J Incl Phenom Macrocycl Chem (2007) 57:211–217
DOI 10.1007/s10847-006-9194-y

ORIGINAL ARTICLE

Interaction between hydrophilic drug and α-cyclodextrins: physico-chemical aspects

Malika Lahiani-Skiba · Yannick Boulet · Ibrahima Youm · Fréderic Bounoure · Philippe Vérité · Philippe Arnaud · Mohamed Skiba

Received: 15 May 2006 / Accepted: 20 October 2006 / Published online: 23 January 2007

Abstract The aim of the study was to evaluate if the complexation of a hydrophilic molecule by cyclodextrins is possible. Cyclodextrins (CDs) are hydrophilic cone shaped molecules, which are used as vehicles able to include organic molecules. Because of the presence of hydroxy groups (OH) outside of the molecule, cyclodextrins are not predisposed to include hydrophilic drugs. They are therefore used to improve the solubility of poor water-soluble molecules. In order to evaluate if the complexation of a hydrophilic molecule by cyclodextrins is possible, lyophilized complexes of cysteamine hydrochloride with α-cyclodextrins (α-CD) have been realized. Six analytical techniques (High performance Liquid Chromatography coupled to UV detection, Thin-Layer Chromatography, Fourier Transform Infrared spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), Mass Spectrometry (SM) and Proton Nuclear Magnetic Resonance (NMR-NOESY spectra)) were used in order to characterize the interaction between the drug and the α-CD. The realization of complex between a cyclodextrin and a water-soluble drug seems feasible. In the case of a hydrophilic molecule, the complexation is not obtained by inclusion of the drug in the cyclodextrin, but by binding to the outside of the cone. This "external complexation" is however sufficient to improve some features of the molecule, such as organoleptic features, and to modifiy measurable parameters (FT-IR, DSC, SM and NMR-NOESY spectra).

Keywords Hydrophilic drug · Cyclodextrins · Cysteamine · Organoleptic features

M. Lahiani-Skiba · Y. Boulet · I. Youm · F. Bounoure · P. Arnaud · M. Skiba (✉)
Laboratoire de Pharmacie Galénique,
ADEN EA 3234, UFR Médecine et Pharmacie,
Université de ROUEN, 22, Bd Gambetta,
76000 Rouen, France
e-mail: mohamed.skiba@univ-rouen.fr

P. Vérité
Laboratoire de Chimie Analytique,
ADEN EA 3234, UFR Médecine et Pharmacie,
Université de Rouen, 22, Bd Gambetta,
76000 Rouen, France

F. Bounoure
In-Cyclo, UFR Médecine et Pharmacie,
Université de Rouen, 22, Bd Gambetta,
76000 Rouen, France

Introduction

Cysteamine hydrochloride (Cyst) is an hydrophilic drug used in the treatment of cystinosis, a genetic disease which affects proteins metabolism and notably the transport of cystein degradation products. Due to strongly unpleasant organoleptic features, a strong hygroscopicity and a poor pharmacokinetic profile ($T_{1/2}$ = 1.75 h), this molecule has been replaced in therapeutics by other cysteamine salts. Cyclodextrins (CDs) are known to be suitable vehicles for poor-water soluble organic molecules through complexation with total or partial inclusion inside their hydrophobic inner core.

By interaction with cyclodextrins, properties of an encapsulated molecule are often notably modified. Besides the increase of obvious solubility of little water-soluble molecules, usually sought-after, the inclusion permits to suppress the bitterness or the odor sickening of molecules [1, 2], to slow down the liberation of

medicines along the gastro-intestinal tractus [1] or to reduce the irritating character of molecules against the gastro-intestinal tractus or the cornea [2, 3].

