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Complexing of Pharmacons with Glycyrrhizic Acid as a Route to the Development of the Preparations with Enhanced Efficiency

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Abstract

Gel chromatographic investigation of the aqueous solutions of glycyrrhizic acid was carried out. A mechanochemical route to obtaining its compositions with poorly soluble pharmaceutical substances was developed, the characteristics of their aqueous solutions were studied, and their pharmacological characteristics were examined.

Key words: complexing, glycyrrhizic acid, pharmaceutical dosage forms, enhancement of drug efficiency

INTRODUCTION

The use of carbohydrate-containing metabolites of biosynthetic and plant origin for the formation of complexes (clathrates) with medicinal substances (pharmacons) becomes a more and more popular approach to the development of new transport forms of known medical preparations. The start of investigations of complexation can be assigned to works [1–3] the authors of which studied the solubilising action of glycyrrhizic acid (GA) and its monoammonium salt glycyrrham on some water-insoluble pharmacons. In the mentioned works, as well as in [4, 5], the authors demonstrated the possibility to obtain aqueous solutions of practically water-insoluble antibiotics oxytetracycline, nystatin, actinomycin C, corticosteroids hydrocortisone and prednisolone, and sulphanilamide preparation sulfazine [5]. It should be stressed that neither of these works mention the ability of GA to form complexes with pharmacons. This statement was formulated for the first time in [6]; the authors of that work emphasize that it is the intermolecular complexes of GA and pharmacons that are the new transport forms of medical preparations. In later works, the opinion was suggested that GA in aqueous solutions in small concentrations can form cyclic dimer structures due to intermolecular hydrogen bonds; such a structures possesses a hydrophobic cavity [7, 8]. Another possible mechanism of the interaction of GA with pharmacons in solution is the inclusion of the molecules of these compounds into self-associated formations (micelles formed in GA solutions within a broad concentration range). However, the existence of micelles was not confirmed by direct proofs and was assumed on the basis of measured concentration dependencies of the viscosity of aqueous GA solutions [9], or on the data obtained by means of dynamic NMR spectroscopy in water-methanol solutions [10]. Also, NMR was used to study complexation between nifedipine and GA; the constants of stability of their intermolecular complexes were estimated [11]. However, due to procedure-based reasons, also water-methanol solutions (30 %) were used as the medium for dissolution. So, in the mentioned studies,

the question concerning the molecular mechanisms of complexation of the molecules of pharmaceutical substances remained unanswered – inclusion into micelles or intermolecular complexes with GA in aqueous solutions, without addition of organic solvents that strongly change the character of interactions between pharmacon molecules and GA.

In the present work, we applied gel-penetrating chromatography to study the structure of the aqueous solutions of GA, in particular in the presence of poorly soluble pharmaceutical substances. This method allows one to determine the presence and size of self-associated formations/micelles and to estimate the concentration range of their existence. On the other hand, to obtain solid composites of GA with pharmaceutical substances,, we used the mechanochemical approach developing at the Institute of Solid State Chemistry and Mechanochemistry, SB RAS (Novosibirsk) [12, 13]. Its advantages include the single-stage synthesis, the absence of liquid phases (solutions or melts), the possibility to obtain solid dispersions of substances having no joint solubility or decomposing during melting. For the comparative evaluation of the strength of binding pharmacon molecules into intermolecular complexes or micelles of GA in aqueous solutions, we used the criterion of the increase in solubility for the studied poorly soluble drug substances [13] for which the pharmacological activity has been studied.

EXPERIMENTAL

Initial substances and experimental procedures

In the study we used GA obtained from the pharmacopeic preparation glycyrrham according to the method described in [14]. The concentration of the major component was (97.1±1.2) %, m. p. 223–224 °C, $[\alpha]_D^{20}$ +62.5° (C 0.02; EtOH). We also used arabinogalactan extracted from Siberian larch according to the procedure described in [15], and pharmacopeic hydroxyethyl starch (GEK-200, 0.5).

1-2-(4-Isobutylphenyl)-propionic acid (ibuprofen, solubility in water 0.030 g/L), 7-chloro-2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-2-on (sibazon, solubility in water 0.050 g/L), 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo-[*b*,*e*] [1,4]-diazepam (azaleptine, solubility in water 0.039 g/L), 1,2-diphenyl-4-*n*-butyl-3,5-pyrazolydynedione (butadione) of "kh. ch." reagent grade (chemically pure) or pharmacopeic, were used without additional purification.

