Chapter 22

Complexes of Polysaccharides and Glycyrrhizic Acid with Drug Molecules – Mechanochemical Synthesis and Pharmacological Activity

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1. Introduction

1.1. Using poly- and oligo-saccharides for drug delivery; Possibility for mechanochemical synthesis of supramolecular systems

Providing efficient ways of delivering active drug molecules to their destinations in target organisms, the so-called drug delivery, is among major challenges in today's pharmacy. An important relevant issue is to enhance the efficacy and safety of pharmaceutical compounds by correcting their solubility [1-3]. Polysaccharides (e.g., derivatives of cellulose, chitosan, and alginic and hyaluronic acids) make part of compositions with controlled or retarded drug release [4-6], while oligosaccharides (alpha-, beta-, and gamma-cyclodextrins and their derivatives) are broadly used to increase solubility and dissolution rates as they can form guest-host supramolecular complexes with poorly soluble drugs [7, 8]. Until recently little was known whether complexes of this kind may result from the activity of natural plant-derived or synthetic water-soluble polysaccharides though these are common elements in dietary supplements or drugs. Polysaccharides have aroused no interest in this respect, possibly because the technology for producing supramolecular complexes requires liquid phases (solutions or melts): The complexes form by molecular interaction in the liquid, and the solid phase is extracted then on drying (solutions) or cooling (melts). However, being easily soluble in water, polysaccharides are almost insoluble in other solvents and, moreover, decompose on heating rather than melt. The target drugs, instead, often dissolve rapidly in non-aqueous solvents but are poorly soluble or insoluble in water. Therefore, the liquid-phase synthesis of polysaccharide-drug complexes has been impeded by the lack of co-solubility.

This difficulty may be surmountable with solid-state chemistry approaches, specifically, with mechanochemical transformations in mixtures of solids [9-11]. Unlike the liquid-phase synthesis, mechanochemical treatment is a simpler single-stage process going without solvents or melts and respective additional procedures. The flow chart in Fig. 1 shows a simplified sequence of transformations the powder mixtures experience during dry milling in various mills.

There may be three types of main products relevant to our study, depending on the properties of starting materials:

1. "molecular dispersions", or solid solutions of drugs in excess filling (dispersion medium);
2. supramolecular complexes or products of chemical reactions between the components;
3. composite materials: aggregates of powdered particles.

In fact, they all are solid dispersions that form supramolecular structures (complexes or micelles) that enclose drug molecules and provide their solubility.

Generally, solid-phase processes have a number of advantages in laboratory and technological uses as they yield, in a shorter time, materials which the classical liquid-phase technology can never provide and allow avoiding problems associated with melts or solvents and side reactions. The high potentiality of mechanical activation was proven in our previous studies [12-14], e.g., on quick-dissolving pharmaceutical compositions [15-18] and synthesis of polyfluorinated aromatic compounds [19, 20].

In this synopsis we present techniques for synthesizing supramolecular complexes of poorly soluble drugs with water-soluble polysaccharides or with glycyrrhizic acid (a plant-derived glycoside), describe physicochemical properties of their solid forms and solutions, and report the results of pharmacological testing.

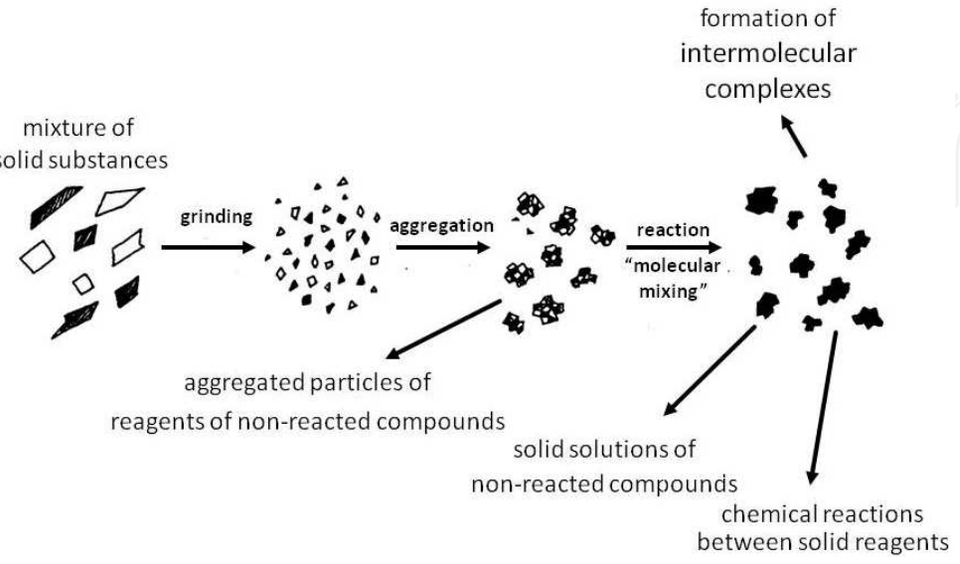


Figure 1. Mechanochemical transformations in mixtures of solids organic substances.

2. Physicochemical description of supramolecular systems including polysaccharides and glycyrrhizic acid with drug molecules

2.1. Synthesis of supramolecular systems of water-soluble polysaccharides and their stability in aqueous solutions

In the course of this study, different conditions of mechanochemical synthesis and various complexing agents have been tried and compared in terms of efficacy. The complexing agents were: arabinogalactan (AG), a water-soluble larch polysaccharide derived from *Larix sibirica* Ledeb. and *Larix gmelinii* (Rupr.), fibregum (FG), a glycoprotein of acacia gum, fruit pectin (PC), hydroxyethyl starch (HES200/0,5), dextrans (D) 10. 40. 70, and ß-cyclodextrin (CD), the latter chosen as standard for being widely used in pharmaceutics. The mixtures of powdered components (polysaccharides/complexing agents and drugs) were dispersed in ball mills at greater or lower intensities in laboratory planetary- and rotary-type mills, respectively. Milder rotary milling was predominantly applied because the molecular-level mixing in a planetary mill, common to laboratory studies of mechanochemical modification of drugs [9], may partly destroy the material and pose scaling problems. The materials processed by nondestructive rotary milling [10, 11, 21], instead, interact to produce solid dispersed systems of components (composite aggregates of superdispersed particles), and the process is easily scaled onto industrial flow mills.

The obtained compositions were checked for drug contents to avoid unwanted chemical reactions. Formation of supramolecular complexes was identified from changes in solubility of drugs in the water solution of the compositions [22].

Dissolution and complexing of poorly water-soluble drugs can be illustrated by such simplified equations:

$$Drug_{solid} \leftrightarrow Drug_{solution} \quad (1)$$

$$Drug_{solution} + CA_{solution} \leftrightarrow (Drug \cdot CA)_{solution} \quad (2)$$

Equilibrium, according to (2), is given by

$$K_{DCA} = [(Drug \cdot CA)_{solution}] / [Drug_{solution}] \times [CA_{solution}], \quad (3)$$

where $Drug_{solid}$ is the drug in a crystalline solid phase, in equilibrium with the solution; $Drug_{solution}$ is the drug existing in the free form in the solution; $CA_{solution}$ is the free complexing agent in the solution; $(Drug \cdot CA)_{solution}$ is the complexing agent-drug complex in the solution; K_{DCA} is the constant of supramolecular complexing.

The value $Drug_{solution}$ corresponds to the thermodynamic equilibrium solubility in the absence of complexing agents. In the case of complexing, the total concentration of the dissolved drug C_{drug} equals the sum its free and bound forms.

$$C_{Drug} = [Drug_{solution}] + [(Drug\ CA)_{solution}] \quad (4)$$

Thus, the solubility increase of a drug in the solution (X) in the presence of a complexing agent is

$$X = C_{Drug} / [Drug_{solution}] = 1 + K_{DCA} \cdot [CA_{solution}] \quad (5)$$

In our view, X is a good proxy of binding strength in the supramolecular complexes drugs may form with various water-soluble polymers.

All poorly soluble drugs we studied have shown a notable solubility increase when became incorporated into compositions with complexing agents. Table 1 below shows solubility data reported in [22-32] as far as published for the first time in this review.

The binding strength in the complexes grows in the series "dextran 70 < dextrans 40 and 10 ~ < HES < ß-cyclodextrin, fibregum < pectin < arabinogalactan". Complexing of pectin with mezapam and clozapine most probably occurs by acid-base reactions, which accounts for quite a high binding strength. However, other complexing agents lack acid-base groups and the interaction mechanism is most likely "hydrophobic", as in the case of cyclodextrin complexes. Thus, the mechanochemical treatment strengthens considerably the drug binding in compositions. The solubility of drugs increases, depending on the way of mixing, in the series "mixing without milling < high-rate milling < low-rate milling.

