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Mechanocomposites based on 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide

Abstract. Current paper discusses the results of complex formation of 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-dien-6,12-olide with polyvinylpyrrolidone, disodium salt of glycyrrhizin acid and magnesium carbonate. Inclusion complexes with disodium salt of glycyrrhizic acid were obtained by mechanochemical treatment into micelles formed by the associated molecules of glycyrrhizic acid. Polyvinylpyrrolidone and magnesium carbonate form host-guest type complexes with 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide. Mechanocomposites were obtained in laboratory ball mill MSHL-1 (Itomak, Russia) with 1 to 6 hours of processing time. Studies on water solubility of obtained mechanocomposites demonstrate that water solubility of 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide in a mechanocomplex with disodium salt of glycyrrhizic acid after 2-hour mechanochemical treatment increased by 4.61 times, in the mechanocomplex with polyvinylpyrrolidone by 4.42 times, and with magnesium carbonate by 1.66 times. The surface morphology of the obtained mechanocomposites was studied by scanning electron microscopy (magnification x500). After mechanochemical treatment, the original shape of the particles of the initial components has changed and it is impossible to isolate individual components, except for the formed agglomerates. The resulting substances are polydisperse powders with particles (5-20 μm in size) and their aggregates. The results obtained indicate that the increase in the water solubility of the substance based on 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide has been achieved by the formation of supramolecular complexes after mechanochemical treatment with polyvinylpyrrolidone, disodium salt of glycyrrhizic acid and magnesium carbonate.

Keywords: 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide, mechanocomposites, complexing agents, glycyrrhizic acid disodium salt, polyvinylpyrrolidone, magnesium carbonate, water solubility, photomicrographs.

Introduction

Natural compounds with immunomodulatory activity are widely used in treatment of various diseases, including autoimmune and inflammatory diseases in addition to cancer. Immunomodulators are agents that have the ability to enhance the host defense response and can be used prophylactically in combination with other therapeutic agents. The anticancer activity of these immunomodulators is due to their anti-inflammatory, antioxidant action, as well as the induction of apoptosis, antiangiogenesis, and antimetastasis [1–2].

An analysis of the available literature data [1-4] indicates that the sesquiterpene lactone 1(10) β-epoxy-5,7α,6β(H)-guai-3(4),11(13)-diene-6,12-olide (1), which has in its structure, in addition to the γ-lactone ring, an epoxy function and an olefinic double bond in the carbon backbone, can be considered as a potential immunomodulator. It is a colorless crystalline substance of the composition $C_{15}H_{18}O_3$ with Tm 101-104°, $[\alpha]_D$ +45° (with 0.3 chloroform).

The main disadvantage of 1(10)β-epoxy-5,7α,6β(H)-guai-3(4),11(13)-diene-6,12-olide, as well as other natural sesquiterpene lactones, is its poor solubility in water, which has a negative effect on its bioavailability and reduces the specific pharmacological activity in the body. Therefore, it is considered practically important to modify the natural guaianolide molecule by converting it into water-soluble complexes.

One of the modern ways to increase the solubility of a medicinal substance is a mechanochemical method of processing, which includes physicochemical transformations of solid components and their mixtures under conditions of intense shock-attrition effects [5-7]. The essence of the technology is to obtain solid dispersions of medicinal substances with

excipients of various chemical nature. At the same time, there is an increase in the solubility of the starting substances and, accordingly, the effectiveness of their pharmacological action. Depending on the physicochemical properties of the substrate, solid dispersions are obtained by dispersing medicinal substances in molecular form or in an amorphous state with the formation of water-soluble inclusion complexes with excipients of the "guest-host" type [8].

Current paper discusses the results of complex formation of 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-dien-6,12-olide with polyvinylpyrrolidone, disodium salt of glycyrrhizin acid and magnesium carbonate. Inclusion complexes with disodium salt of glycyrrhizic acid were obtained by mechanochemical treatment into micelles formed by the associated molecules of glycyrrhizic acid.

