ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 4, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

PREPARATION AND ANALYSIS OF THE NMR SPECTRA OF THE PHARMACEUTICAL SUBSTANCE OOSE-11, -12, -13

BERKENOV K*, DATKHAYEV UM

Department of Pharmacy, S D Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan. Email: berkenov_aidar@mail.ru

Received: 21 December 2016, Revised and Accepted: 30 December 2017

ABSTRACT

Objective: The main issue of this work is obtained, to researching of supramolecular complexes of 20-hydroxyecdysone (20 E) with α-, β-, and γ-cyclodextrins (CD).

Methods: The foundation of substance phytoecdysteroid 20 E was isolated from an ethanol extract plant Otites orae-syvaschicae Klok (OOSE). A method of the development of pharmaceutical substances was used during complexed organic relationship in the way of intense mechanical mixing 20 E with α-, β- or γ-CD. The complexation of 20 E with α-, β-, and γ-CD was studied by nuclear magnetic resonance (NMR) method of spectroscopy.

Results: In the study of the integrated intensities molecules signals steroid guest and host CD complex stoichiometric ratio 1:1 was proved. The formation of clathrates was established after A and B nuclei ecdysterone had been arranged in the internal cavity of CD.

Conclusion: Supramolecular complexes of 20 E with α-, β-, and γ-CD were received during made works. The objects of research were analyzed and described with the help of NMR spectroscopy. The developed technology will be the main device for operating and identification of pharmaceutical substance OOSE-11, -12, and -13.

Keywords: Substance Otites orae-syvaschicae Klok-11, -12, -13, 20-hydroxyecdysone, α-, β-, γ-cyclodextrins inclusion complex nuclear magnetic resonance spectroscopy.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i4.16729

INTRODUCTION

To be pharmacologically active, all drugs must possess some degree of aqueous solubility, and most drugs should be lipophilic to permeate the biological membranes via passive diffusion. The poor solubility and low dissolution rate of poorly water soluble drugs in the aqueous gastro-intestinal fluids often cause insufficient bioavailability. Poorly water-soluble compounds with dissolution rate limited low oral bioavailability present one of the major challenges in pharmaceutical formulation development. The water solubility of any drug is determined by its potency and its type of formulation. For pharmacological response to be shown the solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation.

With the high biological activity of polyhydroxylated steroids isolated from plants is of great interest among scientists and pharmacologists. Proved manifestation phytoecdysteroids adaptogenic, anabolic, radioprotective, anti-ischemic, and tonic action were set efficiency of their use in blocking the inflammatory and immunological processes in the healing of wounds and burns [1-5].

The most famous and widespread representative of phytoecdysteroids is ecdysterone (20-hydroxyecdysone [20 E]) (Fig. 1), found in plants such as *Rhaponticum carthamoides, Serratula coronata,* and *Spinacia oleracea* [6]. The prospect of studying the mechanism of action of 20 E is due to the lack of toxicity, and a manifestation of the tonic, anabolic, stress protective, gastroprotective, antihypoxic, and adaptogenic effects [7-10].

At present time, the richest families on the composition of ecdysteroids and content of 20 E are *Asteraceae* and *Caryophyllaceae*, and type of the most studied in detail the composition of ecdysteroids *S. coronata*-vegetable raw materials for the production of medicaments anabolic, adaptogenic, tonic and hepatoprotective action such as "Ekdifit" and "Serpisten" [11-13].

One of the similar complex investigations in which the article is uses a one-dimensional (1H and ^{13}C) and two-dimensional (COSY and HMQC) nuclear magnetic resonance (NMR) spectra of cytisine, β-cyclodextrin (CD) and their inclusion complexes were interpreted. The changes in the values of 1H and ^{13}C chemical shifts of receptor and substrate in the free state and in the complex were determined. It was found that complexation of cytisine with β-CD is accompanied by full entry of the substrate molecules in the inner sphere of the receptor to form supramolecular complexes of one substrate molecule per receptor molecule [14].