The obtained compositions were analyzed by X-ray powder diffraction and thermal methods. All non-processed mixtures showed X-ray and thermal features typical of crystalline drugs, which disappeared or decreased markedly after milling. Therefore, drugs in the ground mixtures partly or fully loose their crystallinity, possibly, as their solid phase becomes disordered and their molecules are dispersed into the excess solid phase of complexing agents, with formation of solid solutions or supramolecular complexes. In the latter case, the solubility changes evidence that the analyzed compositions form more strongly bound complexes when form in the solid phase than in the solution.

2.2. Molecular dynamics and structure of arabonigalactan complexes

AG-drug systems were investigated by ^{1}H NMR spectroscopy [22] for the molecular dynamics of complexes and the mobility of arabinogalactan (AG) molecule fragments. NMR relaxometry is applicable to molecular complexes as the spin-lattice and spin-spin relaxation times (T_1 and T_2, respectively) are highly sensitive to interactions and diffusion mobility of molecules. As a molecule becomes bound in a complex, its diffusion mobility slows down, and the proton relaxation times decrease notably. In the case of rapid complex-solution molecular exchange, the NMR signal decays according to the mono-exponential law. Otherwise, if the exchange is slower than the relaxation time, the kinetics is biexponential:

$$A(t) = P_1 \cdot \exp(-t / T_{21}) + P_2 \cdot \exp(-t / T_{22}) \quad (6)$$

API	Complexing agent/drug mass ratios mass	Solubility pure drug [Drugsolution} g/l / Solubility by complexation CDrug g/l	Solubility increase, X[1]	Reference
Diazepam	Arabinogalactan (1/10)[2]	0.048/0.058	1.2	[22,24]
	Arabinogalactan (1/10)[3]	0.048/0.115	2.4	[22,24]
	Arabinogalactan (1/10)[4]	0.048/2.31	48.2	[23]
	Pectin (1/10)[3]	0.048/0.67	14.2	[23]
	Hydroxyethylstarch (1/10)[4]	0.048/0.075	1.53	[23]
	Beta-cyclodextrin (1/10)[3]	0.048/0.086	1.8	[23]
	Glycyrrhizic acid (1/10)[3]	0.048/0.16	3.4	[29]
	Dextran 10[4]	0.048/0.09	1.9	[23]
	Dextran 40[4]	0.048/0.092	1.9	[23]
	Dextran 70[4]	0.048/0.057	1.2	[23]
Indomethacin	Arabinogalactan (1/10)[2]	0.04/0.044	1.1	[22,24]
	Arabinogalactan (1/10)[3]	0.04/0.396	9.9	[22,24]
	Arabinogalactan (1/10)[4]	0.04/1.59	39.7	[23]
	Hydroxyethylstarch (1/10)[4]	0.04/0.54	13.5	[23]
	Beta-cyclodextrin (1/10)[3]	0.04/0.096	2.4	[23]
Mezapam	Arabinogalactan (1/10)[2]	0.02/0.98	4.9	[22,24]
	Arabinogalactan (1/10)[3]	0.02/0.382	19.1	[22,24]
	Arabinogalactan (1/10)[4]	0.02/2.81	140.6	[23]
	Pectin (1/10)[3]	0.02/1.54	77.1	[23]
	Hydroxyethylstarch(1/10)[4]	0.02/0.04	2.0	[23]
Clozapine	Arabinogalactan (1/10)[2]	0.04/0.176	4.4	[22,24]
	Arabinogalactan (1/10)[3]	0.04/0.82	20.5	[22,24]
	Arabinogalactan (1/10)[4]	0.04/4.32	107.9	[23]
	Pectin (1/10)[3]	0.04/1.63	40.8	[23]

	Hydroxyethylstarch(1/10)[4]	0.04/0.222	5.5	[23]
	Beta-cyclodextrin (1/10)[3]	0.04/0.60	15.1	[23]
	Glycyrrhizic acid (1/10)[3]	0.04/0.088	2.2	[29]
Nifedipine	Arabinogalactan (1/10)[3]	0.18/1.24	6.9	[26]
	Arabinogalactan (1/20)[3]	0.18/2.46	13.7	[26]
	Glycyrrhizic acid (1/10)[3]	0.18/0.92	5.1	[30]
Dihydro-quercitin	Arabinogalactan (1/10)[4]	0.65/3.75	5.9	[23,27]
	Hydroxyethylstarch(1/10)[4]	0.65/1.97	3.0	[23]
	Fibregum(1/10)[4]	0.65/5.72	8.8	[23,27]
Quercitin	Arabinogalactan (1/10)[3]	0.019/0.21	11.6	[28]
	Arabinogalactan (1/20)[3]	0.019/1.28	71.0	[28]
Ibuprofen	Arabinogalactan (1/10)[2]	0.03/0.036	1.2	[29]
	Arabinogalactan (1/10)[4]	0.03/0.85	28.4	[29]
	Hydroxyethylstarch (1/10)[4]	0.03/0.08	2.6	[29]
	Glycyrrhizic acid (1/10)[3]	0.03/0.441	14.7	[29]
Beta-Carotene	Arabinogalactan (1/40)[3]	< 0.001/2.65	> 2000	[25], This article
Warfarin	Arabinogalactan (1/40)[3]	0.021/0.111	5.3	[31]
Contaxantine	Arabinogalactan (1/40)[3]	< 0.001/2.64	> 2000	[25], This article
Albendazol	Arabinogalactan (1/10)[4]	0.003/0.174	58.0	[32]
	Hydroxyethylstarch(1/10)[4]	0.003/0.094	31.3	[32]
Carbenazim	Arabinogalactan (1/10)[4]	0.009/0.146	16.2	[32]
	Hydroxyethylstarch(1/10)[4]	0.009/0.020	2.1	[32]
Simvastatin	Glycyrrhizic acid (1/10)[3]	0.0012/0.314	260	This article
	Arabinogalactan (1/10)[3]	0.0012/0.044	36,7	This article

1 – To determine the solubility of the drug, machined mixture of complexing agent/drug, in amounts of 0.4 grams, as well as the linkage of individual substances which are equivalent to their content in the above mixture was dissolved in 5 ml of water while stirring with a magnetic stirrer at +25 ° C till reaching constant concentration. The concentration of drug in the solution was analyzed by HPLC.
2 – mixing without mechanical treatment;
3 – treatment in a planetary mill, acceleration 40 g;
4 – treatment in a rotary ball mill, acceleration 1 g;

Table 1. Increase in water solubility of some drugs as a result of complexing.

The fast component P_1 and the slow component P_2 correspond, respectively, to the shares of molecules in the complex and in the solution. Typical T_2 values are 0.5-1 s for molecules in the solution and 0.03-0.09 s for those bound in the complex. Shorter T_{21} times mean lower mobility of drug molecules in the latter case.

Similar considerations apply to the mobility within polymers when parts of a macromolecule differ in mobility, possibly, controlled by their spin and conformations.

2.2.1. T_2 measurements

Arabinogalactan shows biexponential relaxation patterns. The calculated parameters for arabinogalactan and AG-drug complexes are listed in Table 2.

Sample	P_1 %	T_{21} msec	P_2 %	T_{22} msec
Arabinogalactan, native[2]	80	17	22	250
Arabinogalactan, treated in planetary mill[2]	65	25	35	250
Clozapine/Arabinogalactan 1/20 w/w No treatment[3]	88	90	12	1000
Clozapine/Arabinogalactan 1/20 w/w Mixture treated in planetary mill[3]	90	40	10	1000
Mezapam/Arabinogalactan 1/20 w/w No treatment[3]	55	50	45	250
Mezapam/Arabinogalactan 1/20 w/w Mixture treated in planetary mill[3]	90	30	10	250
Diazepam/Arabinogalactan 1/20 w/w No treatment[3]	mono	150	-	-
Diazepam/Arabinogalactan 1/20 w/w Mixture treated in planetary mill[3]	20	60	80	800
Indomethacin/Arabinogalactan 1/20 w/w No treatment[3]	58	50	42	900
Indomethacin/Arabinogalactan 1/20 w/w Mixture treated in planetary mill[3]	67	40	33	900

1 - T2 measurements were performed for the aromatic protons of drug molecules, to an accuracy of ± 10%
2 – solvent D_2O;
3 - solvent 70% D_2O + 30% CD_3OD;

Table 2. Spin-spin relaxation times of protons for arabinogalactan and drug molecules in solutions[1]

The short relaxation times may correspond to the interior protons and the long times may represent the exterior protons of the polymer compound. Mechanical activation in a planetary mill increases the molecular mobility of the interior fragments but decreases their percentage. A relatively narrow ~ 6 kHz band in the ^1H NMR spectra of AG powder, which stands out against a broad line associated with dipole-dipole interaction non-averagable in solids, represents a mobile phase with its integral intensity up to ~ 15% of the number of

hydrogen nuclei in the sample. The mobile phase may correspond to fragments of AG macromolecules, possibly, side chains, as one may reasonably hypothesize given that water content in AG never exceeds 2 wt.%. This very fact appears to facilitate AG-Drug molecular complexing on mechanical activation of solids.