Materials and methods

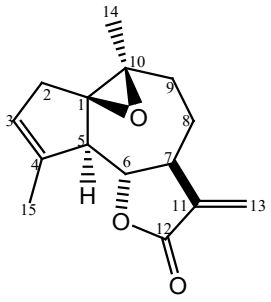
Preparation of solid dispersions. The preparation of solid dispersions was carried out in a MSHL-1 ball mill (Itomak, Russia) with a drum with a fluoroplastic lining. Processing mode: total loading of the components of the processed mixture from 18 to 22 g, drum volume – 300 ml, grinding media – steel balls (diameter 22 mm, loading 675 g). The processing time ranged from 1 to 6 hours. Optimal mass ratios of components: guaianolide/Na_2GA – 1:10, guaianolide/$MgCO_3$ – 1:5 and guaianolide/PVP – 1:10.

Complexing agents. The following were used as complexing agents:

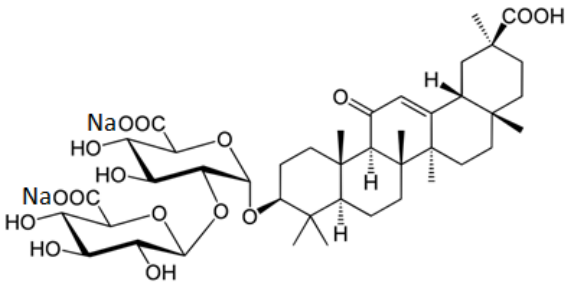
- Disodium salt of glycyrrhizic acid (2) (Na_2GA) – a derivative of plant saponin, (CFS, 98%) manufactured by Shaanxi Sciphar Biotechnology Co. Ltd. (Xi'an, China). The gross formula is $C_{42}H_{60}O_{16}Na_2$. It is a gray powder with a mustard tint. Doesn't melt. Sublimates at a temperature of ~400 °C. Easily soluble in water.

- Polyvinylpyrrolidone (3) (PVP) – a synthetic polymer manufactured by Huangshan Bonsun Pharmaceuricals Co., Ltd. (Huangshan, China). General formula $(C_6H_9NO)_n$. It is a white, yellowish-white powder, odorless. Has a sweetish taste. T_m = 150 °C. Well dissolved in water, ethanol and methanol.

- Substance of basic magnesium carbonate ($MgCO_3$) produced by Biochem Chemopharma (France) of pharmacopoeial purity (Manufacturer's pharmacopoeial monograph 42-3989-08). It is a white powder, odorless and tasteless, practically insoluble in water.



(1)



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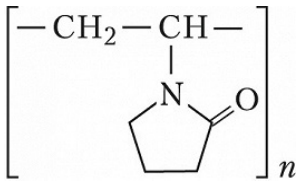

(3)

Results and discussion

The preparation of solid dispersions of 1(10) β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide was carried out in a MSHL-1 ball mill with a drum with a fluoroplastic lining. Processing mode: acceleration of grinding media – 1g, total loading of components of the processed mixture from 18 to 22 g, drum volume – 300 ml, grinding media – steel balls (diameter 22mm, loading 675g). The processing time ranged from 1 to 6 hrs.

As can be seen from Figure 1, the best result of the dissolution of 46.6 ± 0.13 seconds is a two-hour mechanochemical treatment of 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide with disodium salt of glycyrrhizic acid at a ratio of 1:10. 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide and its mechanocomposite with magnesium carbonate are practically insoluble in water.

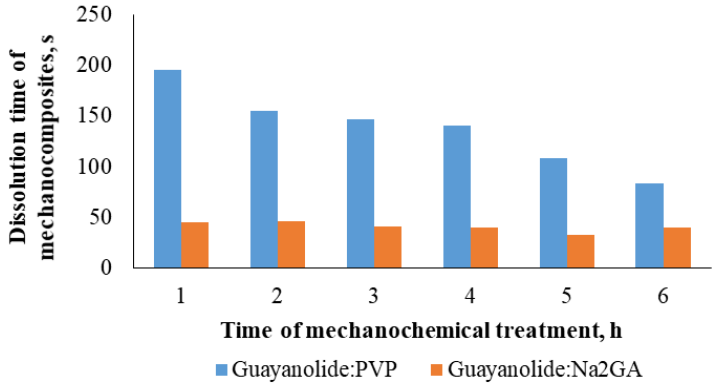

Figure 1 – Dependence of the dissolution of mechanocomposites 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide with polyvinylpyrrolidone and disodium salt glycyrrhizic acid from the time of mechanochemical processing