Nevertheless, because of the presence of hydroxyl groups (OH) outside of the molecule, CDs are not predisposed to include hydrophilic drugs. Inclusion of water-soluble molecules has however already been described, essentially with the beta-cyclodextrin and its derivatives. The encapsulation is obtained in inserting directly the molecule to encapsulate in an aqueous solution of cyclodextrins. Inclusion of benzocaïne, procaïne, as well as salicylic acid were described [4, 5]. Haeberlin et al obtained complexes of peptides with cyclodextrins: the inclusion has been achieved in ratios of 1:15 with a calcitonine derivative and 1:50 with octreotide (derivative of the somatostatine). The five types of cyclodextrins tested (alpha, beta, gamma, hydroxypropyle-beta and dimethyle-beta-cyclodextrin) were able to encapsulate the two molecules. The inclusion had notable effects on the chemical stability of these molecules and against enzymatic attacks [6].

In order to lengthen its half-life, Uekama et al. have realised a complex of molsidomine with per-acetylated derivatives of beta-cyclodextrin. The encapsulation, confirmed by X-ray diffractometry and differential thermal analysis, has been achieved in ethanol (ratio 1:1). The plasmatic kinetic profile of the molecule was lengthened, especially with the perbutanoyl-beta-CD [7].

To reduce a strong bitterness compromising the formulation of an acceptable drinkable solution, Andersen et al. encapsulated the femoxetine, directly in aqueous phase: buffered solutions (pH 10.5) at different concentrations of beta-CD have been added to saturated solutions of the drug and agitated during six days at 22°C to obtain an equilibrium. The formation of inclusion complexes, molar ratio 1:1 and 1:2, has been confirmed by UV spectroscopy and differential thermal analysis [8].

In order to evaluate possibility of a hydrophilic molecule complexation with CDs, lyophilized complexes of Cyst hydrochloride with alpha-cyclodextrins ((-CD) were synthesized. In order to characterize the interaction between Cyst hydrochloride and α-CD, lyophilized complexes were prepared, and tested by six analytical techniques: High Performance Liquid Chromatography coupled to a UV detection (HPLC), Thin-Layer Chromatography (TLC), Fourier Transform Infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Mass Spectrometry (MS) and Proton Nuclear Magnetic Resonance (^{1}H-NMR).

Experimental

Materials

cysteamine hydrochloride ($H_2N–CH_2–CH_2–SH$, HCl molecular weight 113.6 g/mole, melting point 70.2–70.7°C) was obtained from Pharmacy of Paris Hospitals, lot K605-29. Alpha-Cyclodextrin (α-CD) was obtained from *Wacker*®, lot CA100/90. All chemicals and reagents were USP-NF quality.

Cysteamine quantification by HPLC

Cyst concentration was evaluated by an HPLC method with a Varian® 9010 pump, equipped with an 9050 injector, 9100 UV detector, a Varian Star® software (1990, version C), a Cromasil® C18 (5 μm, 18 cm) reverse phase column. Mobile phase consisted in a mixture of water (70% volume), methanol (30% volume), 0.3 g sodium octane sulfonate, sulfuric acid 10^{-1} M to adjust pH to 2.5. Flow rate was fixed to 1.2 ml/min, injection volume to 20 μL and detection wavelength to 205 nm. In that case, retention time was 15 min.

Preparation of α-CD inclusion complexes (IC) and physical mixtures (PM)

Inclusion complexes were produced from solutions obtained by direct dissolution of Cyst hydrochloride in 25 ml of a 10^{-1} M α-CD solution. Lyophilized products were obtained according to the method of Skiba [9] with molar ratio 1/1, 2/1, 3/1, 4/1 (Cyst hydrochloride/α-CD). Solutions were freeze-dried on a shelf at 50°C for 3 h at least. Lyophilization parameters, validated by preliminary works, were: vacuum <200 mTorr, condensor <–40°C, shelf at +30°C. Corresponding PM were obtained by thoroughly mixing Cyst hydrochloride with α-CD. Because of the difference of granulometry and hygroscopicity between the α-CD and Cyst hydrochloride, we opted for a mixture with mortar and pestle, after previous grinding of Cyst hydrochloride crystals. IC and PM contained 10–32%-Cyst hydrochloride.

