UDC 544-16;678,01;615,31

Mechanochemical Preparation and Properties of Water-Soluble Intermolecular Complexes of Polysaccharides and β-Cyclodextrin with Pharmaceutical Substances

A. V. DUSHKIN[1], E. S. METELEVA[1], T. G. TOLSTIKOVA[2], M. V. KHVOSTOV[2] and G. A. TOLSTIKOV[2]

[1]*Institute of Solid State Chemistry and Mechanochemistry, Siberian Branch of the Russian Academy of Sciences, Ul. Kutateladze 18, Novosibirsk 6300128 (Russia)*

E-mail: dushkin@solid.nsc.ru

[2]*Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, Pr. Akademika Lavrentyeva 9, Novosibirsk 630090 (Russia)*

E-mail: tg_tolstikova@mail.ru

(Received March 3, 2010)

Abstract

Comparative investigation of obtaining conditions and the "stability" of water-soluble intermolecular complexes of low-soluble pharmaceutical substances and different polysaccharides with β-cyclodextrin was carried out. Substantial improvement of the pharmacological characteristics of resulting complexes in comparison with initial pharmaceutical substances was demonstrated.

Key words: water-soluble supramolecular complexes, arabinogalactan, β-cyclodextrin, polysaccharides, pharmacological properties, mechanical destruction, low-soluble pharmaceutical substances

INTRODUCTION

Many pharmaceutical substances (PS) are poorly soluble in aqueous solutions, which often decrease the biological availability and efficiency of medications based on them. In this connection, to achieve the basic therapeutic effect, it is necessary to apply increased doses of PS, which causes an increase in the probability of undesirable side effects. The methods frequently used to improve the solubility in aqueous / physiological media and toxicopharmacological properties of PS are based on the formation of their complexes /conjugates with water-soluble synthetic or natural polymers or oligomers [1, 2]. Previously we demonstrated the possibility of mechanochemical preparation of intermolecular complexes of the plant polysaccharide arabinogalactan (AG), which is water-soluble polysaccharide of *Larix sibirica* Ledeb. and *Larix gmelinii* (Rupr.) with poorly soluble PS (indometacin, sibazon, mezapam and azaleptin) [3]. In all the cases we achieved substantial increase in water solubility, a decrease in doses (up to 20 times) and toxic side effects of PS. The mechanochemical route to obtain these complexes or solid dispersed systems forming these complexes during dissolution in water has advantages in comparison with the conventional liquid-phase method in the aspect of the strength of formed complexes and their solubility in water.

Dissolution and complex formation for poorly soluble PS can be represented as [3]

$$PS_s \leftrightarrow PS_{sol} \quad (1)$$

$$PS_{sol} + C_{sol} \leftrightarrow (PS \cdot C)_{sol} \quad (2)$$

Equilibrium according to equation (2) is described as

$$K_f = [(PS \cdot C)_{sol}]/[PS_{sol}] \cdot [C_{sol}] \quad (3)$$

where PS_s is the solid crystal phase of the pharmaceutical substance in equilibrium with solution; PS_{sol} is the pharmaceutical substance in solution in the free form; C_{sol} is complex forming agent in solution in the free form; $(PS \cdot C)_{sol}$ is the complex between the complex forming agent and the pharmaceutical substance in solution; K_f is the constant of formation of intermolecular complex.

The $[PS_{sol}]$ value corresponds to the equilibrium (thermodynamic) solubility without complex forming agents. In the case of complexation, the total concentration of PS in solution (C_{PS}) will be determined as a sum of the concentrations of free PS and PS in the complexes:

$$C_{PS} = [PS_{sol}] + [(PS \cdot C)_{sol}] \quad (4)$$

Thus, an increase in the solubility of PS in solution (X) in the presence of complex forming agent can be calculated using equation

$$X = C_{PS}/[PS_{sol}] = 1 + K_f[C_{sol}] \quad (5)$$

In our opinion, it is convenient to use X value to characterize the "strength" of intermolecular water-soluble complexes of PS with various water-soluble polymers.