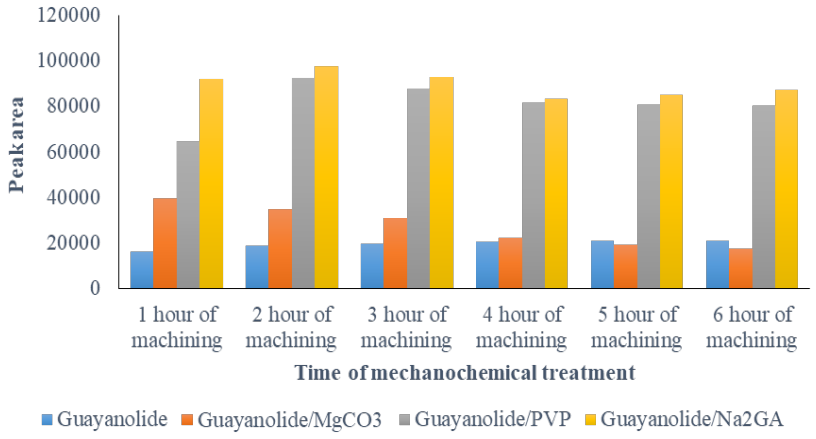

Figure 2 – Effect of mechanochemical treatment time on the complexation of 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide

The results presented in Figure 2 show that during a 2-hour mechanochemical treatment, the water solubility of 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene- 6,12-olide in the mechanocomplex with the disodium salt of glycyrrhizic acid increased by 4.61 times, and in the mechanocomplex with polyvinylpyrrolidone by 4.42 times, and with magnesium carbonate by 1.66 times.

The results obtained indicate that the increase in the water solubility of the substance based on 1(10) β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide has been achieved by the formation of supramolecular complexes by the method of mechanochemical processing. The following were used as complexing agents: a water-soluble derivative of plant saponin – disodium salt of glycyrrhizic acid and synthetic polymer polyvinylpyrrolidone.

The micrographs shown in Figure 3 characterize the surface morphology of the obtained samples. 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide consists of crystalline particles and their agglomerates. The disodium salt of glycyrrhizic acid consists of spherical hollow particles with a smooth surface. After mechanochemical treatment, the original shape of the particles of the initial components has changed and it is impossible to isolate individual components, except for the formed agglomerates.

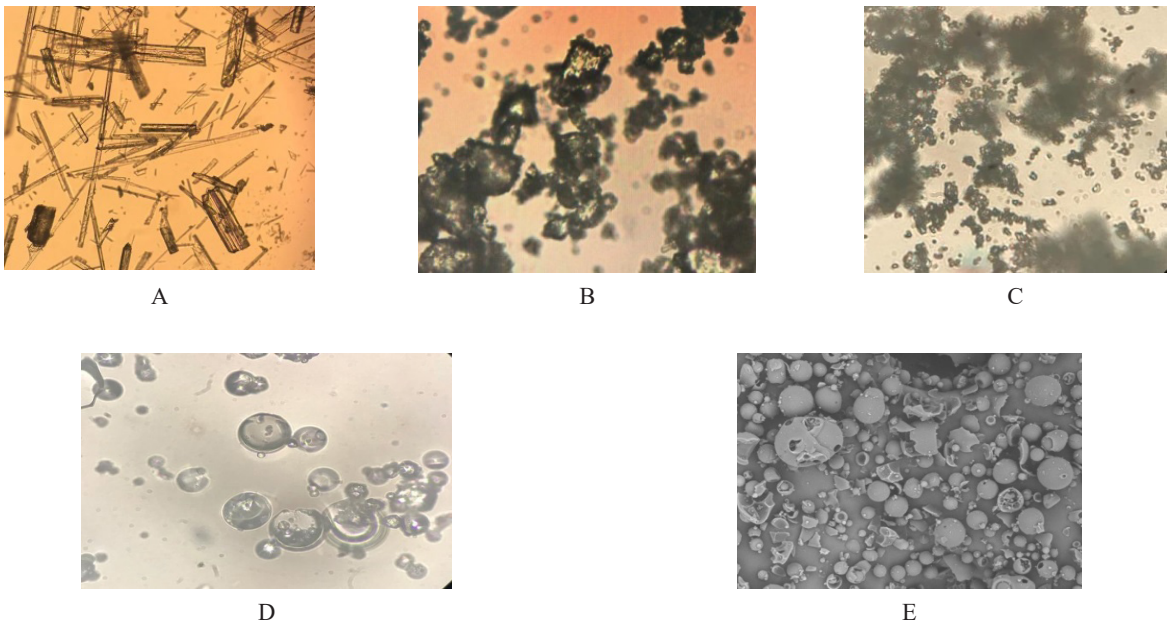

Figure 3 – Electron micrographs of the initial components and the complexes obtained on their basis.
Note: A – 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide;
B – 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide + polyvinylpyrrolidone (m/t time=2 h);
C – 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide + disodium salt of glycyrrhizic acid (time m/t=2 h;);
D – Polyvinylpyrrolidone; E – Disodium salt of glycyrrhizic acid (x500). Magnification in all cases: x500