Differential scanning calorimetry (DSC)

After a stabilization plateau (1 min at 30°C/20 mWatt), samples were heated from 30 to 150°C at a 10°C/min speed, under nitrogen flow. Thermograms were expressed in °C = f (mW) (temperature of the sample according to heating energy).

Mass spectrometry (MS)

Mass spectrometry analysis was performed on a Hewlett Packard S973 mass spectrometry. Samples were introduced by a direct insertion probe heated with increasing temperature (2 min at 30°C, rise in temperature until 450°C at a 10°C/min rate and 2 min stabilization at 450°C). Analysis was achieved by electronic impact (70 eV). Two types of diagrams were analyzed: total ionic flow (expressed in abundance according to the time) and mass spectrum at accurate time during temperature increase. Computer analysis allowed extraction of specific fragments abundance. Because of heating gradient, compounds were sublimated in the increasing order of their boiling point and therefore separated before analysis by MS.

^{1}H-nuclear magnetic resonance (^{1}H-NMR)

^{1}H-NMR spectra were recorded at 297 K, using a presaturation sequence of the residual water resonance, on a spectral window of 10 ppm, constant total concentration (CD + host = 5 mM) in deuterium water (D_2O). Freeze-dried sample with α-CD were also tested by NOESY technique (Nuclear Overhauser Effect Spectroscopy), to evaluate protons spatial proximity.

Fourier transform infrared spectroscopy (ATR-FT-IR

Samples were analyzed in dried phase. The fourier transform infrared spectra were obtained from a Perkin Elmer IR spectrometer. Spectra were achieved from 4000 to 650 cm^{-1}, with H_2O and CO_2 signals ponderation, 8 cm^{-1} resolution, 0.2 cm^{-1} scanning speed, 16 scans.

Results and discussion

Differential scanning calorimetry

An endothermic peak at 70.5°C (Fig. 1) was observed in Cyst hydrochloride thermogram, due to its melting. α-CD presented two endothermic peaks at 88 and 103°C.

IC 1/1 showed disappearance of Cyst hydrochloride melting endotherm, and of the two α-CD endothermic peaks. However, an exothermic peak appeared at approximately 112°C indicating the formation of a new chemical entity, with thermal features different from those of the two raw materials. PM 1/1 presented the superposition of the two pure products thermograms. In the same time, the second endothermic peak described at 103°C for the α-CD disappeared, suggesting a slight interaction between drug and CDs, but different from that existing in IC 1/1 as no trace of the exothermic peak at 112°C, described for the IC, was observed.

Mass spectrometry analysis

Cyst hydrochloride presented two peaks at 4 and 22 min (Fig. 2). For the first peak at 4 min, characteristic fragments were at m/z 77 and 59 due to Cyst. For the second peak, characteristic fragments were at m/z 77, 109 and 135 indicating the degradation of Cyst into cystamine (Fig. 2). α-CD signal appeared later (>25 min). α-CD spectrum also produced small size fragments in the ionization cell, but if fragment m/z 59 was well represented, m/z 77 was very low. In IC freeze dried samples, the Cyst hydrochloride peak at 4 min disappeared (Fig. 3). The concomitant presence of m/z 59 and 77 appeared only at approximately 20 min. This fact seems to prove that there is an interaction between Cyst hydrochloride and α-CD.

