

SYNTHESIS AND CHARACTERIZATION OF FUROSEMIDE COMPLEX IN β -CYCLODEXTRIN

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It is reported the improving of the solubility and UV stability of furosemide, a poorly water soluble diuretic, by complexation with β -cyclodextrin. There is presented the synthesis of the inclusion complexes of furosemid with β -cyclodextrin by co-precipitation, and freeze drying methods, and their characterization. The results confirm the inclusion of the phenyl ring of furosemide within the nonpolar cavity of β -cyclodextrin. Improvement of the aqueous solubility is expected to improve the bioavailability of the drug in oral administration.

Keywords: Furosemid, β -cyclodextrin (β CD), 1:1 furosemid- β CD complex, FT-IR, DSC, SEM

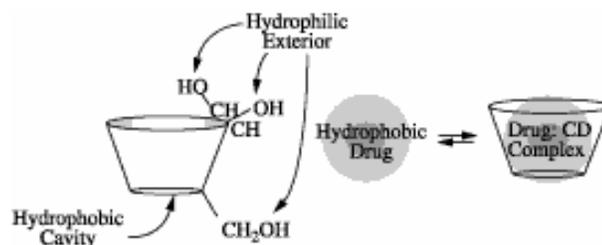
1. Introduction

Cyclodextrins form a number of inclusion complexes with biologically and pharmaceutically important substances. These inclusion complexes (ICs) have been extensively used to improve the solubility, stability and bioavailability of various drugs. They are molecular compounds, having the characteristic structure of adducts, in which one of the molecules (*the host*) spatially encloses another one. The enclosed molecule (*the guest*) is located in the cavity of the host molecule without affecting the framework structure of the host [1, 2]. Some chemical and physical properties of guest molecules are affected by the complex formation. These include alteration of the solubility of the guest compound, stabilization against the effects of light, heat, and oxidation, masking of unwanted physiological effects, reduction of volatility, and others. In some applications, more benefits are obtained by complexation with cyclodextrins [3]. The solubility of a guest compound can be changed upon complexation with a cyclodextrin. On the other hand, cyclodextrins also form crystalline complexes [3]. Cyclodextrins have been reported in a number of studies to interact with many drug molecules by formation of inclusion complexes (Scheme 1) [4-12].

Furosemide, a sulfonamide-type drug, is used to cure hypertension and edema associated with congestive heart failure, cirrhosis, and renal disease. This drug can be used for the treatment of hypertension by itself or in combination with other antihypertensive agents. Among the various physico-chemical properties of this drug, poor water solubility is noticed, that is detrimental to its efficiency. Indeed, furosemide is a very slightly water soluble drug (0.006 mg/mL at pH=5, and 37 °C temperature) and presents low stability against light..

The present study will describe the inclusion complex formation of the diuretic furosemide drug with β -cyclodextrin (β -CD). The complex formation and its molecular/supramolecular structure have been investigated by ¹HNMR, FT-IR, X-ray diffraction, SEM and DSC measurements. The obtained complex exhibits high aqueous solubility and stability against the illumination.

The preparation of the complex was performed in two different ways co-precipitation and freeze drying.



Scheme 1: Schematic view of the process of drug cyclodextrin inclusion.

2. Experimental

2.1 Materials

Furosemide and β -CD were purchased from Aldrich Chemical Co. Other reagents and chemicals were of analytical reagent grade.

Synthesis of furosemide / β -CD by co-precipitation

To a 10 mL round bottom flask equipped with magnetic stirrer was dissolved at room temperature (0.0459 g, 0.0404 mmol) of β -CD in distilled water (3.0 mL) and after that (0.0127 g, 0.03839 mmol) of furosemide dissolved in ethanol (3.0 mL) was added drop by drop to the aqueous solution of β -CD. The mixture was stirred for 3 days. The precipitated was filtered, washed with cold water once (2.0 ml) and dried. 0.0496 g of white solid product was obtained. The yield was 76.5%.

Synthesis of furosemide / β -CD by freeze drying method

The β -CD was dissolved, in a 50 mL round bottom flask equipped with magnetic stirrer, at room temperature (0.1380 g, 0.121 mmol), in distilled water (9.0 mL) and finally (0.0381 g, 0.115 mmol) of furosemide was added. The mixture was stirred for 3 days and the complex was obtained by freeze drying method.

