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Cocrystals of Betulin with Dicarboxylic Acids: Solid State Synthesis and Properties

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Abstract

Cocrystals of betulin with dicarboxylic acids were obtained using mechanochemical treatment with the addition of small amounts of organic solvents. The formation of cocrystals was confirmed by powder X-ray diffraction, thermal analysis, and IR spectroscopy methods. The presence of a solvent, the optimal components ratio, and the duration of mechanical processing are important for the formation of cocrystals. In order to choose a solvent for preparing betulin cocrystals, the solvents of different polarity were compared. It has been shown that cocrystals are formed if solvents that can effectively dissolve dicarboxylic acid are used during mechanical processing. For comparison with the mechanochemical method, betulin cocrystals were obtained by heating a mixture of starting reagents. Morphological changes observed by scanning electron microscopy upon heating the reaction mixtures also indicate the formation of cocrystals. It has been shown that when cocrystals are dissolved in water, solutions with an increased concentration of betulin are formed, and an increase in the length of the aliphatic chain of the acid leads to a decrease in the rate of betulin release into the solution.

Keywords: betulin, cocrystals, dicarboxylic acids, mechanochemical synthesis, solubility of betulin

INTRODUCTION

Betulin and its derivatives are attracting increasing interest due to the broad range of their biological and pharmacological activity, including antiviral, antibacterial, anti-inflammatory, anti-tumour, neuroprotective, etc. [1–4]. However, betulin, similarly to many derivatives, possesses poor solubility in water, which pulls down its bio-availability. Various methods have been used to improve the solubility and/or dissolution rate of betulin and its derivatives: preparation of solid dispersions with water-soluble polymers [5], liposomes [6], cyclodextrin derivatives [7], incorpo-

ration into nanosystems [8], etc. A disadvantage of these methods is the low stability of resulting systems, because the pharmaceutical substance is in the amorphous state as a rule, and is bound to the carrier through weak hydrogen or Van der Waals bonds.

Among other approaches used to improve the solubility of active pharmaceutical ingredients (API), preparation of their crystalline forms is also considered to be promising. These may be polymorphous modifications, salt forms, solvates and hydrates, as well as cocrystals exhibiting increased dissolution rates [9]. Preparation of cocrystals, that is, multicomponent crystal systems

containing API and non-toxic partner molecules bound with the medicinal molecules, through hydrogen bonds as a rule, is employed in pharmacy as a strategy to alter the physicochemical properties (in particular dissolution rate and solubility) of medicinal substances [10–12].

Dicarboxylic acids are widely used in pharmacy to prepare cocrystals. The structural formulas of betulin and dicarboxylic acids are shown in Fig. 1. Betulin contains secondary and primary hydroxyl groups, which can form hydrogen bonds with the functional groups of dicarboxylic acid during the formation of a cocrystal.

There are many methods to obtain cocrystals [13]. The most widespread method is crystallisation from solution. For the components that do not decompose when heated, spray drying procedure is used. Joint melting can be used if the components melt without decomposition. An alternative method to obtain cocrystals is a mechanical treatment in a ball mill using small amounts of a solvent [14, 15]. The mechanochemical method possesses a number of advantages, such as promptitude, efficiency, and environmental safety. In addition, though solvate formation is possible during mechanochemical synthesis, this is observed not so frequently as during cocrystallisation from solution.

Betulin solvates were obtained in our works [16] by crystallisation from different solvents, namely acetone, methanol, ethanol, propanol, butanol, ethylacetate, chloroform, and dichloromethane. Other betulin solvates are also known [17–19]. Classical crystallisation from solution to obtain cocrystals is poorly suitable for betulin because of its clearly pronounced ability to form solvates.

Using mechanochemical treatment and adding small amounts of organic solvents, we have obtained betulin cocrystals with a series of dicarboxylic acids: adipic [20–22], suberic [23], terephthalic [24], characterised by the improved solubility of betulin in comparison with the initial substance.

