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PREPARATION AND PHYSICOCHEMICAL PROPERTIES OF SUPRAMOLECULAR COMPLEXES OF ECDYSTERONE

© ***E.S. Meteleva[1*], N.E. Polyakov[2], B.I. Tuleuov[3], B.S. Temirgaziyev[3], A.V. Dushkin[1], S.M. Adekenov[3]***

[1] Institute of Solid State Chemistry and Mechanochemistry SB RAS, Kutateladze st., 18, Novosibirsk, 630128 (Russia), e-mail: mete@ngs.ru
[2] Institute of Chemical Kinetics and Combustion SB RAS, Institutskaya st., 3, Novosibirsk, 630090 (Russia)
[3] International Research and Production «Holding Phytochemistry», Gazalieva st., 4, Karaganda, 100009 (Kazakhstan)

The supramolecular complexes of ecdysterone (20E), an active ingredient of many adaptogenic and anabolic drugs, with 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) and disodium salt of glycyrrhizic acid (Na_2GA) were prepared by mechanochemical method throw the formation of solid dispersion (SD) of components. These complexes have been studied, both in solutions and solid state by various physicochemical methods, ^{1}H NMR spectroscopy, HPLC, differential scanning calorimetry and X-ray diffraction analysis in comparison with the initial ecdysterone. It was found that the water solubility of 20E at 37 °C was increased by 3.0 and 2.7 times for 20E/2-HP-β-CD and 20E/Na_2GA complexes, respectively. Also, the transmembrane permeability of E20 and its supramolecular complexes through artificial membranes has been evaluated by PAMPA assay. The results indicate the prospects of using 20E/2-HP-β-CD and 20E/Na_2GA complexes for increasing absorption in the gastrointestinal tract. In addition, the advantages of green mechanochemical technology of inclusion complexes preparation have been demonstrated.

Keywords: Ecdysterone, Cyclodextrins, Glycyrrhizic acid, Mechanochemistry, Pharmaceutics Solid Dispersions, NMR spectroscopy, Solubility, Permeability.

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Introduction

Solubility and bioavailability of drugs are usually the main and interrelated factors in the development and creation of new potentially effective dosage forms. Ecdysterone – (20-hydroxyecdysone, β-ecdysone, (22*R*)-2β,3β,14,20,22,25-hexahydroxy-5β-cholest-7-en-6-one) (Fig. 1). Unfortunately, the extremely low solubility of ecdysterone (20E) in water [1] leads to slow and incomplete absorption in the gastrointestinal tract, which significantly reduces the effectiveness of drugs based on it.

Ecdysterone has adaptogenic, anabolic, and psychostimulating activity while showing extremely low toxicity and does not have the androgenic, antigonadotropic, or thymolytic effects inherent in most steroid anabolics [2].

Currently, according to the international database Ecdybase.org [3] more than 335 pharmacological substances based on ecdysterone and ecdysteroids (Cytodyne, Ecdybol, Power Health, Muscle Drive HP, Methoxy HG – Chrysin, Z-mass, Activator 1 and others) have been created, most of which are biologically

Elizaveta Sergeevna Meteleva – Candidate of Chemical Sciences, senior researcher, e-mail: mete@ngs.ru
Polyakov Nikolay Eduardovich – Doctor of Chemical Sciences, head of the laboratory, e-mail: polyakov@kinetics.nsc.ru
Tuleuov Borash Iglikovich – Doctor of Chemical Sciences, Professor, Head of the Laboratory of Chemistry of Steroid Compounds, e-mail: Tulbor@mail.ru
Temirgaziev Bakhtiyar Serikovich – Master of Technical Sciences, researcher, e-mail: b.s.temirgaziev@gmail.com
Dushkin Alexander Valerievich – Doctor of Chemical Sciences, Chief Researcher, e-mail: dushkin@solid.nsc.ru
Adekenov Sergazy Mynzhasarovich – Doctor of Chemical Sciences, Academician of the National Academy of Sciences of the Republic of Kazakhstan, Professor, General Director, e-mail: arglabin@phyto.kz

[*] Corresponding author.

active additives and have the status of drugs: Ecdystene and Ecdyphyte. Ecdysten is a tonic drug in tablets of 0.005 g (96% ecdysterone), and Ecdyphyt is an anabolic and adaptogenic preparation in tablets of 0.24 g, based on the sum of ecdysteroids (up to 1.5%) and flavonoids (up to 5.0%). [4]. However, there is no water-soluble dosage form of ecdysterone that could be extremely useful in the development of new-generation drugs that are promising for relieving general and chronic fatigue, reducing nervous and muscular fatigue, improving memory, lowering sugar levels, restoring post-infarction and post-stroke conditions and increasing overall vitality. In this regard, the creation of a water-soluble dosage form with high bioavailability is a rather popular and actual task.

To enhance the water solubility and bioavailability of drugs, the technology of obtaining so-called drug delivery systems (DDS) of biologically active molecules with various carriers is widely used. At present, this direction is leading the world pharmaceutical market in sales volume. One of the important directions is the creation of supramolecular delivery systems, containing molecules of poorly soluble biologically active compounds [5].

