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Mechanochemical Modification of the Properties of Antihelminthic Preparations

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Abstract

The processes of joint mechanical activation of some benzimidazole antihelminthic preparations with water-soluble polymers in the mills of shock-attrition type were studied. The conditions of synthesis of supramolecular complexes with different components ratios and energy strain of activator mills were discussed. The products of mechanochemical synthesis were characterized on the basis of water solubility and particle size. The products obtained possess higher water solubility and smaller particle size than the initial benzimidazole compounds; the products are of interest as potential antihelminthic preparations with increased efficiency.

Key words: antihelminthic preparations, carbendacim, albendazole, polymers, polysaccharides, grinding, mechanical activation, supramolecular complexes water solubility, particle size, nanotechnology, mechanochemical synthesis, echinococcosis

INTRODUCTION

It is known that grinding processes are among the most important processes in the pharmaceutical industry because they allow obtaining powder with controlled particle size [1]. Positive and negative consequences of the application of grinding processes to pharmaceutical substances were described with numerous examples in review [2]. The most significant changes in the properties of pharmaceutical substances were observed in the case of the joint mechanical treatment of the substances with polymers. These studies were carried out since the 70s of the last century by two groups of Japanese scientists [3, 4]. Investigations performed by various schools of Russian scientists in the field of mechanochemistry of organic compounds are presented in [5]. At present, the processes of obtaining finely dispersed powders relate to the area of nanotechnology, which is getting introduced into various branches of science and technology. In this regard, obtaining nanometer-sized supramolecular systems of biologically active substances with the help of mechanochemical methods can solve the problems both of scientific and technological aspects. Thus, supramolecular complexes prepared by means of the mechanochemical modification of the properties of known pharmaceutical substances were obtained that allow achieving the necessary pharmacological effect in multiply reduced doses with respect to the officially recommended doses, and therefore can reduce the toxic effects of drugs in the living organism.

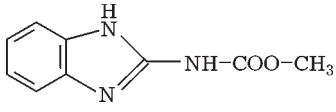
Previously, we obtained complexes using the methods of mechanochemical modification of antihelminthic substance medamin. The formation of complexes was confirmed by the data of IR spectroscopy, DTA and X-ray phase studies [9]; the solubility and dialysis processes were studied. We succeeded not only in achieving higher efficiency of the preparation against round and tape worms but also in significant expansion of the range of action (including the action against labrax echinococcosis).

The present work deals with the study of antihelminthic preparations in benzimidazole series (carbendacim, albendazole), and in particular with the mechanochemical preparation of supramolecular complexes of antihelminthic preparations with water-soluble polysaccharides and β-cyclodextrin. These preparations are poorly absorbed in the gastrointestinal tract (GIT) and have low bioavailability, probably due to low solubility in aqueous solutions. We supposed that biologically active molecules in the complexes will have a higher pharmacological effect due to the increased ability to achieve biologically active sites of endoparasites and affect them.

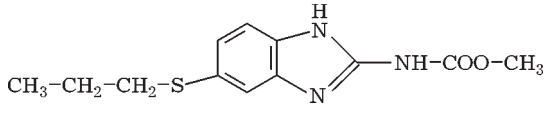
EXPERIMENTAL

We chose the following polymers and reagents for the study:

1) Carbendacim – methyl(1*H*-benzimidazol-2-yl)carbamate, the substance of antihelminthic medamin, pharmacopeia purity). Gross formula: $C_9H_9N_3O_2$, structural formula:



2) Albendazole – methyl[6-(propylthio)-1*H*-benzimidazol-2-yl)carbamate, the substance of antihelminthic albendazole, pharmacopeia purity. Gross formula: $C_{12}H_{15}N_{30}O_2S$, structural formula:



3) Arabinogalactan (Fibrolar C) – amorphous powder, light-cream colour, manufactured by Khimiya Drevesiny Ltd. (Irkutsk) TU-9363-021-39004141–08, 19092010 series.

4) Hydroxyethyl starch (HES 200/0.5) and β-cyclodextrin of pharmacopeia purity.

A roller ball mill VM-1 was used to carry out mechanochemical synthesis. The advantages of this mill for mechanochemical preparation of water-soluble compositions was demonstrated by us previously in [8].

