

INCLUSION COMPOUNDS OF β -CYCLODEXTRIN-PITOFENONE HYDROCHLORIDE. INVESTIGATIONS OF SOLID FORMS

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ABSTRACT. Pitofenone hydrochloride (PF.HCl) is a pharmaceutical substance used as an antispasmodic drug. The inclusion compounds of β -cyclodextrin (β -CD) with pitofenone hydrochloride (PF.HCl) have been prepared by co precipitation and freeze-drying methods in order to increase the capacity user acceptance to wafer interest in terms of their taste. For physical chemical characterization the inclusion compounds were analyzed by powder X-ray diffraction, FTIR and ss-NMR spectroscopy, DSC, SEM and the structural architecture of the inclusion compound was proposed by molecular modeling techniques. The new product obtained can be used in the pharmaceutical industry because it provides improved smell and taste as compared to the starting substance.

Keywords: *pitofenone hydrochloride, β -cyclodextrin, SEM, DSC, magnetic and FTIR spectroscopy, molecular modeling*

INTRODUCTION

Pitofenone hydrochloride (methyl 2-[4-(2-piperidin-1-ylethoxy) benzoyl]benzoate) with molecular formula $C_{22}H_{25}NO_4 \cdot HCl$ (Fig.1a) and molecular weight 403.9 g/mol is a drug with an antispasmodic activity that is currently available in a combination product with fempiverinium bromide and metamisol sodium. The combination drug product is called Spasmalgon and is used for release of pain and spasms of smooth muscles despite its availability on the market. The attempts to grow crystals of pitofenone hydrochloride suitable for single crystal analysis were unsuccessful and therefore, we

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reported [1-2] the crystal structure as determined from X-ray powder diffraction. Cyclodextrins are formed by the action of the enzyme glucosyltransferase upon starch. Three cyclodextrins are typically formed: α -, β -, and γ -cyclodextrins which contain 6, 7 (Fig. 1b) and 8 α -1,4 linked glucose monomers, respectively. Cyclodextrins are ring or torus-shaped molecules and possess a hydrophobic cavity and a hydrophilic exterior. The hydrophobicity of the cavity allows cyclodextrin to associate with non polar organic molecules or portions of organic molecules to form inclusion complexes [3]. We decided to use β -CD because is very cheap, quite soluble in water and its geometrical dimensions can accommodate guest molecule such as PF.HCl.

Several inclusion compounds of various cyclodextrins with different molecules having practical applications were already reported [3-31].

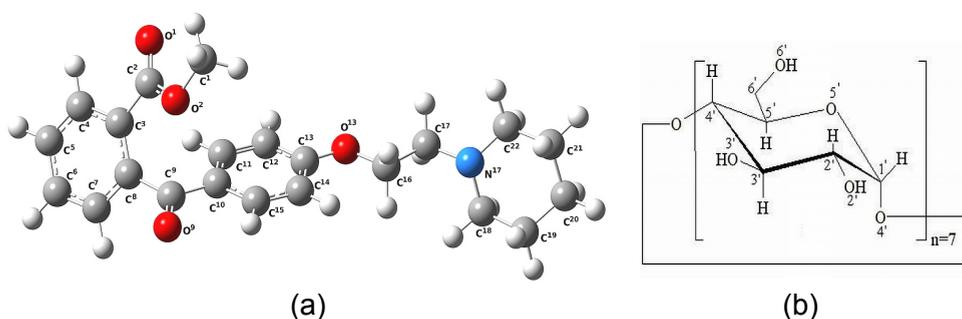


Figure 1. Molecular structure of methyl 2-[4-(2-piperidin-1-ylethoxy) benzoyl] benzoate hydrochloride (pitfenone hydrochloride, PF.HCl) (a), Chemical structure of β -cyclodextrin (b)

The aim of this paper was to prepare inclusion compounds of PF.HCl with β -cyclodextrin by co precipitation and freeze-drying techniques and to characterize them by employing powder-X-ray diffraction, FTIR, ss-NMR spectroscopy, DSC, SEM and molecular modeling DFT techniques.