In the present work we carried out comparative studies of the efficiency of different conditions of mechanochemical synthesis and the use of different complex forming agents – arabinogalactan, fibregum, pectin, hydroxyethyl starch, dextran 10, 40, 70, and β-cyclodextrin. All the complex forming agents are allowed for use in food or pharmaceutical industry. As a reference, we chose β-cyclodextrin which is widely used in pharmaceutical industry to enhance solubilisation of poorly soluble PS. Two modes of mechanical treatment were used: intense (in the planetary laboratory mil) and a softer one (in a rotary ball mill). The intense mode is more popular in laboratory studies of mechanochemical modification of medicines [4] and allows one to mix the components at the molecular level; however, in this case partial destruction of the substances under treatment is possible. In addition, it is a problem to scale the application of such a high-intensity mechanochemical process. Therefore, we also used low-intensity mechanochemical action realized in a usual rotary ball mill. We demonstrated previously [5] that this kind of treatment allows one to obtain solid dispersed systems of components – composite aggregates of ultrafine particles the hydration of which is accompanied by the interaction of the treated substances. This allows one to decrease the energy strain of the mechanical treatment process and to provide the possibility of scaling in for flow industrial mills.

EXPERIMENTAL

Initial compounds and experimental procedures

We used arabinogalactan (AG) extracted from Siberian larch using the procedure described in [6], certified according to TU 363-015-39094141–03, registered as a raw material for the production of biologically active additive (BAA) under the trademark of Fibrolar, additionally purified through redeposition in ethanol. The content of the major substance in the product exceeded 99.5 %, humidity was 0.01 %, and phenol admixtures (flavonoids) were present in the amount of 0.15 %. In addition, we used fibregum (glycoprotein from acacia gum; its macromolecules are composed of arabinogalactan by 95–99 % and proteins by 1–5 %) manufactured by CNI (France), pharmacopeic β-cyclodextrin, citrus pectin, dextran 10, 40 and 70, and hydroxyethyl starch (HES).

Reagents: 1-2-(4-isobutylphenol)-propionic acid (ibuprofen, water solubility 0.030 g/L), 1-(*para*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid indometacin, water solubility 0.036 g/L), 7-chloro-2,3-dihydro-1-methy-5-phenyl-1H-1,4-benzodiazepin-2-on (sibazon, water solubility 0.05 g/L), 7-chloro-2,3-dihydro-1-methy-5-phenyl-1H-1,4-benzodiazepin (mezapam, water solubility 0.020 g/L), (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[*b*,*e*],[1,4]-diazepin (azaleptine, water solubility 0.039 g/L), dimethyl ester of 2,6-dimethyl-4(2′-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid (nifedipine, water solubility 0.179 g/L), 1,2-diphenyl-4-*n*-butyl-3,5-pyrazolidinedione (butadione), sodium 2,3-dimethyl-1-phenyl-4-methylaminopyrazolone-5-N-methanesulphonate hydrate (analgin) of "kh. ch." reagent grade or pharmacopeic were used without additional purification. We also used dihydroquercetin (DHQ) substance (Diod-Taksifoliya Co., Belgorod, Russia), water solubility 0.633 g/L.

Two modes of mechanochemical treatment were used:

1. Soft treatment was carried out in a jar roller BM-1 with the cylinder equipped with fluoroplastic lining. Milling tools were steel balls (ShKh-15 grade) 15 mm in diameter, total ball mass was 675 g. The acceleration of milling bodies was 1*g* (free fall). The volume of the cylinder was 300 mL. Total mass of the components of mixture under treatment was 20 g. The duration of the joint mechanical treatment was 1 to 24 h.

2. Planetary mill AGO-2 was used for intense treatment. Treatment mode: acceleration of milling bodies 60*g*, the mass of the mixture under treatment 3 g, cylinder volume 40 mL, milling bodies were steel balls 6 mm in diameter, load mass 75 g. Treatment time was 3 to 10 min. Longer treatment caused partial decomposition of samples, while shorter time of mechanical activation can be the reason of insufficient homogenization of the sample.

