the resulting X-ray amorphous powder to the original carvedilol.

Purity assessment (thin-layer chromatography and HPLC methods) showed that the resulting amorphous carvedilol has a total impurity content less than 1%, being in compliance with the appropriate normative document ND 42-11503-01 for carvedilol drug substance.

Thus, the analytical data (NMR spectroscopy, thin-layer chromatography, and HPLC) show that the cryomodified substance is carvedilol.

According to the XRD data, the cryomodified carvedilol occurs in the amorphous state. As to the original carvedilol substance, its XRD pattern contains a set of diffraction maxima, indicative of its crystalline state. The corresponding diffraction patterns are shown in Fig. 2.

The IR spectra of the original and amorphous carvedilol differ in the 1000–800 cm^{-1} region.

The original carvedilol substance exhibits a single melting endothermic peak at 114°C, while the carvedilol synthesized by the low-temperature route has no fixed melting point (according to the differential scanning calorimetry data).

Examination of the particle size distribution for the original and cryomodified carvedilol shows that, along with amorphization, the cryomodification procedure leads to the particle size reduction from >10 μm to <1 μm.

To test the biological activity of the amorphous carvedilol, two groups of rats were administered the original crystalline and the cryomodified amorphous carvedilol. Systemic blood pressure measurements showed that the amorphous substance causes the pulse interval in animals to increase, evidently due to blocking of β_1-adrenergic receptors of the heart. This leads to reduction of the baroreflex coefficient in animals, caused by phenylephrine and isoprenaline administration, and thereby indicates an increased bioactivity of the cryomodified carvedilol compared to the original drug.

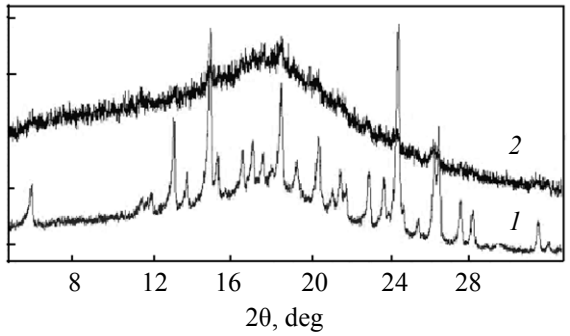
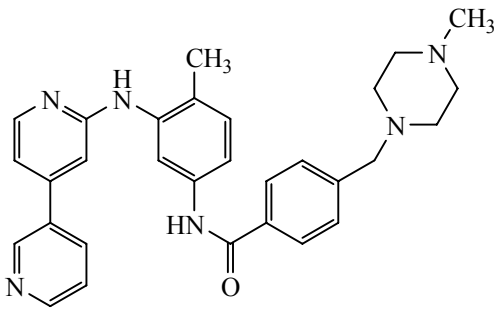


Fig. 2. X-ray diffraction patterns of the (*1*) original and (*2*) cryochemically modified carvedilol samples.



Imatinib mesylate [13]

Imatinib mesylate is an antileukemic cytostatic drug belonging to the class of targeted cytostatics that selectively attack cells having certain gene defects characteristic of tumors.

Imatinib mesylate occurs in several crystalline modifications, each characterized by its specific melting point and micron-sized crystallite shape. Also known is an X-ray amorphous hydrate modification of imatinib mesylate whose X-ray diffraction pattern exhibits an amorphous halo; this modification has a water content of 2.2–3.2 wt % [14].

Cryochemical modification of imatinib mesylate was carried out by the high-vacuum sublimation–low-temperature condensation route and yielded an amorphous form.

The ^{1}H NMR spectroscopic and thin-layer chromatographic examinations revealed identity of the original imatinib mesylate and the new X-ray amorphous modification. The water content, as estimated by the Fischer method, was 0.5 ± 0.2 and 0.3 ± 0.1 wt % for the original substance and X-ray amorphous modification, respectively.

The NMR spectra of the original and prepared substances are virtually identical; they do not contain bands corresponding to hydrate water molecules.

It follows from the NMR spectroscopic and thin-layer chromatographic data that the substance prepared by us is anhydrous imatinib mesylate. The purities of