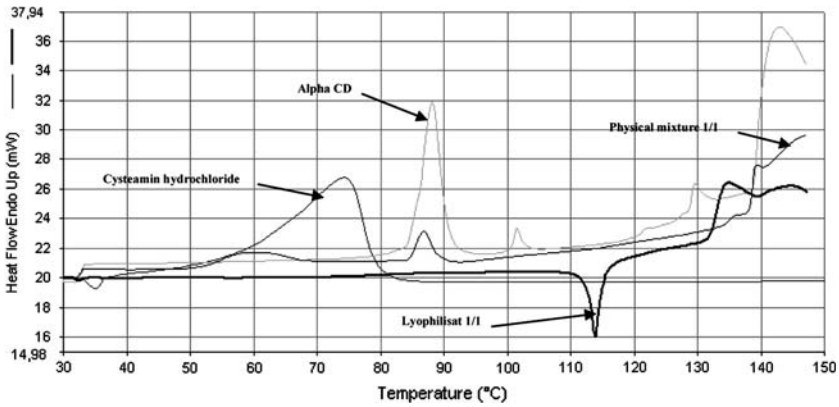

Fig. 1 DSC thermograms of Cyst, α-CD, freeze-dried IC 1/1 and PM 1/1

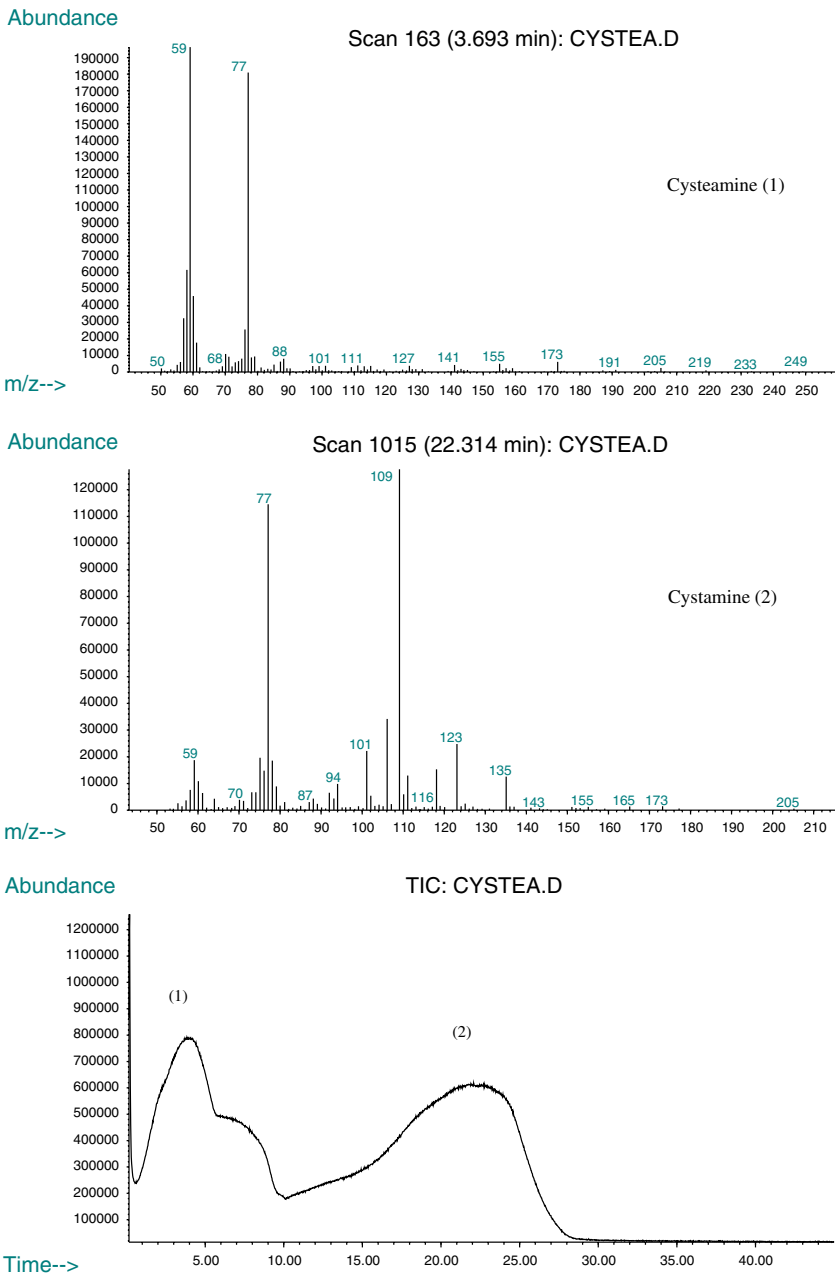

Fig. 2 Mass spectra of cysteamine hydrochloride at 4 and 22 min

^{1}H-nuclear magnetic resonance

Single ^{1}H-NMR spectra of pure Cyst hydrochloride, α-CD and IC freeze-dried sample 1/1 did not show proton displacement. In the same way, there was no proton displacement between freeze-dried sample 1/1 and the corresponding PM spectra (Fig. 4). No H_3 and H_5 proton displacement was found (H_3 and H_5 being the protons usually implied in case of molecule inclusion inside the CDs cavity).

In contrast, ^{1}H-NMR showed the Cyst dimerisation into cystamine in IC samples: this phenomenon was not observed in the PM.