To determine the solubility of PS, mechanically treated mixtures of a complex forming agent with PS in the amount of 0.4 g, as well as weighted portions of individual substances equivalent to their content in the above-indicated mixtures, were dissolved in 5 mL of water under stirring with a magnetic mixer (frequency 600 min^{-1}) for 6 and 24 h at a temperature of 24 °C. The concentration of PS in sampled taken from the mixtures was analyzed with the help of HPLC. Constant concentrations in solutions were achieved within dissolution time <<6 h. In all the cases, PS in solution was present in equilibrium with non-dissolved PS precipitate. Complex forming polysaccharides and β-cyclodextrin passed into solution completely.

Physicochemical analysis of the samples

The HPLC analyses were carried out on an Agilent 1200 chromatograph with Zorbax Eclipse XDB-C18 column, 4.6×150 mm. Column temperature was 30 °C. Diode matrix detector was used. The HPLC procedure was used to determine the water solubility of PS from the compositions complex forming agent/PS. The eluent was 25 % acetonitrile + 75 % acetate buffer (pH 3.4); detection was carried out within the range 254 to 280 nm. The concentrations of PS under investigation were determined with respect to their specially prepared solutions in ethanol.

X-ray phase analysis (XPA) of the resulting products was carried out on a DRON-3 diffractometer using CuK_α radiation; the frequency of counter rotation was 2 degrees per minute.

Molecular mass distribution (MMD) of samples was determined on an Agilent 1200 with PL aquel-OH 30 column, 300×7.5 mm. Column temperature was 25 °C. Refractometric detector was used. A solution of 0.1 M $LiNO_3$ was used as a solvent and eluent; flow rate was 1 mL/min. Calibration was carried out using dextran standards from Sigma-Aldrich with molecular masses 1, 5, 12, 25, 80, 150, 270 and 410 kDa. The results were processed and MMD was calculated using Agilent GPC Data Analysis software) assuming the linear dependence of log MM on retention time.

Pharmacological characteristics

The work was performed with white outbred mice with body mass 20–25 g, and rats of Wistar line with body mass 200–220 g from the Laboratory of Experimental Animals of the Institute of Cytology and Genetics, SB RAS (Novosibirsk, Russia). The animals were kept under standard vivarium conditions with free access to water and standard granulated food. Experimental groups were formed by taking 8–10 individuals of the same mass.

Standard tests were used to determine pharmacological activity [7].

To study analgesic activity of the complexes, we used two models of experimental pain: acetic convulsions (0.75 % acetic acid in the amount of 0.1 mL per one animal) and thermal irritation hot plate (54 °C).

Anti-inflammatory activity was determined using the standard carrageenan model (1.5 % solution, 0.05 aponeurosis). The effect was estimated from the percentage of a decrease in edema caused by the flogogenic agent.

Along with the positive properties, many nonsteroidal anti-inflammatory means cause injury of the mucous coat of stomach (ulcerogenic action), which substantially limits their application for curing inflammation processes. We estimated the effect of complex formation

on the degree of ulceration of the mucous coat of stomach under the action of nonsteroidal anti-inflammatory means. For this purpose, we carried out separate experiments to estimate the number of ulcerous damage sites on the mucous coat of stomach after intragastric introduction of the complexes under investigation in comparison with the individual substance of the medical preparations.

RESULTS AND DISCUSSION

Complexes AG—PS. Dependence on the conditions of mechanochemical preparation

The data on the changes of PS solubility from the mixtures with arabinogalactan are presented in Table 1. In all the cases under investigation, a substantial increase in the solubility of poorly soluble PS was detected, which demonstrates the high efficiency of AG as a complex forming agent. Also in all the cases, the solubility of PS increases depending on the method of preparing mixtures in the following sequence: mixing without mechanical treatment < intense mechanical treatment < mechanical treatment under soft conditions.

We studied X-ray diffraction patterns of the resulting powdered mixtures AG-PS. The results of X-ray phase analysis are illustrated for the system indometacin–AG as example (Fig. 1). Other studied systems (see Table 1) have similar characteristics.

