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Research Article

Development of A water soluble inclusion complex hydroxypropyl-β-cyclodextrin with disulfiram

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Abstract

Disulfiram (DSF) was successfully used to treat cataract – one of the most common eye disease. Unfortunately the usage of the DSF in ophthalmology is limited due to its low solubility in water. The objective of this article is to propose a new way to product an inclusion complex of DSF with hydroxypropil-β-cyclodextrin (HPβCD) that allows enhancing drug's solubility in water. The inclusion complex was prepared by dissolving of dry samples of HPβCD and DSF in ethyl alcohol with the molar ratio 2:1. To confirm complex formation and establish the structure we used differential scanning calorimetry, nuclear magnetic resonance spectroscopy and X-ray powder diffraction.

We compared typical thermograms of disulfiram, physical mixture of DSF with HPβCD, inclusion complex of HPβCD and DSF. Thermogram of complex showed no melting peak for DSF. Complex shows the presence of signals on diffractogram that characterize the amorphous state of inclusion complex. The obtained product can be classified as freely soluble in water. We validated the molar ratio of HPβCD and DSF in the inclusion complex and proposed structure of the inclusion complex based on NMR.

Keywords: host-guest complex; new method; structure studies

1. Introduction

The main function of eye is to collect and focus light on the retina. It is necessary for lens to remain clear to make this process successful. Cataract is a disease characterized by dysfunction of the lens due to opacification[1]. Cataract is one of the most common eye diseases. It affects about 10 million people in the Russian Federation[2]. Nagai et al. showed that disulfiram (DSF) can be an effective drug for cataract treatment[1]. DSF reduces the action of hydroxyl radicals and inhibits lipid peroxidation. It is acts in cells as an inhibitor of superoxidedismutase (SOD)[3]. Sodium diethyldithiocarbamate (DETC) is the main metabolite of DSF. DETC is chelating intracellular Cu2+[3]. DETC inhibits lipid peroxidative damage induced by ascorbate / Fe++ / ADP, Fe++ / ADP / carbon tetrachloride or glutathione depletion[4]. Unfortunately, use of the DSF in ophthalmology is limited due to its low solubility in water. In order to enhance the solubility of the DSF Nagai et al. proposed to use cyclodextrins (CD)[1]. CD increases the water solubility by formation of inclusion complexes with "host - guest" interactions. The potential benefits of DSF usage are the reason why the synthesis and study of the composition and properties of inclusion complexes is an urgent task for the treatment of cataracts. There are several types of CD, depending on the number of glucopyranose units and substitution degree. β-CD is often used in pharmaceutical practice, especially its hydroxypropyl derivatives [6].

2. Materials and Methods

2.1 Materials

In this work we used hydroxypropyl-β-cyclodextrin (HPβCD) with substitution degree 0.6 (by company ASHLAND Cavitron w7hp5 pharma) and DSF (manufactured by Synthexim).

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2.2 preparation of HPβCD/ DSF inclusion complex

We prepared inclusion complex by dissolving dry samples of HPβCD and DSF in ethanol with the molar ratio of HPβCD to DSF 2: 1. This mixture was stirred at 65˚C for 30 minutes at 450 min^{-1} until the achievement of DSF complete dissolution. The product was filtered through a membrane filter with a pore size of 0.22 µm. The organic solvent was removed on a rotary evaporator. The inclusion complex was evaluated as "freely soluble in water" according to the European Pharmacopoeia [7].

As a reference, a physical mixture of HPβCD and DSF was used which was obtained by mixing of the dry and unprocessed ingredients at the same stoichiometric ratio (2:1).

2.3 Characterization of HPβCD/ DSF inclusion complex

Study of inclusion complex was carried out by differential scanning calorimetry (DSC), nuclear magnetic resonance spectroscopy (NMR) and X-ray powder diffraction (XRPD).

2.3.1 Differential scanning calorimetry (DSC)

DSC experiments were carried out on a differential scanning calorimeter DSC-500 in the dynamic mode at a heating rate of 10 ˚C/min (gas argon flow rate at 50 ml / min) and with the temperature range 50-330 ˚C.