The NOESY technique done on IC 1/1 samples in deuterium water, showed the proximity of Cyst methylenes groups protons with protons H_2 and H_4, located at the outside of the α-CD. This proximity has been interpreted as a ''binding'' between Cyst methylene groups and the outside of α-CD (Fig. 5A, B).

Fourier transform infrared spectroscopy studies

Cyst hydrochloride presented several characteristic peaks at 3400 cm^{-1} (N–H elongation in amino groups), 2890 and 2950 cm^{-1} (C–H elongation in methylene groups), 1600 cm^{-1} of N–H angular dis-

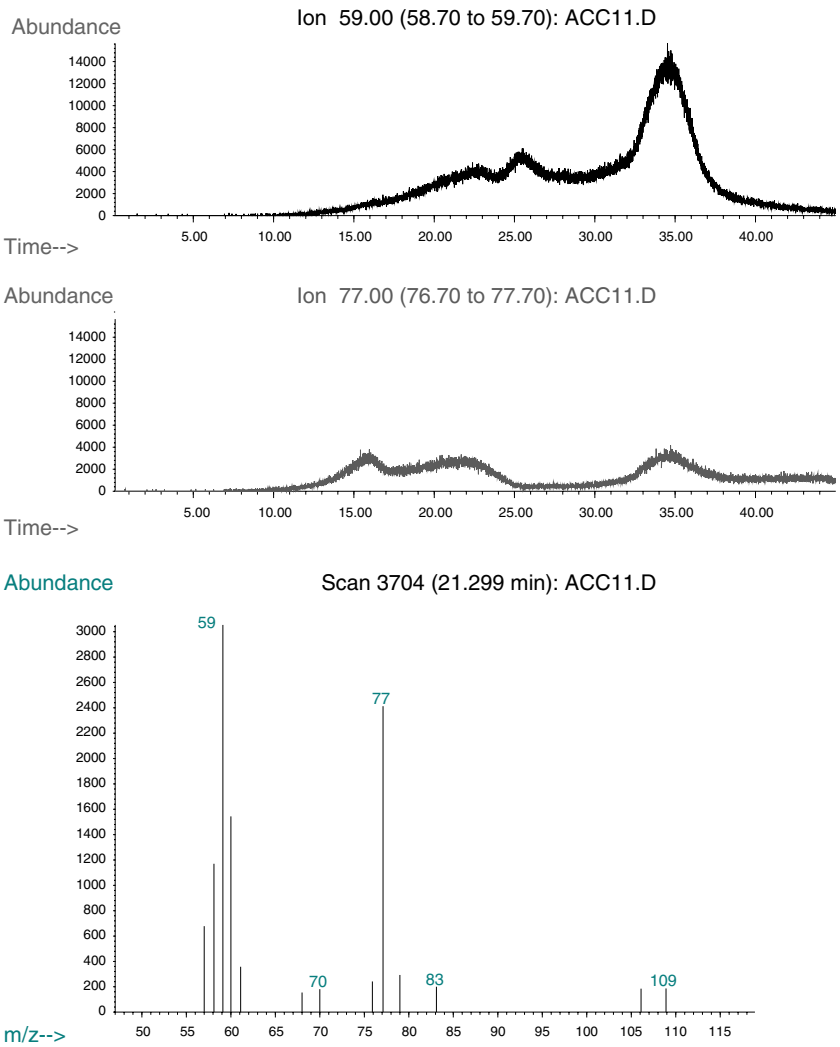

Fig. 3 Total Ionic flow (fragments m/z = 59 and 77) and mass spectrum of freeze-dried IC 1/1 at t = 21 min