Mechanical treatment of the initial medical substances, as well as AG, did not cause significant changes of their diffraction patterns. At the same time, these changes are observed after mechanical treatment of the mixtures of all the studied PS with AG. In all the cases, the patterns of untreated mixtures contain reflections characteristic of the crystal phase of

TABLE 1

Increase in the water solubility of pharmaceutical substances (PS) from compositions with arabinogalactan (arabinogalactan/PS = 10 : 1) mechanically activated under different conditions

PS	Method of obtaining*	Solubility (C_{PS}), g/L	Increase in solubility (X)
Sibazon	Without mechanical activation	0.058	1.2
	AGO-2	0.130	2.6
	VM-1	2.408	48.2
Indometacin	Without mechanical activation	0.044	1.1
	AGO-2	0.144	4.0
	VM-1	1.587	39.7
Mezapam	Without mechanical activation	0.097	4.9
	AGO-2	0.215	10.8
	VM-1	2.812	140.6
Azaleptin	Without mechanical activation	0.174	4.4
	AGO-2	0.656	16.4
	VM-1	4.317	107.9
Nifedipine	Without mechanical activation	0.322	1.8
	AGO-2	1.234	6.9
	VM-1	2.457	13.7
DHQ	Without mechanical activation	1.030	1.6
	AGO-2	2.850	4.5
	VM-1	3.751	5.9
Ibuprofen	Without mechanical activation	0.036	1.2
	VM-1	0.853	28.4

* Without mechanical activation – the mixture of powdered initial substances that were not subjected to mechanical treatment; AGO-2 – intense mode of mechanical treatment in the planetary mill AGO-2; VM-1 – soft mode of mechanical treatment in a rotary ball mill VM-1.

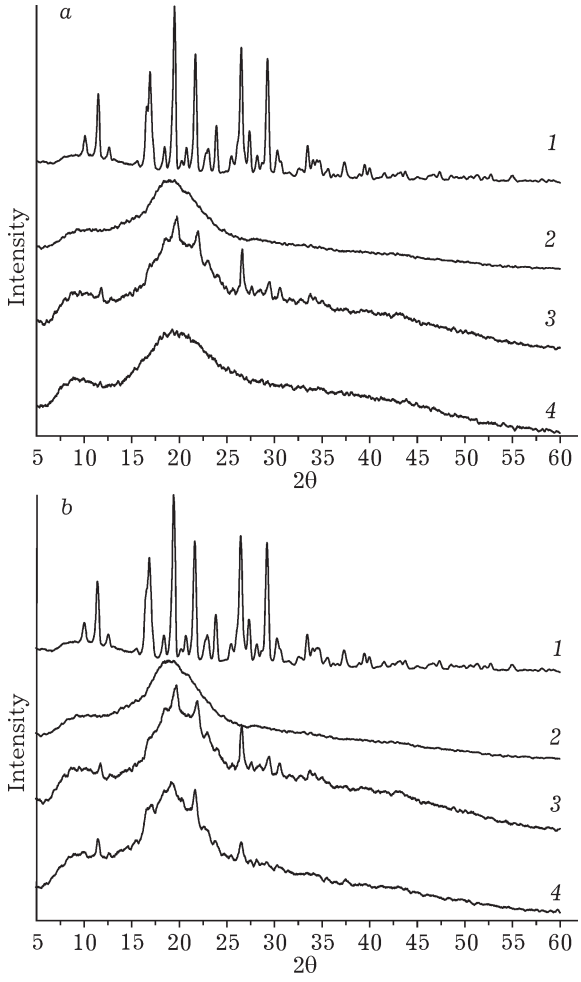

Fig. 1. X-ray diffraction patterns of initial indometacin (*1*), arabinogalactan (*2*), a mixture of arabinogalactan and indometacin (1 : 10) before (*3*) and after (*4*) mechanical treatment: *a* – AGO-2, treatment time 10 min; *b* – VM-1, 2 h.

PS, and there is a phase transition of melting in thermograms; these features disappear after mechanical treatment in AGO-2 mill. It follows from these data that characteristic signs of the crystal phase of studied PS disappear in mechanically treated mixtures. Evidently, either complete disordering of the solid PS phase or molecular dispersing of PS into the excess of the solid phase of AG occurs with the formation of solid solutions or intermolecular complexes. Mechanical treatment of a mixture of AG and PS in the roller mill VM-1 causes less substantial changes of X-ray diffraction patterns and DSC thermograms. Only relatively small decrease in the intensity and broadening of the reflections of crystal PS phases is observed. Only partial disordering of the crystal structure of PS is likely to occur. So, the soft mechanochemical treatment results in the formation of a solid dispersed system of components composed of ground partially disordered crystal particles of PS and amorphized arabinogalactan particles.