The most effective drugs were created and resorted to the possibilities of supramolecular chemistry. The ability to create nanocapsules complexes of biologically active component improves the solubility and physical and chemical stability of the substrate to improve its bioavailability and local tolerability [15,16]. Extensive use toroidal molecules of α-, β-, and γ-CD (Fig. 1) as the host molecules explains their structural features, the ability to bind hydrophobic molecules guest, nontoxicity and also possibility produce from renewable raw materials-starch [17].

According to the results of similar work, it was concluded that the bicalutamide -β-CD complexation results in an increase in the solubility and dissolution rate of the bicalutamide, suggesting a possible enhancement of its oral bioavailability [18]. Also from the results of the study, it is concluded that the solubility of lornoxicam was highly dependent on the pH of the medium as well the CD. Solubility was found to be the lowest in acidic medium which increased progressively with increase in pH of the media and a drastic increase in solubility was observed in alkaline media (pH 10.0). Phase solubility studies revealed that presence of HP β-CD can efficiently increase the solubility of laboratory response network than the β-CD with the same molar concentrations used [19,20]. In this connection with the above, this work has been devoted to the research of encapsulated

complexes phytoecdysteroids 20 E with the most common methods of CD-dimensional and two-dimensional NMR spectroscopy.

In turn, we by the biological screening flora of Republic of Kazakhstan, by us there was defined a new kind of in excess of concentrated ecdysteroid containing plants Otites orae-syvaschicae Klok (OOSE) from the family of *Caryophyllaceae*, which grows in the territories: The republic of Kazakhstan, Northern Mongolia, and China. This plant allows us to produce phytoecdysteroids on an industrial scale. Dissolution studies were performed separately in 900 ml water with 0.25% sodium lauryl sulphate maintained at 37°±0.5°C using USP XXII type II dissolution test apparatus at a speed of 50 rpm. Samples of 10 ml were withdrawn at regular intervals and replaced the same with fresh dissolution medium. The samples were estimated for the amount of dissolved by measuring their absorbance at 231 nm [21, 22]. The amount of released was calculated and plotted against time and compared with the pure drug (Fig. 5).

METHODS

The foundation of substance phytoecdysteroid 20 E was isolated from an ethanol extract plant OOSE. A method of the development of pharmaceutical substances was used during complexed organic relationship in the way of intense mechanical mixing. Dissolved EtOH and 20E was added to dissolve α-, β-, and γ-CD and they were mixed during 5 hrs. Obtained substance 20 E with α-CD will be called OOSE-11 on the basis of their production in turn 20 E with β-CD would be named as the OOSE-12, and the last representative of a number of substances with γ-20 E CD will be called OOSE-13.

Then, the resulting compound was dried, and crystallization was carried out naturally. The method of formation of a complex between of the 20 E with α-, β-, and γ-CD are numbers of substances that were obtained "OOSE-11,12,13." The complexation of 20 E with α-, β-, and γ-CD was studied by NMR method of spectroscopy. Substrate atoms and receptors determined with the help of one-dimensional and two-dimensional spectra.

NMR spectra were obtained starting compound and supramolecular complexes were recorded 5 mm tube at room temperature on a high-resolution NMR spectrometer JNN-ECA 400 of «Jeol». Operating frequency is 399.78 and 100.53 MHz spectrometer at the nuclei ^{1}H and ^{13}C, respectively. Spectrum sweep width was approximately 5000 (^{1}N) and 22000 Hz (^{13}C). As a solvent used DMSO-d_6 production qualification "Sigma-Aldrich." Chemical shifts are measured relative to signals of residual protons or carbon atoms deuterated dimethyl sulfoxide.

Registration dimensional spectra COSY, HMQC, HMBC, and ROESY were carried out using the appropriate pulse sequences included in the software package "Delta V4.3.6."