Activation in roller ball mill VM-1

The steel cylinder 300 mL in volume was charged with 2.1 g of the pharmaceutical substance, 21.0 g of the complex-forming agent (arabinogalactan, HES or β-cyclodextrin) and 675 g of metal balls (ball diameter: 15 mm). The cylinder is mounted on rollers; the mixture is activated by cylinder rotation with a frequency of 90 min^{-1} for 1–6 h. The composition of the pharmaceutical substance with the complex-forming agent is obtained (with the mass ratio of 1 : 10) as loose white-coloured powder. The optimal time of mechanical activation was established on the basis of the entire set of data on the solubility of resulting compositions; it was 2 h.

Determination of the solubility of antihelminthics

To determine the solubility of antihelminthics, 0.44 g of mechanically treated mixture of polymer/antihelminthic (10 : 1) and the weighted portions of individual substances equivalent to their content in the indicated mixtures were dissolved in 5mL of distilled water under stirring with a magnetic mixer (600 min^{-1}) for 6 and 24 h at a temperature of 25 °C. The concentrations of antihelminthics in the samples were analyzed by means of HPLC. Constant concentrations in solutions were achieved within dissolution time essentially shorter than 6 h. In all the cases, antihelminthics in solution were in equilibrium with the precipitate of non-dissolved substance. In this situation, the polymers/complex-forming agents passed into solution completely. The HPLC analysis was performed using an Agilent 1200 chromatograph with a Zorbax Eclipse XDB-C18 column, 4.6 × 50 mm. Column temperature was 30 °C, an UV diode array detector was used. The HPLC

method was used to determine the concentration/solubility of antihelminthics in water from the compositions of polymer/antihelminthic. The system acetonitrile-water (at a ratio of 25 : 75) was used as the eluent, detection was carried out within the wavelength range 230–280 nm. The concentrations of studied antihelminthics were determined with respect to specially prepared solution in alcohol.

Determination of particle size/dispersed composition of the particles suspensions

The granulometric/disperse composition of the particles in the suspensions of initial antihelminthics and their complexes was determined using a Microsizer-201a laser particle size analyzer (VA Instalt, Russia). The powder under study (1–5 g) was put into the sample preparation unit (the volume of liquid: 150 mL) in the amount sufficient to achieve 70–75 % light transmittance through the cell. Measurements were carried out after stirring for 1–2 min with the simultaneous ultrasonic treatment of the suspension to destroy agglomerates. Data processing was carried out using the calculation programme built in the analyzer. The results were presented in the form of histograms of mass distribution over the particle size.

RESULTS AND DISCUSSION

After oral introduction of the resulting compositions, the pharmaceutical substance will be present in the gastrointestinal tract of the animal both in dissolved form and as non-dissolved fine particles. It may be assumed that an increase in water solubility will promote an increase in the biological availability and the preparations under study [6, 8]. On the other hand, the dispersity of the suspensions affects the efficiency of the contact action of particles on biologically sensitive centres of helminths [11, 12] located in the gastrointestinal tract. In this regard, special attention was paid to the studies of water solubility and disperse composition of suspensions.

The data obtained in the measurements of water solubility of initial substrates, as well as their compositions with complexing agents are presented in Table 1. Water solubility increases to the most substantial extent for arabinogalactan (AG) in comparison with the increase of water solubility for hydroxyethyl starch (HES), β-cyclodextrin (CD) and compositions obtained by mechanochemical processing.

Previously it was shown [6–8] that an increase in water solubility of poorly soluble low mass molecular organic substances occurs due

TABLE 1

Increase in the solubility of antihelminthics in water from the compositions with various complexing agents (duration of mechanical processing in the VM mill: 2 h)

Composition of samples (mass ratio)	Preparation method	Solubility $C_{p.s}$, g/L	Increase in solubility (X)
Carbendacim	Initial substance	0.009	–
Carbendacim – CD (1 : 6)	Without mechanical treatment*	0.013	1.4
	Mechanical treatment	0.013	1.4
Carbendacim – HES (1 : 10)	Without mechanical treatment	0.010	1.1
	Mechanical treatment	0.020	2.1
Carbendacim – AG (1 : 10)	Without mechanical treatment	0.073	8.1
	Mechanical treatment	0.146	16.2
Albendazole	Initial substance	0.003	–
Albendazole – CD (1 : 6)	Mechanical treatment	0.009	3.0
Albendazole – HES (1 : 10)	» »	0.094	31.3
Albendazole – AG (1 : 10)	» »	0.174	58.0

Note. CD – β-cyclodextrin, HES – hydroxyethyl starch, AG – arabinogalactan.

* Mixtures of the powders of initial substances not subjected to mechanical treatment.