The goal of the work is to summarise the results obtained, compare them with the data obtained for betulin cocrystals with sebacic acid, and reveal the features of synthesis and properties of betulin cocrystals under variations of synthesis conditions and a coformer. To compare with the mechanochemical method, we studied crystallisation of the mixtures of betulin with acids from the same solvents that were used during mechanochemical synthesis, and synthesis from components during heating.

EXPERIMENTAL

Materials

Betulin was isolated from birch bark according to the procedure developed for this purpose [25] at the Institute of Chemistry and Chemical Technology SB RAS (Krasnoyarsk, Russia). Betulin was purified by recrystallisation from ethanol, followed by drying in the air. Thus obtained product was betulin semihydrate [26]. According to the data obtained by gas-liquid chromatography in combination with mass spectrometry (GLC-MS), lupeol content in the final product did not exceed 1.5–2.5 wt%.

Dicarboxylic acids (Riedel-de Haen, Switzerland; Reachim, Russia) were used without preliminary purification and drying. Ethylacetate and chloroform (Reachim, Russia) were purified by distillation. Other solvents (Reachim, Russia, or Sigma-Aldrich, USA) were of analytical grade and could be used without additional purification.

Obtaining cocrystals using mechanochemical methods

Mechanical treatment of betulin mixtures with dicarboxylic acids in the molar ratios of 1 : 1, 1 : 2, 2 : 1 was carried out in an agate mortar by crushing manually (treatment time 20 min, sam-

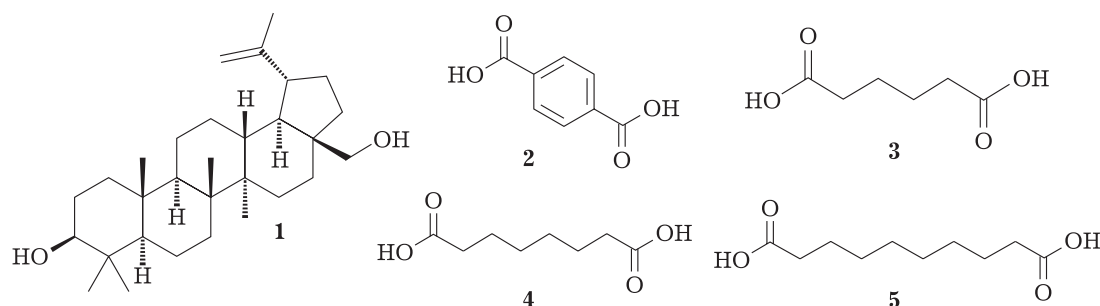


Fig. 1. Molecular structures of betulin **1**, terephthalic **2**, adipic **3**, suberic **4** and sebacic **5** acids.

ple mass about 100 mg), and in different kinds of mills to compare the effects, in particular, in a vibratory micromill Vibrator DDR-GM 94-58 (Narva, Germany), in a SPEX 8000 mill (Certiprep Inc., USA) and in the planetary centrifugal mill AGO-2 (Institute of Solid State Chemistry and Mechanochemistry SB RAS, Russia). The conditions of treatment in the vibratory micromill were: amplitude 5 mm, frequency 50 Hz, cylinder volume 6 mL, ball diameter 15 mm, ball mass 14 g, sample mass 100 mg. Treatment time was 30 min. The conditions of treatment in the SPEX 8000 mill: cylinder volume 60 mL, ball diameter 6 mm. The ratio of sample mass to the ball mass was 1 : 40, the load on a ball was 8–10 g. Treatment time was 15 min for the acids with chain length C_2 – C_6 and 20 min for C_6 and larger. The conditions of treatment in AGO-2 mill were: cylinder volume 40 mL, ball diameter 6 mm, the ratio of sample mass to ball mass 1 : 30, load on a ball 20 g. Treatment time was 15 min. The cylinders and balls of all mills were made of steel. Treatment was carried out in the absence of solvents and with the addition of small amounts of organic solvents. In the latter case, at first the mixtures were treated in the dry state for 5 min, and then a solvent was added (in the mortar – 100 μ L, in the Vibrator micromill – 10 μ L, in the SPEX mill – 300 μ L), and treatment was continued. The solvent was completely evaporated during the treatment, and the product was dry.