Dissolution rate studies

Dissolution studies were performed separately in 900 ml water with 0.25% sodium lauryl sulphate maintained at 37 ° ±0.5 °C using USP XXII type II dissolution test apparatus at a speed of 50 rpm. Samples of 10 ml were withdrawn at regular intervals and replaced the same with fresh dissolution medium. The samples were estimated for the amount of dissolved by measuring their absorbance at 231 nm [21, 22]. The amount of released was calculated and plotted against time and compared with the pure drug (Fig. 5)

RESULTS AND DISCUSSION

The proton spectrum of the 20 E characteristic signals of 5 methyl groups observed in the strong field: 0.70 ppm (S, 3H, H-18), 0.78 ppm (S, 3H, H-19), and 1.01 ppm (D, *J* = 10.1 Hz, 9H, H-21, H-25.26). In the area of 1.20-2.20 ppm, unresolved signals total integral intensity steroid 14H methylene groups were observed [21]. A widened singlet and a doublet at a frequency of 2.94 and 3.06 ppm (J=4.6 Hz) can be attributed to the protons at 12 and 16. Signals 1 H methine groups appear in a weak field. Protons H-5 and H-17 resonates in ppm 3.54 (D, 2H, and *J* =12.5 Hz). Br s at a frequency of 3.71 ppm corresponds to H-9. Mis-screening of protons H-2 and H-3 methine groups of A (δ=4.33-4.43 ppm, dd, 2H, and *J*=4.6 and 6.0 Hz) were explained by the effect of neighboring OH groups [22]. The most high-frequency signal doublet (5.57 ppm, 2H, and *J*=2.5 Hz) can be attributed to the proton of the atom C-7, coupled with the 6-keto group [23].

Correlation of these signals was produced being based on the spin-spin interaction spin–spin coupling constant (SSCC). It is known that for protons 6-membered rings A, B, and C values are within SSCC J (ax, ax) =10.5-13.5 Hz, J ($_{ax, eq}$) =3.5-5.0 Hz, and J ($_{eq, eq}$) =2.5-4.0 Hz.

More detailed information about the structure of the sample can be extracted from the carbon and DEPT-spectra. The presence of methyl groups of 5 confirmed signals in the silnopolnoy part of the spectrum (17.68, 21.49, 24.40, 29.48, and 30.58 ppm). Also in the strong field (δ=20.60-41.90 ppm) signals appear that can be attributed to the 8 methylene groups [21].

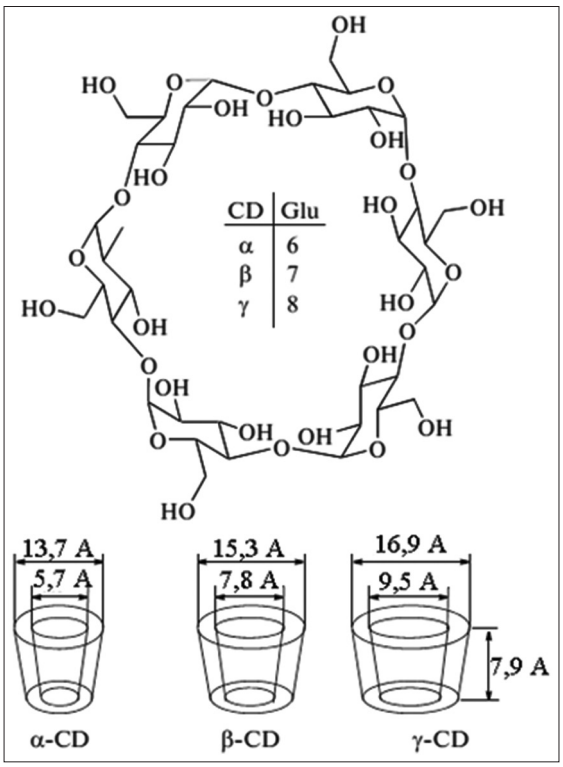

Fig. 1: Schematic representation of the structure of molecules of α-, β-, and γ-cyclodextrins