AG-Drug systems most often exhibit distinct biexponential kinetics as evidence that the drug molecules are either free or bound in complexes with AG. The bound molecules are more abundant and less mobile in milled samples, while the free ones keep almost invariable NMR relaxation times. The characteristic ^{1}H NMR bands of clozapine and mezapam move to low field on complexing, possibly because the molecules become protonated at the account of minor remnant uronic acid present in AG, the shift being likewise greater in the milled samples. However, no complexing-related shifting appears in the cases of indomethacin and diazepam. The life time of drug molecules in complexes with AG must to be ~$\geq$ 100 ms, judging from the conditions of slow exchange.

The system AG-diazepam offers an illustrative example. Solutions of these mixtures not subjected to mechanical treatment show mono-exponential relaxation behavior, but with shorter times than in free diazepam, likely as a result of rapid solution-complex molecular exchange. The milled mixtures, on the contrary, have biexponential kinetics corresponding to slower exchange of molecules and stronger binding.

Thus, dynamic NMR spectroscopy of all Drug-AG solutions indicates formation of supramolecular drug-polysaccharide complexes, like the data on solubility increase. Most likely, the complexing sites are at side chain spaces in the branching macromolecules. Unlike cyclodextrins, ensembles of polysaccharide molecules (including arabainogalactan) are micro-heterogeneous in mass and structure. As a result, molecular modeling of the complex is very difficult. The binding mechanism appears to lie mainly with hydrophobic interactions [33, 34] which are typical of guest-host cyclodextrin complexes. A certain support to this hypothesis comes from stronger binding of highly lipophilic drugs which are almost insoluble in water. In this case, the branched structure of AG macromolecules [35, 36] is especially favorable for complexing. However, Coulomb interactions may contribute as well in the presence of acid-base groups in polysaccharides and drugs [37].

2.3. Transformations of polysaccharides in solid state and in solutions

Macromolecules characteristically have broadly varying molecular weights, from ~10^3 to ~10^7 Da. Macromolecules in polymers involved in technological production of various materials may experience mechanical action and partial destruction (breakdown of chains) whereby their molecular weight becomes ever more heterogeneous and diminishes on average [38]. The destructive change may be especially prominent in “dry” technological processes, such as pulverization, pelleting, or mixing, e.g., in mechanochemical solid-state complexing of drugs with water-soluble polymers (polysaccharides). Partial destruction of polymers may change their toxicological properties which have to be cautiously monitored when making new drugs and food products.

Molecular weight patterns were studied [39] in polysaccharides (dextrans 10, 40, and 70, HES 200/0.5, and larch AG and acacia FG gum) by gel permeation chromatography (GPC) [40] of samples treated in rotary and planetary mills; the obtained materials were tested for their toxicity.

See Fig. 2 for example chromatograms of AG and Table 3 for calculated molecular weights of the analyzed polysaccharides before and after mechanical treatment.

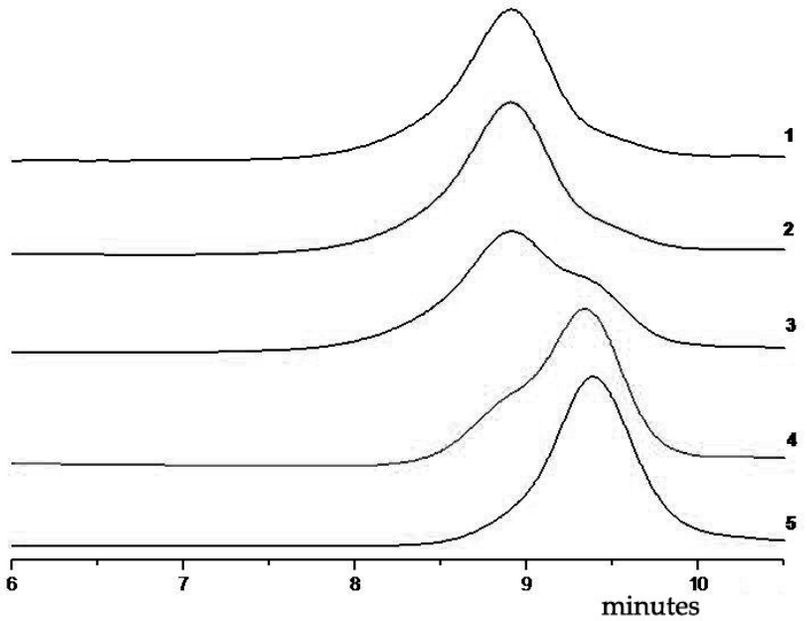


Figure 2. GPC chromatograms of 0.02 wt.% arabinogalactan water solution. 1 = native; 2 – 5 = subjected to mechanical treatment: ball mill, 2 hours (2), ball mill, 24 hours (3), planetary mill, 10 min (4), extremely intense treatment, mixed ball loading (5); Eluent: H_2O/0.1 N $LiNO_3$

Polysaccharides ground in a high-rate planetary mill diminish markedly in molecular weight and change slightly their polydispersity index Mw/Mn. The mechanical destruction is stronger in polymers with larger molecular weights, which agrees with published evidence [39]. Note that highly branching macromolecules (HES and AG) break down into roughly equal fragments. The Mw/Mn ratios in polysaccharides do not grow much, possibly, because destruction mostly affects their high-molecular fractions. Destruction is apparently controlled by the structure of polysaccharide molecules and physicochemical chain breakdown mechanisms. According to a model for linear synthetic polymers [41], the chains that occur in the middle of macromolecules are especially prone to failure. Destruction of HES and AG is qualitatively similar to that model, though dextrans and fibregum may deform by a different mechanism.

The results for larch arabinogalactan are worth of special consideration. High-rate treatment in a planetary mill, especially with mixed ball loading reduces strongly the AG molecular weight. According to chromatograms (Fig. 2), its Mw 17.3 kDa macromolecules split quantitatively into two almost equal parts of Mw = 8.3 kDa, while their Mw/Mn ratio decreases to 1.08 [23]. Therefore, the native AG molecules may consist of two relatively weakly bonded fragments of equal molecular weights and easily break down on milling

[23,39]. Note that AG macromolecules with MM (Molecular Mass) ~9 kDa are likewise the main product of chemical destruction of Canadian larch AG [42].

Furthermore, the analyzed polysaccharides experience almost no mechanical failure on low-rate grinding in a rotary mill (Table 3). Thus, ball rotary milling appears to be most often preferable, as molecular mass changes in technologically produced polymers are commonly unwanted in view of their further use in dietary supplements and drugs, otherwise additional tests and standardization may be required.

Sample	Treatment	Mn, kDa	Mw, kDa	Mw/ Mn	Weight shares of macromolecules, kDa	
					10%	90%
Fibregum	Native	146.6	256.7	1.8	<75.9	<528.2
	Planetary mill, 20g, 20min	31.4	55.2	1.8	<16.3	<113.5
	Ball mill, 1g, 4hours	120.3	231.6	1.9	<60.3	<478.4
Arabinogalactan	Native	13.9	17.3	1.2	<9.0	<27.9
	Planetary mill, 20g, 20min	9.3	11.2	1.2	<6.1	<18.4
	Ball mill, 1g, 4hours	13.1	16.3	1.2	<8.1	<26.2
Hydroxyethyl starch 200/0,5	Native	47.9	116.9	2.4	<20.9	<265.3
	Planetary mill, 20g, 20min	26.6	55.2	2.1	<12.7	<118.9
	Ball mill, 1g, 4hours	45.6	105.5	2.3	<20.0	<237.6
Dextran 70	Native	30.9	76.4	2.5	<14.0	<174.7
	Planetary mill, 20g, 20min	22.7	54.8	2.4	<10.4	<123.5
	Ball mill, 1g, 4hours	29.6	73.5	2.5	<13.4	<169.2
Dextran 40	Native	24.6	38.0	1.5	<13.3	<72.3
	Planetary mill, 20g, 20min	19.5	31.9	1.6	<10.4	<61.3
	Ball mill, 1g, 4hours	24.3	37.4	1.5	<13.0	<71.2
Dextran 10	Native	8.3	13.4	1.6	<4.2	<26.4
	Planetary mill, 20g, 20min	8.0	12.1	1.5	<41.9	<22.7
	Ball mill, 1g, 4hours	8.3	13.4	1.6	<4.2	<26.3

Table 3. Molecular mass distribution of polysaccharides

Toxicological tests of the milled polysaccharides show that a single intragastric injection administered at doses from 500 to 6000 mg/kg body weight caused no death in experimental animals. Their appearance, behavior, and state were within the background over the whole dose range; no statistically significant changes in body temperature relative to the control was observed, and body weight growth was uniform in all groups. Injections of the tested polysaccharides neither induced any considerable effect on the central nervous system of the mice. Patomorphological postmortem examination of mice in 14 days after polysaccharide administration revealed no pathology in thoracic and abdominal cavities. The median lethal dose LD_{50} for all polysaccharides was over 6000 mg/kg body weight on single intragastric injection.