Molecular mass characteristics of arabinogalactan before and after mechanochemical treatment under different conditions

One can see from the data presented in Table 1 that the solubility of PS from dispersions with AG is essentially dependent on the method of mechanochemical treatment. In our opinion, to verify the choice of the modes of mechanical treatment, it is necessary to take into account possible transformations of polysaccharide macromolecules. It is known that polymer macromolecules under intense mechanical action can undergo partial destruction accompanied by a decrease in molecular mass. To study this phenomenon int eh systems under investigation, we used gel filtration chromatography (Fig. 2). Calculated data on molecular mass distributions are presented in Table 2.

It follows from these results that the use of the intense (rigid) mode of mechanical treatment (AGO-2 mill) involves substantial decrease in the molecular mass of AG macromolecules. In fact, according to the chromatographic data obtained, its macromolecules with MM ~ 17.5 kDa get broken into two practically equal fragments with MM ~ 8 kDa. It is inter-

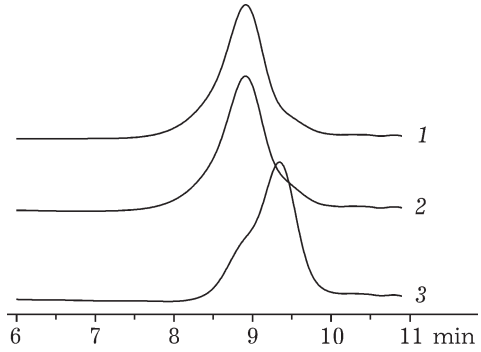

Fig. 2. Gel chromatograms of 0.02 mass % solution of arabinogalactan: *1* – initial; *2*, *3* – after treatment in mills: VM-1 (2 h) and AGO-2 (10 min), respectively; eluent: 0.1 M $LiNO_3$ solution.

TABLE 2

Molecular mass characteristics of arabinogalactan (AG) samples before and after mechanochemical treatment

Parameters	Method of obtaining AG		
	Initial	VM-1 (2 h)	AGO-2 (10 min)
t_1	8.9	8.9	9.0
M_w/M_n	17 515/14 070	17 230/13 790	15 930/15 900
S	163 443	165 129	35 367
t_2			9.3
M_w/M_n			8150/7740
S			120 397
ΣS	163 443	165 129	155 764

Note. t_1, t_2 are times of peak appearance; M_w is mass-average molecular mass; M_n is number-average molecular mass; S is peak area, relative units; ΣS is a sum of the areas of all the peaks in the chromatogram.

esting that such a phenomenon is observed during chemical destruction of AG from Canadian larch [8]. In our opinion, it is this phenomenon that can explain the differences in PS solubility and therefore in the strength of complex formation. It is likely that native AG macromolecules form stronger complexes with PS molecules than the macromolecules formed as the product of mechanochemical destruction. From the viewpoint of using the studied dispersions in BAA and medicines, in the technological processes of their preparation it is desirable to avoid noticeable changes of the molecular mass characteristics of polymer materials because otherwise additional tests and standardization might be necessary. As a consequence, on the basis of the entire set of data obtained, it is reasonable to use preferentially soft mode of mechanical treatment to obtain water-soluble dispersions of PS with AG.

Complexes of medical substances with different polysaccharides and β-cyclodextrin

For comparative estimation of the possibilities to use other oligo- and polysaccharides than AG to obtain intermolecular complexes of low-soluble PS, we studied oligosaccharide β-cyclodextrin (M_w = 1135 Da) and polysaccharides pectin (M_w = 38 kDa), fibregum (M_w = 526 kDa), dextrans (M_w = 10, 40, 70 kDa) and hydroxyethyl starch (M_w = 180 kDa). The data on the changes of solubility of PS from mixtures with indicated poly- and oligosaccharides are listed in Table 3.