Intermolecular complexes can be prepared by various methods such as co-precipitation, melt fusion, hot melt extrusion, solvent evaporation, kneading, spray drying, and freeze-drying. Recently, the use of the solid-state mechanochemical method, which transferred mechanical energy to the solid-state compounds and may cause plastic deformation and concurrent changes in the crystal structure, along with crystalline phase transitions and amorphization [6], has become very attractive in the field of the pharmaceutical industry to enhance the solubility and dissolution rate of poorly water-soluble active pharmaceutical ingredients (APIs) [7, 8]. Compared with a previously mentioned liquid-phase method, the mechanochemical process offers significant advantages such as a one-stage technological process, absence of solvents or melts and respective additional procedures, lowering of unwished admixtures, high stability of formed complexes in water solution, and low operating cost [9, 10]. The more recent publications confirm the benefits of the mechanochemical approach on the examples of various BCS class II and IV drugs (for example, see [11–15]). In the present paper, this green chemistry mechanochemical approach was applied to synthesize intermolecular complexes of 20E with disodium salt of glycyrrhizic acid (Na_2GA) and 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD). The advantage of Na_2GA is that it is very soluble in water but does not form a gel in contrast with pure GA. Similarly, the advantage of HP-β-CD is better solubility compared to previously used β-CD.

Modification of 20E solubility and absorption has been the subject of several research publications, which used inclusion complexes with cyclodextrins [16]. One of the ways to increase the solubility of drugs that are difficult to dissolve in water is the inclusion of the latter in complexes with cyclodextrins (CD), which are cyclic glucose oligomers. CDs have found wide application in the pharmaceutical industry due to their non–toxicity, biocompatibility, availability (produced from a renewable source - starch), and the ability to form guest-host inclusion complexes with many organic and natural biologically active compounds, including steroid compounds [17–20]. The inclusion complex 20E with 2-hydroxypropyl-β-cyclodextrin (20E/2-HP-β-CD) has a better solvent solubility and transdermal permeability than the free 20E. According to published data, oral bioavailability and transdermal bioavailability of 20E/2-HP-β-CD, respectively, increased by 2.97 and 1.92 times compared to the initial 20E [16].

Glycyrrhizic acid and its disodium salt (disodium 30-hydroxy-11,30-dioxoolean-12-en-3β-yl 2-*O*-β-D-glucopyranuronosyl-β-D-glucopyranosiduronate; Na_2GA, fig. 1) are also effective natural low molecular weight complexing agents with increased biodegradability and bioavailability [21–23]. Glycyrrhizic acid (GA) is a triterpene glycoside extracted from liquorice root and demonstrates antiviral, anti-inflammatory, and antitumor properties [23]. Due to its amphiphilic property, GA can form complexes with various hydrophobic molecules, significantly increasing their solubility and increasing the permeability of the drug through cell membranes [22–29]. The advantage of Na_2GA solution is its lower viscosity compared with GA solutions. We can expect a synergistic effect from the use of Na_2GA as a drug delivery system with 20E. Given that mechanochemical technology and Na_2GA have the potential to improve the bioavailability of water-insoluble drugs, we then estimate the permeability of 20E as an amorphous SD in a matrix consisting of Na_2GA.

Thus, the purpose of this work was the development of mechanochemical technology for the preparation of water-soluble solid pharmaceutical dispersions of 20E with 2-HP-β-CD and Na_2GA, which forms supramolecular structures under dissolution in water and to study their physicochemical and biophysical properties.

Materials and methods

Na_2GA (Shaanxi Pioneer Biotech Co., Ltd, China) was used as an auxiliary substance, the content of the main substance >91%; 2-HP-β-CD was used as pharmaceutical grade [30]. 20E (with 99% purity according to HPLC data)

was isolated from aboveground organs (leaves, stems, flowers) of *Silenewolgensis* (Hornem.) Collected near the village of Kyzylkayyn of Bukhar-Zhyrau district of Karaganda region in June 2021 in the flowering phase (Fig. 1).

Preparations of SDs were executed by the joint mechanochemical treatment of 20E and excipients powders in the mass ratio 1 : 10 in a VM-1 ball mill (acceleration 1g). 1.8 g of substance 20E and 18 g of auxiliary substance were placed in a metal drum with a fluoroplastic lining (300 ml capacity, loading of grinding steel balls with a diameter of 23 mm, 675 g), and processing was carried out for 8 hours.

The 20E AFI as far as obtained physical mixtures and mechanochemically obtained SDs were analyzed by the HPLC method on an Agilent 1200 chromatograph with a Zorbax Eclipse XDB-C18 column, 4.6×50 mm; column temperature 30 °C; diode-array detector. The acetonitrile-water system (20 : 80) was used as an eluent, the flow rate was 1 ml/min, and the sample volume was 5 µl, detection at a wavelength of 242 nm. Concentrations of 20E were determined relative to its specially prepared solution in ethanol. The standard's deviation of measurements not exceeded ±3%. The similar HPLC conditions were used for measurement of content the active substance (20E) and its solubility in water from the obtained compositions.

To determine the solubility of 20E, 0.5g was placed in a conical flask, and 5 ml of water was added and stirred on a shaker at a speed of 200 rpm 37 °C. After certain periods, 200 µl of the solution was taken for analysis.

To determine the phase solubility of 20E solid dispersion, 2.75 g of SD was placed in a conical flask and 5 ml of water was added. The mixture was for 1 hour on an orbital shaker at a speed of 200 rpm at 37 °C. Then the solutions were centrifuged, filtered, and analyzed.

The formations of 20E complexes in water solutions were investigated by ^{1}H NMR spectroscopy. NMR spectra were recorded in D_2O solutions (99.9% D, Aldrich) on a Bruker AVANCE III 500 spectrometer (Germany) at a frequency of 500 MHz. The T_2 relaxation time was measured using the standard CPMG sequence.