RESULTS AND DISCUSSION

X-ray diffraction

Powder X-ray diffraction patterns of PTF.HCl, of β -CD and of the inclusion compounds obtained co precipitation and freeze-drying (lyophilization) are presented in Fig. 3. One can observe that X-ray patterns for *co* and *fd* products are different as compared to the patterns of starting compounds, *i.e.* inclusion compounds are obtained. The most important reflections that appear for PF.HCl- β -CD do not belong to β -CD or to PF.HCl. The crystallite

sizes were evaluated using Scherrer relationship [31]. We have obtained for crystallite size $D=300\text{\AA}$ for PF.HCl- β -CD while crystallite size for β -CD are $D=1200\text{\AA}$ and for PF.HCl $D=1300\text{\AA}$.

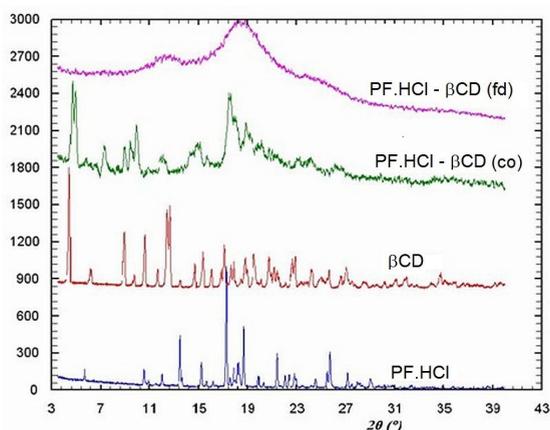


Figure 3. Powder X-ray diffraction patterns of PTF-HCl, of β -CD and of the inclusion compounds obtained co precipitation (*co*) and freeze-drying (*fd*)

The amorphous character of *fd* product and also partially amorphous character for *co* product is confirmed.

DSC

The DSC thermograms of pure PF.HCl, β -CD and PF.HCl- β -CD inclusion compounds, respectively are presented in Fig. 4.

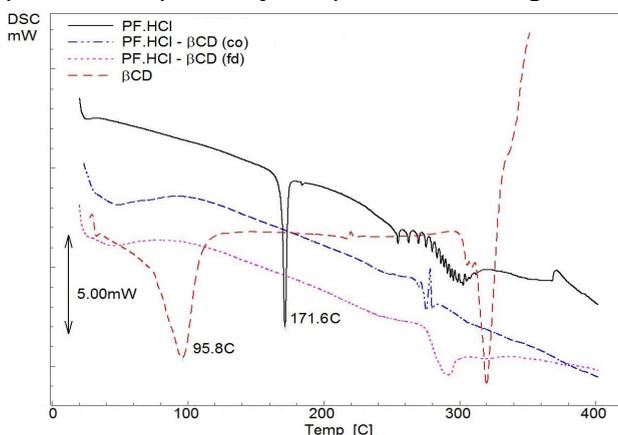


Figure 4. DSC curves of PF.HCl, of β -cyclodextrin and of their inclusion compounds obtained by co precipitation and freeze drying methods

The DSC trace of the β -CD reveals a broad endothermic signal between 74 - 118 °C, with $\Delta H = 190$ kJ/mol, that corresponds to the loss of the water molecules existing as residual humidity, as well as those are included in the cavity [27, 28]. From 290 °C the melting followed by decomposition of the compound occurs [5, 29]. The DSC curve of the PF.HCl presents a sharp endothermic peak at 171.6 °C, with $\Delta H = 117.7$ kJ/mol, corresponding to the melting of the drug, compound shows thermal stability up to 230 °C when degradation begin. In the case of the co-precipitated and freeze-dried PF.HCl- β -CD compounds the disappearance of dehydration endothermic peak of β -CD and of melting peak of the PF.HCl were observed. These facts are indications for the occurrence of the inclusion process between PF.HCl and β -CD [30].

ss-NMR Analysis

The solid state ^{13}C CP-MAS NMR spectra of PF.HCl, β CD and those two compounds of PF.HCl- β CD, obtain by co-precipitation (*co*) and freeze-drying (*fd*) techniques are presented in Figure 5.