2.3.2 Nuclear magnetic resonance spectroscopy (NMR)

C^{13} NMR spectra were recorded on a Avance Bruker DPX-300 at 313 K with the operating frequency of 75 MHz. Spectra were recorded in the Inverse Gate mode. Since the integrated intensity is also affected by relaxation effects, the delay between impulses according to the rule 5T1 was 15 s. The quantity of scans taken was 1000.

2.3.3. X-ray powder diffraction (XRPD)

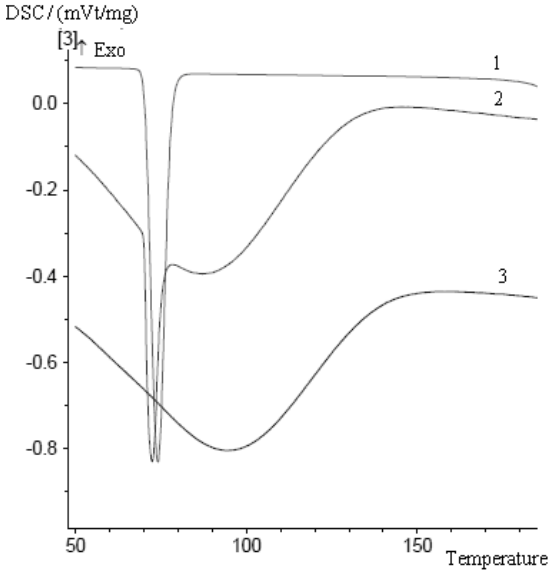
XRPD studies were carried out on powder diffractometer X'Pert Pro MPD. The experiment was conducted under the same conditions for all four sample - voltage of 50.0 kV and a current of 40.0 mA (2kW) at a scan rate at 2θ 0,008˚/min in the range of angles 2θ 5˚ - 60˚

3. Characterization of DSF inclusion complex

3.1. DSC analysis

Typical DSC thermograms of disulfiram, its physical mixture with HPβCD and DSF, inclusion complex of HPβCD and DSF presented in Figure 1.

Fig.1. DSC thermograms of a pure DSF (1), the physical mixture of the HPβCD and DSF (2), and inclusion complex of HPβCD and DSF (3).

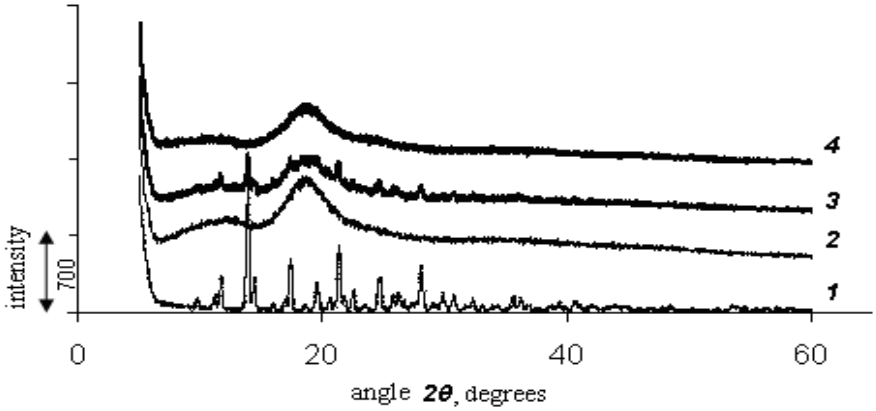


We can see endothermic peaks on the DSC thermograms of pure DSF (1) and of physical mixture of the HPβCD and DSF (2). On the DSC thermogram of inclusion complex (3) this endothermic peak is missing. There is also a broad endothermic peak on the curves (2) and (3) with a maximum at 100 ˚C.

3.2 X-ray powder diffraction (XRPD)

Typical X-ray diffraction spectra of the DSF (1), HPβCD (2), their mechanical mixture (3) and the inclusion complex of HPβCD and DSF (4) are shown on the Fig.2.