Mechanochemical treatment was carried out in the planetary mill AGO-2. Treatment mode: acceleration of milling bodies 60*g*, mass of the mixture under treatment 3 g, cylinder volume 40 mL, milling bodies – steel balls 6 mm in diameter, the mass of loaded balls 75 g. Treatment time was 3 to 10 min. Longer treatment caused partial chemical decomposition of samples, while after treatment for shorter time insufficient homogenization of samples is possible.

To determine the solubility of pharmacons, mechanically treated mixtures GA/pharmacon in the amount of 0.4 g and weighted portions of individual substances equivalent to their content in the indicated mixtures were dissolved in 5 mL of distilled water under agitation with a magnetic mixer (rotation frequency 600 min^{-1}) for 6 and 24 h at a temperature of 24 °C. The concentrations of pharmacons in sampled portions were determined with the help of HPLC. Constant concentrations in solutions were achieved within dissolution time <<6 h. In all the cases, pharmacon was in equilibrium with the solid non-dissolved pharmacon. Complex-forming agents passed into the solution in whole.

Physicochemical analysis of solutions

The analyses by means of HPLC were carried out on an Agilent 1200 instrument with a Zorbax Eclipse XDB-C18 column (4.6 × 50 mm). Column temperature was 30 °C. Diode matrix detector was used. The HPLC technique was used to determine the solubility of pharmacons in water from the compositions complex-forming agent/pharmacon. Elution was carried out with the system 25 % acetonitrile + 75 % acetate buffer (pH 3.4); detection was carried out within the range 254–280 nm. The concentrations of pharmacons under investigation were determined with respect to specially prepared solutions of the pharmacons in alcohol.

Thermal analysis of samples under investigation was carried out by means of differential scanning calorimetry (DSC) with the help of DSC 550 (Instrument Specialists Inc., USA) in the atmosphere of Ar. Temperature programme was 20–250 °C, heating rate 5 °C/min.

The molecular mass distribution (MMD) of the samples was determined with Agilent 1200 chromatograph with gel chromatographic column PL aquagel-OH 30 (300 × 7.5 mm). Column temperature was 30 °C. Refractometric detector was used. The solution of 0.02 % NaN_3 was used as the solvent and eluting agent, the flow rate was 1 mL/min. Calibration was carried out with the standards of dextrans (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 Date Analysis software (assuming the linear dependence of log MM *versus* retention time).

Pharmacological studies

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

To determine the pharmacological activity, we used standard tests [16].

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

Along with the positive properties, many Nonsteroid anti-inflammatory means cause injury of the mucous membrane of stomach (ulcerogenic action), which substantially limits their application to treat inflammatory processes. In this connection, we also estimated the effect of complex formation on the degree of ulceration of the mucous membrane of stomach under the action of nonsteroid anti-inflammatory means. Separate experiments were carried out to measure the amount of ulcerous damage of the mucous membrane of stomach caused by the intragastric introduction of complexes under investigation, in comparison with the effects of the individual pharmaceutical substances.

To determine the effect of complexation between GA and psychotropic means on the central nervous system, we used the "open field" test allowing one to take into account the locomotor and investigative activity of mice, as well as the number of vertical struts for 3 min. The locomotor acts were recorded automatically in Truscan setup (Coulbourn Instruments, USA). Using the model of chloral hydrate sleep we studied the effect of complexes on the duration of the somnifacient action of chloral hydrate. This test allows one to estimate the change of the sedative effect of psychotropic drugs caused by complexation.

Statistical treatment of the data was carried out using the software package Statistica 7.0. Results are presented as an average value ± standard error. The reliability of differences was assessed using the Student's *t*-criterion.

RESULTS AND DISCUSSION

Gel chromatographic investigation of the aqueous solutions of GA

Gel chromatograms of the aqueous solutions of GA are presented in Fig. 1. Within all the concentration ranges studied, we observed the

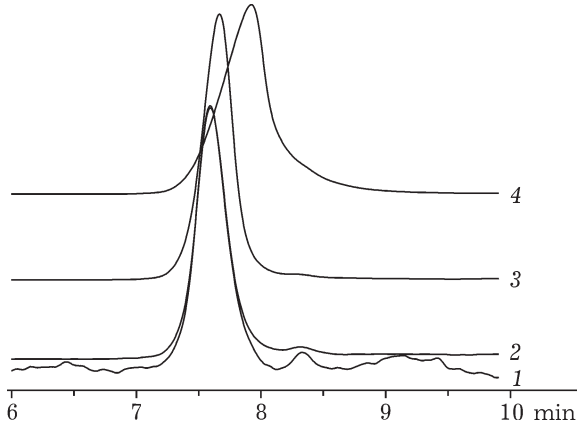

Fig. 1. Gel chromatograms of the solution of glycyrrhizic acid. Concentration in the solution, mass %: 0.001 (*1*), 0.01 (*2*), 0.1 (*3*), 0.5 (*4*).