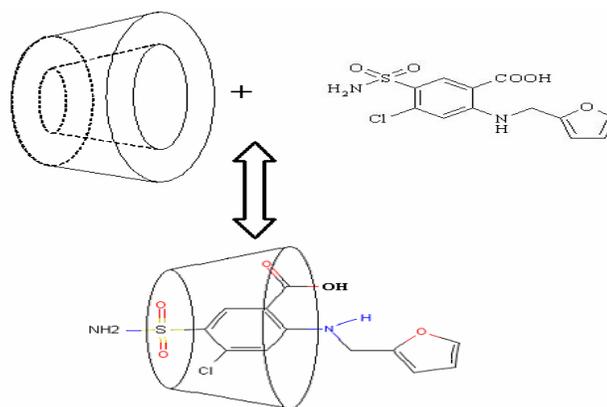
2.2. Methods

The $^1\text{H-NMR}$ spectra were obtained using a Bruker Avance 400 DRX spectrometer at 400 MHz using DMSO-d_6 or D_2O as a solvents. DSC measurements were performed with a Mettler DSC-12E apparatus in air and at a heating rate of $10^\circ\text{C}/\text{min}$. The infrared (IR) spectra were obtained on a SPECORD Carl Zeiss Jena IR spectrometer (KBr pellets).

The microphase structure was investigated by X-Ray diffraction (XRD) with Philips Xpert XRD System. The molecular structure was investigated by InfraRed (IR) with a Nicolet FT-IR 320. The surfaces morphology of materials was examined by scanning electron microscope Tesla BS 3001. The samples were fixed on a brass stub using two-sided tape and then gold coated in vacuum by a sputter coater. The pictures were then taken at an excitation voltage of 20 kV

3. Results and discussion

Furosemide and β -CD complexes were prepared in a molar ratio of 1:1 by two methods i.e. co-precipitation and freeze drying method (Scheme 2).



Scheme 2. Synthesis of inclusion complex furosemide / β -CD

¹H-NMR characterization

Fig. 1 presents the ¹H-NMR spectra of the β -cyclodextrin / furosemide inclusion complex. The stoichiometry (0.6:1) of the inclusion complex was proved by the integrals ratio.

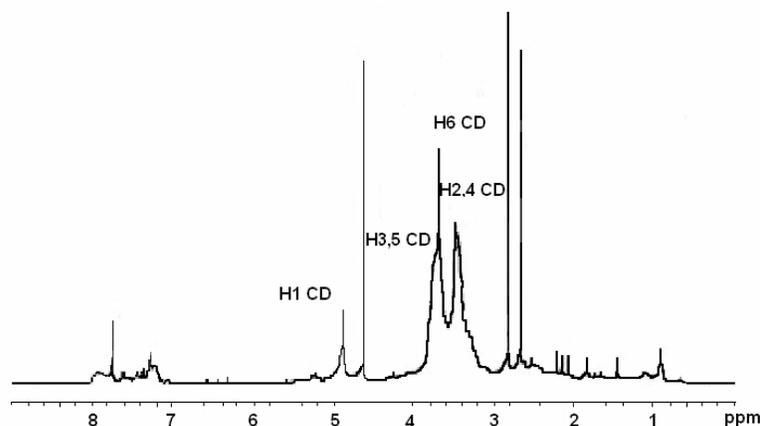


Fig. 1. ¹H NMR spectra (D_2O , 25 °C) of furosemide / β -CD complex

The characteristic absorptions of aromatic nuclei from furosemide (¹H NMR spectra, DMSO, 25 °C) are situated in the range 7.2-7.9 ppm domain. After inclusion of the aromatic protons of furosemide the peaks become more or less shifted. The signals characteristic for β -cyclodextrin are present only in the spectra of the inclusion complex. The inclusion complex shows a marked contrast of its solubility as compared to the furosemide. While the furosemide was characterized using DMSO as solvent the inclusion complex was characterized by using D_2O as solvent.

Infrared (IR) characterization

In the IR spectrum (not shown) the characteristic absorption of β -CD are superposed over the furosemide ones. This phenomenon is due to the differences between molecular weights of the components even if the molar ratio of the inclusion is 0.6:1. The infrared spectrum of the inclusion complex shows specific absorption peaks at 3370 cm^{-1} (OH stretching H-bonded), 2930 cm^{-1} (OH stretching), 1627 cm^{-1} (OH bending), 1368 cm^{-1} (OH deformation), 1243 cm^{-1} (OH bending), 1156 cm^{-1} (COC stretching and OH bending), 1079 and 1032 cm^{-1} (COC stretching) characteristic for β -cyclodextrin. Weak absorption peaks found at 1560 cm^{-1} and those at 650 cm^{-1} , attributed to N-H link vibrations, led us to the conclusion that furosemide is included in the β -cyclodextrin. Also, the

FT-IR spectra (not shown) of the furosemide, β -CD/furosemide 1:1 physical mixture, and β -CD/furosemide 1:1 complex show the most interesting signals: wide band at $3300\text{--}3600\text{ cm}^{-1}$ due to OH bonds stretching; a strong and complex band at $1000\text{--}1260\text{ cm}^{-1}$ due to C-O- bonds stretching. The sharp signal at 1556 cm^{-1} , due to sulphonamide group drastically reduces its intensity and sharpness in the complex.