tortion, 1500 cm^{-1} C–H vibrations, between 1400 and 860 cm^{-1}, C–H angular distortions and C–N elongation (Fig. 6). S–H binding, theoretically present between 2600 and 2550 cm^{-1}, did not clearly appear in the spectrum. However this signal often has a weak intensity, and can be covered by CH_2 elongation band. SH can be also engaged due to Cyst spontaneous dimerisation into cystamine. α-CD was characterized by a large band of weak intensity between 3500 and 3200 cm^{-1}, due to O–H elongation, a well individualized peak, at 1150 cm^{-1} and a band of strong intensity culminating at 1030 cm^{-1}, characteristic of C–O elongation, often present in CDs. The strong intensity bands at approximately 2980 and 1600–1500 cm^{-1} notably disappeared, in spite of the presence of 10.5% Cyst hydrochloride in the 1/1 freeze-dried IC. IC molar ratio 2/1 and 3/1 did not show an obvious signal of the presence of Cyst, in spite of stronger organoleptic features. IC molar ratio 4/1 presented a signal at 3400–3300 cm^{-1}, and only two small signals at 1600 and 1500 cm^{-1}, confirming the organoleptic features (smell, taste) changes. PM produced the Cyst signal, more intense at the end of the mixing process than after some hours of storage: interaction between CDs and the drug appeared during the drying phase, or an unmixing phenomenon occured. Therefore PM tests were done only on freshly prepared samples. PM 1/1–4/1 presented all the characteristic bands from Cyst but not as intense as the two bands of the Cyst hydrochloride alone, increasing with the molar ratio.

The evolution of the organoleptic features are in favor of the realization of a complex: the disappearance of strong taste of drugs after inclusion in cyclodextrins has already been noticed by Andersen et al. with the femoxetine hydrochloride, Uekama et al. with the clofibrate and Miyaji et al. with the fenbufen [8, 10, 11].