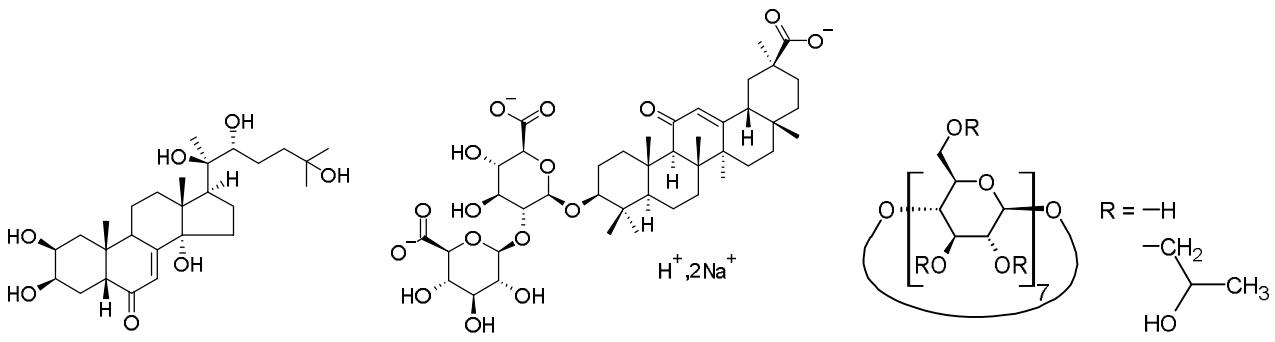
Thermal analysis of the studied samples was carried out using the DSC-550 instrument (USA) in the Ar atmosphere. Temperature program 20–300 °C, heating speed 5 °C min^{-1}.

X-ray diffraction analysis (XRD) of the solid powdered substances was conducted on a DRON-4 instrument (Burevestnik, St. Petersburg, Russia) using CuKα irradiation with the counter speed 2 °C/min and the range of intensity measurement 1000.

Thermal analysis of the samples was carried out by differential scanning calorimetry (DSC) on a DSC-550 device (Instrument Scientific Specialists Inc., Omaha, NE, USA) in Ar atmosphere, with the temperature regimen 20–260 °C and the heating rate 10 °C/min. Sampling weight was 10 mg.

Electron micrographs were obtained using a HITACHI TM-1000 microscope. Magnification up to 2500 times.

Transmembrane permeability was measured on artificial hexadecane membranes [Mccallum MM. (2013). High-throughput approaches for the assessment of factors influencing bioavailability of small molecules in preclinical drug development [PhD thesis]. The University of Wisconsin – Milwaukee] using the PAMPA method. Special 12-position Transwell cells with a polyester membrane with a diameter of 12 mm, pore sizes 0.4 µm and an area of 1.12 cm^2 were used for the analysis (Corning Incorporated, art. 3401). The test sample was placed in the donor cell – a sample of the test material (in terms of 2 mg 20E) in 0.5 ml of distilled water, and 1.5 ml of water was placed in the "acceptor" cell. The assembly of these cells was incubated in an orbital shaker at 37 °C. At certain time intervals, 1 ml of the solution was taken from the acceptor cell and replaced with an equal amount of water. The concentration of 20E in the selected solutions was measured by HPLC according to the previously specified method.



20E **Na_2GA** **2-HP-β-CD**

Fig. 1. Structures of ecdysterone (20E); disodium glycyrrhizinate (Na_2GA); and 2-hydroxypropyl-β-cyclodextrine (2-HP-β-CD)

Results and discussion

Physicochemical study of compositions in solid state. Physicochemical changes in the solid phases of the starting materials and compositions obtained by mechanochemistrywere investigated by the methods of DSC, X-ray diffraction analysis and microscopy.

The thermogram of differential scanning calorimetry (DSC) is shown in Figs. 2 and 3. Results of X-ray phase analysis (XRD) are shown in Figs. 4 and 5.

In all cases, the untreated mixtures contain X-ray reflexes characteristic of the crystalline phase 20E that intensities sufficiently lowered or disappeared after mechanical processing.

DSC thermogramms of initial 20E shows m/p nearly 240 °C which is very familiar to known date of PUB CHEM. The additional endothermic peak at ~220 °C possibly relates to admixture, which appears in HPLC assay and may be isomeric form of 20E. Mixtures without milling have decreasing of intensities of E20 melting, but appear a relatively low temperature peaks, seems by formation of eutectics with auxiliary substances. Any case after mechanochemical treatment, we observe no pronounced thermal effects. In additions with XRD dates we may conclude loosing of 20E cristallinity , possibly by amorphisation or dispersion 20E molecules into an excess volume of the solid phase of excipients.

Electronic micrographs of the starting pure materials powders and the resulting SD powders are shown in Fig. 6. It can be seen that the starting substances are quite large. However, during mechanochemical processing, the particles of the starting substances are destroyed and a polydisperse powder consisting of irregularly shaped particles is formed.

SDs solubility study in water solution. To begin with, the solubility kinetics of the initial 20E was investigated. The results are presented in Table 1. It can be seen that after an hour of dissolution, the concentration becomes equilibrium.

Data on the changes in solubility of 20E of the compositions after 3 hours of dissolution are presented in Table 2. In all the studied cases, there is an increase in solubility of 20E, which demonstrates high-efficiency Na_2GA and 2-HP-β-CD as the complexing agents.