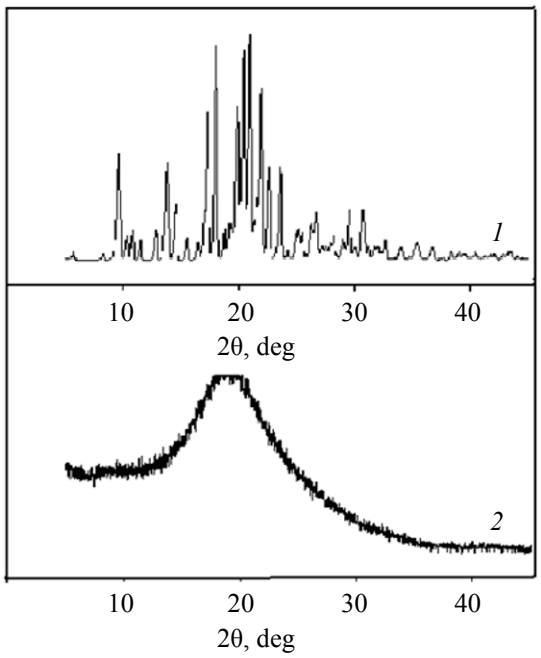


Fig. 3. X-ray diffraction patterns of the (*1*) original and (*2*) cryochemically modified imatinib mesylate samples.

the original imatinib mesylate and anhydrous amorphous modification, as estimated by thin-layer chromatography, are virtually identical, 0.27 against 0.18% impurity content. Therefore, modification does not cause changes in the original imatinib mesylate substance.

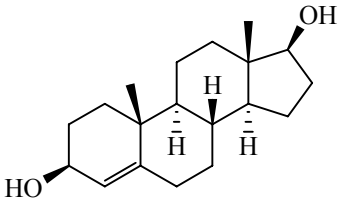
According to the X-ray diffraction data, the resultant substance is an X-ray amorphous modification of imatinib mesylate; its diffraction pattern is characterized by an amorphous halo. The X-ray diffraction patterns of the original and cryochemically modified imatinib mesylate are shown in Fig. 3.

The mid- and near-IR spectra (1100–1400 cm^{-1}) of the original and modified samples are different. The lack of intense absorption in the 3200–3400 cm^{-1} region suggests that the samples do not contain hydrate water.

The melting point of the resulting X-ray amorphous anhydrous modification is 135–137 against 214–216°C for the original substance.

The specific surface area of the original imatinib mesylate substance is 0.32 $m^2\ g^{-1}$, and that of the X-ray amorphous anhydrous imatinib mesylate modification (depending on the preparation conditions), 25–47 $m^2\ g^{-1}$, which range corresponds to the average particle diameter of 110–200 nm. The bulk density of the original substance powder is 0.5 against 0.005–0.055 g cm^{-3} (depending on the preparation conditions) for the resulting modification.

The new, cryomodified drug, dissolves in a hexane–methanol system two times faster compared to the original substance. Enhanced dissolving power of the X-ray amorphous anhydrous imatinib mesylate modification will be responsible for reduced time of its absorption into the body and increased biological activity compared to the original substance.



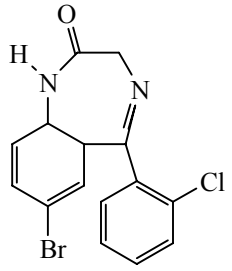
Androstenediol [15, 16]

Δ^5-Androstenediol-3β,17β is a natural hormone with pronounced immunostimulatory and radioprotective properties [15]. By the sublimation with a carrier gas–low-temperature condensation route we prepared nanoscale androstenediol monohydrate. Using thin-layer chromatography, was estimated that the original and cryochemically modified samples has the content of impurities less than 0.5%.

Differential scanning calorimetry and thermogravimetric analysis showed that the original and cryochemically modified Δ^5-androstenediol-3β,17β samples are monohydrates characterized by a dehydration peak at 110–147 and 102–128°C, respectively. The melting points of the original and cryochemically modified androstenediol are practically identical, 187 against 188°C.

The crystallographic data obtained from the cryochemically modified androstenediol correspond to the known structure from the Cambridge Structural Database: a = 6.250 Å, b = 12.143 Å, c = 23.440 Å, α = β = γ = 90°, V = 1779.0 $Å^3$, Z = 4, ρ_{calc} = 1.152 mg m^{-3}, space group $P2_12_12_1$.

The initial particle size lies in the 8–594 μm range (optical-microscopic data). The particle size and shape for the cryochemically modified Δ^5-androstenediol-3β,17β were determined by scanning electron microscopy. As seen from Fig. 4, the cryomodified substance is comprised of highly monodisperse elongated rod-shaped particles with rounded ends, whose average longitudinal size is 219 ± 9 nm [16].



Phenazepam [17–19]

Phenazepam is a psychotropic drug (synthesized and developed in the USSR in the 1970s) for which only one crystalline modification is known that was characterized by a set of X-ray diffraction maxima and their intensities [20].

Cryochemical modification of phenazepam was carried out by the sublimation with a carrier gas–low-temperature condensation route and yielded a new nanostructure.

The ^{1}H NMR and ^{13}C NMR spectra of the original and resulting substances revealed their identity. According to the ascending thin-layer chromatographic data, both samples have the same chemical purity.

Using the spectrophotometric technique presented in FS (Pharmacopoeia Monograph) 42-2411-99, the authenticity of the resulting cryochemically modified phenazepam was confirmed.