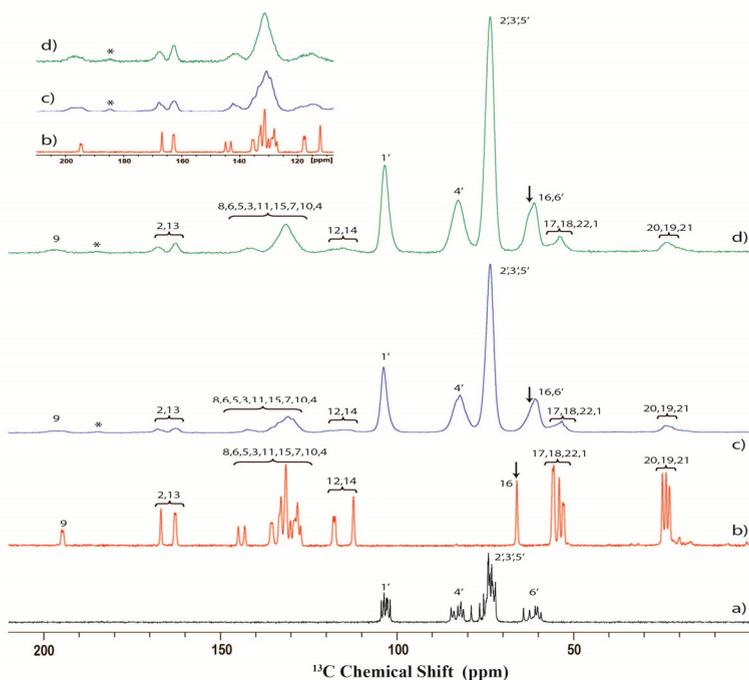


Figure 5. ^{13}C CP-MAS NMR spectra of: a) β -CD, b) PF.HCl, c) PF.HCl- β CD (*co*), d) PF.HCl- β CD (*fd*). In the inset figure are the corresponding lines of PF.HCl in the 110 – 200 ppm range. The spinning sidebands are shown by asterisks.

The compound PF.HCl- β CD obtained by co-precipitation (see Fig. 5.c) is a mix of amorphous and crystalline phase whereas that obtained by freeze-drying is an amorphous one (see Fig. 5.d). Despite the amorphous character of the spectra, the most obvious difference observed between free state and complexed state of PF.HCl, is the upfield shift of the line situated at 66.1 ppm in the PF.HCl spectrum, marked with black lines in the NMR spectra (Figs. 5b, 5c, 5d) this indicating a different chemical environment for the corresponding C16 atom of pitofenone and the possibility of inclusion compounds formation.

FTIR spectroscopy

FTIR spectra of PF.HCl, of β -cyclodextrin and of their inclusion compounds obtained by *co* and *fd* are presented in Fig. 6.

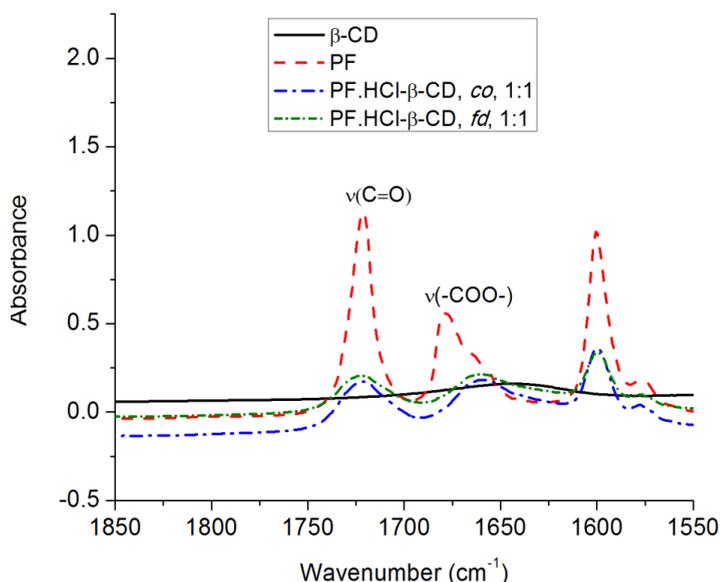


Figure 6. FTIR spectra of pitofenone hydrochloride, β -cyclodextrin, of pitofenone hydrochloride- β -cyclodextrin inclusion compounds obtained by co precipitation (*co*) and by freeze-drying (*fd*) methods, 1850-1550 cm^{-1} spectral domain