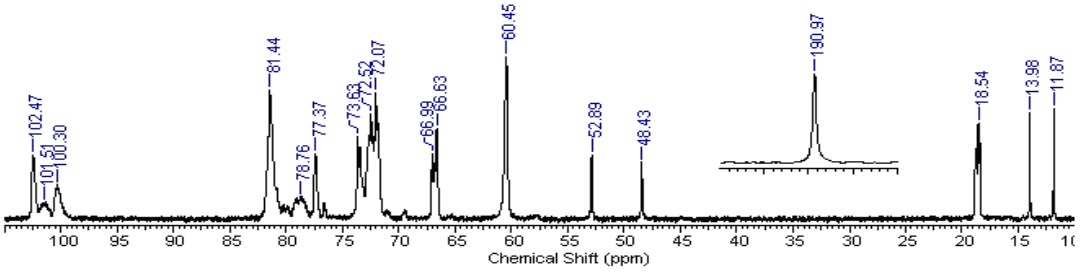
Fig.2. X-ray diffraction spectra of pure DSF (1), HPβCD (2), a mechanical mixture of HPβCD and DSF (3), and inclusion complex of HPβCD and DSF (4).



There is a series of intense peaks on the diffractogram of pure DSF (1). At the diffraction pattern of pure HPβCD (2) intense peaks are absent. Diffraction pattern of a physical mixture of HPβCD and DSF (3) combines difractograms (1) and (2). However, at the diffraction pattern of inclusion complex of HPβCD and DSF (4) intense peaks are missing.

3.3 nuclear magnetic resonance spectroscopy (NMR)

Figure 3: A typical C^{13} NMR spectrum of the inclusion complex HPβCD and DSF



4. Result and Discussion

4.1 DSC analysis

Endothermic peaks in the thermograms (1) and (2), described in 3.1, can be assigned to the melting of DSF (73.0 °C and 72.4 ˚C). This peak is absent on the thermogram (3) while the DSF is not available in a crystalline form and is completely included into the complex. The broad endothermic peak described in 3.1 seems to correspond to the removing of adsorbed water while it is absent when scanning is repeated.

4.2 X-ray powder diffraction (XRPD)

Intense peaks on the difractogram (1), mentioned in section 3.2, indicate the crystal structure of the DSF. Absence of intense peaks at diffraction pattern (2) shows the amorphous state of HPβCD. Diffraction pattern (3) shows both a series of intense peaks DSF and amorphous state of HPβCD, corresponding to the presence of both compounds. However, absence of intense peaks at pattern (4) allows assuming that the crystal form of DSF does not exist in the complex and that DSF is full included in the internal cavity of HPβCD.

4.3 Nuclear magnetic resonance spectroscopy (NMR)

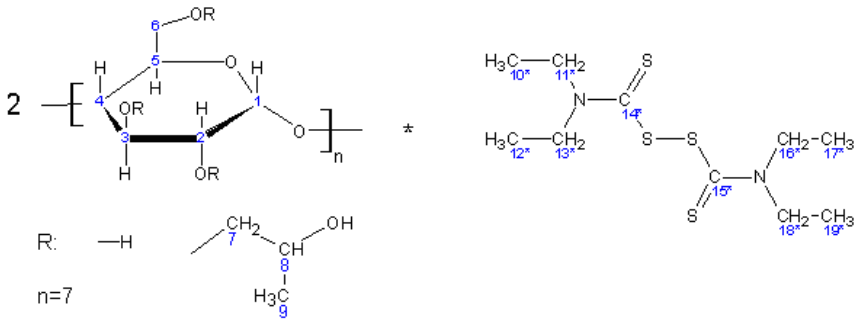
Interpretation of NMR ^{13}C signals of HPβCD - DSF complex were carried out with the help of the spectral SDBS [8] database and are presented in table 1.

Table 1: Assignment of signals or group of signals in NMR ^{13}C spectra for HPβCD — DSF inclusion complex (*)

№	Assigned signals or group of signals	Chemical shift, ppm (comparing to DSS)	
		HP-β-CD	DSF
1.	Signal C14*, C15*		191.0
2.	Signals C1	99.0-103.0	
3.	Signals C2-C5, C7	69.0-82.0	
4.	Signals C8	66.0-67.5	
5.	Signal C6 (in case of unsubstituted glycopyranose ring or substituted on C2 atom)	60.4	
6.	Signal C11* , C18*		52.9
7.	Signal C13* , C16*		48.4
8.	Signals C9	18.1-19.1	
9.	Signal C10* , C19*		14.0.
10.	Signal C12* , C17*		11.9

Assigned numbers for carbon atoms in the molecules HPβCD and DSF are shown on Figure 3.