Thus, no conversion of the chemical nature of substance is involved in cryochemical modification of pharmacopoeial phenazepam.

As shown by comparison of the X-ray diffraction data for the original and cryochemically modified phenazepam samples, we obtained a new crystalline β-modification (Fig. 5).

Crystal structure examination of the resulting substance by powder X-ray diffraction (Cu$K_{\alpha 1}$ radiation, λ = 1.54059 Å, transmission mode, 2θ = 5°–80°, 8 h) yielded the following crystal lattice data: monoclinic, a = 14.792(5) Å, b = 11.678(3) Å, c = 8.472(2) Å, β = 93.677(19)°, V^3 = 1460.4(7) Å^3, ρ_{calc} = 1.59 g cm^{-3}, Z = 4, space group $P2_1/c$.

Comparison of our results with the published data from [20] confirms preparation from pharmacopoeia phenazepam of a new crystalline modification, β-modification. For details on this phenazepam modification, see [19]; those data were entered into the Cambridge Structural Database.

The IR spectra of the original substance and of β-modification of phenazepam differ in the 600–700, 800–900, 1350–1400, and 1600–1700 cm^{-1} regions.

Thermoanalytical examinations (argon stream, heating rate 10 deg min^{-1}) showed the following. The calorimetric curves for the original sample and β-polymorph of phenazepam, obtained by cryomodification, are different; the curve for the pharmacopoeia phenazepam contains an additional endothermic peak at 207–212°C. The melting points derived from the calorimetric curves of the samples and also established according to National Pharmacopoeia, vol. XI (Method 1A, in a capillary), were 226–227°C.

Using the electron micrographs obtained with a JSM 6380 LA scanning electron microscope at ×1000–20000 magnifications, the particle diameter was estimated at 10–120 μm for the original phenazepam and 50–300 nm (depending on the preparation conditions) for the cryomodified sample. According to the X-ray diffraction data, the particle size for the latter sample lies within the same range.

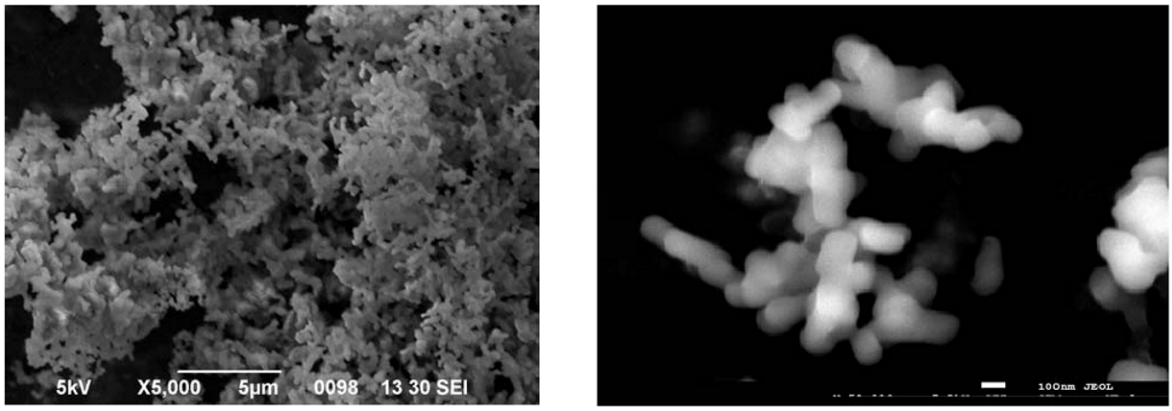


Fig. 4. Electron micrographs of Δ^5-androstendiol-3β,17β (scanning electron microscopic data).

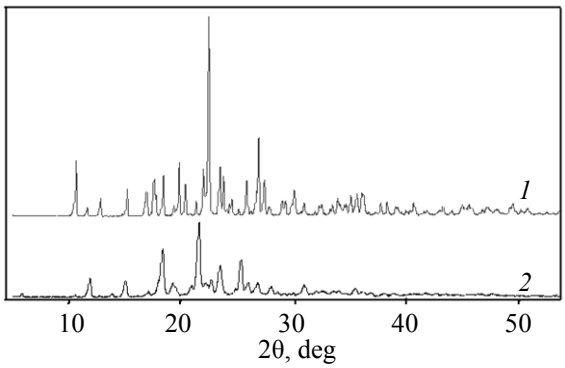


Fig. 5. X-ray diffraction patterns of the (*1*) original and (*2*) cryochemically modified (β-form) phenazepam samples.