In all the cases studied, an increase in the solubility of poorly soluble PS occurs. In this situation, the strength of intermolecular complexes increases in the row: dextran 70 < dextrans 40 and 10 < HES < β-cyclodextrin, fibregum < pectin < arabinogalactan. In the case of pectin with mezapam and azaleptini, complex formation occurs most probably due to acid-base interactions, which explains relatively high bonding strength. However, other complex forming agents do not contain acid-base functional groups; therefore, the most probable mechanism is the interaction due to the formation of hydrogen bonds PS–complex forming agent. In all the examples described above, mechanochemical treatment of the mixtures causes a substantial increase in the strength of bonding. In the opinion of the authors, this phenomenon is connected with the difference in the dynamics of mixture dissolution. Hydration of mechanochemically synthesized solid dispersed systems involves simultaneous transfer of all the components of PS complex former mixtures into the solution. Thus, under experimental conditions, a supersaturated PS solution is formed at first, and then intermolecular complexes are rapidly formed. During the dissolution of mixtures that were not subjected to mechanochemical treatment, the dynamics of dissolution is different: at first, the complex forming agent is dissolved rapidly,

TABLE 3

Increase in the water solubility of pharmaceutical substances (PS) from compositions with different polysaccharides and β-cyclodextrin at a ratio of 10 : 1

Sample composition	Solubility of PS, g/L	Increase in solubility
	AGO-2*	
β-Cyclodextrin/sibazon	0.090	1.8
β-Cyclodextrin/mezapam	0.092	4.6
β-Cyclodextrin/azaleptine	0.604	15.1
β-Cyclodextrin/indometacin	0.096	2.4
Pectin/mezapam	1.542	77.1
Pectin/sibazon	0.711	14.2
Pectin/azaleptine	1.632	40.8
Fibregum/DHQ	4.491	4.4
	VM-1**	
Fibregum/DHQ	5.550	8.8
Dextran 10/sibazon	0.094	1.9
Dextran 40/sibazon	0.096	1.9
Dextran 70/sibazon	0.059	1.2
HES/azaleptine	0.222	5.4
HES/sibazon	0.075	1.5
HES/mezapam	0.042	2.0
HES/indometacin	0.525	13.5
HES/DHQ	1.972	2.3
HES/ibuprofen	0.079	2.6

* Intense mode of mechanical treatment in planetary mill AGO-2.

** Soft mode of mechanical treatment in rotary ball mill VM-1.

then the crystal PS particles get slowly dissolved until the saturation concentration is achieved. The rates of the formation of intermolecular complexes and their strengths differ. The described phenomenon is of substantial interest from the viewpoint of governing the mechanisms of intermolecular interactions and the choice of technological processes for obtaining the described solid systems, and requires further investigation.

Specific choice of complex forming agents for the development of new medicines and BAA depends not only on the strength of water-soluble complexes but also on a number of other parameters, such as biological activity of the complex forming agent, its cost, purity, the existence of corresponding certifying documents *etc*. In our opinion, all the studied polysaccharides can be used to increase the solubility of poorly soluble biologically active substances along with popular complex-forming agent β-cyclodextrin which was included in our study as a reference. However, the use of AG allows one to achieve the highest PS water solubility and in the majority of cases also to increase their pharmacological activity substantially.

Pharmacological properties of intermolecular complexes

In this paper we present the results of pharmacological investigations confirming the effect of complex formation between pharmacons (PS) and water-soluble polysaccharides on the basic properties of PS.

The subjects of investigation were nonsteroidal anti-inflammatory medicines (butadion), non-narcotic analgesic agents (ibuprofen and analgin). In the first series of experiments with two models of experimental pain (chemical irritation "acetic convulsions" and thermal irritation "hot plate"), we studied the analgesic