Methods of investigation

Powder X-ray diffraction analysis was carried out using a D8 Advance diffractometer (Bruker, Germany) with a one-dimensional detector Lynx-Eye, CuK_{α} -radiation, $2\theta = 5$ – 70° .

The IR spectroscopic studies were carried out using the attenuated total internal reflection method (ATR) without special sample preparation, with a Fourier spectrometer Digilab Excalibur 3100 (Varian, USA) and a single-reflection ATR measurement attachment Miracle ATR with a ZnSe crystal (Pike Technologies, USA).

The morphology was studied using electron microscopy. The studies by scanning electron microscopy (SEM) were performed with a TM-1000 microscope (Hitachi, Japan).

Thermal analysis was carried out using the system for simultaneous thermal analysis STA 449 F1 Jupiter (Netzsch, Germany). Measurements were performed in the flow of argon (99.990 %) with the volume flow rate of 50 mL/min. A weighted

portion (30–50 mg) was placed in the cell, which was then evacuated to 1 Pa with a roughing pump. Then argon was passed through the cell. Heating rate was 10 $^\circ$ C/min.

Measurements by differential scanning calorimetry (DSC) were made using a DSC 200 F3 MAIA calorimeter (Netzsch, Germany). Solid samples in the amount of about 5 mg were placed in the closed aluminium crucibles 40 μ L in volume, scanned within the temperature range from 25 to 250 $^\circ$ C in argon at a heating rate of 6 $^\circ$ C/min.

The dissolution of betulin cocrystals was studied with a solubility tester Varian 705 DS (USA) according to the procedure described in [23].

RESULTS AND DISCUSSION

Effect of the conditions of mechanochemical treatment on the formation of cocrystals

To obtain cocrystals with the help of mechanochemical methods, along with comminuting the reagents mixture in a mortar, we used mechanical treatment in a Vibrator micromill and in SPEX and AGO mills. It is known that the efficiency of mechanical treatment of solid mixtures depends on the mechanical characteristics (softness, hardness, fragility, plasticity) and chemical properties (acidity, basicity, the presence of functional groups) of the components. High-energy planetary mill AGO-2 is often efficient in amorphising pharmaceutical substances, because mechanical action may involve local contact melting of the substances, followed by melt solidification. On the other hand, the mill SPEX 8000 is more preferable for the interaction between components due to the sharing stress at the contact between particles, and better components mixing.

In the case of adipic [21] and suberic [23] acids, it was demonstrated that the formation of cocrystals was not observed as a result of dry treatment, both in a mortar and in the SPEX mill. The formation of new phases was not detected also after the treatment of mixtures in a mortar with the addition of ethanol. With an increase in the intensity of mixture treatment in mills with the addition of small amounts of the solvent, the appearance of new reflections is observed in the diffraction patterns, while the intensity of reflections related to initial reagents decreases (Fig. 2).

The IR spectra of mechanically treated mixtures in comparison with initial betulin are shown

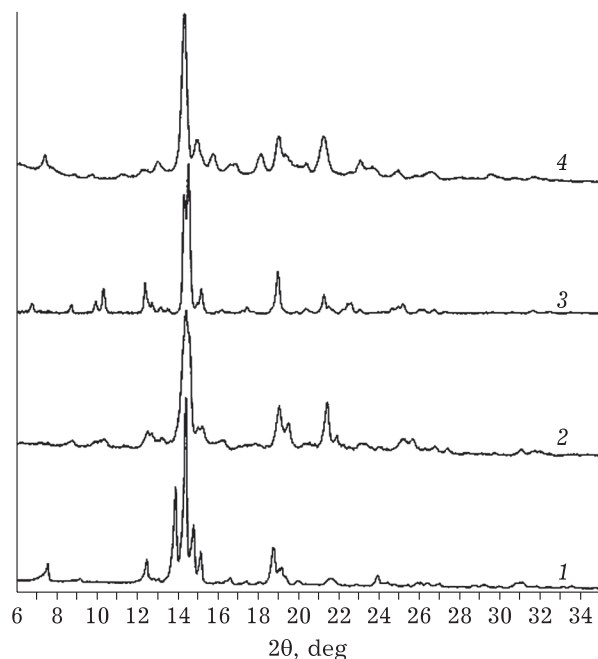


Fig. 2. Diffraction patterns of initial betulin (1), betulin cocrystals with adipic (2), suberic (3) and sebacic (4) acids.