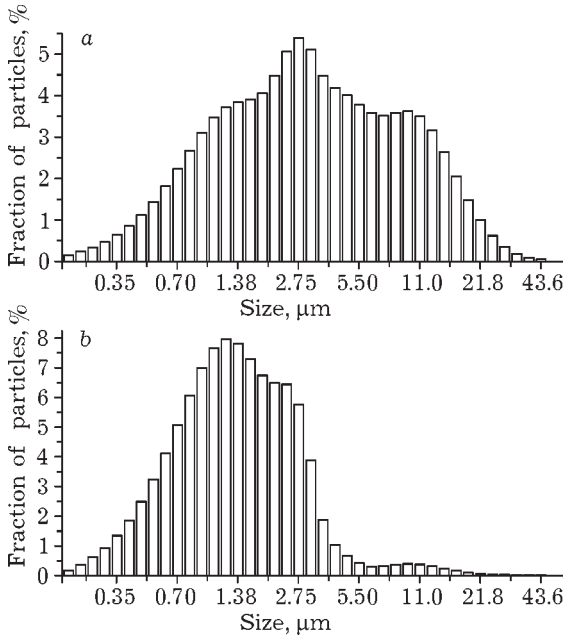


Fig. 1. Histograms of the mass particle distribution in the suspensions of albendazole (*a*) and mechanochemically prepared composition albendazole/arabinogalactan (1 : 10) (*b*).

to the formation of water-soluble supramolecular complexes of the type of "guest – host", where "host" is soluble oligomers and polymers (arabinogalactan, hydroxyethyl starch and β-cyclodextrin). It was found that water solubility depends on the strength (stability) of complexes. It follows from the results obtained that arabinogalactan is the most efficient complex-forming agent, and this is in agreement with the data obtained previously for other poorly soluble substances [8].

The results of particle size analysis of the aqueous suspensions of obtained powdered products are shown in Fig. 1 for albendazole as example. Cumulative granulometric measurement data are shown in Table 2. It should be noted that in all cases we measure only non-dissolved particles of pharmaceutical substances, while the complex-forming agent is completely dissolved.

One can see in the data presented in Table 2 that the joint grinding of carbendacim and albendazole with polysaccharides leads to a significant reduction in the size of non-dissolved particles. These data support our assumption that the mechanical treatment of their mixtures with polymers allows one to obtain micro- and possibly nanosized particles of medical substances [10]. The largest effect is achieved by suspending the compositions including arabinogalactan. At the same time, the same samples demonstrate the maximal increase in the solubility of medical substances (see Table 1). It is likely that the compositions of the studies antihelminthics and arabinogalactan obtained mechanochemically are the most promising ones for further pharmacological studies.

Thus, we obtained the compositions of antihelminthic medicines (albendazole and carbendacim) possessing significantly improved water solubility and dispersity, and consequently increased antihelminthic activity.

Preliminary studies carried out *in vivo* under laboratory conditions with the infective ini-

TABLE 2

Data of granulometric composition (mass distribution over particle size) for aqueous suspensions of the compositions of antihelminthics obtained by means of mechanical treatment in VM mill for 2 h

Sample composition (mass ratio)	Maximal particle size in separate fractions, μm			
	Fraction of total mass of samples, %			
	30	50	70	90
Albendazole – AG (1 : 10)	1.00	1.41	2.06	3.22
Carbendacim – AG (1 : 10)	0.86	1.19	1.66	2.74
Albendazole – HES (1 : 10)	1.86	3.13	5.31	10.40
Carbendacim – HES (1 : 10)	1.17	1.74	2.63	4.76
Albendazole – CD (1 : 6)	1.73	2.85	4.46	8.25
Carbendacim – CD (1 : 6)	1.13	1.65	2.47	4.24
Albendazole – initial substance	1.82	3.24	6.17	13.46
Carbendacim – initial substance	2.31	3.78	6.43	13.44

Note. CD – β-cyclodextrin, HES – hydroxyethyl starch, AG – arabinogalactan.

tial forms of nematodes (eggs, larvae) point to the inhibitory effect of the synthesized complexes. This allowed starting up the industrial tests of these preparations in the livestock farms of Baimakskiy District of the Republic of Bashkortostan.

Test results obtained by present confirm the promising character of the preparations proposed by us for dehelminthization of agricultural animals.

CONCLUSION

By means of the mechanochemical modification of antihelminthic preparations (carbendacim, albendazole) through their joint mechanical activation with polysaccharides and cyclodextrin, the possibility to increase their water solubility and dispersity of aqueous suspensions was demonstrated. The results showing an increase in their antihelminthic activity were obtained.

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