J Incl Phenom Macrocycl Chem (2011) 70:415–419
DOI 10.1007/s10847-010-9910-5

ORIGINAL ARTICLE

Complexation of ebastine with β-cyclodextrin derivatives

T. Maddens · I. Vélaz · R. Machín · J. R. Isasi · C. Martín · M. C. Martínez-Oharriz · A. Zornoza

Received: 11 June 2010 / Accepted: 29 November 2010 / Published online: 14 December 2010

Abstract Complexation of ebastine (EB) with hydroxypropyl and methyl-β-cyclodextrin (HP-β-CD and Me-β-CD) was studied in aqueous solutions and in the solid state. The formation of inclusion complexes in aqueous solutions was analysed by the solubility method. The assays were designed using low CD concentrations compared with the solubility of these derivatives in order to avoid non-inclusion phenomena and to obtain a linear increase in EB solubility as a function of CD concentration. The values of complexation efficiency for HP-β-CD and Me-β-CD were 1.9×10^{-2} and 2.1×10^{-2}, respectively. It seems that the non polar character of the methyl moiety slightly favoured complexation. In relation to solid state complexation, 1:1 EB:CD systems were prepared by kneading, and by heating a drug-CD mixture at 90 °C. They were analysed using X ray diffraction analysis by comparison with their respective physical mixtures. A complex with a characteristic diffraction pattern similar to that of the channel structure of β-CD was formed with Me-β-CD in 1:1 melted and 1:2 EB:CD kneaded systems. Complexation with HP-β-CD was not clearly evidenced because only a slight reduction of drug crystallinity was detected. Finally, the loading of EB in two β-CD polymers cross-linked with epichlorohydrin yielded 7.3 and 7.7 mg of EB/g polymer respectively.

Keywords Cyclodextrin · Solubility isotherms · Ebastine · Inclusion complexation

T. Maddens · I. Vélaz · R. Machín · J. R. Isasi · C. Martín · M. C. Martínez-Oharriz · A. Zornoza (✉)
Departamento de Química y Edafología, Facultad de Ciencias, University of Navarra, Irunlarrea s/n, 31080 Pamplona, Navarra, Spain
e-mail: azornoza@unav.es

Introduction

The most widely employed drug formulations are solid forms prepared for oral administration. The main properties that determine the biopharmaceutical behaviour of a drug in the oral route are its aqueous solubility and its ability to permeate biologic membranes [1]. Ebastine (Fig. 1) is an antihistaminic drug that is effective for the treatment of allergic diseases. It is a basic compound that contains a tertiary amine group with pKa 8.8. This drug presents a very low water solubility and a high hydrophobicity, with a partition coefficient (logP) of 7.64 [2], therefore, according to the biopharmaceutical classification system (BSC), this drug belongs to the class II group of pharmaceuticals. These compounds are relatively lipophilic and poorly soluble in water and the rate-limiting step in the absorption process is drug dissolution, because they can permeate well through the gastrointestinal tract [3]. In general, drugs with the above mentioned characteristics are good candidates for achieving a bioavailability enhancement through complexation with cyclodextrins [4].

Taking into account the chemical structure of ebastine, the CD cavity that best fits the dimensions of the terminal benzene rings is that of β-CD [5], therefore, the cyclodextrins of election were methyl-β-CD and hydroxypropyl-β-CD, two derivatives which are more soluble than the parent compound and also with pharmaceutical interest [6].

The evaluation of complexation through solubility assays is often problematic due to the presence of non inclusion effects that contribute to solubilisation, but this technique becomes important when it is not possible to analyse complexation by spectroscopic techniques. This paper focuses the solubility studies from two different points of view: firstly, the analysis of the solubilisation that can be attributed to inclusion complexation and allows the

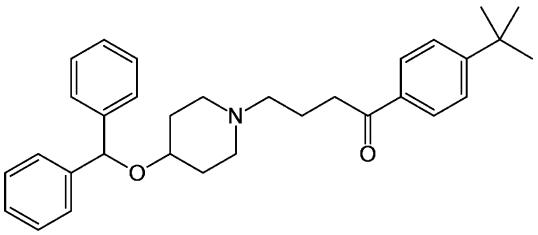

Fig. 1 Chemical structure of ebastine

characterization of the host–guest inclusion interaction and secondly, the achievement of a maximum solubilisation for practical purposes. In addition, the formation of complexes in the solid state has been discussed.