2.4. Supramolecular structures of glycyrrhizic acid (GA) and poorly soluble drugs water solutions: Synthesis and properties

Biosynthetic and natural plant-derived carbohydrate-bearing metabolic agents have been increasingly used for obtaining complexes (clathrates) with drugs in drug delivery research. The mechanism of GA-pharmacon interaction in solutions may consist in involving drug molecules into self-associates (micelles) that exist in a wide range of concentrations in GA solutions. Until recently however, the existence of micelles in GA solutions had no direct proof but was either inferred from measured concentration dependences of solution viscosity [43] or was studied by dynamic NMR spectroscopy in water-methanol solutions [44]; the latter (30% concentration) were at the same time used as solvent for technical reasons. Thus, the molecular mechanism of drug complexing remained unclear, whether it was incorporation into micelles or supramolecular complexes with GA in water solutions, without organic solvents which change notably the GA-pharmacon reactions.

GA water solutions, with and without the presence of poorly soluble drugs, were investigated in [29] using gel permeation chromatography, which allows detecting self-associates/micelles and estimating their sizes and concentrations. On the other hand, solid GA-drug dispersions were obtained with the mechanochemical approach developed earlier [10, 11]. The binding strengths in GA-pharmacon supramolecular complexes or GA micelles in water solutions were compared using the criterion of solubility increase in poorly soluble drugs [22] and studied in terms of pharmacological activity.

Chromatograms of GA water solutions (Fig. 3) show peaks of high-molecular (~ 46-67 kDa) forms over all studied concentration ranges, while the GA molecular weight is 836.96 Da (Table 4).

The peak areas, being proportional to the solution concentrations and calculated relative to the known amounts of standard dextrans, show that almost all GA is stored in the solution. This, in our view, is evidence for the existence of GA self-associates (micelles). The critical concentration of micelle formation (CMC) was estimated earlier [43] from viscosity change in GA solutions to be 0.004 wt. % (0.05 mM). In our case estimating exact CMC is difficult for the limited sensitivity of refractometric detector and for dilution in the chromatographic column. However, it may be inferred from the time to the chromatographic peak (~0,5 min) and the elution rate. The solution we studied underwent about 10-fold dilution, and the derived CMC is ~≤ 0.0001 wt.%, (0.001mM), or far less than in water-methanol solution (0.04-0.08 wt.% or 0.5-1.0 mM) [44]. In diluted 0.01-0.001 wt. % solutions, there is only one type of micelles (~ 66 kDa) with a very low Mw/Mn ratio of 1.08-1.06. As the GA solutions reach concentrations of 0.5 wt.%, micelles decrease in weight to form ~ 46 kDa bodies and increase in Mw/Mn ratios. Therefore, almost all glycyrrhizic acid in water solutions from 0.0001 to 0.5 wt.% exists in the self-associated form of micelles, out of which the ones with MM= ~ 66 kDa consisting of about 80 GA molecules are most stable.

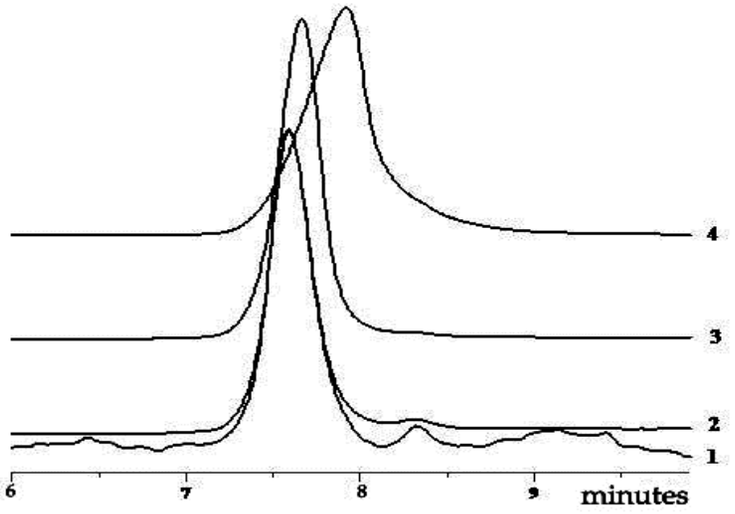


Figure 3. GPS chromatograms of glycyrrhizic acid water solution. 1 - concentration - 0.001, 2 - 0.01, 3 - 0.1, 4 - 0.5 wt.%.

COMPOSITION	Molecular masses	Solution concentration, wt. %			
		0.001	0.01	0.1	0.5
GLYCYRRIZIC ACID	Mw/Mn kDa	65.93/61.0	66.2/62.5	60.7/57.3	45.3/36.5
GLYCYRRIZIC ACID/ /IBUPROFEN 10/1 w/w Treated in planetary mill 3 min	Mw/Mn kDa	69.0/67.2	69.4/65.3	65.2/61.7	48.6/39.0

Table 4. Molecular-mass characteristics of micelles in solutions of glycyrrhizic acid and its composition with ibuprofen

2.4.1. GA solid dispersions with poorly water-soluble drugs

Solid GA dispersions with ibuprofen, phenylbutazone, clozapine, and diazepam were obtained by milling with GA (10:1 mass, or 2.5/1 – 4/1 molar ratios). Their thermal analysis data indicate that the crystalline phase of the drugs becomes disordered, until complete loss of crystallinity. In our view, the molecules of drugs may disperse into the excess solid GA with formation of solid solutions. Other investigated systems behave in a similar way.

As the dispersions dissolve, the drugs become more soluble in water (Table 1), this being evidence of the efficiency of GA as a solubilizing agent and the mechanochemical treatment as a tool for synthesis of water-soluble solid dispersions. GA has a nearly intermediate solubilizing effect higher than HES but lower than AG.

2.4.2. GPC of dissolved GA-drug solid dispersions

The GPC data for GA-ibuprofen dispersions in water are shown in Table 4. Similar results of MM increase in micelles were obtained for the GA-diazepam, GA- phenylbutazone, and GA-clozapine systems. The peak areas, proportional to the concentrations of the analyzed solutions, indicate that they bear almost the entire mass of GA-drug samples. Thus, the dissolved drugs in the GA-drug complexes are likewise self-associated in micelles which are stable in a broad range of concentrations, as well as in the solutions of native GA. Therefore, we suggest that poorly soluble drugs increase their solubility by incorporating into GA micelles/self-associates. GA molecules contain a hydrophilic (two glucuronide residues) and a hydrophobic (triterpene) components (Fig. 4).

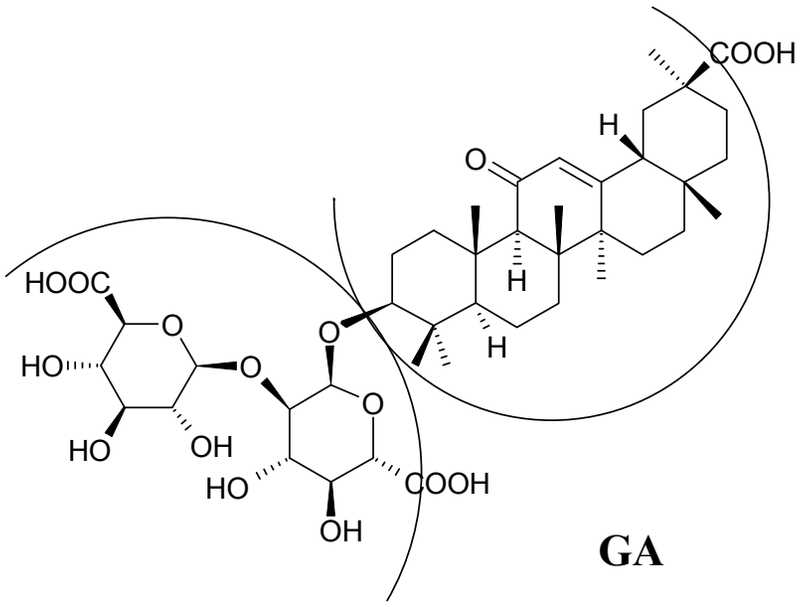


Figure 4. The structure of glycyrrhizic acid

In micelles, the latter are most likely oriented inward and the former toward the outer surface of the self-associate, while the drug molecules may occur either in the interior hydrophobic part or complex with the exterior hydrophilic part of the micelles. Unfortunately, the experimental evidence is insufficient to judge about these subtle GA-drug interaction mechanisms. Generally, the MM of GA-pharmacon micelles are 5-7% higher than those in GA water solutions, all over the studied range of concentrations. Another possibility is that drug molecules may substitute for some GA molecules while the micelles generally grow in size. As the solution concentration increases, the size difference of micelles grows correspondingly. Note that the region of high GA concentrations is proximal to the conditions in which the solubility of drugs was measured, this being additional evidence for the suggested mechanism of water solubility increase. An explanation for the decreasing MM differences on dilution may be that drug molecules can escape from GA micelles during GPC process to diluted solutions and appear in chromatograms as individual substances eluted at different rates [45]. Anyway, further investigation is needed to gain more insights into the GA-drug interaction.