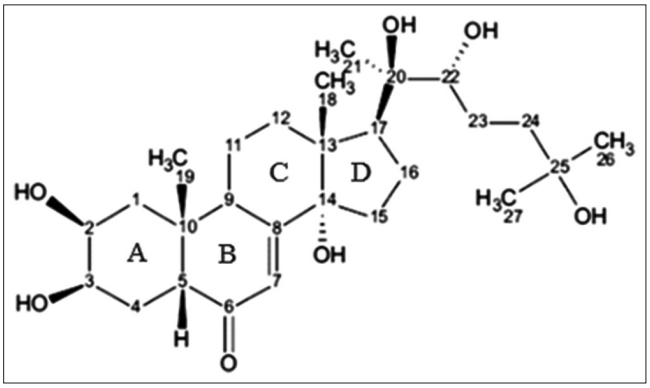

Fig. 2: The structural formula of 20-hydroxyecdysone

Characteristic for CH-group of resonating at the following chemical shifts: δ (C-9) =33.66 ppm, δ (C-17) =49.19 ppm, δ (C-5) =50.59 ppm, δ (C-3) =67.09 ppm, δ (C-2) =67.30 ppm, and δ (C-22) =76.66 ppm. Quaternary carbons give less intense signals at frequencies 38.13 (C-10), 47.35 (C-13), 69.24 (C-25), 76.19 (C-20), and 83.49 ppm (C-14).

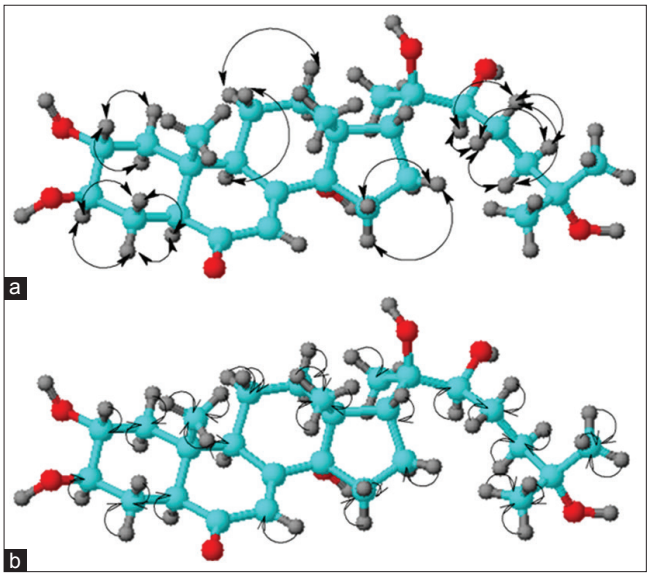

Fig. 3: Correlation, (a) COSY (^{1}H-^{1}H), (b) HMBC (^{1}H-^{13}C) in a molecule of 20-hydroxyecdysone

In the downfield (δ=203.25 ppm) carbon signal manifests a carbonyl group (C-6). With 165.80 and 120.97 ppm observed signals carbon atoms C-8 and C-7 in the state sp^2 hybridization (fragment -C=CH-) [21].

The total number of identified methyl, methylene and methine groups, and quaternary carbon atoms fully consistent with structure 20 E (Fig. 2).

To confirm the interpretation of one-dimensional spectra there were determined produced spin-spin correlation via heteronuclear two-dimensional homo-and spectroscopy (Fig. 3).

To establish the type of ecdysterone formed inclusion complexes with CDs there have been studied ^{1}H chemical shifts of the substrate and receptor in the free state in the composition of supramolecules (Tables 1 and 2).

Based on the above-tabulated data, it can be noted that the greatest displacement of the protons experiences inner sphere CDs - H-3 and H-5. It is assumed the formation of internal complex with ecdysterone. The study of the integrated intensities of the signals and a host of guest molecules leads to the conclusion of a stoichiometric ratio of 1:1. Establish hydroxyecdysone fragment molecules in the inner sphere located conventional sphere decoder studied changes in the chemical shift values (Table 1). Overlapping signals ^{1}H substrate greatly complicates the analysis of NMR data.

The assumption about the formation of complexes by placing the inner CD cavities rings A and B ecdysterone confirmed by two-dimensional spectra of NOESY (Fig. 4), the determination in the interaction of the spins of the molecules ^{1}H guest and host.