TABLE 1

Molecular mass characteristics of self-associates of glycyrrhizic acid (GA) in aqueous solutions

Samples	Concentration in solution under investigation, %							
	0.001		0.01		0.1		0.5	
	t_1, min	M_w/M_n	t_1, min	M_w/M_n	t_1, min	M_w/M_n	t_1, min	M_w/M_n
Initial GA	7.59	77 300/73 300	7.59	77 500/74 900	7.67	73 300/70 900	7.92	49 200/54 800
GA/ibuprofen (10 : 1, AGO 3 min)	7.58	78 800/76 500	7.57	80 000/77 400	7.66	74 800/72 400	7.91	57 000/52 700

peaks of high-molecular formations with the molecular mass approximately equal to 46–67 kDa, while the molecular mass of GA is 836.96 Da. The characteristics of MMD are presented in Table 1. Peak areas are linearly proportional to the concentrations of the analyzed solutions. Calculation of peak areas with respect to the known amounts of dextran standards shows that they contain almost the whole mass of GA in the samples under investigation. Thus, in our opinion, self-associated formations of GA – micelles – are observed in gel chromatograms. Previously [9] the critical concentration of micelle formation (CCMF) was estimated from the changes in the viscosity of GA solutions; it was equal to 0.004 mass % (0.05 mM). In our case, it is difficult to determine CCMF exactly: first, because of the limited sensitivity of refractometric detection, second, due to the dilution of the GA solution under analysis during its elution in the chromatographic column. However, this value may be estimated from the retention time of the peak and elution rate. According to our estimates, elution of the solution under investigation causes its dilution by a factor of 10. So, the calculated CCMF value is not more than 0.0001 mass % (0.001 mM). At the same time, for water-methanol solutions CCMF value is equal to 0.04–0.08 mass % (0.5–1.0 mM) [11], which substantially exceeds the CCMF value for aqueous media. In diluted solutions (CCMF = 0.01–0.001 mass %) only one type of micelles is observed; their mass is approximately equal to 66 kDa, and they are characterized by very low degree of polydispersity (M_w/M_n = 1.08–1.06). With an increase in the concentrations of GA solutions up to 0.5 mass % (see Fig. 1, Table 1), micelle mass decreases, polydispersity increases; micelles with the mass approximately equal to 46 kDa. So, it may be concluded that GA is almost completely self-associated in aqueous solutions within concentration range 0.0001–0.5 mass %; the most stable micelles are those with MM 66 kDa, composed of approximately 80 GA molecules.

Compositions of GA with difficulty soluble pharmaceutical drugs

Solid dispersions of GA with ibuprofen, butadione, azaleptine and sibazon were prepared using the mechanochemical method. The mass excess of GA was 10/1, which corresponds to molar ratios (2.5/1)–(4/1). The data of thermal analysis for the system GA/butadione as example (Fig. 2) allow one to conclude that mechanochemical treatment involves disordering of the crystal phase of studied pharmacons, up to the complete loss of crystallinity. In our opinion, it is possible to disperse pharmacon molecules into the excess of the solid phase of GA with the formation of solid solutions. Other studied systems have similar characteristics.

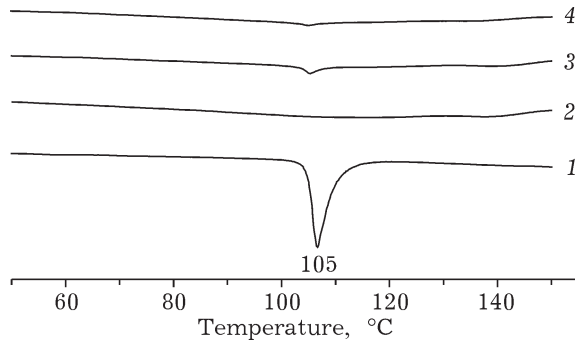

Fig. 2. DSC thermograms of initial butadione (*1*), GA (*2*), mixture of butadione with GA (1/10) before (*1*) and after (*4*) mechanical treatment.