3. Pharmacological activity of supramolecular complexes

The pharamacological activity of the complexes was investigated *in vivo* on females and males of outbred white mice and on Wistar rats, obtained from the SPF vivarium of the Institute of Cytology and Genetics, Novosibirsk. All animal procedures and experiments followed the 1986 Convention on Humane Care and Use of Laboratory Animals. The activity was determined using standard pharmacological tests [46]. All studied complexes, with some exceptions, are AG-Drug or GA-Drug compositions of 10:1 weight ratio, which was found out to be of greatest efficacy [22].

3.1. Arabinogalactan-drug complexes

As a special study has shown, AG complexing with nonsteroidal anti-inflammatory drugs and non-narcotic analgesics of various action mechanisms can reduce the required dose for 5-100 times and, hence, avoid the side effects typical of the drugs.

For instance, complexing with indomethacin allows 10 to 20 times dose reduction relative to the standard and induces twice fewer cases of lesion to gastric mucous membrane, with the same high anti-inflammatory activity [22, 24].

Administration of phenylbutazone in a complex with AG, at ten times lower pharmacon content, stimulated analgesic activity, both in the model of chemical effect and on thermal action, which may expand its applicability scope (Table 5).

Compounds	Hot plate, s	Acetic acid writhing model, amount
Control	28.73±2.34	4.76±0.26
Phenylbutazone:AG 1:10. 120 mg/kg per os	42.20±5.20*	2.00±0.09*
Phenylbutazone, 12 mg/kg per os	11.40±0.80	4.25±0.50
Ibuprofen:AG 1:10. 200 mg/kg per os	23.50±2.43	0.75±0.01*
Ibuprofen, 200 mg/kg per os	19.5±2.45	1.50±0.03*
Metamizole sodium: AG 1:10. 50 mg/kg per os	32.50±3.70	1.75±0.08
Metamizole sodium, 50 mg/kg per os	30.40±3.20	0.63±0.10
Metamizole sodium, 5 mg/kg per os	17.70±2.30	4.25±0.75
*p <0.05 relative to control		

Table 5. Analgesic activity of AG complexes with non-narcotic analgesics

AG complexes with 10 times smaller doses of ibuprofen and metamizole sodium showed a high analgesic activity in visceral pain models (the former) and in two models of test pain.

Studies in this line were continued, with the above approach and proceeding from the obtained results, on AG complexes with drugs that involve the central nervous system. Specifically, AG complexing with diazepam allowed reducing the dose for ten times and enhanced the anxiolytic effect. AG complexes with mezapam acted as standard anxiolytics

at 20 times lower doses of the phamacon. The AG-clozapine complexing provides a two-fold dose decrease at a higher sedative action.

Another objective was to study drugs involving the blood coagulation and cardiovascular systems. The AG-warfarin (WF) complex was tested on intragastric injection in females of Wistar rats, with prothrombin time (PT, in seconds) as the principal criterion of the action. PT is the classical laboratory test of the exterior blood coagulation pathway used to evaluate the system of hemostasis in general and the efficacy of warfarin therapy in particular. The complex was administered once, in a dose of 20 mg/kg body weight, which is equivalent to 2 mg/kg warfarin. In 24 hours after the injection, PT increased considerably (to 30 s against the 11.63 s for the intact control). With free warfarin, this time was 42 s, or 28.5% longer than with the AG-warfarin complex, but it equalized (21 s) for both agents in 48 hours after a single injection (Fig. 5,6).

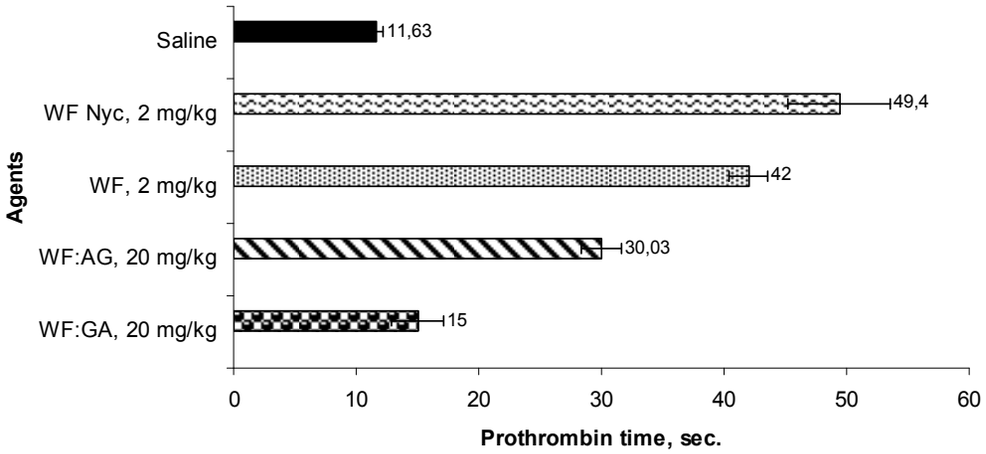


Figure 5. Prothrombin time, 24 hours after single dosing of WF:AG and WF:GA.

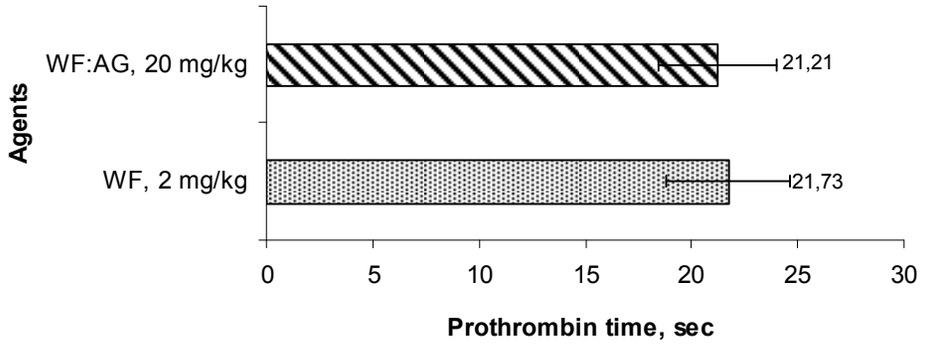


Figure 6. Prothrombin time, 48 hours after single dosing of WF:AG and WF.

The difference in pharmacokinetics between the AG:WF complex and free warfarin was further explored after a single administration of 20 and 2 mg/kg body weight, respectively. Blood was sampled in 1, 8, 10, 12, 24, 48, and 72 hours after injection on decapitation. Fig. 7 shows average plasma warfarin contents, and pharmacokinetic parameters are listed in Table 6. The concentrations of the compounds increase in a similar way but free warfarin

reaches C_{max} seven hours sooner (T_{max}) than in complexes with AG, and the concentrations become equal in 24 hours after single dosing. Excretion, on the contrary, is slower in pure warfarin than in the complex, which is consistent with 27 % higher clearance (CL) in the complex than in the free drug. Thus, warfarin increases more smoothly when bound with AG than in the free form and thus poses lower bleeding risks associated with its abrupt rise during dosage adjustment. Furthermore, shorter mean retention times (MRT) for the AG:WF complex may secure the following injection and accelerate warfarin excretion in the case of drug withdrawal.

Thus, complexing of warfarin with AG increases its safety and reduces unwanted bleeding risks in the case of anti-coagulant treatment.

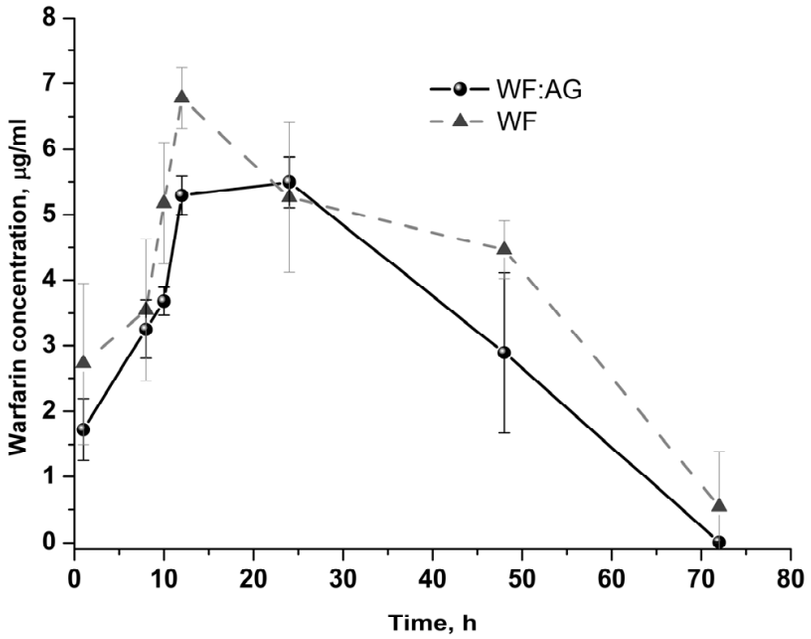


Figure 7. Mean plasma concentration–time profile of WF:AG and blank WF after single oral administration at a dose of 20 mg/kg (dose of WF is equal to 2 mg/kg) and 2 mg/kg, respectively.