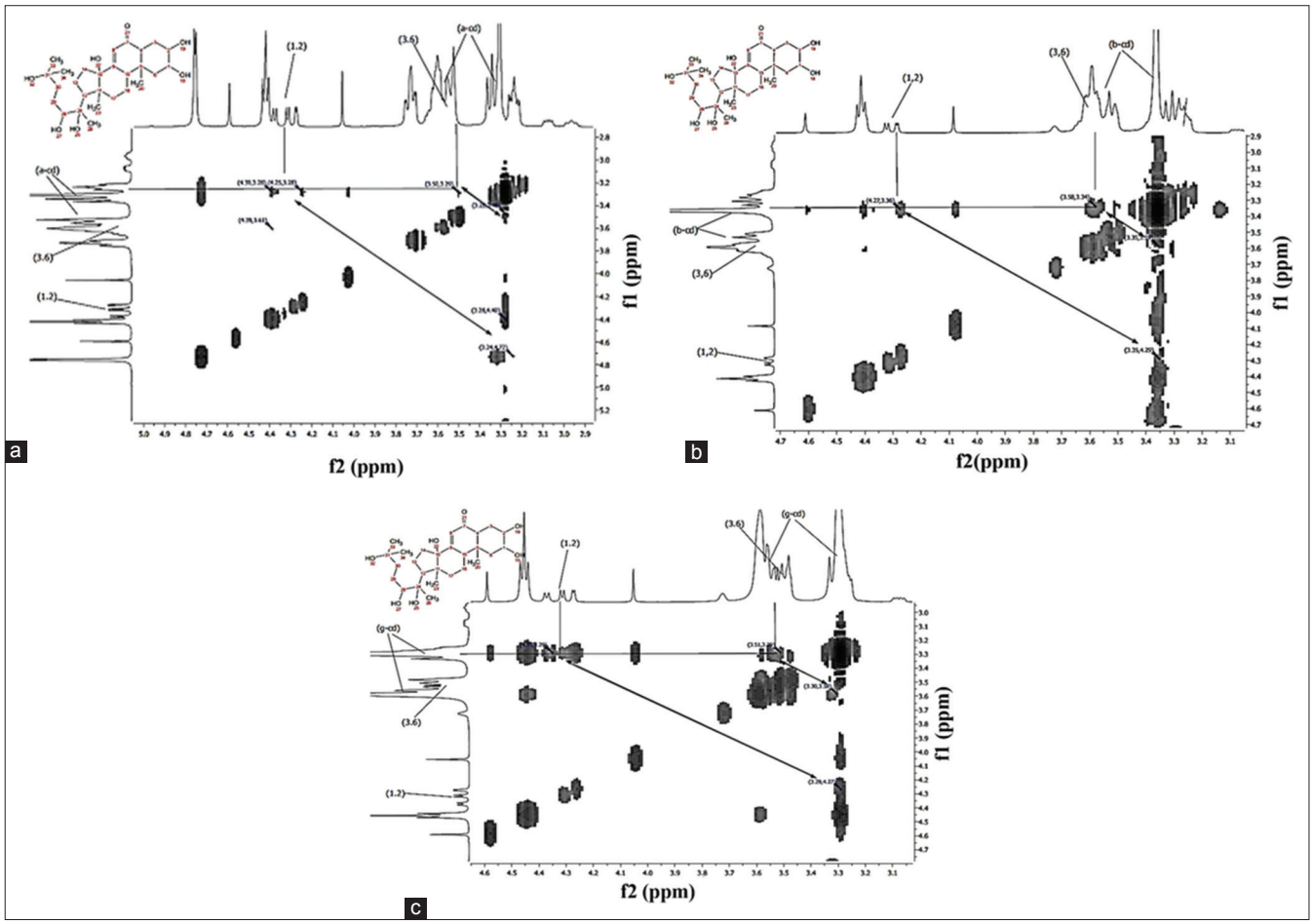

Fig. 4: NOESY spectra of supramolecular complexes OOSE-11(a), -12(b), and -13(c)

Table 1: Chemical shift values 20 ^{1}H-hydroxyecdysone in a free state (δ_0, ppm) and the complexes (δ, ppm) with α-, β-, and γ-CD