Safety of 20e was calculated as relations of its calculated initial content to observed in SD's.

Table 1. Solubility 20E of time at 37 °C

Dissolutiontime, h	Concentration 20E, g l^{-1}
0.5	14.0
1	14.4
2	14.4
3	14.2

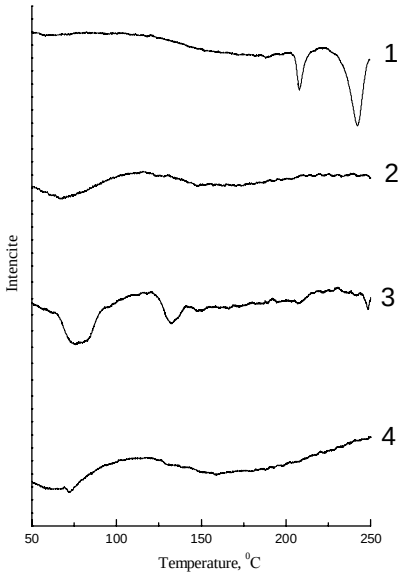


Fig. 2. DSC thermograms of DSC of the initial 20E (1), 2-HP-β-CD (2), as well as physical mixture of 20E and 2-HP-β-CD 1 : 10 (3) and its SDs (4)

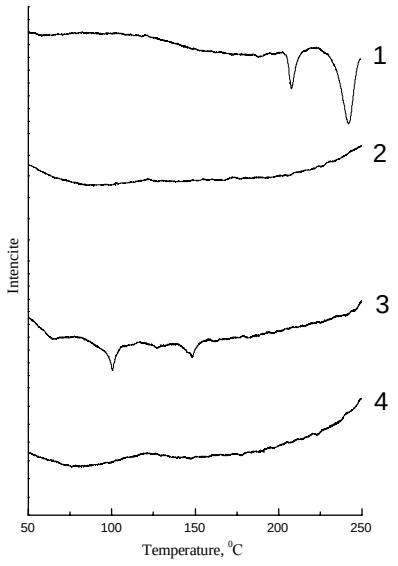


Fig. 3. DSC thermogramsof the initial 20E (1), Na_2GA (2), as well as physical mixture of 20E and Na_2GA 1 : 10 (3) and its SDs (4)

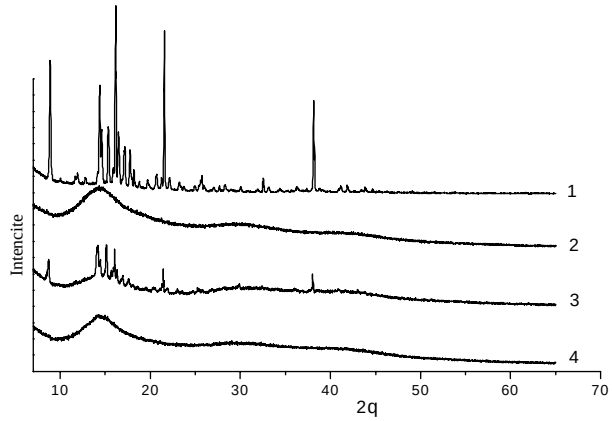


Fig. 4. X-Ray diffractograms of the initial 20E (1), Na_2GA (2), as well as physical mixture of 20E and Na_2GA 1 : 10 (3) and its SDs (4)

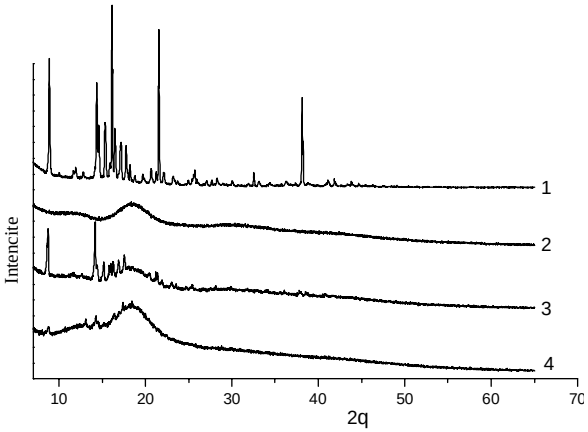
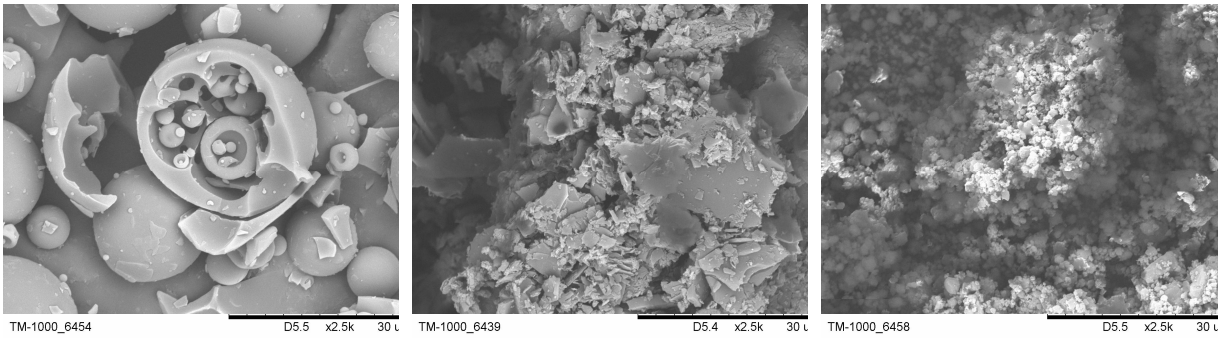