TABLE 4

Analgesic activity of the complexes of pharmaceutical substances

Complexes	Hot plate, s	*P*	Acetic convulsions, number	*P*
	Hydroxyethyl starch (HS)/ibuprofen			
Reference, H_2O, 0.2 mL/10 g per os	12.7±0.9		7.1±1.2	
Ibuprofen, 200 mg/kg per os	10±0.6	<0.05	0.75±0.4	<0.004*
HES/ibuprofen (10 : 1), 200 mg/kg per os	12.5±1.4	<0.9	0.6±0.26	<0.001*
HES, 180 mg/kg per os	9.75±0.5	<0.05*	2.7±0.9	<0.02*
	Arabinogalactan/ibuprofen			
Reference, H_2O, 0.2 mL/10 g per os	26.6±2.5		4.25±0.94	
Arabin/ibuprofen (10 : 1), 200 mg/kg per os	23.5±2.4	–	0.75±0.01	<0.05
Ibuprofen, 200 mg/kg per os	19.5±2.45	–	1.5±0.03	<0.05
	Arabinogalactan/analgin			
Reference, H_2O, 0.2 mL/10 g per os	26.2±2.95		5.12±1.14	
Arabin/analgin (10 : 1), 50 mg/kg per os	32.5±3.7	–	1.75±0.08	<0.004*
Analgin, 50 mg/kg per os	30.4±3.2	–	0.63±0.1	<0.004*
Analgin, 5 mg/kg per os	17.7±2.3	<0.02*	4.25±0.75	<0.56
	Arabinogalactan/butadione			
Reference, H_2O, 0.2 mL/10 g per os	33.4±3.0		4.9±1.4	
Arabinogalactan/butadion (10 : 1), 120 mg/kg per os	42.2±5.2	<0.05	2.0±0.09	<0.05
Butadion, 12 mg/kg per os	11.4±0.8	–	4.25±0.5	–

*$P < 0.05$ with respect to reference.

activity of the complex of HES with ibuprofen in the mass ratio of 10 : 1, which corresponds to the molar ratio of 4 : 1. The dose of ibuprofen in the complex was 20 mg/kg. For correctness of experiment, we also tested HES itself. The reference for comparison was ibuprofen introduced in the dose of 200 mg/kg. All the agents were introduced intragastrically 1 h before the reproduction of models.

In the second series we estimated the anti-inflammatory activity of butadion with the standard carrageenin (1.5 % solution, 0.05 aponeurosis) model. For correctness of experiment, butadion was introduced intragastrically in the same doses in which it was present in the complexes (Table 4).

One can see in the data shown in Table 4 that the use of HES as the complex forming agent helped a ten-fold decrease in ibuprofen dose with the conservation of the high analgesic activity only for the model of visceral pain "acetic convulsions". HES itself, unlike ibuprofen, demonstrated reliable analgesic activity with both models.

In the next series of experiments, using the above-described design, we studied the analgesic action of the complexes of plant polysaccharide AG with nonsteroidal anti-inflammatory means and non-narcotic analgesic agents (ibuprofen, analgin and butadione) possessing different mechanisms of action.

TABLE 5

Anti-inflammatory activity of the complexes arabinogalactan/butadion (10 : 1) with the model of carrageenan edema

Reference (carrageenan)	Butadion, 50 mg/kg	Complex, 100 mg/kg	Reference	Butadion, 30 mg/kg	Complex 300 mg/kg	500 mg/kg
72.2±5.3	49.7±4.0*	70.9±4.8	58.3±5.4	40.8±4.7*	36.4±5.5*	53.9±2.5

* $P < 0.05$ with respect to the reference.

The data on analgesic activity of complexes with ibuprofen, butadion and analgin are presented in Table 4.

One can see that the complexes with ibuprofen in a decreased pharmacon dose (10 times) exhibit rather high analgesic activity only with the model of visceral pain.

The introduction of butadione in the form of the complex in which pharmacon content is decreased by a factor of 10, too, caused the manifestation of analgesic activity not only for chemical irritation model but also for thermal action, which can broaden the range of its application as analgesic means (see Table 4). A noteworthy fact for this PS is that complexing caused levelling of one of the basic effects (anti-inflammatory).

Analgin occupies a special place among the studied pharmacons due to its high water solubility. As a consequence, it is impossible to confirm the formation of a complex between it and polysaccharides on the basis of an increase in its water solubility, unlike for other pharmacons. However, NMR studies (similar to [3]) of the relaxation of aqueous solutions of mechanochemically prepared solid dispersions with AG point to the presence of intermolecular complexes of analgin molecules and AG. According to the data shown in Table 5, in the case of complexing of analgin with AG with a decrease in dose (10 times) the high analgesic activity was conserved with two models of experimental pain.