Figure 3: Assigned numbers for carbon atoms in HPβCD and DSF molecules



Due to the isolation of signals in order to calculate the molar ratio of HPβCD to DSF we used the C1 signals deriving from methine group of α-D-glucopyranose units belonged to HPβCD and methyl groups of DSF (C10 *, C19 * or * C12, C17 *).

To make it possible to determine the molar ratio of HPβCD to DSF in the inclusion complex it is necessary to pay attention to the following factors:

1) Since the solubility in water (D_2O) of pure DSF is extremely low (4.09 mg/l for 25ºC), and the content of a sample of the DSF for spectrum's registration is about 2 grams / liter (clear solution), we can conclude that the content which is not included in the set of the DSF can be neglected and it is possible to register only an inclusion complex.

2) The integrated intensities of the signals in the ^{13}C NMR spectrum are registered in Inverse Gate mode proportional to molar ratio between them.

According to these statements, the stoichiometric ratio of HPβCD to DSF can be calculated by using the formula 1:

$$n = \frac{I_{C1}/7}{I_{(C10^*,C19^*)}/2} \quad (1), \text{ where}$$

I_{C1} - integral intensity of C1 signals deriving from methine group of α-D-glucopyranose units belonged to HPβCD

$I_{C10^*,C19^*}$ - integral intensity of signals deriving from methyl groups of DSF (C10 *, C19 * or * C12, C17 *).

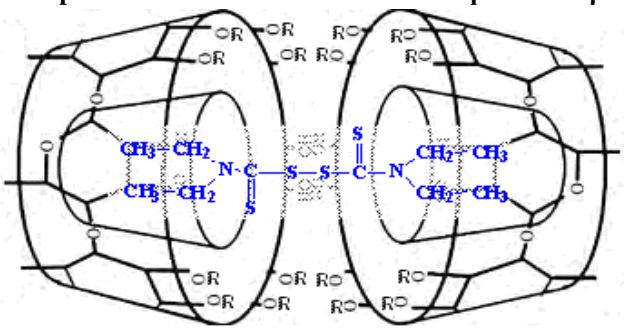
Values of integral intensities for C1 and C10*, C19* signals for three series of HPβCD - DSF inclusion complexes are summarized in Table 2.

Table 2: The values of the integrated intensities of the signals in NMR ^{13}C spectra of HPβCD -DSF inclusion complex (*)

№	Signal	Seria 1	Seria 2	Seria 3
1.	Signal C1 (99.0-103.0 ppm)	8.12	8.05	7.90
2.	Signal C10*, C19* (14 ppm)	1.10	1.09	1.11
Stoichiometric ratio of HPβCD to DSF (n)		2.11	2.07	2.03

Stoichiometric ratio of HPβCD to DSF is validated by C^{13} NMR. It shows the ratio 2 to 1 (HPβCD to DSF), suggesting that the structure of the inclusion complex as follows the figure 4. More detailed description the structure of the inclusion complex will be described in the following papers.

Fig. 4: Proposed structure of the inclusion complex of HPβCD and DSF.



5. Conclusions

A new method to obtain the inclusion complex of HPβCD with DSF by using ethyl alcohol as solvent was elaborated.

According to the Ph. Eur., the obtained product was classified as freely soluble in water. DSC method confirmed the formation of inclusion complex by the absence of the melting peak of crystalline DSF. Powder diffraction method confirmed the formation of an inclusion complex by the absence of a series of intense peaks that characterize the crystalline state of the DSF and the presence of signals on diffractogram that characterize the amorphous inclusion complex. The molar ratio 2:1 of HPβCD and DSF in the inclusion complex was validated by using NMR method. We also proposed structure of the inclusion complex.

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