As can be seen in Figure 3, the resulting substances are polydisperse powders with particles 5–20 μm in size and their aggregates. In this case, in the above complexes, hydrogen bonds are formed in the intermolecular space. As a result of mechanical treatment of mixtures of powders after the initial grinding, the process of aggregation of microparticles takes place. Microcomposites are formed, consisting of submicron particles and having a very developed contact between the phases. In this case, the inclusion of the substrate molecule into micelles, which are formed due to the associated molecules of glycyrrhizic acid. And polyvinylpyrrolidone and 1(10) β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide form guest-host complexes.

Mechanical treatment of guaianolide with basic magnesium carbonate, polyvinylpyrrolidone and disodium salt of glycyrrhizic acid transforms the crystalline substance into an amorphous state. The process is accompanied by the formation of coordination bonds between the molecules of 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide and complexing agents by donor acceptor mechanism. The process of obtaining complexes occurs in the solid phase, which makes it possible to exclude the use of organic solvents. The results obtained correlate with existing information discussed below.

Carrying out mechanical activation in mills is the most common operation in mechanochemistry. The advantages of this method are one-step, relative simplicity, the ability to increase bioavailability without changing the molecular structure. The process takes place in the solid phase, which allows the exclusion of toxic solvents. High-intensity mechanical treatment can lead to the breaking of strong covalent bonds, while low-intensity mechanochemical treatment allows the molecules of biologically active substances to “penetrate” into the space inside the macromolecule or self-associates of the excipient, forming a supramolecular complex due to hydrogen bonds and van der Waals forces. The absence of covalent interaction between the molecules of biologically active substances and complexing agents indicates that the structure of the original substance does not change. To improve the solubility of simvastatin lactone and increase its oral bioavailability, its complexes with arabinogalactan and disodium salt of glycyrrhizic acid were obtained by mechanochemical activation. Pharmacokinetic tests *in vivo* on laboratory animals show a significant increase in the bioavailability of simvastatin after its administration in the form of a complex with the disodium salt of glycyrrhizic acid or with arabinogalactan [9].

By mechanochemical treatment of triterpene betulin diacetate with arabinogalactan (ratio 1:9), mechanocomposites were obtained. It was established by gel permeation chromatography that mechanochemical treatment leads to a change in the molecular weight distribution [10]. Mechanical activation of a mixture of betulin diacetate with aerosil leads to homogenization of the mixture as a result of dispersion of the components and the formation of mechanically activated composites. Using physicochemical methods, electron microscopy, Infrared spectroscopy and X-ray phase analysis, it was proved that mechanical activation leads to the formation of composites of betulin diacetate with aerosil and amorphization of crystalline diacyls. The water solubility of mechanically activated betulin diacetate composites increased from 0.8 to 6.1 g/mL [11]. Pharmaceutical solid dispersions of curcumin polyphenol with macromolecular polysaccharide arabinogalactan were obtained by mechanical processing. The complexes obtained by mechanochemical treatment demonstrated an increased solubility of curcumin up to 10.5 times compared to pure curcumin [12]. Composites of praziquantel with disodium salt of glycyrrhizic acid were obtained by mechanochemical processing at mass ratios of components 1:5, 1:10, and 1:20. In this case, the greater the mass ratio, the greater the increase in the solubility of praziquantel in water [13].

Conclusion

The results of the study show that both disodium salt of glycyrrhizic acid and polyvinylpyrroldone can be used as complexing agents in the mechanochemical treatment of sesquiterpene lactone 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide. The obtained mechanocomplexes of 1(10)β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-dien-6,12-olide with polyvinylpyrrolidone and disodium salt of glycyrrhizic acid have an increased water solubility. This does not change the molecular structure of 1(10) β-epoxy-5,7α(H),6β(H)-guai-3(4),11(13)-diene-6,12-olide, which is an important factor to preserve the pharmacological activity of the sesquiterpene lactone molecule.

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