in Fig. 3. These data confirm the formation of cocrystal. After mechanical treatment, a noticeable shift of the vibrations of betulin is observed: the stretching vibrations of O–H ($3600\text{--}3300\text{ cm}^{-1}$) and C–O (about 1020 cm^{-1}), and bending vibrations of OH groups at about 1400 cm^{-1} (see Fig. 3). These data provide evidence that the hydroxyl groups of betulin take part in the formation of intermolecular hydrogen bonds with the carboxylic groups of the acid.

The same is also observed in the IR spectrum of betulin cocrystal with sebacic acid, which is shown in Fig. 4, in comparison with the spectra of betulin and sebacic acid. In spite of the superposition of betulin and acid bands, after the formation of cocrystals one can see also the changes in the vibration bands of sebacic acid in the region of stretching vibrations of C–O and bending vibrations of OH groups at 1400 and 900 cm^{-1} , respectively [27].

It is well known that the properties of solvents used for mechanochemical treatment are essential for the formation of cocrystals [13, 14, 28]. In particular, in the case of solvents in which neither of the components is soluble, cocrystals were not obtained as a result of mechanical treatment [21].

The use of solvents with different polarity (Table 1) shows that there is no direct correlation between the dielectric constants of the solvents

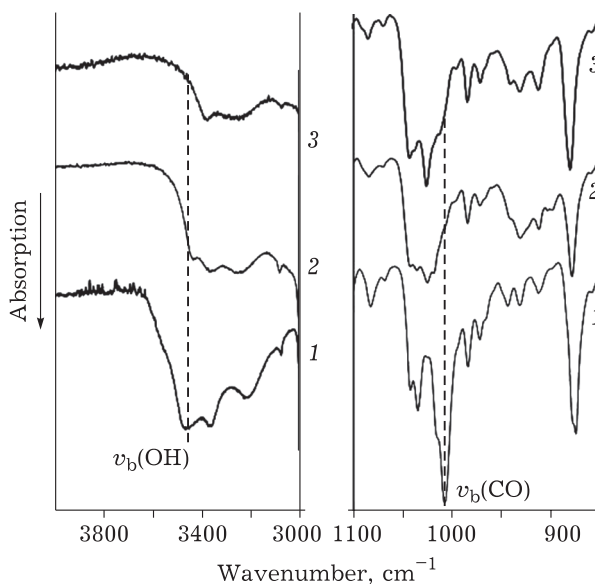


Fig. 3. IR spectra of initial betulin (1), betulin cocrystals with adipic (2) and suberic acids (3).

and the results of cocrystal synthesis. Nevertheless, it turned out that cocrystals were obtained using the solvents in which the acids had higher solubility.

It is known that the synthesis of organic compounds in the solid phase, including cocrystals, is hindered by diffusion limitations for organic molecules. The synthesis proceeds successfully if it goes through a fluid phase in which one or both reagents possesses sufficient mobility, and components mixing can occur at the molecular level. For this reason, in the presence of liquids in which at least one of the reagents is soluble, mechanical action leads to the mass transfer and mixing at the sub-molecular level. Short-term ($10^{-2}\text{--}10^{-3}\text{ s}$) pulsed heating arising during mechanical treatment promotes molecular mixing.

It can be assumed that the addition of a solvent causes partial dissolution of the acid in it, and this solution wets the surface of betulin particles. In addition, mechanical action can lead to disordering of the surface betulin layers, which accelerates dissolution and subsequent formation of betulin cocrystals with the dicarboxylic acid.