One can conclude (see Fig. 6B) that the $\nu(\text{C}=\text{O})$ vibrational frequency located at $\sim 1721 \text{ cm}^{-1}$ is not shifted by complexation, whereas 1679 cm^{-1} the vibrational mode - $\nu(-\text{COO}-)$ is shifted to 1658 cm^{-1} by complexation with β -CD. It is an indication of the inclusion compound formation both by co precipitation and by freeze-drying processes.

SEM

SEM images of pitofenone hydrochloride, β -cyclodextrin and of pitofenone hydrochloride- β -cyclodextrin inclusion compounds obtained by co precipitation (*co*) and freeze-drying (*fd*) methods are presented in Figs. 7 A-D. On the SEM images of the same size it can be seen that the dimensions of the crystallites are different for pure substances (crystalline substances) and for inclusion compounds.

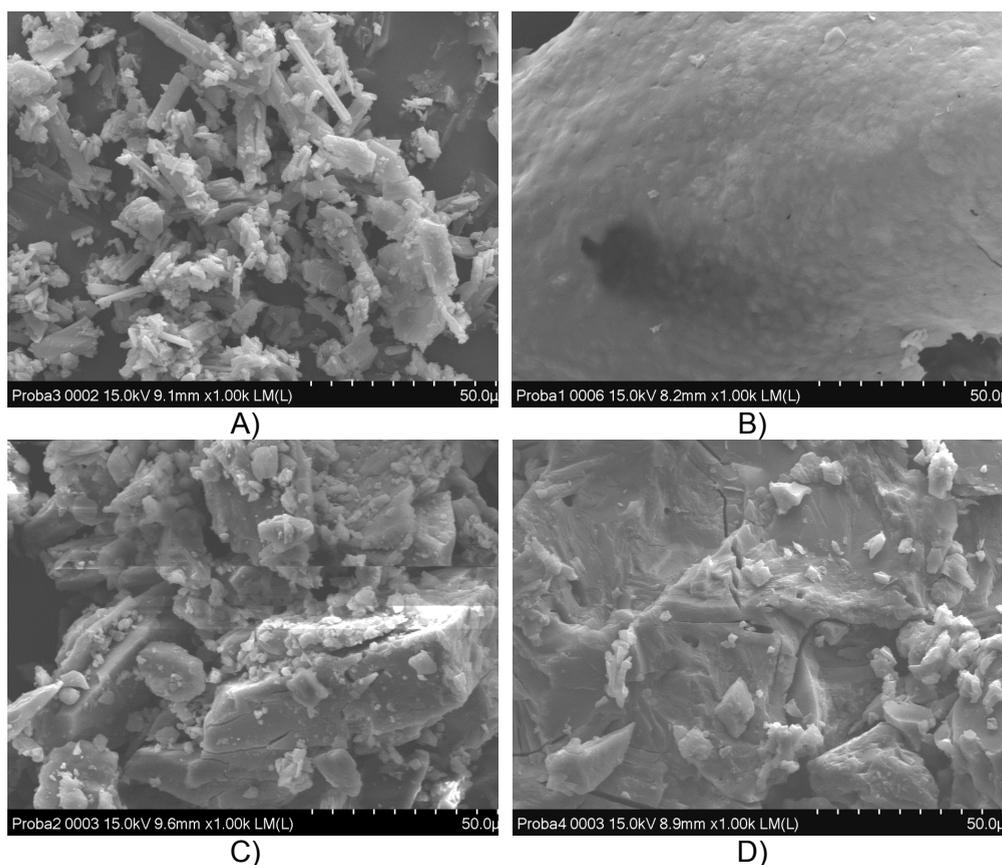


Figure 7. Scanning electron microphotographs: A) β -CD, B) pitofenone HCl, C) PF.HCl- β CD (*co*) and D) PF.HCl- β CD (*fd*)