Compounds	WF, 2 mg/kg	WF:AG, 20 mg/kg
CL, ml/h	1.52±0.03	1.93±0.18*
MRT, h	31.39±1.82	21.81±2.38*
Terminal half life, $T_{1/2}$, h	5.11±0.24	6.38±2.55
T_{max}, h	11.00±1.41	18.00±8.49
C_{max}, μg/ml	6.47±0.91	5.64±0.19
AUC, μg h/ml	263.01±0.02	208.34±20.03*
*p<0.05 against WF		

Table 6. Pharmacokinetic parameters of WF and WF:AG

Another drug in which we studied the pharmacological effect of complexing with AG was nifedipine (NF). NF is a dihydropyridine blocker of slow calcium channels that dilates coronary and peripheral vessels and reduces the oxygen demand in myocardium.

Nifedipine exerts a minor negative inotropic effect and a very weak antiarrhythmic action. Intravenous injection of 3.5 mg/kg AG:NF complex (0.35 mg/kg NF) caused 26 % drop of blood pressure, measured via a carotid cannula, while 0.35 mg/kg NF can provide only a 9% decrease.

Being aware that nifedipine has a pleiotropic antiarrhythmic effect, besides the basic hypotensive action, the NF:AG complex was investigated in this respect on intravenous injection in a model of arrhythmia induced with 250 mg/kg calcium chloride. The complex administered in a dose of 0.175 mg/kg body weight (0.0175 mg/kg NF) arrested lethal heart rate disorder in 100% and 65% of cases, respectively, when applied prior to and after exposure to the arrhythmogen. Pure NF at 0.0175 mg/kg had no antiarrhythmic effect in the model of calcium chloride arrhythmia.

Thus, the NF:AG complex has demonstrated a stronger hypotensive and antiarrhythmic action than pure NF on intravenous administration, while its effective hypotensive dose is ten times smaller. Arabinogalactan itself does not induce any statistically significant decrease in blood pressure and cardiac rates. Furthermore, it is important that the new method adds another water-soluble form of NF as there is the only soluble nifedipine (adalate) available in the market.

A similar hypotensive effect was obtained with nisoldipine, another dihydropyridine.

Complexing of hypoglycemic drugs (metformin, rosiglitazone, insulin) with AG increased their solubility but allowed no dose reduction, though it improved notably the state of animals exposed to a toxic dose of alloxan, an agent simulating trial hypergclycemia.

The reported pharmacological data on the AG complexes with these pharmacons agree with the results on complexing with terpenoids (glycirrhizic acid, stevioside, and rebaudioside). In both cases, complexing increases the basic activity of drugs, allows dose reduction and forms new properties. We suggest to call this effect complexing or clathration of pharmacons with plant-derived carbohydrate-bearing metabolic agents.

3.2. Glycyrrhizic acid (GA)-drug complexes

3.2.1. Nonsteroidal anti-inflammatory drugs (NSAID)

GA:NSAID complexes with acetylsalicylic acid (ASA), diclofenac (OF), phenylbutazone (BD), and indomethacin (IM) were synthesized in solutions [24] and in solid state [23]. Complexing was confirmed by spectrometry. In IR spectra, the bands of hydroxyl and carbonyl groups of the glycoside were shifted to short wave numbers.

All mentioned complexes show anti-inflammatory action in smaller doses than the primary drug, and have 3-11 times larger therapeutic index (LD_{50} /ED_{50}) [47,48] (Table 7).

GA complexes with aspirin and diclofenac (GA:ASA, GA:OF) exerted a prominent anti-inflammatory action in six models of acute inflammation induced by carrageenan, formalin, histamine, serotonin, Difko's agar, and trypsin, as well as in the cases of

chronic inflammation (cotton and pocket granulomas) in intact and adrenalectomized animals [48].

Compounds	Dose range of complex*	Dose range of free NSAID
GA : ASA (1:1)	4500/82=54.8	1900/98=19.4
GA: OF (1:1)	1750/12.5=140	310/8=33.7
GA : BD (1:1)	3150/62=50.8	880/56=15.7
GA : AN (1:1)	8000/68=117.6	570/55=10.3

Table 7. Anti-inflammatory effects of GA complexes and free NSAID. *LD_{50} /ED_{50}; ED_{50} – effective dose

The anti-inflammatory action of the GA:IM complex is stronger than of the free drug at equal dosing (10 mg/kg body weight). GA:NSAID complexing also potentiates other (analgesic, antipyretic) biological activities [29]. The GA:OF complex exerted a more prominent anesthetic action than diclofenac in electric and thermal stimulation (57.5±2.0 and 43.2±2.6) and exceeded the amidopyrine effect in the case of thermoalgesic stimulation (23.4±1.1 and 18.5±1.4). The anesthetic activity of the GA: metamisole sodium (GA:AN) complex is 11.4 times higher relative to metamisole sodium (AN) alone [23] while and that of the GA:ASA complex exceeds the effect of aspirin in animals exposed to thermoalgesic stimuli. The GA complexes with ASA and OF are 3 and 2.3 times more potent pain relievers than the respective NSAID in acetyl choline writhing model. The GA:ASA complex demonstrates a 4 times higher therapeutic index than aspirin in the acetic acid writhing model. The GA:ASA and GA:OF complexes show high antipyretic activity, twice larger than in the pure pharmacons [47,48].

Thus, water-soluble GA:ASA and GA:OF complexes evoke prominent anti-inflammatory and antipyretic effects, their spectrum of pharmacological activities and therapeutic ratio being larger than in the respective NSAID. The complexes also induce a marked membrane-stabilizing effect and reduce accumulation of primary and secondary products of lipid peroxidation in animals with chronic inflammation.

Complexing of glycyrrhizic acid with ibuprofen increases the analgesic action of the latter at twice lower doses.

Furthermore, GA complexes irritate less strongly the gastric mucosal membrane than their NSAID counterparts. For instance, the GA:ASA complex promotes reparation of ulcers though the ulcerogenic activity of GA:OF is minor. Both complexes diminish E1 and E2 blood prostaglandins in animals with chronic inflammation. The complexes can be recommended for clinical trials as anti-inflammatory agents, including for patients that suffer from ulcer of stomach and duodenum. The acute toxic effect of GA:NSAID complexes is 2 to 14 times as low as in the respective pharmacons (Table 8) [47, 48].

GA:NSAID complexing obviously exerts a synergetic effect of higher biological activity along with lower toxicity and weaker ulcerogenic action on the gastrointestinal tract, and

has a higher water solubility. It is evident on comparing the pharmacological activities of the ASA:GA and OF:GA supramolecular complexes obtained by dissolution and solid-state mehcanochemical synthesis that the latter opens a promising perspective of a technologically preferable and saving way of producing highly active NSAI drugs [23].

Complexes	LD_{50}, mg/kg	Pharmacon LD_{50}, mg/kg
GA : ASA (1:1)	4500	1900
GA: OF (1:1)	1750	310
GA : BD (1:1)	3150	880
GA : AN (1:1)	8000	570

Table 8. Acute toxicity of GA:NSAID complexes in mice (per os)

The GA:Rofecoxib complex at doses 50 and 100 mg/kg body weight shows no active anti-inflammatory and analgesic effects but is more highly soluble.

3.2.2. Prostaglandins

Prostaglandins have been of broad use in human and veterinary medicine for their ability, in small doses, of stimulating womb muscles.

Veterinary uses of prostaglandins have been especially important: prostaglandin-based drugs are employed in swine breeding for farrow synchronization and are highly potent in preparing cattle and horse females for artificial insemination; these drugs allowed solving many problems with puerperal complications in cows and horses.

Kloprostenol, one main veterinary drug, is made by multistage synthesis, like other prostaglandins, which imposes its high price. It is urgent to reduce the effective dose and at the same time to increase the stability of labile prostaglandins in finished drugs. Both solutions have been found through complexing E and F prostaglandins (PGE1, PGE2, PGF2α), sulprostone (SP) and kloprostenol with GA. The complexes were synthesized and tested for uterotonic activity (Table 9).

In experiments on rats and guinea pigs, the GA:PGE1 (1:1) and GA:PGF2α (1:1) complexes changed the amplitude of uterine actions twice more strongly than the same concentration of PGE1 sodium (10-8 g/ml). SP and PGE2 as complexes with GA (1:1) induced three times greater amplitudes and increased uteric tonicity [49].