Fig. 5. X-Ray diffractogramsof the initial 20E (1), 2-HP-β-CD (2), as well as the physical mixture of 20E and 2-HP-β-CD 1 : 10 (3) and its mechanochemical obtained SDs (4)



Na_2GA 20E Solid dispersion Na_2GA/20E 10/1 VM-1 8h

2-HP-β-CD 20E Solid dispersion 2-HP-β-CD/20E 10/1 VM-1 8h

Fig. 6. Electronic micrographs of starting materials and solid dispersions

Table 2. Increase of solubility 20E in water from compositions with auxiliary substances and their decomposition in solution

№	Samplecomposition, massratios	Concentration of 20E in solution, g l^{-1}	Increaseinsolubility	Safety of initial 20E, %
1	20E	14.4	n/a	100.0
2	20E/Na_2GA 1/10	38.4	2.7	99
3	20E/2-HP-β-CD 1/10	42.6	3.0	98

NMR study in water solution. Currently, NMR spectroscopy is one of the most informative methods for studying the structure and intermolecular interactions in various complexes of steroid compounds, so this research method was chosen to study ecdysterone complexes. In this work, we studied 1% solutions of 20E complexes with 2-HP-β-CD (1 : 10) and with Na_2GA (1 : 10) at pH=5.1. Relaxation times were measured for several protons of 20E, whose signals did not intersect with signals from additives (2-HP-β-CD and Na_2GA). The corresponding protons are marked in the ^1H NMR spectra (Fig. 7) by numbers.

It is known that the rate of proton relaxation is very sensitive to the rotational mobility of the molecule, which makes it possible to use this method to prove the formation of inclusion complexes of a medicinal substance. As an example, the use of the NMR relaxation method to prove the interaction of the "guest" molecule with the 2-HP-β-CD macromolecule or Na_2GA micelle. Fig. 8 shows the kinetics of the NMR signal decay in the CPMG experiment for various protons 20E indicated in Fig. 7.

It can be seen that the presence of Na_2GA significantly reduces the relaxation time of T_2, from 2 to 4 times, which indicates a decrease in the mobility of molecule 20E. At the same time, for complexes with 2-HP-β-CD, the changes in relaxation times are rather weak, which may be a consequence of the weak stability of the complex due to the good solubility of 20E itself. Nevertheless, the formation of the complex is indicated by a change in the chemical shifts of some 20E protons. The greatest changes in chemical shifts are observed for methyl protons 20E in the region of 0.8–1.2 m.d. (Fig. 9).

To obtain additional confirmation of the formation of the 20E complex with 2-HP-β-CD, selective NOESY experiments were carried out. The presence of cross-peaks between protons 20E and 2-HP-β-CD is unambiguous proof of the formation of the complex (Fig. 10).

The most intense cross-peak is observed at the signal from the propyl tail of the 2-HP-β-CD (1.1 ppm), which indicates the predominant localization of 20E closer to the exit from the cavity of the CD. Peaks of lower intensity are also observed on the remaining CD protons (3.5–4.1 ppm). This is consistent with the assumption of weak stability of the complex.

Thus, it can be concluded that 20E forms more stable complexes with Na_2GA than with 2-HP-β-CD. The reason may be the inclusion into micelles of GA (that is a product of hydrolysis of Na_2GA) and 20E molecules, which are more stable than complexes with 2-HP-β-CD. One more conclusion can be drawn from the T_2 relaxation experiments. As can be seen from the observed kinetics of the echo signal decay (Fig. 9), that for pure 20E they are not mono-exponential, this is especially noticeable for protons 2 and 3. The presence of an initial kinetic region with shorter T_2 times indicates the formation of 20E aggregates in aqueous solutions.

An important feature of glycyrrhizic acid and its salts is the high sensitivity of its ability to self-association with the pH of the medium [31]. The carboxylates of glycyrrhizic acid have, according to the ACD/Percepta prediction software (GALAS) three stages of dissociation with pKa values of 2.6 and 3.2 for sugar carboxylates, and 4.9 for the terpenic one; sugar hydroxyls between 12.8 and 15.7 [32]. In an acidic environment, it forms micelles and gels at concentrations above the critical micelle formation concentration (~0.3 mM). At pH>6, GA micelles [5] become unstable due to Coulomb repulsion, however, recent studies show that supramolecular associates of some drugs with GA exist even at pH 6–7.4 [27, 33].

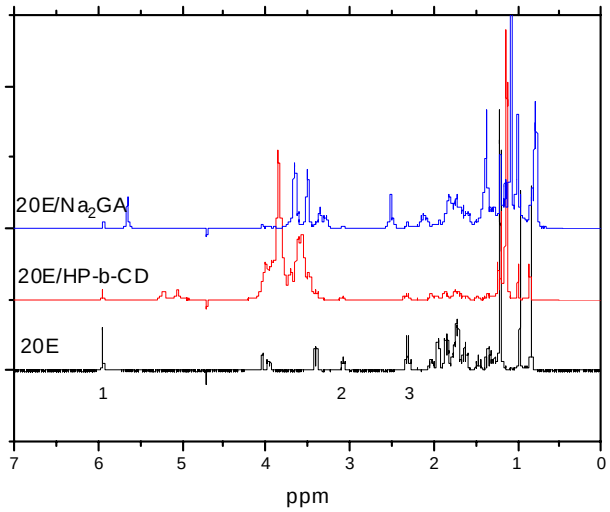


Fig. 7. NMR spectra of ^1H (500 MHz) of the studied samples in D_2O solutions, pH=5.1. Numbers 1–3 marked protons 20E for which relaxation times T_2were measured