TABLE 2

Increase in pharmacon solubility in water from mechanochemically prepared compositions with different complex-forming agents (mass ratio of complex-forming agent to pharmacon = 10/1)

Pharmacons	Complex-forming agent	Solubility of pharmacon, g/L	Increase in solubility (X)
Butadione	Glycyrrhizic acid	>0.010*	>1.1
Azaleptine	« «	0.081	2.0
Sibazon	« «	0.170	3.4
	Arabinogalactan	0.130	2.6
Ibuprofen	Glycyrrhizic acid	0.440	14.7
	Arabinogalactan	0.853	28.4
	Hydroxyethyl starch	0.079	2.6

*Calculated over the keto form of butadione; the enol forms and butadione bound with GA residues are not taken into account.

A substantial increase in aqueous solubility of the pharmacons is observed during the dissolution of the resulting dispersions. This demonstrates the high efficiency of GA as a solubilising agent and the efficiency of the mechanochemical method of obtaining water-soluble solid dispersions. The data on the aqueous solubility of pharmacons are presented in Table 2.

It is interesting to compare the efficiency of GA as a solubilising agent with polysaccharides studied by us previously [13, 17]. For sibazon and ibuprofen as examples, Table 2 shows the comparative data on aqueous pharmacon concentrations achieved due to the formation of water-soluble complexes/associates with GA and polysaccharides arabinogalactan (from Siberian larch) and hydroxyethyl starch (HES). One can see that GA is more efficient as a solubilising agent in comparison with HES but less efficient than arabinogalactan.

Gel chromatography examination of the aqueous solutions of compositions GA/pharmacon

Gel chromatograms of the aqueous solutions of dispersions, for the GA/ibuprofen system as an example, are shown in Fig. 3. The data on their MMD are presented in Table 1. Similar data were obtained with the systems GA/sibazon, GA/butadione, and GA/azaleptine. The areas of the peaks are linearly proportional to the concentrations of the solutions under analysis. Calculation of peak areas with respect to the known amounts of standards (dextrans) shows that they contain almost the whole mass of GA/pharmacon samples under investigation. So, the substances dissolved in the aqueous solutions of GA/pharmacon compositions are self-associated in micelles that are stable within a broad concentration range, similarly to the solutions of initial GA. An increase in the water solubility of difficulty soluble pharmacons is likely to occur due to their inclusion in micelles/self-associates of GA. The GA molecule contains a hydrophilic part (two glucuronide residues) and a hydrophobic part (triterpene fragment) (Fig. 4). Most probably, GA molecules in a micelle are oriented with their hydrophobic fragments inside, while their hydrophilic parts are directed to the external surface of the self-associate. Pharmacon molecules can be either in the internal hydrophobic part of the micelle or they can form complexes with the external hydrophilic fragments. Unfortunately, experimental data do not allow us to make conclu-

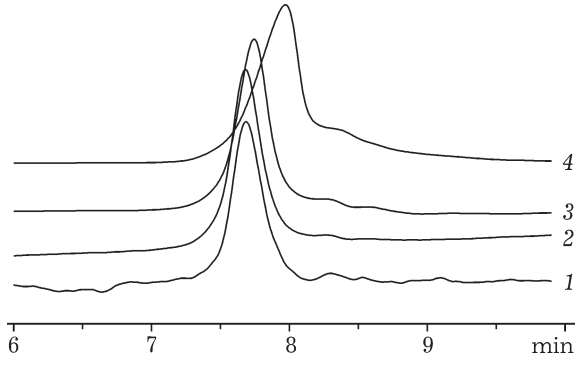

Fig. 3. Gel chromatograms of the solution of GA/ibuprofen (10/1) mixture. Concentration in the solution under investigation, mass %: 0.001 (*1*), 0.01 (*2*), 0.1 (*3*), 0.5 (*4*).