An efficient veterinary drug of klatraprostin has been developed on the basis of GA complexing with kloprostenol, a well known synthetic luteolitic prostaglandin, at doses five times as low as in the world practice [48, 49]. The drug is cheaper than its imported analogs while its action is more physiological than in the best foreign counterparts.

Klatiram, which contains an aminoacid (tyrosine) besides GA and kloprostenol [48,49], has a still greater potency. It is more effective than estrofan though bears 100 times less prostaglandin (kloprostenol).

Compound	Change of uterine contraction amplitude, %	P	Change in uterus tonus, %	P
GA: PGE1	53.4±5.0	<0.002	49.4±1.2	<0.002
PGE1	24.3±1.5	<0.05	30.7±2.2	<0.05
GA : SP	150.0±11.0	<0.001	135.0±10.0	<.001
SP	50.0±5.0	<0.001	115.0±9.5	<0.001
GA : PGE2	63.5±6.0	<0.001	40.7±4.0	<0.002
PGE2	20.0±2.8	<0.05	33.5±2.4	<0.05
GA: PGF2α	55.6±5.0	<0.001	61.0±5.6	<0.001
PGF2α	27.8±1.5	<0.05	39.4±5.3	<0.02

Table 9. Uterotonic activity of prostaglandins and their GA complexes (1:1) in rats *ex vivo*, phosphate buffer C = 10-8 g/ml. PGE1 , PGE2, SP, PGF2α.

3.2.3. Cardiovascular drugs and anticoagulant warfarin

Pharmacological activity was also investigated in GA complexes with antiarrhythmic Lappaconitine hydrobromide (LA) and antihypertensive nifedipine (NF) drugs.

Lappaconitine hydrobromide belongs to the group of clinically used antiarrhythmic drugs and is administered to patients with various rhythm disorders, especially, ventricular arrhythmia, paroxysmal ciliary arrhythmia, and monofocal atrial tachycardia, but it has a drawback of high toxicity.

In special experiments on antiarrhythmic action of LA:GA complexes, the one patented as alaglizin [50] showed the highest efficacy. Alaglizin, being ten times less toxic than LA, induced antiarrhythmic effects in models of calcium chloride and aconitine arrhythmia and had the highest antiarrhythmic therapeutic index (LD_{50}/ED_{50}) among all available drugs of this kind. When administered at 0.125 mg/kg and 0.250 mg/kg body weight, alaglizin causes no influence on electrocardiogram parameters, according to an extended study in models of calcium chloride and adrenal arrhythmia. Intravenous injection of 0.125 mg/kg alaglizin prior to exposure to a lethal dose of calcium chloride blocked arrhythmia in 80% of rats; 0.250 mg/kg of alaglizin applied after arrhythmogenic $CaCl_2$ stopped the already developed arrhythmia in 50% of animals. A single injection of 0.125 mg/kg and 0.250 mg/kg alaglizin in a model of adrenal arrhythmia prevented full development of arrhythmia; 50% and 100 % of animals recovered normal ECG parameters on receiving 0.125 mg/kg and 0.250 mg/kg alaglizin, respectively. Alaglizin in the model had an ED_{50} of 0.125 mg/kg body weight against 0.290 mg/kg in LA and thus contained 14 times lower lappaconitine.

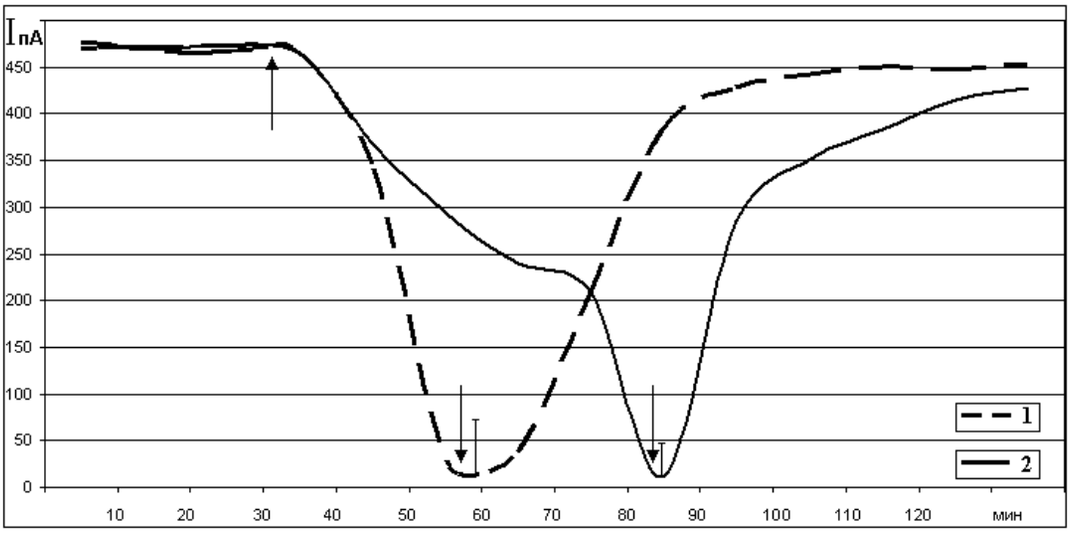
The NF:GA complex exerted hypotensive action on intravenous injection of its water solution in rats at a dose with ten times lower NF [51, 52]. The hypotensive effect of GA complexing with nisoldipine (another dihydropyridine) was similar to that of complexes with AG.

Complexing can have an important consequence of amplifying the pleiotropic effect of pharmacons (see above), such as NF. The wanted antiarrhythmic effect of NF can be only

achieved with a dose that causes almost critical blood pressure drop, but the NF:GA complex induces the same effect with 29 times lower NF than the hypotensive dose.

Thus, the NF:GA complex is a promising parenteral drug with universal activity against hypertensive crises attendant with arrhythmia.

A presumable action mechanism of the NF:GA complex was studied *in vitro* on neurons isolated from peripharyngeal ganglia of *Lymnacea stagnalis* molluscs. The provoked responses are arrested completely with an NF concentration as high as 3.0 mM but with 30 times as low concentration (0.1 mM) of NF:GA. The responses to NF are blocked faster and recover sooner after neuron washing than the responses induced by the complex, which indicates stronger binding of the latter with receptors and its more prolonged action. The higher receptor affinity of the complex is corroborated by comparing the NF and NF:GA effects on calcium channels [51] (Fig. 8)



1. amplitude of calcium currents induced by Nifedipine.
2. amplitude of calcium currents induced by Nifedipine clathrate with glycyrrhizic acid.
Upward arrows show start point of the blocker action. Downward arrows show start point of blockers washout.
X axis set as time in minutes.
Y axis set as amplitude of incoming current in pA.

Figure 8. Averaged changes in calcium current amplitude induced by action and washout of blockers within a group of neurons (12 cells in each group).

The propranolol-GA complex studied in terms of hypotensive activity ensured 14% blood pressure drop in normotensive animals at a dose of 0.2 mg/kg body weight (minimum dose 0.0025 mg/kg gave a 11% decrease). Pure propranolol decreases blood pressure for 11% in a dose of 0.2 mg/kg; a similar pressure drop may be achieved by the minimum dose 0.0025 mg/kg, but it is statistically unconfident. Therefore, complexing enhances and stabilizes the hypotensive action of propranolol and allows 12.5-fold reduction of its dose.

Furthermore, complexing amplifies the antiarrhythmic action (pleiotropic effect) of propranolol. Namely, 100% of animals exposed to 0.3 mg/kg arrhythomogenic 0.1 % adrenalin survived on administration of 0.0025 mg/kg GA:Propranolol, while only 40 %

survived among those who received 0.0002 mg/kg propranolol (this being the dose contained in the complex). Control animals responded to adrenalin by fatal disrhythmia grading into ventricular fibrillation, and 100% of them died [53].

The complex of GA with anticoagulant warfarin was investigated in a dose of 20 mg/kg body weight, which corresponds to 2 mg/kg WF. Prothrombin time was first measured six hours after a single intragastric injection. The interval was chosen proceeding from pharmacokinetics of warfarin: its plasma metabolites reach the maximum in 6-12 hours in rats [54]. In our experiments, PT has shown a confident change only in the positive control group with 2 mg/kg WF, but it is not clinically significant as it fails to ensure the required increase in coagulation time. A significant PT increase was observed on single intragastric administration of WF, while the WF:GA complex induced a smooth PT rise as late as in 30 hours (two injections) after the beginning of the experiment and the values corresponding to the positive control (WF) were reached only in 54 hours (three injections). Thus, WF:GA complexing made WF more soluble in water but slowed down its wanted anticoagulant action, possibly because GA molecules screened its active centers (Fig. 9).