Suberic acid, due to a longer hydrophobic fragment than that of adipic acid, is soluble not only in polar solvents but also in those with low polarity. As a result, in contrast to the synthesis of betulin cocrystals with adipic acid [21], cocrystals with suberic acid were obtained by mechani-

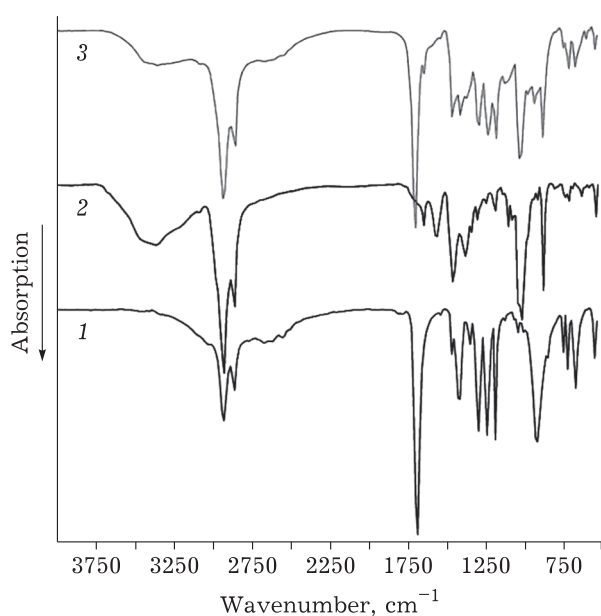


Fig. 4. IR spectra of initial sebamic acid (1), initial betulin (2), betulin cocrystal with sebamic acid (3).

cal treatment in the presence of any of the used solvents. At the same time, the low interaction rate in the case of some solvents can be due to the low solubility of components in this solvent. For instance, the time of treatment in the SPEX mill was 15 min for betulin mixtures with adipic acid and 20 min with suberic acid.

Screening of the formation of betulin cocrystals with dicarboxylic acids involved the following molar ratios: 1 : 1, 1 : 2, 2 : 1. As a rule, cocrystals with saturated carboxylic acids (adipic, suberic, sebamic) had 1 : 1 stoichiometry, while with terephthalic acid it was 2 : 1, which is likely due to the molecular structure of the latter acid.

It has been demonstrated using thermal methods [22, 23] that after mechanical activation, the thermal effects of initial components are not observed, which confirms the completeness of their interaction at the components ratio involved.

Synthesis of betulin cocrystals upon heating

In the case of adipic [22], suberic [23] and sebamic acids, cocrystals were obtained by heating the initial mixtures of reagents to the melting point of an acid. The data of X-ray diffraction analysis show that the same cocrystals as those from the mechanochemical synthesis were obtained.

It is known that the reactions participated by solids can proceed through the gas phase as the solid + gas processes, especially if one of the reagents is rather volatile. Heating of betulin mixtures with adipic acids within the temperature region of cocrystal formation (70–120 °C) can also involve the endothermic process of adipic acid sublimation, followed by vapour interaction with the surface of betulin particles [22]. In turn, within temperature range 130–160 °C, in which unreacted adipic acid melts, the interaction of the solid + liquid type may be possible [22]. It is known that cocrystallisation from the melt is one of the methods to obtain pharmaceutical cocrystals [10]. Nevertheless, it should be stressed that in the case of a physical mixture of betulin with adipic acid, only the latter component was present in the molten state. It is possible that the reaction of the solid + liquid type leading to the formation of cocrystals could proceed in this case.

TABLE 1

Physicochemical properties of solvents used for the mechanochemical synthesis of betulin cocrystals with dicarboxylic acids, and the result of synthesis

Solvent	Dielectric constant [29]	Betulin solubility [30], $10^4 x_i$	Adipic acid solubility [31, 32], $10^4 x_i$	Synthesis result	Suberic acid solubility [33, 34], $10^4 x_i$	Synthesis result
Ethanol	24.3	8.98	326	Yes	281	Yes
Isopropanol	18.3	14.63	287	Yes	271	–
Ethylacetate	6.02	20.66	36.8	No	31	Yes
Chloroform	4.7	43.70	20.95	No	s	Yes
1,4-Dioxane	2.21	–	347	Yes	546	Yes
Acetone	20.7	15.78	130	Yes	128	Yes
Toluene	2.4	~24	0.45	–	s	Yes

Notes. 1. x_i – solubility, molar fraction. 2. s – soluble. 3. Yes – cocrystal was obtained. 4. No – cocrystal was not obtained. 5. Dash – no data.