DFT molecular modeling

The equilibrium geometry of the β -CD – pitofenone host-guest supramolecular complex was obtained at DFT level of theory using the M11 [24] exchange-correlation functional considering the def2-TZVP [25] basis

set implemented in the Gaussian09 quantum chemistry program package [26]. The geometry configuration of the pitofenone hydrochloride- β -CD inclusion compound can be seen from two points of views: side view and bottom view, see Figs. 8A and B, respectively. In order to estimate the thermodynamic effects on the inclusion process it was further performed a normal mode analysis to calculate the Gibbs free energy. This calculation was possible to be done only by using the smaller size def2-SVPP [25] basis set. Accordingly, the calculation of intermolecular interaction energy was performed using both the smaller and larger basis sets. The intermolecular interaction energy obtained at M11/def2-SVPP level of theory is -61.41 kcal/mol, while the similar value obtained with the M11/def2-TZVP method is -41.87 kcal/mol. As we can see, with the increasing of the basis set's size the intermolecular interaction energy drastically decrease. In order to take into account the thermodynamic effects, we have computed also the Gibbs free energy considering the M11/def2-SVPP level of theory.

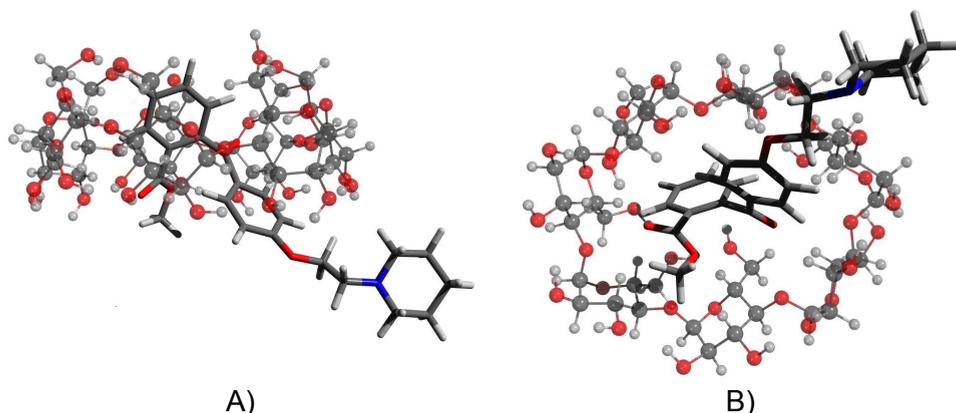


Figure 8. A) Side view of the PF.HCl- β CD, B) Bottom view of the PF.HCl- β CD inclusion compound

For this value we have obtained -6.36 kcal/mol showing a spontaneous complexation process, but it should be accepted with some reserve because of the basis size effects. On the other hand it is important to know effects which can hinder the complexation. In this way, we have computed the deformation energy, which is the energy difference between the energy values of the molecular constituents obtained at the equilibrium geometry configuration in isolated and complexed cases. The deformation energy of the β -CD is +29.28 kcal/mol for M11/def2-SVPP case and +19.67 kcal/mol for M11/def2-TZVP, respectively. The similar values for the pitofenone are +6.04 kcal/mol for M11/def2-SVPP case and +2.81 kcal/mol for M11/def2-TZVP,

respectively. It can be observed that the inclusion process also induce a strong deformation of the β -CD cavity, while that of the pitofenone is relatively small. Another interesting feature of the complexation is that the pitofenone molecule makes only two hydrogen bonds with the OH groups of the β -CD, the most part of the interaction is given by the long-range contribution of the dispersion forces. At the same time the line shift in ss-NMR spectra for the C16 carbon atom could be induced by one of the hydrogen bonds made by the O13 atom with an OH group from the bottom part of the β -CD.