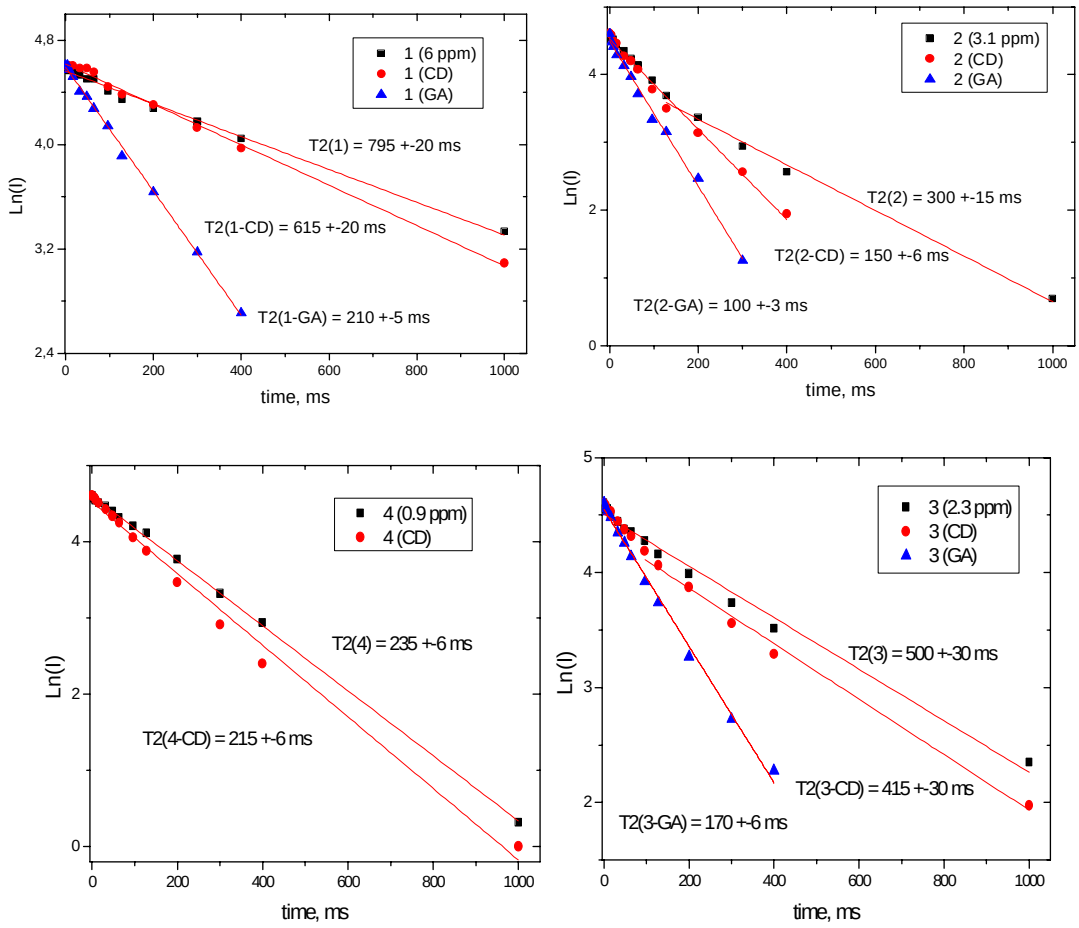


Fig. 8. The kinetics of the echo signal decay (on a logarithmic scale) and the relaxation times T_2 of protons 20E (1–4) in the absence and the presence of 2-HP-β-CD (CD) or Na_2GA (GA) in D_2O at T=30 °C

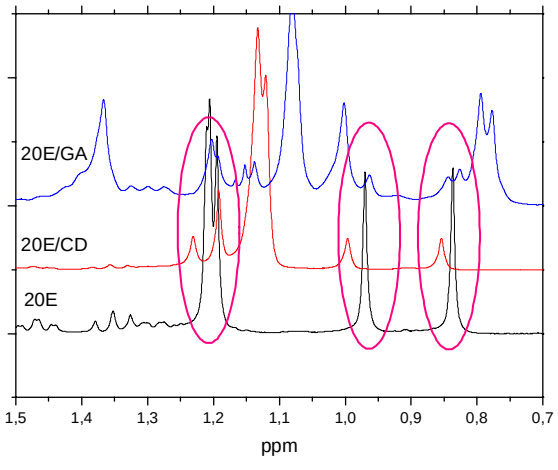


Fig. 9. Fragments of ^1H (500 MHz) NMR spectra of the studied samples in D_2O solutions, pH=5.1. Ovals are marked protons 20E for which changes in chemical shifts or width due to complexation are observed