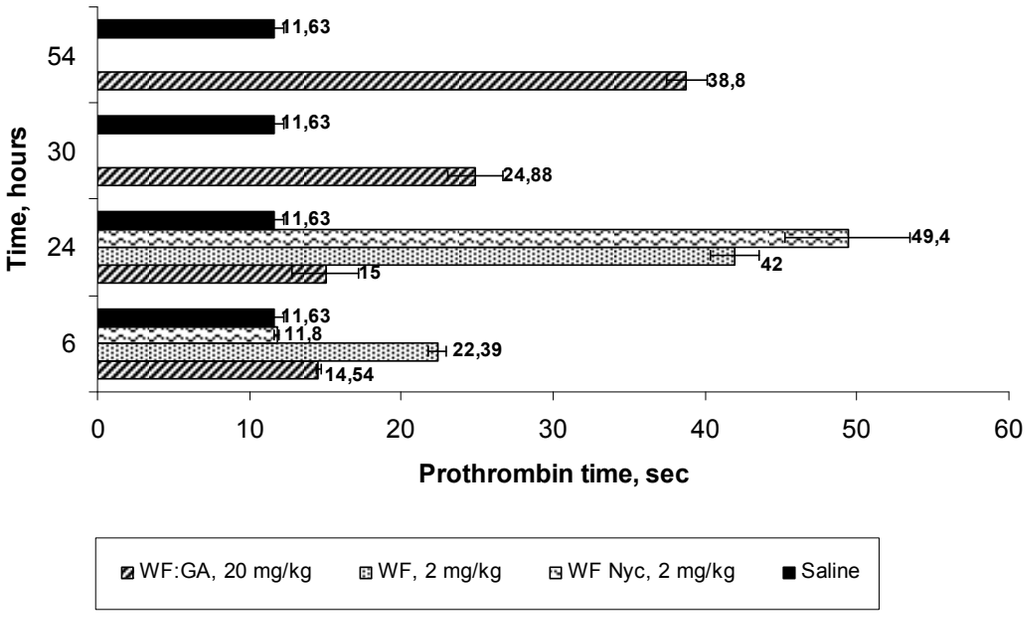


Figure 9. Prothrombin time of WF:GA

3.2.4. Psychotropic drugs

The complexation effect was discovered in a pharmacological study of GA complexes with antidepressant fluoxetine and with anxiolytic gamma-amino-beta-phenylbutirate hydrochloride. Fluoxetine (FL), N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine acts as a depression antagonist by inhibiting serotonin neuronal uptake in CNS. Antidepressants are known to have many drawbacks, such as large doses, a narrow activity spectrum, high toxicity and prolonged elimination that involves liver cells and exerts deleterious effects on the renal function.

The study of fluoxetine complexes with GA (hereafter fluaglizin) aimed primarily at checking the possibility to alleviate the side effects of the pharmacon. In preliminary testing, the 1:10 FL:GA complex showed the highest activity. The complex, with 0.072 pharmacon parts in its one weight part, was patented under the name of fluaglizin (FG) [55,56]. Note that its LD_{50} exceeds 5000 mg/kg body weight against LD_{50} =248 mg/kg in fluoxetine.

Fluaglizin exerts a more prominent antidepressant effect than fluoxetine on single-dose administration in the Porsolt test and is a stronger serotonin uptake inhibitor. For instance, it suppressed the action of chloral hydrate more effectively than fluoxetine in a test with 5-hydroxytryptophan. Like fluoxetine, fluaglizin lacks anxiolytic activity.

The antidepressive effect of fluaglizin was identical to that of fluoxetine in a model of social depression in mice, namely, the animals became twice more communicative toward familiar and unfamiliar partners (had twice greater frequency and time of contacts). The dose of fluoxetine in the FL:GA complex is 1.08 mg/kg body weight against the standard 15 mg/kg. Fluaglizin, like fluoxetine, prevents blood glucose drop and attenuates peroxidation normalizing the antioxidant status of depressed individuals [55, 56].

In order to understand the mechanism of FL:GA action and compare it with fluoxetine, we studied its effect on contents of catecholamines and their precursors in different brain parts on single and therapeutic 25 mg/kg administration. The 17 times lower dose of fluoxetine in FL:GA complexes induced a weaker effect on serotonin uptake and triggered dophamine exchange in brain [57]. The nootropic activity of fluoxetine, first mentioned in [48], shows up also in the FL:GA complex.

Fluoxetine (30 μM) is known to suppress epileptiform activity which is evident in 50% lower-amplitude oscillations of electric potential (reflecting the activity of nerve cells) in response to pulse stimulation of hippocampus on the background of picrotoxin action.

Like fluoxetine, fluaglizin inhibits bikukulin-induced epileptiform activity in hippocampus sections in rats and acts as epilepsy antagonist [48].

Phenybut (FB), 4-amino-3-phenyl-butyric acid, is a nootropic and tranquilizing drug which relieves stress and anxiety and improves sleep. It is used in clinical practice against asthenia, neurotic anxiety, and sleep disorders, prior to surgery, and for preventing naupathia. It, however, has drawbacks of inducing somnolence and allergic reactions.

The FB:GA complex is twice less toxic than FB and stimulates cognitive activity in the same way as the pharmacon and GABA but, unlike the two latter, it provides a 20% increase in memorizing abilities in animals and attenuates sedative effects [48].

3.2.5. Anticancer drugs

GA complexes (1:1) with 5-fluorouracil, tegafur, and daunorubicin hydrochloride were synthesized in solution [58]. Complexing decreases the toxicity of the drugs and increases

their solubility in water. The GA complex with fluorouracil exerts antineoplastic action with respect to Pliss lymphosarcoma, B-16 melanoma, and Geren carcinoma. The effective indices are, respectively, 3.05; 2.11, and 1.76. The tumor growth inhibition is 67.2%; 53.4 %; 87.1 %.

3.3. Antimicrobial drugs

GA complexes with antibiotics (chloramphenicol) and sulfanilamides (sulfamethoxypyridazine, saladimetoxine, sulfamonomethoxine, sulfadimidine, and sulfaguanidine), as well as with isoniazid and nitrofural, 1:1, were likewise synthesized in solution and compared in terms of their microbial activity. The highest survival rate (90%) was observed on the 10^{th} day after exposure to infection in animals with staphylococcosis that received 50 mg/kg GA-chloramphenicol, while only 30% survived when pure chloramphenicol was given. The percentage of survived animals with *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *E. coli* infections reached 80% on GA: chloramphenicol administration but it was only 20-50% on treatment with free chloramphenicol. The complex was also shown to stimulate humoral and cell immune responses [59].

3.4. Simvastatin (hypocholesterolemic drug)

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (3HMG-CoA) reductase, the so-called statins, are known to successfully reduce low-density lipoproteins, and are used for this in atherosclerosis therapy. However, most statins cause side effects and have to be replaced by safer drugs, with a more prolonged action. NMR spectroscopy of the behavior of simvastatin (SMS) in solutions with GA indicates formation of stable complexes. The synthesized SMS:GA complex is stable in water solutions at GA above 0.2 mM [60]. The complex patented as simvaglysine (SMG) [61] demonstrates uncompetitive inhibition of 3HMG-CoA reductase. SMG, in doses with three times lower statin, turns out to be more potent and safer than SMS.

Thus, simvaglysine acts as an uncompetitive inhibitor/proinhibitor of the 3HMG-CoA reductase reaction by inducing inhibition of cholesterol synthesis in liver microsomal fraction of rats *in vitro*, being no less potent than simvastatin. Within the inhibition constants from 100 to 300 nM, SMS inhibits 37.7- 42.0% mevalonate formation, while SMG provides a 31-33% total blood cholesterol decrease after 14 days of administration in rats with hypercholesterolemia, at doses with 3 times lower SMS, which is as effective as the therapeutic dose of simvastatin. The greater safety of SMG is confirmed by a lower blood CPK (creatine phosphokinase) increase after 14 days of treatment than in the case of SMS: 2.3 lower than with 2-5 times larger SMS doses [60, 61].

4. Conclusion

Thus, water-soluble molecular complexes of various polysaccharides and glycyrrhizic acid with drugs that normally dissolve poorly in water have been synthesized and tested

for binding strength and pharmacological activity in comparison with the constituent drugs.

The suggested mechanochemical synthesis of solid dispersed systems "drug-complexing agent" ensures high strength of the complexes, on condition of low-energy nondestructive treatment of polysaccharide macromolecules.

Arabinogalactan, a water soluble polysaccharide of *Larix sibirica* Ledeb. and *Larix gmelinii* (Rupr.), when used as a complexing agent, provides the highest solubility among other studied poly- and oligosaccharides. Another advantage of arabinogalactan is its exceptional, almost infinite, raw material source in the Northern Hemisphere (in Russia and Canada), as well as the availability of extraction and purification technologies [62].

The molecules of poorly water soluble drugs can be carried by micelles that form in water solutions of glycyrrhizic acid. Complexing with polysaccharides, in the same way as with GA, increases the solubility of pharmacons and allows reducing significantly (up to ten times) the effective dose avoiding, at the same time, some unwanted side effects.

Thus, a new perspective is opening of obtaining highly effective and safe drugs and new ways of drug delivery.

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