Complexing of pharmacons with AG and HES confirmed the previously discovered effect of pharmacon clathration by polysaccharides, which is a decrease in the actual dose of PS with the conservation of the high basic activity and therefore this means a decrease in the degree of apparent side effects [9, 10].

CONCLUSIONS

In the present work, we carried out a comparative investigation of obtaining conditions and strength of water-soluble intermolecular complexes of poorly soluble medical substances and different polysaccharides, as well as β-cyclodextrin; we also demonstrated the effect of complex formation on the pharmacological properties of some PS. On the basis of results obtained, the following conclusions were drawn:

1. It was shown that the mechanochemical route of obtaining solid dispersed systems PS–complex forming agent allows one to achieve the highest strength of intermolecular complexes. Mechanical treatment should be carried out under soft conditions excluding destruction of polysaccharide macromolecules. Additional advantage of this mode of mechanical treatment is the possibility of scaling it to industrial flow mills.

2. The use of arabinogalactan, water-soluble polysaccharide of Siberian larch (*Larix sibirica* Ledeb.) and Gmelin larch (*Larix gmelinii* (Rupr.)) as complex-forming agent allows one to achieve the highest water solubility in comparison with other studied poly- and oligosaccharides. Additional advantages of AG include unique almost unlimited raw material basis at the territory of Russia and the existence of industrial technologies of its isolation and purification [11, 12].

3. Complexing of medical substances (pharmacons) with polysaccharides, similarly to the case of complexing with plant glycosides, allows substantial (10 times) decrease in the actual dose, therefore, this means a decrease in undesirable side effects and increase in the solubility of PS.

The obtained results open outlooks for the development of the technology for pharmaceuticals with increased efficiency and safety.

REFERENCES

1 Nokhodchi A., *J. Pharm. Pharmauceut. Sci*., 8, 1 (2005) 18.
2 Shtilman M. I., *Soros. Obraz. Zh*., 5 (1998) 48.
3 Dushkin A. V., Meteleva E. S., Tolstikova T. G., Tolstikov G. A., Polyakov N. E., Neverova N. A., Medvedeva E. N., Babkin V. A., *Izv. RAN. Ser. Khim*., 6 (2008) 1274.
4 Dubinskaya A. M., *Usp. Khim*., 68, 8 (1999) 708.
5 Dushkin A. V., *Chem. Sust. Dev*., 12, 3 (2004) 251. URL: http://sibran. ru/English/csde.htm
6 Medvedeva E. N., Babkin V. A., Makarenko O. A., Nikolaev S. M., Khobrakova V. B., Shulunova A. M., Fedorova T. E., Eskova L. A., *Khim. Rast. Syrya,* 4 (2004) 17.
7 Fisenko V. P., (Ed.), Rukovedstvo po Eksperimentalnomu (Doklinicheskomu) Izucheniyu Novykh Farmakologicheskikh Veshchestv, Minzdrav RF, IIA "Remedium", Moscow, 2000, p. 304.
8 Prescott J. H., Enriquez P., Jung C., Menz E., Groman Ernest V., *Carbohydrate Res*., 278 (1995) 113.
9 Tolstikova T. G., Tolstikov A. G., Tolstikov G. A., *Vestn. RAN*, 77, 10 (2007) 867.
10 Tolstikov G. A., Baltina L. A., Grankina V. P., Kondratenko R. M., Tolstikova T. G., Solodka. Bioraznoobraziye. Khimiya. Primeneniye v Meditsine, Geo, Novosibirsk, 2007, p. 311.
11 Medvedeva E. N., Babkin V. A., Ostroukhova L. A., *Khim. Rast. Syrya,* 1 (2003) 27.
12 Medvedeva S. A., Aleksandrova G. P., Dubrovina V. I., Chetverikova T. D., Grishchenko L. A., Krasnikova I. M., Feoktistova L. P., Tyukavakina N. A., *Butlerov Commun*., 7 (2002) 45.