The electron microscopic studies of betulin crystal morphology upon heating in the presence of dicarboxylic acids (adipic or suberic) show that the morphological changes in reaction mixtures can serve as a test for cocrystal formation. Thus, in the case of a physical mixture of betulin with adipic acid heated to 110–120 °C, the morphology of betulin crystals differs from the morphology of betulin heated separately from the acid: instead of needle-like crystals, platy crystals are observed [35].

It can be assumed that the interaction upon heating proceeds through the gas phase. Indeed, adipic acid heating to a temperature of 100–110 °C involved its crystallisation on the cool parts of the vessel. The polymorphous transition of betulin within this temperature range [26] most probably promotes the interaction of betulin with the acid according to Hedwall's effect: a solid-phase chemical reaction proceeds at a higher rate in the region of phase transition. Nevertheless, the interaction was incomplete, because, in contrast with the mechanochemical method, heating does not involve mixing, therefore, not the whole crystal surface is available for the gas phase.

Betulin heating in mixture with sebacic acid also caused changes in crystal morphology, likely due to the formation of cocrystals (Fig. 5).

At the same time, in the case of suberic acid, no morphological changes differing from those observed for the polymorphous transitions in individual components were revealed. Therefore, no interaction through the gas phase occurs in this case. This may be because suberic acid is not prone to sublimation to a sufficient extent at this temperature. As a result, betulin undergoes a transition to another polymorphous modification without forming a cocrystal [35].

Synthesis of betulin cocrystals by crystallisation from solution

To compare with the mechanochemical synthesis, crystallisation from solutions was carried out using the same solvents as those used for mechanical treatment. Experiments on crystallisation have shown that a solvate of betulin with ethanol crystallises from the solution of a mixture of betulin with adipic acid [21]. It can be assumed that the formation of this solvate is thermodynamically more favourable than the formation of cocrystal. Nevertheless, a mixture of solvate with cocrystal was crystallised from the solution in isopropanol. Moreover, betulin cocrystal with adipic acid identical to the product obtained using the

mechanochemical method was formed in dioxane solution.

Evidently, in the case of equilibrium crystallisation, a necessary condition for the formation of cocrystals is lower cocrystal solubility in the solvent involved, in comparison with the solubility of individual components or other products formed in the process. For this reason, different products – a solvate or cocrystal – can crystallise from a definite solvent. On the other hand, mechanochemical synthesis often gives the products corresponding to rapid crystallisation [36], which leads to the formation of betulin cocrystals during mechanochemical treatment.

Properties of betulin cocrystals

The curves of dissolution in water for mechanically activated betulin mixtures with dicarboxylic acids are shown in Fig. 6.

According to the data obtained previously [36], betulin solubility in water is $(1.90 \pm 0.55) \cdot 10^{-3}$ mg/mL (37 °C). One can see (Fig. 6) that in the case of mechanical activation of betulin in mixture with adipic acid at the molar ratio of 1 : 1 the rate of betulin release into solution increases in comparison with the mechanically activated mixtures of betulin with suberic and sebacic acids. Previous studies of cocrystal dissolution have shown [37] that not only the energy of intermolecular interactions in the crystal but also the ease of solvent molecule attack on the molecules in the crystal, determined by the crystal structure, are essential for dissolution rate. It appears that an increase in the hydrophobic part of the molecular complex with an increase in the length of the aliphatic chain of the acid causes a decrease in dissolution rate and thus leads to a lower concentration of the substance in solution. This fact indicates that the properties of cofomer molecules hold decisive importance for the formation of the supramolecular structure of betulin cocrystals and their properties.