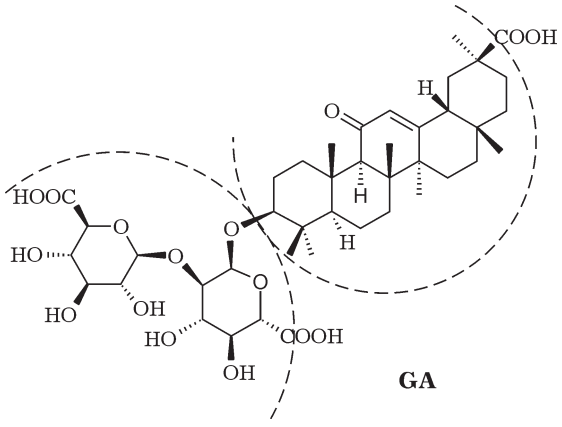

Fig. 4. Structure of glycyrrhizic acid.

sions concerning the intimate mechanisms of the interaction between pharmacon molecules and GA self-associates. In general, the MM of micelles of the compositions of GA with pharmacons exceed the MM in the aqueous solutions of GA by 5–7 % within the whole concentration range studied. The obtained data substantiate the assumption concerning the possibility to include pharmacon molecules into GA micelles. The substitution of a part of GA molecules is also possible, with an increase in the total increase in micelle size. As the concentration of the solution increases, the differences in micelle size increase, too. A decrease in the differences in MM during dilution can be connected with the fact that gel filtration chromagraphy of diluted solutions may involve withdrawal of pharmacon molecules from GA micelles. In such a situation, they are eluted with substantially different rates and should appear in chromatograms as individual substances [18]. In any case, further investigation is necessary to determine the character of pharmacon/GA interactions in more detail.

Pharmacological properties of GA/pharmacon complexes

Examination of the analgesic activity during complexation of GA with ibuprofen and butadione. The analgesic activity of the compositions of GA with ibuprofen, butadione with the mass ratio of 10/1, corresponding to the molar ratio of 4/1, was studied with two models of experimental pain: chemical irritation (acetic convulsions) and thermal irritation (hot plate, 54 °C) (Table 3).

One can see in the data presented in Table 3 that complexation of nonsteroid anti-inflammatory drug ibuprofen with GA by means of mechanochemical treatment helps one to decrease the dose by a factor of 10 with the conservation of the high analgesic activity but only for the model of chemical irritation of peritoneum. This fact also points to the conservation of the anti-inflammatory action of this pharmacon in the complex.

In the case of another nonsteroid anti-inflammatory drug, butadione, complexation did not cause a decrease in the dose (see Table 3); therefore, no increase in the analgesic activity was observed, unlike for ibuprofen. The pharmacological data correlate with the changes of solubility of these pharmacons during complexation with GA (see Table 3). At the same time, evaluation of the ulcerative injury under the

TABLE 3

Analgesic activity of the compositions GA/ibuprofen and GA/butadione in the tests of hot plate and acetic convulsions (peroral introduction)

Agents	Dose, mg/kg	Hot plate, g	Acetic convulsions, number
Reference		26.2±2.9	5.1±1.1
GA/ibuprofen (10/1)	200	13.5±1.0*	2.4±0.9*
Ibuprofen	200	18.0±2.6*	0.4±0.2*
Reference		18.6±0.9	4.3±1.1
GA/butadione (10/1)	120	11.4±0.8	6.4±1.0
Butadione	12	15.0±0.7	6.8±0.9

Note. Dose for reference was 0.2 mL/10 g.

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

TABLE 4

Effect of the complexes of conjugation of glycyrrhizic acid (GA) with sibazon and azaleptine (1 : 10) on the parameters of locomotor activity in the test of open field (peroral introduction)

Groups	Dose, mg/kg	A	B	C	D	E	F	G	H
Reference		14.3±2.1	98.3±3.4	21.8±3.5	155.5±16.3	1.3±0.2	3.0±0.6	3.3±0.6	0.0±0.0
Sibazon	2.5	10.3±1.3	107.0±2.1	20.9±2.9	233.0±26.2*	3.2±0.3	7.0±0.8	8.0±0.9	2.9±0.7
GA/sibazon	2.5	16.9±2.4	93.7±5.4	26.3±5.4	161.3±23.6	2.4±0.8	3.6±0.8	3.8±0.8	0.0±0.0
Azaleptine	25	14.7±1.1	80.1±4.5*	39.8±4.5*	103.5±13.5*	0.9±0.1*	2.1±0.9	2.7±1.3	0.0±0.0
GA/azaleptine	25	12.5±1.1	82.1±8.4	37.9±8.4*	104.4±14.2*	0.8±0.1*	3.9±0.7	4.4±0.8	0.0±0.0

Notes. 1. A – total locomotor activity (number of acts), B – locomotor activity, s; C – immobile time, s; D – motion distance, cm; E – motion velocity, cm/s; F – number of examined holes; G – time of investigative reactions; H – number of vertical stands. 2. The dose of sibazon and azaleptine in complexes with GA was 0.25 and 25 mg/kg, respectively.