No atom C	Group	In a free state	The complex consists of					
			OOSE-11		OOSE-12		OOSE-13	
		δ_0(^{1}H)	δ(^{1}H)	Δδ	δ(^{1}H)	Δδ	δ(^{1}H)	Δδ
1	>CH_{ax}-	1.44	1.42	−0.02	1.45	0.01	1.45	0.01
	>CH_{eq}-	1.80	1.79	−0.01	1.81	0.01	1.82	0.02
2	>CH-	4.33	4.31	−0.01	4.32	−0.01	4.31	−0.02
3	>CH-	4.42	4.38	−0.04	4.44	0.02	4.44	0.02
4	>CH_{ax}-	1.65	1.67	0.02	1.67	0.02	1.67	0.02
	>CH_{eq}-	1.74	1.75	0.01	1.77	0.03	1.77	0.03
5	>CH-	3.54	3.56	0.02	3.57	0.03	3.56	0.02
6	>C=O	-	-	-	-	-	-	-
7	-CH=C<	5.57	5.58	0.01	5.59	0.02	5.58	0.01
8	>C=CH-	-	-	-	-	-	-	-
9	>CH-	3.71	3.71	0	3.73	0.02	3.73	0.02
10	>C<	-	-	-	-	-	-	-
11	>CH_{ax}-	1.65	1.64	−0.01	1.63	−0.02	1.67	0.02
	>CH_{eq}-	1.80	1.79	−0.01	1.79	−0.01	1.81	0.01
12	>CH_{ax}-	3.06	3.06	0	3.07	0.01	3.06	0
	>CH_{eq}-	1.82	1.82	0	1.81	−0.01	1.82	0
13	>C<	-	-	-	-	-	-	-
14	>C<	-	-	-	-	-	-	-
15	>CH_a-	1.97	1.98	0.01	1.98	0.01	1.97	0
	>CH_b-	1.56	1.57	0.01	1.57	0.01	1.56	0
16	>CH_a-	2,94	2.95	0.01	2.96	0.02	2.95	0.01
	>CH_b-	1.74	1.75	0.01	1.74	0	1.74	0
17	>CH-	3.54	3.53	−0.01	3.53	−0.01	3.54	0
18	-CH_3	0.70	0.72	0.02	0.72	0.02	0.72	0.02
19	-CH_3	0.78	0.79	0.01	0.79	0.01	0.80	0.02
20	>C<	–	–	–	–	–	–	–
21	-CH_3	1.02	1.02	0	1.02	0	1.02	0
22	>CH-	3.37	3.37	0	3.36	−0.01	3.36	0.01
23	-CH_2-	1.58	1.58	0	1.58	0	1.58	0
24	-CH_2-	1.44	1.44	0	1.45	0.01	1.44	0
25	>C<	–	–	–	–	–	–	–
26	-CH_3	1.02	1.02	0	1,02	0	1.02	0
27	-CH_3	1.02	1.02	0	1.02	0	1.02	0

*Δδ=δ-δ_0, OOSE: Otites orae-syvaschicae, α-, β-, and γ-CD: α-, β-, and γ-cyclodextrins

Table 2: Chemical shift values 1H α-, β-, and γ-CD in a free state (δ_0, ppm) in the complexes and OOSE-11, 12, 13 (δ, ppm)

No	α-CD			β-CD			γ-CD		
	δ_0	Δ	Δδ	δ_0	δ	Δδ	δ_0	δ	Δδ
H-1	4.76	4.75	−0.01	4.77	4.78	0.01	4.83	4.83	0
H-2	3.22	3.24	0.02	3.27	3.28	0.01	3.30	3.32	0.02
H-3	3.37	3.31	−0.06	3.45	3.36	−0.09	3.37	3.41	0.04
H-4	3.24	3.26	0.02	3.30	3.31	0.01	3.32	3.32	0
H-5	3.34	3.37	−0.03	3.45	3.36	−0.09	3.37	3.41	0.04
H-6	3.60	3.60	0	3.57	3.59	0.02	3.58	3.57	−0.01

OOSE: Otites orae-syvaschicae, α-, β-, and γ-CD: α-, β-, and γ-cyclodextrins

From similar works, we realized that the CD is a good basis for the creation of a hydrophilic substance. We expect that the complex 20 E with CD allow increase the bioavailability and pharmacological effect on the organism. Owing to the good solubility of substance a lower concentration will be used [18-20]. One of the similar studies is the publication of the receipt and study NMR spectra of complex natural alkaloid cytisine with the beta CD [14].