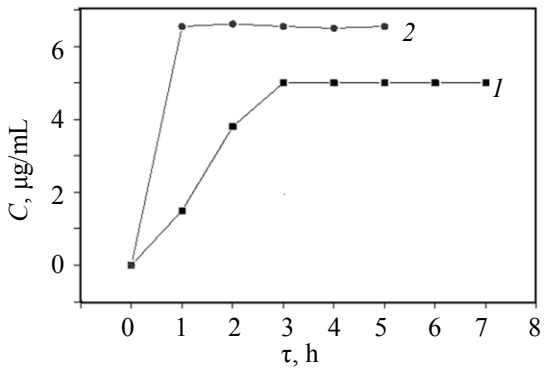


Fig. 6. Kinetic curves of dissolution in water for the (*1*) original and (*2*) cryochemically modified (β-form) phenazepam samples.

Figure 6 shows the kinetic curves of dissolution of the original phenazepam and nanophenazepam. The new crystalline β-modification dissolves in water 3.9 times faster than the original substance and forms supersaturated aqueous solutions. This suggests a higher bioavailability of the crystalline β-modification (faster absorption into the body) and enhanced therapeutic effect compared to the original substance.

Biological tests on rats demonstrated more anxiolytic activity and much less sedative activity for a composition based on the new crystalline β-modification of phenazepam [18].

In the concluding part, we will discuss some issues of development of new drug compounds, in particular, of nanosized drug polymorphs.

Any modification procedure applied to chemical compounds raises the question of possible losses. The method proposed by us comprises the sublimation and condensation steps and provides the yield of 85–90% for the end modified substance. By improving the isolation system for the end product, its yield can be increased.

All pharmaceutical substances are subject to storage stability requirements. Stability is particularly essential for polymorphic crystalline states which can either change from one form to another or be transformed into the initial state. Without interaction with the atmosphere (no water and oxygen vapor), pharmaceutical substances stored in the crystalline form are more stable than suspensions thereof. For example, crystalline β-modification of phenazepam preserved its structure and crystal size for three years.

The cryochemical modification techniques involving controlled conversion of the original substance to the gas phase, followed by controlled vapor condensation onto a cold surface, allow reducing the amount of impurities occurring in the original substance, thereby contributing to stabilization of the cryomodified product.

Of much importance in application of pharmaceutical substances is their bioavailability which depends on the dissolution rate and degree of solubility. As indicated in [21], more than 50% of products in the pharmaceutical industry are poorly soluble substances. Preparation of nanoforms of poorly soluble drugs appears to be one of the ways to increase their solubility (large surface areas and small particle sizes). Relative monodispersity minimizes Ostwald's ripening or recondensation.

Our experiments showed that, for phenazepam, reduction in the particle size to 50 nm causes significant, fourfold, increase of solubility rate. This is probably due to interaction of the nanoparticles and the solvent, as well as to the competition nucleation and dissolution processes.

Another important factor affecting the dissolution process is whether the substance of interest occurs in the crystalline or amorphous state. Drug substances in an amorphous state or in a crystalline form with large surface areas and high internal energies exhibit higher solubilities and enhanced dissolution rates.

Along with bioavailability, toxicity of pharmaceutical substances determines their suitability for application. This factor is particularly important in the case of nanoscale drugs.

For nanoform of phenazepam, we examined its effect on glial cell proliferation. To this end, an MTS test for a culture of C6 rat glial cells was carried out using a standard microplate photometer. We found that, as the original pharmacopoeia phenazepam amount increases from 0 to 100 μM, the cell viability decreases by 25%, while with phenazepam nanoform in identical amounts the cell viability changes negligibly.

Our results are indicate of a lower toxicity of nanoforms.

A classification system subdividing nanoparticles into four classes according to their toxicity, based on their size and biodegradability/nonbiodegradability in the body, was proposed in [22].

The first class of nanoparticles (low/no risk) includes biodegradable nanoparticles with sizes above 100 nm (e.g., nanoemulsions, liposomes, and drug nanocrystals); nonbiodegradable particles with sizes above 100 nm belong to the second class. The third class consists of biodegradable nanoparticles with sizes below 100 nm; high-risk nonbiodegradable nanoparticles with sizes below 100 nm belong to the fourth class.

With safety being the major priority in medicine, determination of toxicity of new drugs is of crucial importance for their registration. It is necessary to control the particle size when drugs are administered by injection, because particles larger than 5 μm may cause capillary clogging, which problem does not arise with nanoparticles. In the case of oral drug administration, small particle size facilitates uniform distribution of a substance in the gastroenterological tract, so local concentration growth is excluded. For nanosized drug particles administered by inhalation the deposition of the substance in the upper respiratory tract is reduced.

ACKNOWLEDGMENTS

The authors are grateful to S.A. Grabeklis and D.V. Chitsyakov from the Department of Biokinetics, Belozerskii Research Institute of Physcicochemical Biology, Lomonosov Moscow State University, for the experiments performed.

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