Finally, the development of new polymeric materials and assemblies containing cyclodextrin moieties is a field of increasing interest because of their particular properties and drug release characteristics [7]. These polymers can also be applied in environmental protection due to their ability to retain organic pollutants from soils and wastewater. In this sense, an additional objective of this paper was to explore the possibility of loading EB in a cyclodextrin polymer, a covalent polymer network obtained by cross-linking cyclodextrins with epichlorohydrin.

Materials and methods

Materials

Ebastine was a gift from Laboratorios Cinfa (Pamplona, Spain). Methyl-β-CD (Me-β-CD) and hydroxypropyl-β-CD (HP-β-CD) were purchased from Cyclolab (Budapest, Hungary) and they had average substitution degrees of 12 and 4, respectively. Two insoluble polymers of β-CD crosslinked with epichlorohydrin were used. One of them was purchased from Cyclolab (Hungary), it was in the shape of spherical beads with size ranging between 100 and 300 μm, its CD content was 55 wt% and its swelling capacity 5 mL/g in water. The other polymer was synthesised in our laboratory as described in a previous paper [8], its CD content and swelling capacity were 62 wt% and 5,8 mL/g, respectively, and the particle size ranged between 100 and 300 μm All other reagents and solvents were from Panreac (Barcelona, Spain).

Methods

Solubility studies

The solubility assays were carried out in water at 25 °C. Two experimental procedures were assayed. The first procedure was performed by adding 2 mg of EB to 20 mL test tubes containing different concentrations of HP-β-CD and Me-β-CD, ranging from 0 to 5×10^{-3}M. After sonicating the test tubes during 30 min they were placed in a bath at 50 °C for one hour and then cooled at 25°C. Finally, the suspensions were seeded with approximately 1 mg of EB to avoid supersaturation and they were shaken in a bath at 25 °C during approximately five days, until equilibrium was reached. Samples were filtered through 0.45 μm cellulose filters and measured at 258 nm with a HP8452A diode array spectrophotometer. The assays were made in triplicate.

The second procedure differed from the above mentioned in a single aspect that was the way by which the drug was added to the solution in the first step. Instead of adding 2 mg EB as a solid, 2 mg EB dissolved in 20 μL of ethanol were added to the test tubes. It was intended to ascertain if 0.1% ethanol could shorten the equilibration time without a significant alteration of the solubility isotherm.

The assays were designed using maximum CD concentrations of 5×10^{-3} M, relatively low values compared with the solubility of these CDs, in order to minimise phenomena of self-aggregation and to enable that the phase solubility diagram presented a linear increase in EB solubility as a function of CD concentration [9].

The phase solubility profiles were constructed from the experimental data. When linear diagrams are obtained (A_L), the following equation can be obtained for 1:1 drug/CD complexes.

$$S_t = S_o + \frac{K_{1:1} S_o [CD]}{1 + K_{1:1} S_o}$$

The apparent stability constant of the complex ($K_{1:1}$) and the complexation efficiency (CE) [10] can be determined from the slope and the intercept of the diagram using the equation:

$$K_{1:1} = \frac{\text{Slope}}{S_o(1 - \text{Slope})}$$

$$CE = S_o \cdot K_{1:1} = \frac{\text{Slope}}{(1 - \text{Slope})}$$

The complexation efficiency (CE) represents the complex to free CD concentration ratio. It is a useful parameter to determine the solubilising potential of cyclodextrins when there is a high experimental error associated to the determination of the solubility of the pure drug.

An additional study was carried out to determine the CD concentrations needed to dissolve doses of both 10 and 20 mg of EB in 250 mL of CD aqueous solution. The concentrations employed were preliminary estimated from a prolongation of the solubility isotherm. Solubilisation was achieved by manual stirring at room temperature combined with sonication during 60 min.

Solid state complexation

Both 1:1 EB:CD and 1:2 solid systems were prepared by kneading and by melting a drug-CD mixture. The kneaded products were obtained by preparing a suspension of the corresponding cyclodextrin (0.4 mmol) in a mortar with 10 mL of water. Then EB (0.4 mmol) was dissolved in 5 mL of ethanol and was added dropwise to the suspension under constant kneading until a thick paste was obtained, which was subsequently dried at 40°. Physical mixtures EB–CD were also prepared for comparison purposes. The 1:2 systems were prepared using 0.4 mmol of EB and 0.8 mmol of CD by the same procedure.