From the result, it concludes that the inclusion complex prepared by modified kneading method of 20E and A G β-cyclodextrin showed a reduction in peak intensity as compared to 20E indicating the formation of an inclusion complex. The dissolution performance of inclusion complex by kneading method was increased as compared to pure 20E, physical mixture and co-grinding complex in water, pH 1.2 and pH 6.8 in

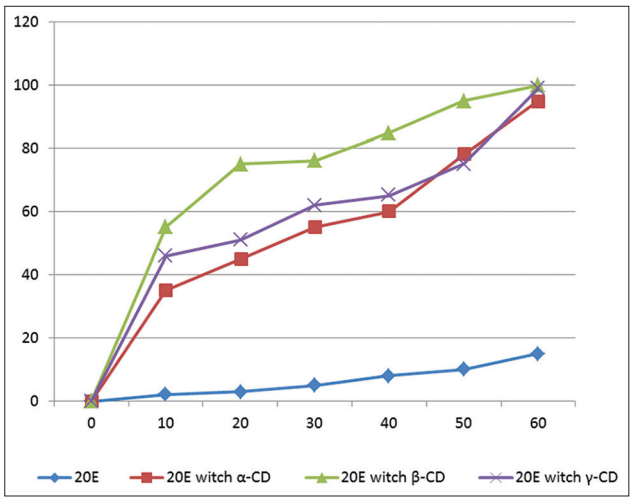

Fig. 5: Schedule dissolution rate studies

particular time course. This may be attributed to improved wettability of drug particles significant reduction particle size during formation of inclusion complex and intrinsically higher rate of dissolution of the selected soluble polymer, which could pull insoluble but finely mixed drug into the bulk of dissolution medium.

Based on the absence of similar works, the production of supramolecular complexes phytoecdysteroid with α-, β-, and γ-CD. Moreover, the results are presented in this article. One could argue that this direction is a perspective and important for the pharmaceutical industry.

CONCLUSION

Supramolecular complexes of 20 E with α-, β-, and γ- CD were received during made works. This method should allow produce to a substance "OOSE-11,12,13." Furthermore, we see in section results spectral analysis and description of the substance. A detailed description of the NMR spectra, allow to use as a standard for identification in the future analog research with steroids.

REFERENCES

1. Laekeman G, Vlietinck A. Phytoecdysteroids: Phytochemistry and pharmacological activity. Nat Prod 2013;3827-49. DOI: 10.1007/978-3-642-22144-6-173.
2. Syrov VN. Comparative experimental investigation of the anabolic activity of phytoecdysteroids and steranabols. Pharm Chem J 2000;34(4):193-7.
3. Dinan L, Harmatha J, Volodin V, Lafont R. Phytoecdysteroids: Diversity, biosynthesis and distribution. Ecdysone Struct Funct 2009;3-45. DOI: 10.1007/978-1-4020-9112-4_1.
4. Mamadalieva NZ, Gamberdieva DE, Lafont R, Girault JP. Phytoecdysteroids and antibacterial activity of the plant *Coronariaflos-cuculi*. Chem Nat Compd 2008;44(3):404-6.
5. Shirshova TI, Politova NK, Burtseva SA, Beshlei IV, Volodin VV. Antimicrobial activity of natural ecdysteroids from *Serratula coronata* L. And their acyl derivatives. Pharm Chem J 2006;40(5):268-71.
6. Dinan L, Savchenko T, Whiting P. On the distribution of phytoecdysteroids in plants. Cell Mol Life Sci 2001;58(8):1121-32.
7. Gorelick-Feldman J, Cohick W, Raskin I. Ecdysteroids elicit a rapid Ca2 flux leading to Akt activation and increased protein synthesis in skeletal muscle cells. Steroids 2010;75(10):632-7.
8. Syrov VN, Saatov Z, Sagdullaev SH, Mamatkhanov AU. Study of the structure - Anabolic activity relationship for phytoecdysteroids extracted from some plants of Central Asia. Pharm Chem J 2001;35(12):667-71.
9. Savchenko RG, Kostyleva SA, Odinokov VN, Akhmetkireeva TT, Benkovskaya GV. Stress - And geroprotective properties of 20-hydroxyecdysone and its derivatives. Adv Gerontol 2015;5(4):247-51.
10. Punegov VV, Sychov RL, Zainullin VG, FedorovVN, Punegova NV. Extraction of ecdysteron-80 from *Serratula coronata* L. And assessment of its pharmacological action. Part I. Adaptogenic,