CONCLUSIONS

Inclusion compounds of PF.HCl with β -cyclodextrin were prepared by co-precipitation and freeze drying. They were characterized in the solid phase by X-ray diffraction, DSC, FTIR, ss-NMR, SEM and molecular modeling. X-ray diffraction pattern shows a compound in the amorphous state if it is obtained by freeze-drying. By co-precipitation a partially amorphous compound with an important crystalline phase was obtained. DSC curves indicate the formation of different compounds as compared to the starting ones. FTIR spectroscopy indicates the COO⁻ group's involvement in the complexion process. ss-NMR indicates the formation of an amorphous compound (those obtained by freeze drying) and a partially crystalline compound (those obtained by co-precipitation) with the possibility of inclusion compounds formation (a shifted line of PF.HCl in the ¹³C CP-MAS spectra of the new compounds as compared to the spectrum of PF.HCl). Based on PXRD and ss-NMR results, the so obtained compounds (by co-precipitation and lyophilization) have partially amorphous and amorphous character, respectively. There are indications that the X-ray diffraction pattern of the obtained inclusion compounds by co precipitation is different as compared to starting one diffractograms. DFT molecular modeling technique gives the spatial architecture of the inclusion compound.

EXPERIMENTAL

Powder X-ray diffraction (PXRD) patterns were collected in the $2\theta=3.5-40^\circ$ angular domain with a BrukerD8Advance diffractometer, using Cu $K\alpha_1$ radiation ($\lambda = 1.5406 \text{ \AA}$) (40 kV; 40 mA). In order to increase the resolution, a Ge 111 monochromator was used to eliminate the $K\alpha_2$ radiation. Data collection was performed with the DIFFRAC plus XRD Commander programs' package at room temperature. The step scan mode was performed with a step

of 0.01° at a rate of 1 step/s. The samples were mildly pre-ground in an agate mortar in order to control crystals size and to minimize the preferred orientation effects.

FTIR spectroscopy. FTIR spectra were recorded in the 4000 to 400 cm^{-1} spectral range with a resolution of 4 cm^{-1} with JASCO 6100 FTIR spectrometer by employing KBr pellet technique; the spectra were processed with Spectral Analysis software.

DSC analysis. DSC thermograms were registered with a DSC60 Shimadzu differential scanning calorimeter by heating about of the 1.5 mg of samples from room temperature up to 400°C , in a crimped aluminium pan, under flowing nitrogen flux, the heating rate being $10^\circ\text{C}/\text{min}$. For data collection the Shimadzu TA-WS60 and TA60 2.1 software were employed.

Solid-State NMR, ss - ^{13}C NMR spectra were obtained on a Bruker Avance III 500 MHz wide-bore NMR spectrometer operating at room temperature, using a 4mm CP-MAS probe head. Standard RAMP CP-MAS spectra were acquired at 14 kHz spinning frequency, 2 ms CP contact time, high-power (100 kHz) proton decoupling under TPPM, recycle delay of 5 s by averaging 1200 transients for pitofenone hydrochloride and β -cyclodextrin, and 15.500 transients for pitofenone-hydrochloride- β -cyclodextrin. The recorded spectra are calibrated relative to the CH_3 line in TMS (tetramethylsilane), through an indirect procedure which uses α form of L-Glycine as external standard ($\text{C} - \text{O}$ of Glycine at 176.5 ppm).

SEM was performed on a UHR-SEM HITACHI 8230. The samples were drop casted and evenly distributed on silicon wafers. The measurements advantage: high resolution at low default does not destroy evidence and extract real surface. The samples were analyzed in their natural state without coverage with the conductive layer. The SEM analyzes are performed in high vacuum. The advantages of EDX (EDS) energy dispersive X- ray spectroscopy: xMax were made with 80 mm^2 SDD Oxford instrument type (silicon drift detector). The SEM parameters: 15 kV voltage, more vacuum or equal $\geq 10^{-4}$, Mars and secondary electrons of images were used.

Molecular modeling. Full geometry optimization and frequency calculations for βCD , PF.HCl molecules and their host-guest compound have been carried out in the gas phase at the DFT level of theory considering the M11 [24] exchange-correlation functional together with the def2-SVPP [25] basis set implemented in the Gaussian 09 [26] program package. No negative wavenumbers were obtained for all three cases, proving that true minima of the potential energy surfaces were found in the optimizations.

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