Differential scanning calorimetry (DSC)

The DSC plot of pure furosemide drug powder (Fig. 2) shows a sharp endothermic peak near 210°C , which is attributed to furosemide melting temperature. In the thermogram of β -CD, two endothermic peaks were observed in the temperature range between $100\text{--}110^\circ\text{C}$ due to the loss of water and near 330°C , it is observed and endothermic peak that corresponds to the β -CD fusion. The DSC pattern of furosemide/ β -CD inclusion complexes (1:1) prepared by physical mixing show the presence of peaks of both pure compounds. It is remarkable that the drug melting endotherm is slightly shifted from its original position of 210°C to 218°C . The thermogravimetric plots of furosemide/ β -CD complex prepared by freeze drying method (1:1) evidences the vanishing of the endothermic peak characteristic of furosemide, thus suggesting the end of complex formation.

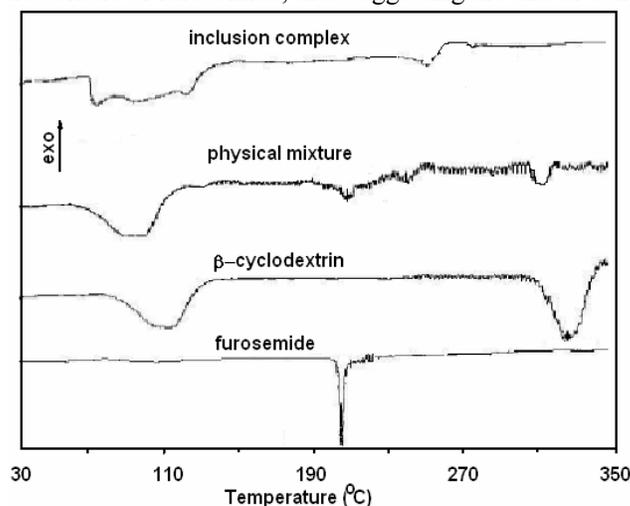


Fig. 2. DSC thermograms of furosemide, β -CD, furosemide/ β -CD physical mixture, and furosemide/ β -CD inclusion complex

X-Ray Diffraction

The XRD pattern of furosemide shows intense and sharp peaks that prove the crystalline nature of the compound (Fig. 3). The inclusion complexes of furosemide with β -CD physical mixture (1:1) show undefined, broad, diffuse peaks of low intensities. The inclusion complexes of furosemide with β -CD prepared by freeze drying (1:1) show a broad large background under the crystalline peaks. This feature indicates the formation of a significant amount of amorphous material. β -CD in its crystalline form exhibits diffraction peaks at 2θ values of 4.75° , 12.7° , 19.7° , 21.1° , 22.8° , 24.3° , and 35.9° . The peak corresponding to 12.6° (2θ) in β -CD decreases in intensity only in the case of physical mixture and inclusion complex and this is a proof for the intimate changes at the lattice level during interpenetrations of the two substances. New peaks at 5.4° and 13.6° are observed in diffractograms of both physical mixture and inclusion complex.

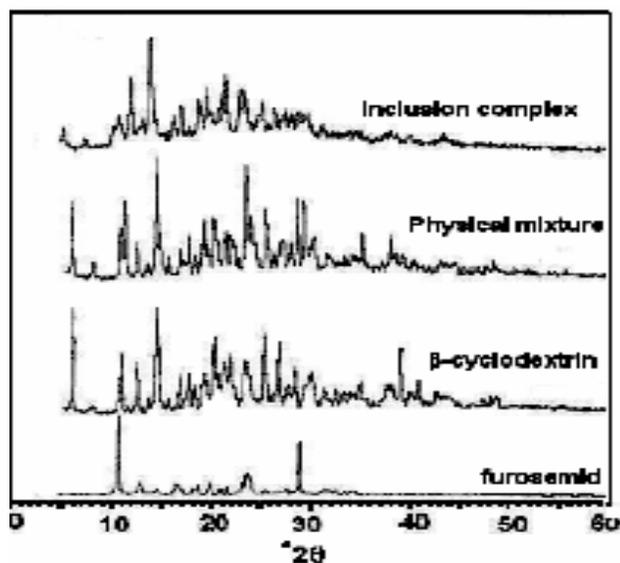


Fig.3. XRD patterns of furosemide, β -CD, furosemide/ β -CD physical mixture, and furosemide/ β -CD inclusion complex.