Betulin cocrystal with terephthalic acid demonstrates an increase in the rate of betulin dissolution in comparison with the dissolution of betulin cocrystal with adipic acid, which gives an increased betulin concentration in water, conserved for not less than 2 h. Betulin concentration in solution reaches about 0.025 mg/mL, which is 13 times higher than betulin solubility. It is assumed that cocrystals of the medicinal product with a cofomer, formed due to hydrogen bonds between the components, dissociate in the aqueous medium

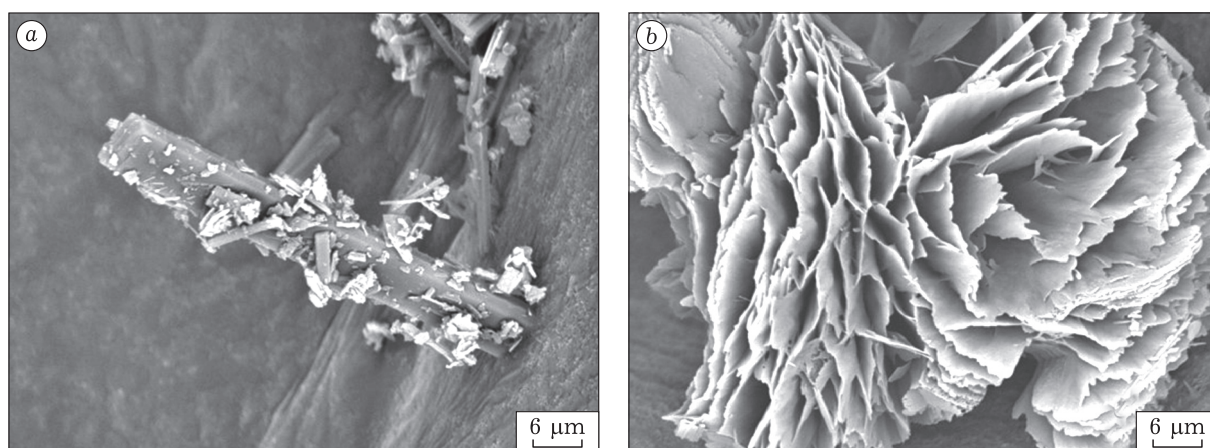


Fig. 5. SEM images of initial betulin (a) and a mixture of betulin with sebacic acid after heating to 120 °C (b).

within a short time interval (from several minutes to an hour) because heteromolecular interactions in the cocrystal can be weaker than homomolecular interactions in the phases of individual components [37]. Inhibition of the precipitation of hydrophobic pharmaceutical molecules, followed by crystallisation of the drug, is provided due to the use of coformer affecting crystal nucleation and growth.

Stability of betulin cocrystals

One of the essential characteristics of cocrystals is their stability because they can decompose at increased temperature or even during storage under normal conditions [38].

Investigation of betulin cocrystal behaviour at increased temperatures reveals that the hydrate of betulin cocrystal with adipic acid obtained by mechanochemical treatment is transformed into

the anhydrous form upon heating up to 70 °C. Endothermic effect on the DSC curve in the region of 70–110 °C corresponds to this process (Fig. 7, a). Then a structural transformation proceeds at 120 °C, related to structure disordering, which is confirmed by the presence of endothermic effect on the DSC curve and a decrease in the intensity of reflections in the diffraction patterns, with subsequent decomposition of the cocrystal at 180 °C, accompanied by the evolution of gaseous products as a result of adipic acid sublimation [22]. Betulin melting is not observed, because the product of decomposition contains the residue of non-evolved adipic acid. This kind of behaviour during heating is observed for betulin cocrystal with suberic acid (see Fig. 7, b) [39].

Thus, betulin cocrystals are unstable at increased temperature, and their decomposition is preceded by crystal structure disordering. Following the assumption formulated by the authors of [38], who explain the decomposition of cocrystals by the presence of plastic phases as the components, the decomposition of betulin cocrystals can be also associated with the properties of betulin molecules, which requires further investigation.