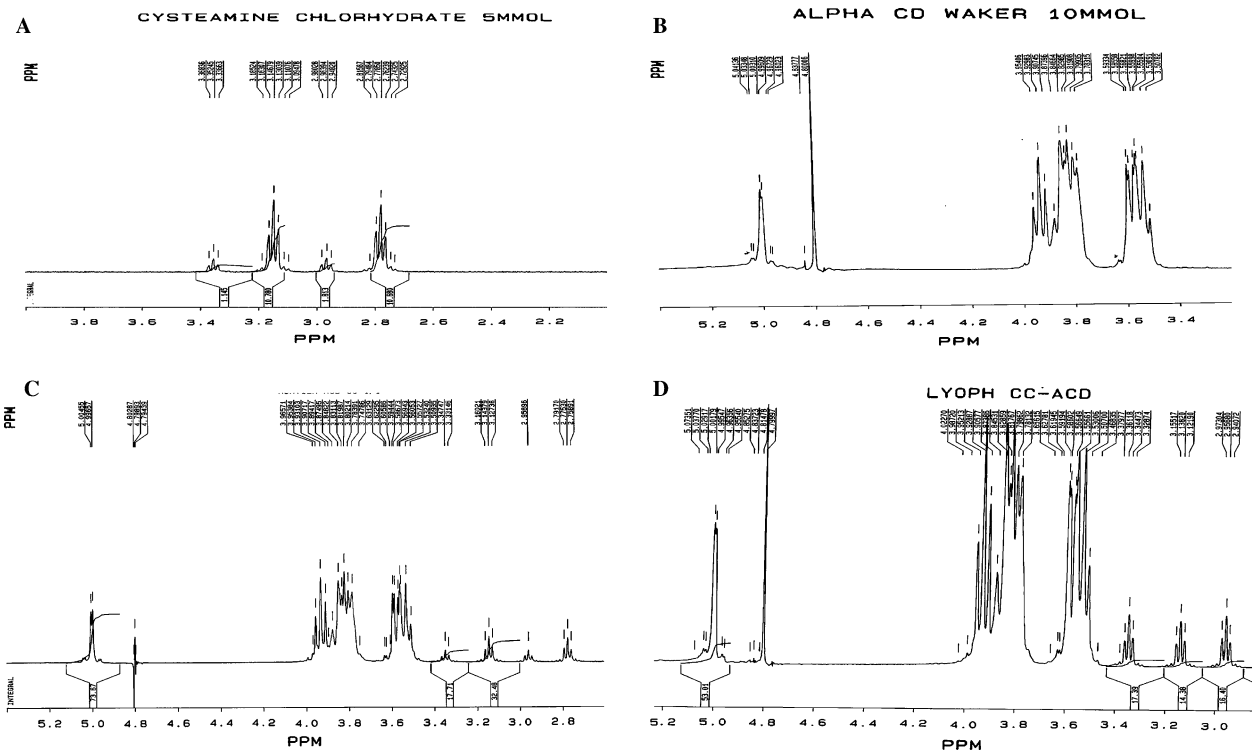

Fig. 4 ^{1}H-RMN spectra of: (**A**) pure cysteamine hydrochloride, (**B**) α-CD, (**C**) PM spectra (1/1) and (**D**) IC freeze-dried sample (1/1)

The disappearance of the odor of the cysteamine rejoins the results of Gal-Füzy et al. and Hou and Wang after complexation of essential oil solutions and ranitidine [12, 13].

Conclusion

By complexation of cysteamine hydrochloride with α-CD, we obtained in fact an odorless powder, of moderate flavor, stockable at room temperature and moisture, and allowing a direct compression.

Disappearance of Cyst hydrochloride IR and DSC peaks supported the hypothesis of complex formation. Increasing retention time and disappearance of MS fragments m/z = 77 and 59 after 4 min were two additional arguments in favor of an interaction between the drug and α-CDs. NMR-NOESY allowed to propose a model describing binding between Cyst methylene groups and α-CD H_2 and H_4 protons in

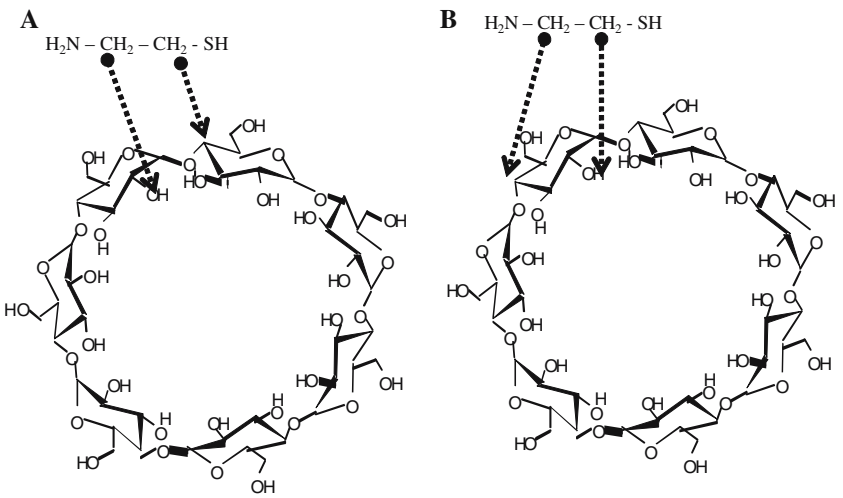

Fig. 5 Representation proposed emphasing interactions between Cyst and α-CD, according to the results of NMR NOESY technique (**A**) interactions of CH_2 of cyst. with H_2 and H_4 of two different glucopyranose (**B**) interactions of CH_2 of cyst. with H_2 and H_4 of a same glucopyranose