gastroprotective, thermoprotective, and antihypoxic activity. Pharm Chem J 2008;42(8):446-51.
11. Adekenov S.M. Method for producing an anabolic and adaptogenic means "Ekdifit" from Serpukhov crowned *Seratula coronota* L." Innovative patent of Republic of Kazakhstan №23093, issued November 15, 2010.
12. Volodin VV, Pchelenko LD, Volodina SO, Kudryasheva AG, Shevchenko OG, Zagorskaya NV. Pharmacological ecdysteroid substance "Serpisten". Plant Resour (Russia) 2006; 42(3):113-129.
13. Tuleuov BI. The steroid compounds of plants and drugs based on them. Search, chemical modification and practical aspects of the application. Karaganda: Glasir; 2009. p. 208.
14. Nurkenov OA, Seilkhanov TM, Fazylov SD, Issayeva AZ, Kabieva SK, Takibayeva AT. Synthesis and studies of supramolecular inclusion complexes of cytisine with β-cyclodextrin by NMR spectroscopy. Int J Appl Fundam Res 2015;7:223-6.
15. Potopova AA. Study of Acute Toxicity of the Extract of Baikal Skullcap (*Scutellaria baicalensis* Georgi) and an Inclusion Complex with 2-Hydroxypropyl. The Collection of Scientific Papers "Development, Research and Marketing of New Pharmaceutical products". Vol. 69. Pyatigorsk; 2014. p. 296-8.
16. Seylhanov TM. NMR spectroscopy of the cyclodextrin inclusion complexes. Kokshetau: KSU Sh Ualihanova; 2015. p. 155.
17. Valle EM. Cyclodextrins and their uses: A review. Process Biochem 2004;39:1033-46.
18. Srikanth MV, Babu GV, Rao NS, Sunil SA, Balaji S, Ramanamurthy S. Dissolution rate enhancement of poorly soluble bicalutamide using β-cyclodextrin inclusion complexation. Int J Pharm Pharm Sci 2010;2(1):191-8.
19. Shrishailgouda S, Patil V, Gupta M, Gupta KS, Doddayya H. Effect of pH, selected cyclodextrins and complexation methods on the solubility of lornoxicam. Int J Pharm Pharm Sci 2014;6(8):324-7.
20. Hayder1 MA, Sunderland VB. Effect of hydroxypropyl-ß-cyclodextrin complexation on the aqueous solubility and stability of artesunate. Int J Pharm Pharm Sci 2014;6(7):598-601.
21. Fedorov VN, Punegova NV, Zainullin VG, Punegov VV, Sychev RL. Extraction of ecdysterone-80 from *Serratula Coronata* L and evaluation of its pharmacological properties. Effects on hormone-transmitter balance in chronic cardiac failure. Pharm Chem J 2009;43(1):231-5.
22. Mamadalieva NZ, Ramazanov NS, Girault JP, Lafont R, Saatov Z. Preparation of 20-hydroxyecdysone-22-benzoate. Chem Nat Compd 2004;40(5):488-91.
23. Bobaev ID, Bobakulov KM, Ramazanov NS, Abdullaev ND. Synthesis of new 20-hydroxyecdysone o-chlorobenzoyl derivatives. Chem Nat Compd 2006;51(4):706-10.