CONCLUSION

Mechanochemical treatment was applied to obtain betulin cocrystals with dicarboxylic acids: adipic, suberic, sebacic, terephthalic. It has been established that the presence of a small amount of a solvent is necessary for the synthesis to proceed. Experiments with solvent variations showed that the necessary condition was acid solubility in the solvent involved in the synthesis. It is shown that mechanical treatment with the addition of small amounts of organic solvents is a more preferable

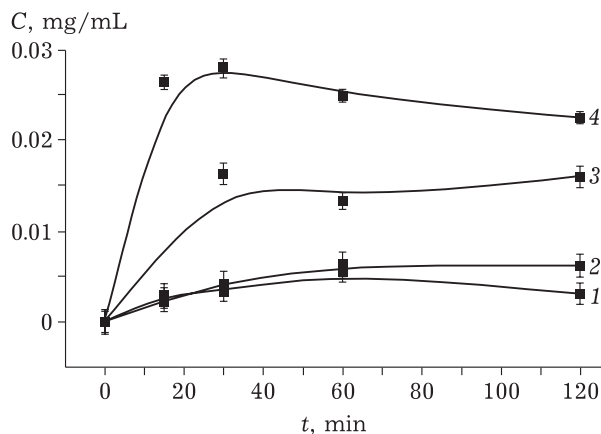


Fig. 6. The curves of betulin release into solution during the dissolution of mechanically activated mixtures with suberic (1), sebacic (2), adipic (3) and terephthalic (4) acids.

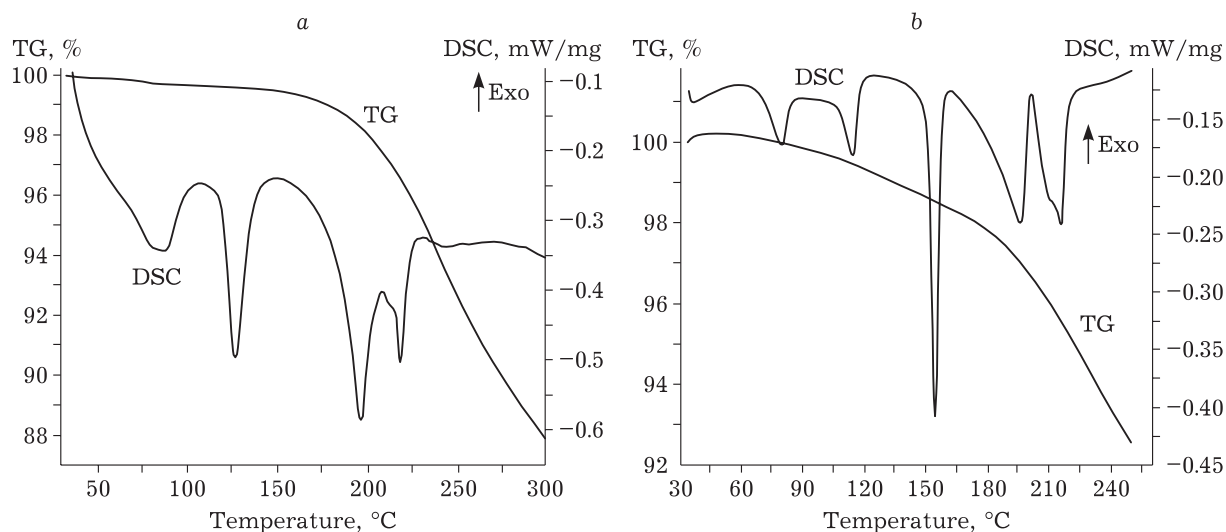


Fig. 7. TG and DSC curves of betulin cocrystals with adipic acid (a) and with suberic acid (b).

method for obtaining cocrystals than crystallisation from solution because of the high inclination of betulin to form solvates. Changes in the crystal morphology in reaction mixtures with the formation of betulin cocrystals with adipic and sebacic acids upon heating were revealed. The interaction of adipic acid vapour with the surface of betulin crystals is assumed. On the contrary, the morphological changes in betulin mixture with suberic acid during heating point only to the polymorphous transformations of the components. The results of experiments on the dissolution of betulin cocrystals with suberic and sebacic acids in comparison with the data for betulin cocrystals with adipic acid show that an increase in the length of aliphatic chain in the acid leads to a decrease in the rate of betulin release into solution. Investigation of the effect of coformer structure allows choosing proper partner molecules for the solubilisation of betulin-based pharmaceutical dosage forms by cocrystallisation.

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