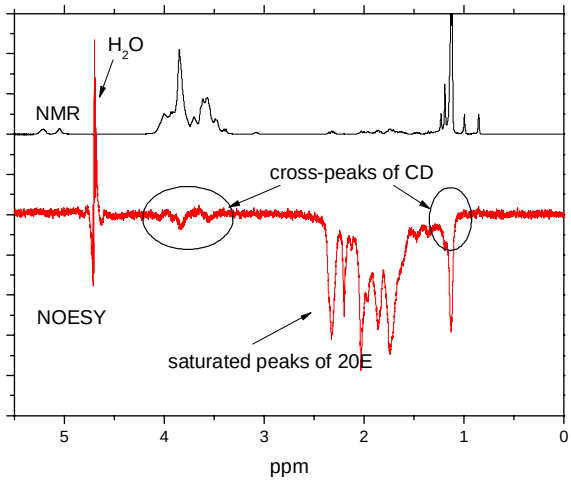


Fig. 10. ^1H NMR and NOESY spectra of complex 20E with 2-HP-β-CD (1 : 10) in D_2O solutions at pH=5.1

In this paper, we measured proton relaxation times 20E at three different pH values of the medium, 3.5, 5.1 and 7.4. The results are presented in Table 3.

At pH=3.5, gel formation is visually observed, and the measured short relaxation times reflect the low mobility of 20E molecules in gel media. At pH=5.1, a significant reduction in T_2 compared to the initial 20E means the inclusion of the latter in the micelles. At pH=7.4 (phosphate-salt buffer), micelles are destroyed, however, lower values of relaxation time compared to the initial compound indicate the presence of complexes. It can be assumed that in this case 20E complexes with individual glycyrrhizic acid molecules are formed [23].

Parallel Artificial Membrane Permeability Assay (PAMPA). The results of measurements of transmembrane permeability using the PAMPA method described in the experimental part are shown in Fig. 11. It can be seen that the diffusion rate of E20 molecules increases from solutions of its complexes, compared with the original substance E20. This justifies an increase in the bioavailability of 20E from its above-described solid dispersions (SD) with complexing agents.

Table 3. Proton relaxation times T_2 (in msec) of various protons 20E in complex with Na_2GA (1 : 10) at three pH values of the medium, 3.5, 5.1 and 7.4. *The last column shows the values of T_2 of the initial 20E at pH=5.1. The calculation error does not exceed 5%

№ proton	pH=3.5	pH=5.1	pH=7.4	20E*
1 C(6)-H	8.0	210	620	795
2 C(9)-H	8.3	100	240	300
3 C(17)-H	8.6	170	450	500

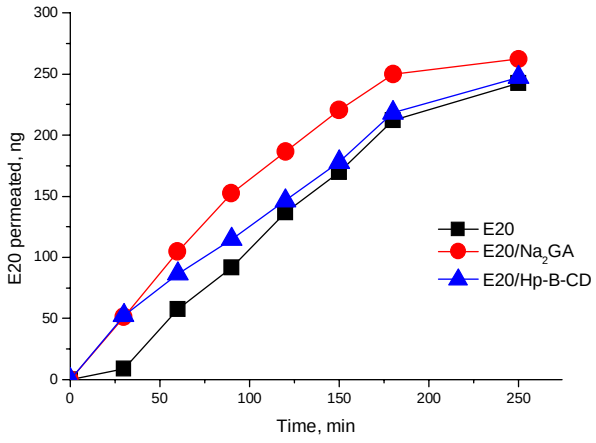


Fig. 11. Dynamics of the transfer of 20E from SDs solutions through an artificial membrane

Conclusions

In the present work, amorphous solid dispersions of 20E with 2-HP-β-CD and Na_2GA were obtained by solid-state mechanochemical method. This technology offers significant advantages such as a one-stage technological process, absence of solvents or melts and respective additional procedures, lowering of unwished admixtures, high stability of formed complexes in water solution, and low operating cost.

Physical properties of SDs powders have been characterized using electron micrographs (SEM), DSC thermograms and X-ray diffraction. The properties of solutions as far as the inclusion of 20E in complexes with 2-HP-β-CD and micelles of Na_2GA were confirmed by increasing phase solubility (intrinsic solubility) and by ^{1}H NMR spectroscopy. The solubility of 20E from SDs in water is more than 3 and 2.7 times higher than the solubility of the initial substance 20E. The results of this study indicate the possible increase of bioavailability of using mechanochemically synthesized supramolecular delivery systems based on intermolecular complexes 20E with 2-hydroxypropyl-β-cyclodextrin or the inclusion of its molecules in micelles formed by salts of glycyrrhizic acid to formulate new dietary supplements and drugs of increased efficacy.

References

1. Temirgaziyev B.S., Kučáková K., Baizhigit Y.A., Jurášek M., Džubak P., Hajdúch M., Dolenský B., Drašar P.B., Tuleuov B.I., Adekenov S.M. *Steroids*, 2019, vol. 147, pp. 37–41.