Scanning Electron Microscopy

Scanning electron microscopy is used to assess the microscopic aspects of the drug, of the complexing agent, of the physical mixture and of the complex formation (Fig. 4). Although this method is not a decisive method to confirm complex formation, nevertheless, it helps to assess the existence of a single component in the preparation products.

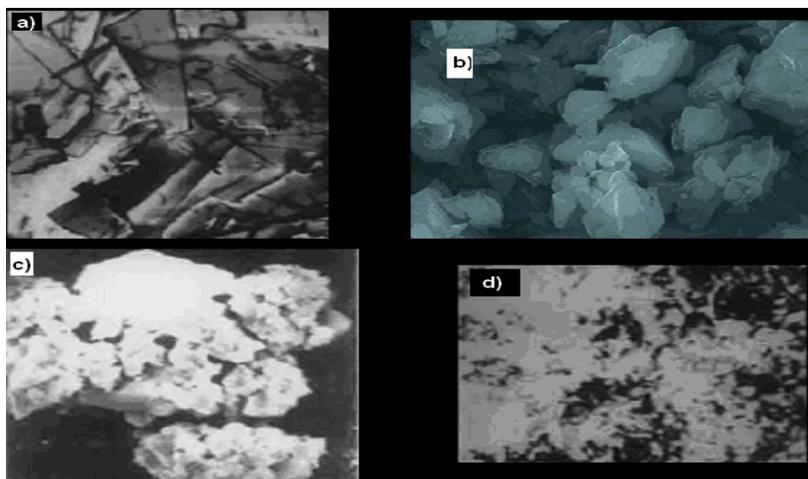


Fig. 4. SEM images of: (a) β -CD, (b) furosemide, (c) furosemide- β -CD inclusion complex, (d) furosemid- β -CD physical mixture.

Pure furosemide is characterized by the presence of crystalline particles of regular size. Pure β -CD also appears as crystalline particles without a definite shape. The physical mixture of furosemide/ β -CD showed the crystalline structure of both furosemide and β -CD. Crystals of furosemide mixed with CD crystals were seen adhering to their surfaces. The photomicrographs of

freeze drying inclusion complex show small size particles with the tendency to aggregation, suggesting the existence of an amorphous product with the presence of a single component in the complex. This indicates a maximum or complete complex formation.

4. Conclusions

An inclusion complex of furosemide / β -CD was prepared successfully by freeze drying and co-precipitation method in a molar ratio of 1:1. Furosemide/ β -CD complex has physicochemical characteristics differentiating it from furosemide. Complexes formation has been studied. The powder X-ray diffraction patterns of the inclusion complex formed by furosemide and β -CD, of the physical mixture of the crystalline substances in the 1:1 ratio, point out to the variations of the crystalline structure which proves the formation of the inclusion complex. The differences in DSC curves, of furosemide/ β -CD and of the physical mixture of furosemide and β -CD indicate that the interaction of the drug with β -CD, i.e. the formation of complex took place. The aqueous solubility of furosemide was increased by inclusion in β -CD. The results obtained by different characterization techniques clearly indicate that the co-precipitation and freeze drying method leads to formation of complexes between furosemide and β -CD. The preliminary data allows to hope that complexation of furosemide with β -CD will lead to a better therapeutic efficacy.

Acknowledgements

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References

- [1] J. Szejtli, Chem. Rev. **98**, 1743 (1998).
- [2] E. Redenti, C. Pietra, A. Gerloczy, L. Szente, Advanced Drug Delivery Review **53**, 235 (2001).
- [3] A. R. Hedges, Chem. Rev. **98**, 2035 (1998).
- [4] M. Kimura, H. Kakogawa, EP 0 709 099 **A2** (1996).
- [5] K. Nakanishi, T. Masukawa, T. Nadai, K. Yoshii, S. Okada, K. Miyajima, Biol. Pharm. Bull., **20**, 66 (1997).
- [6] J. K. Ong, V. B. Suderland, C. McDonald, J. Pharm. Pharmacol., **49**, 617 (1977).
- [7] J. Mielcarek, Pharmazie, **51**, 477 (1996).
- [8] B. Haraoui, Clin. Drug Invest, **19(2)**, 33 (2000).
- [9] J. McEwen, Clin. Drug Invest, **19(2)**, 27 (2000).
- [10] E. Gamiz, M. Soriano, G. Delgado, J. Parraga, R. Delgado, Ars Pharmaceutica, **43:1-2**; 173 (2002).
- [11] J. A. Castro-Hermida, I. Pors, E. Ares-Mazas, C. Chartier, Revue Méd. Vét., **155**, 8-9, 453 (2004).
- [12] C. P. Rossel, J. S. Carreño, M. Rodríguez-Baeza, J. B. Alderete, Quimica Nova, **23(6)**, 749 (2000).