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

TABLE 5

Effect of the compositions with the conjugation GA/sibazon and GA/azaleptine (10/1) on the somnifacient action of chloral hydrate (350 mg/kg, intraperitoneal introduction)

Agents	Dose, mg/kg	Time to fall asleep, min	Sleep duration, min
Reference		4.4±0.2	74.0±3.7
Sibazon	2.5	4.3±0.2	94.0±11.8
GA/sibazon	2.5	3.8±0.3	74.0±3.7
GA/sibazon	25	3.5±0.2	240.0±1.0
Azaleptine	2.5	4.0±0.3	254.2±16.8
Azaleptine	25	3.8±0.3	300.0±1.7
GA/azaleptine	25	4.0±0.3	200.0±20.4

action of individually introduced butadione and in complex with GA showed that complexation promotes protection of the mucous membrane of stomach from injury. For example, after the peroral introduction of butadione in the doses of 200 and 20 mg/kg the number of ulcerative affections was 54.0±1.7 and 5.0±0.9, respectively, while after the introduction of GA/butadione compositions (10/1) no ulcerative affections were observed.

Pharmacological properties during complexation of GA with sibazon and azaleptine. Investigating the effect of complexation of the medical substances under examination with arabinogalactan, it was shown that the pharmacological characteristics of preparations get improved, the dose decreases, therefore the by-effects are reduced [13, 19]. In this connection, it was interesting to continue the comparative studies of GA complexation with psychotropic agents (Tables 4, 5).

It was established (see Table 4) that the complexation of sibazon with GA promotes the recovery of all the parameters of locomotor activity to the normal level, which is connected with the stimulating action of GA [20].

For complexation with azaleptine as an example, another effect on pharmacon properties was demonstrated: the effect of azaleptine is reproduced completely with the dose decreased by a factor of 10.

The next test characterizes one more basic activity of psychotropic agents: sedative action (see Table 5). A direct dose dependent effect was discovered; it manifests itself as an increase in the duration of somnifacient action of chloral hydrate in comparison with sibazon.

In the case of azaleptine (see Table 5), complexation caused a decrease in the duration of somnifacient action in comparison with the individual substance.

Ambiguous data obtained in the study are likely to be due to the individual properties of

GA itself (psychostimulant action) and its ability to enter the allosteric interaction of a pharmacon with specific receptors.

Thus, complexation of difficulty soluble pharmacons due to the inclusion of their molecules into micelles with GA in aqueous medium allows one to improve their pharmacological characteristics.

CONCLUSIONS

In the present work, we carried out gel chromatographic investigation of the aqueous solutions of glycyrrhizic acid. A mechanochemical method of obtaining its composites with difficulty soluble medical substances was developed, the characteristics of their aqueous solutions were examined, and their pharmacological properties were studied.

1. It was demonstrated that glycyrrhizic acid in aqueous solutions within concentration range 0.001–0.5 mass % is self-associated in micelles with MM ~ 46–66 kDa; in diluted solutions the most stable micelles are those with MM ~ 61–66 kDa.

2. By means of mechanochemical treatment of the component mixtures, compositions were obtained. They are solid dispersions of glycyrrhizic acid with a medical substance (ibuprofen, azaleptine, sibazon, butadione). It was shown that the water solubility of medical substances from the obtained materials increases by a factor of 2–14.7.

3. It was shown that in aqueous solutions of the mixtures of glycyrrhizic acid and medical substances within the concentration range 0.001–0.5 mass % the dissolved substances are self-associated in micelles with MM ~ 49–69 kDa, which somewhat exceeds the MM of micelles of the initial glycyrrhizic acid. It is concluded that an increase in the solubility of difficulty soluble medical substances occurs due to their inclusion into micelles/self-associates of glycyrrhizic acid.

4. The use of the studied pharmacons in the form of water-soluble compositions with glycyrrhizic acid substantially improves their pharmacological characteristics. In particular, a decrease in the actual dose and undesirable by-effects is achieved. In the case of psychotropic medical preparations, ambiguous data obtained in the investigation can be connected with the individual pharmacological properties of glycyrrhizic acid itself (psychostimulant) and its ability to perform the allosteric interaction of a pharmacon with specific receptors.

The data obtained are interesting for the development of pharmaceuticals with increased efficiency and safety.

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