2. Lafont R., Balducci C., Dinan L. *Encyclopedia*, 2021, vol. 1, pp. 1267–1302.
3. Lafont R., Harmatha J., Marion-Poll F., Dinan L., Wilson I.D. *The Ecdysone Handbook. 3rd Edition*, 2002. URL: https://ecdybase.org/.
4. Zibareva L.N. *Farmatsevticheskiy byulleten'*, 2015, no. 3-4, pp. 40–48. (in Russ.).
5. Dushkin A.V., Meteleva E.S., Tolstikova T.G., Khvostov M.V., Polyakov N.E., Lyakhov N.Z. *Chem. Sustainable Dev*., 2019, vol. 3, pp. 206–216.
6. Boldyrev V.V. *J. Mater. Sci.*, 2004, vol. 39, pp. 5117–5120.
7. Dushkin A.V. *Chem. Sustainable Dev*., 2004, vol. 12, pp. 251–273.
8. Dushkin A.V. *High-energy ball milling. Mechanochemical processing of nanopowders*. Woodhead Publishing Limited Oxford, 2010, pp. 249–273.
9. Barzegar-Jalali M., Valizadeh H., Shadbad M.R.S., Adibkia K., Mohammadi G., Farahani A., Arash Z., Nokhodchi A. *Powder Technol*., 2010, vol. 197, pp. 150–158.
10. Dushkin A.V., Meteleva E.S., Tolstikova T.G., Khvostov M.V. *Chem. Sustainable Dev.*, 2010, vol. 18, pp. 631–640.
11. Chistyachenko Y.S., Meteleva E.S., Pakharukova M.Y., Kotokhin A.V., Khovstov M.V., Varlamova A.I., Glamazdin I.I., Khalikov S.S., Polyakov N.E., Arkhipov I.A., Tolstikova T.G., Mordvinova V.A., Dushkin A.V., Lyakhov N.Z. *Curr. Drug Delivery*, 2015, vol. 12, pp. 477–490.
12. Kong R., Zhu X., Meteleva E.S., Chisyachenko Y.S., Suntsova L.P., Polyakov N.E., Khvostov M.V., Baev D.S., Tolstikova T.G., Yu J., Dushkin A.V., Su W. *Int. J. Pharm.*, 2017, vol. 534, pp. 108–118.
13. Meteleva E.S., Chistyachenko Y.S., Suntsova L.P., Khvostov M.V., Polyakov N.E., Selyutina O.Yu., Tolstikova T.G., Frolova T.S., Mordvinov V.A., Dushkin A.V., Lyakhov N.Z. *J. Drug Delivery Sci. Technol*., 2019, vol. 50, pp. 66–77.
14. Kong R., Zhu X., Meteleva E.S., Dushkin A.V., Su W. *J. Drug Delivery Sci. Technol*., 2018, vol. 46, pp. 436–445.
15. Zhang Q., Polyakov N.E., Chistyachenko Yu.S., Khvostov M.K., Frolova T.S., Tolstikova T.G., Dushkin A.V., Su W. *Drug Delivery*, 2018, vol. 25, pp. 198–209.
16. Wang L., Li S., Cai H., Liu X., Feng T., Zhao X. *J. Microencapsulation*, 2022, vol. 39, pp. 145–155.
17. Temirgaziev B.S., Tuleuov B.I., Romanova M.A., Seidakhmetova R.B., Seilkhanov T.M., Seilkhanov O.T., Salkeyeva L.K., Adekenov S.M. *Russ. J. Gen. Chem*., 2019, vol. 89, pp. 424–428.
18. Uekama K., Hirayama F., Arima H. *J. Inclusion Phenom. Macrocyclic Chem*., 2006, vol. 56, pp. 3–8.
19. Forgo P., Gondos G. *Monatsh. Chem*., 2002, vol. 133, pp. 101–106.
20. Mu K., Jiang K., Wang Y., Zhao Z., Cang S., Bi K., Li Q., Liu R. *Molecules*, 2022, vol. 27, pp. 1–14.
21. Lu M., Wei W., Hu W., Polyakov N.E., Dushkin A.V., Su W. *Polymers*, 2022, vol. 14, pp. 1–21.
22. Dushkin A.V., Tolstikova T.G., Khvostov M.V., Tolstikov G.A. *The Complex World of Polysacchraides*. InTechOpen London, 2012, pp. 573–602.
23. Selyutina O.Yu., Polyakov N.E. *Int. J. Pharm*., 2019, vol. 559, pp. 271–279.
24. Selyutina O.Yu., Shelepova E.A., Paramonova E.D., Kichigina L.A., Khalikov S.S., Polyakov N.E. *Arch. Biochem. Biophys*, 2020, vol. 686, 108368.
25. Selyutina O.Yu., Apanasenko I.E., Kim A.V., Shelepova E.A., Khalikov S.S., Polyakov N.E. *Colloids Surf. B*, 2016, vol. 147, pp. 459–466.
26. Selyutina O.Yu., Polyakov N.E., Korneev D.V., Zaitsev B.N. *Drug Delivery*, 2016, vol. 23, pp. 848–855.
27. Selyutina O.Yu., Mastova A.V., Shelepova E.A., Polyakov N.E. *Molecules*, 2021, vol. 26, 1270.
28. Kim A.V., Shelepova E.A., Selyutina O.Yu., Meteleva E.S., Dushkin A.V., Medvedev N.N., Polyakov N.E., Lyakhov N.Z. *Mol. Pharmaceutics*, 2019, vol. 16, pp. 3188–3198.
29. Kim A.V., Shelepova E.A., Evseenko V.I., Dushkin A.V., Medvedev N.N., Polyakov N.E. *J. Mol. Liq*., 2021, vol. 344, 117759.
30. 2-Hydroxy Propyl Beta Cyclodextrine for Synthesis. URL: https://www.chemicalbook.com/ProductChemicalPropertiesCB111058615_EN.htm.
31. Petrova S.S., Schlotgauer A.A., Kruppa A.I., Leshina T.V. *Z. Phys. Chem*., 2017, vol. 231, pp. 839–855.
32. ACD/Percepta 14.53.0 (Build 3577).
33. Franca M.T., Martins Macros T., Paulo P.F., Gerola A.P., Stulzer H.K. *J. Mol. Liq.*, 2020, vol. 301, 112336.

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