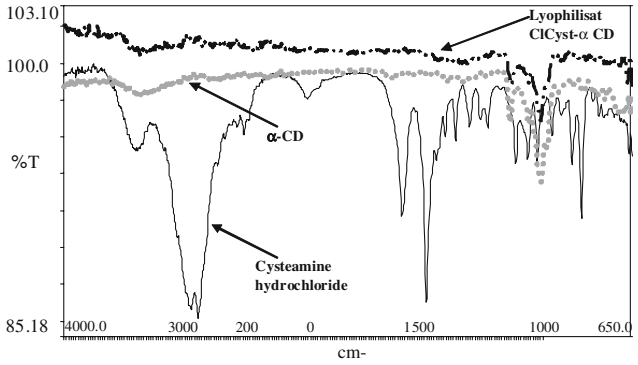

Fig. 6 FTIR spectra of Cyst, α-CD and freeze-dried IC

freeze-dried products. Therefore NMR-NOESY confirmed an interaction between α-CD and an hydrophilic molecule and explained that the first signal recovered in FT-IR when the molar ratio of Cyst increased or when PM were prepared, was the signal at 3400 cm^{-1} characteristic of the amino group, a group not involved in the interaction.

References

1. Saenger, W.: Cyclodextrin inclusion compounds in research and industry. Angew. Chem. Int. Ed. Engl. **19**, 344–362 (1980).
2. Rajewski, R.A., Stella, V.J.: Pharmaceutical applications of cyclodextrins. 2. In vivo drug delivery. J. Pharm. Sci. **85**(11), 1142–1169 (1996).
3. Loftsson, T., Brewster, M.E.: Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. **85**(10), 1017–1025 (1996).
4. Lach, J.L., Chin, T.F.: Inhibition of hydrolysis by means of molecular complex formation. J. Pharm. Sci. **53**, 924 (1964).
5. Frömming, K.H., Sandman, R., Weyermann, I.: Stabilitätsprobleme bei der Verarbeitung von Einschluβverbindungen der Desoxycholsäure und des β-Cyclodextrins in Arzneiformen. Dtsch. Apoth.-Ztg, **112**, 707 (1972).
6. Haeberlin, B., Gengenbacher, T., Meinzer, A., Fricker, G.: Cyclodextrins, useful excipients for oral peptide administration? Int. J. Pharm. **137**, 103–110 (1996).
7. Uekama, K., Horikawa, T., Yamanaka, M., Hirayama, F.: Peracylated β-cyclodextrins as novel sustained-release carriers for a water-soluble drug, Molsidomine. J. Pharm. Pharmacol. **4**, 714–717 (1994).
8. Andersen, F.M., Bundgaard, H., Mengel, H.B.: Formation, bioavailability and organoleptic properties of an inclusion complex of femoxetine with β-cyclodextrin. Int. J. Pharm. **21**, 51–60 (1984).
9. Skiba, M., Fessi, H., and Puisieux, F. French Patent N° 94.02.312 (1994).
10. Uekama, K., Oh, K., Otagiri, M., Seo, H., Tsuruoka, M.: Improvement of some pharmaceutical properties of clofibrate by cyclodextrin complexation. Pharm. Acta Helv. **58**(12), 338–342 (1983).
11. Miyaji, T., Inoue, Y., Acartürk, F., Imai, T., Otagiri, M., Uekama, K.: Improvement of oral bioavailability of fenbufen by cyclodextrin complexations. Acta Pharm. Nord. **4**(1), 17–22 (1992).
12. Gal-Füzy, M., Szente, L., Szejtli, J., Harrangi, J.: Cyclodextrin stabilized volatile substances for inhalation therapy. Pharmazie, **39**, 558–559 (1984).
13. Hou, S., Wang, P.: The preparation of inclusion compound of ranitidine - β?cyclodextrin. Chinese Pharm. J. **31**(8), 479–481 (1996).