The melted systems were prepared by heating a 1:1 EB:CD mixture at 90 °C, above the melting point of the drug (88 °C).

The resulting solid systems were characterised by powder X ray diffraction analysis using a Brucker D8 Advance diffractometer with CuK_{α} radiation, 40 kV and 30 mA.

Sorption in a β-CD polymer

Two β-CD polymers cross-linked with epichlorohydrin have been employed for loading EB: a commercial product and other synthesised in our laboratory by a procedure described in a previous paper [8]. The former was obtained by suspension and the last was synthesised in our laboratory by block polymerisation. These polymers have been compared in order to evidence the reproducibility of the sorption properties of similar polymers synthesised by different polymerization procedures.

EB was dissolved in a 40% v/v ethanol–water mixture and subsequently added to an accurately weighed amount of polymer that had been previously allowed to swell. The mixture was continuously stirred and samples were taken from the supernatant until equilibrium was reached. The residual concentrations were determined spectrophotometrically.

Results and discussion

Solubility studies

The solubility studies comprised two types of assays, those that employed low CD concentrations with the aim of evaluating inclusion complexation and those that involved high enough concentrations to achieve a degree of solubilisation for pharmaceutical application.

Solubility isotherms become an important tool for the evaluation of complexation of non fluorescent drugs whose poor solubility precludes the use of other spectroscopic techniques. The first objective of the solubility study was to quantify the extent of complexation from the solubility assays. The solubility isotherms of EB in the presence of Me-β-CD and HP-β-CD are shown in Fig. 2. They are linear, as expected because of the relatively low CD concentrations employed. The solubility of EB increased about 35-fold in the presence of 5.0×10^{-3} M concentrations of the CDs assayed. The values of complexation efficiency derived from the slope of the diagrams were $(2.1 \pm 0.2) \times 10^{-2}$ and $(1.9 \pm 0.2) \times 10^{-2}$ for Me-β-CD and HP-β-CD, respectively. The extent of complexation with the two cyclodextrins assayed is similar, although it seems that it is slightly favoured with Me-β-CD, the hydrophobic derivative, in comparison with the hydrophilic HP-β-CD. Taking into account that the determination of the low intrinsic solubility of EB $(S_o = 3.1 \pm 0.5) \times 10^{-6}$ M involves a considerable error, only a rough estimation of the apparent stability constants could be made from the experimental data. The apparent stability constants for both complexes were estimated to be in the range of $K_{11} = (6 \pm 2) \times 10^3$ M^{-1}. This result shows that K_{11} is strongly affected by the inaccuracy of the value of intrinsic solubility; for this reason, the complexation efficiency results a better parameter to evaluate complexation in the case of very poorly soluble drugs like EB.

The analysis of the initial portion of the isotherm enables a way to standardise the comparison of inclusion complexation data arising from different host–guest systems because the interactions that take place are mainly based on inclusion phenomena.

With respect to the methods employed to reach equilibrium, the results obtained when 20 μL of ethanol were used to incorporate the drug were similar to those obtained with the first method.

A second aspect of the solubility study was the use of high concentrations of cyclodextrin to achieve a solubility enhancement high enough to include EB in the Class I of the BCS. In this system, the solubility classification is referred to the highest dose strength of the drug present in an immediate release product. A drug is considered soluble if the highest dose strength is soluble in 250 mL of aqueous media through the pH range of 1.2–6.8 [1]. It was found that a dose of 10 mg of EB was dissolved in 250 mL of 1.2×10^{-2} M aqueous solutions of either Me-β-CD or HP-β-CD at room temperature, although dissolution was faster with the former. It was necessary to increase CD concentration up to 3×10^{-2} M to dissolve the highest dose strength of EB, which is 20 mg. The behaviour of EB in the presence of Me-β-CD and HP-β-CD closely reaches that of class I drugs of the BCS. Although the amount of CD necessary to dissolve the maximum dose is high for practical applications, the use of cyclodextrins can be considered a good approach to improve the pharmaceutical behaviour of EB by means of supramolecular interactions.

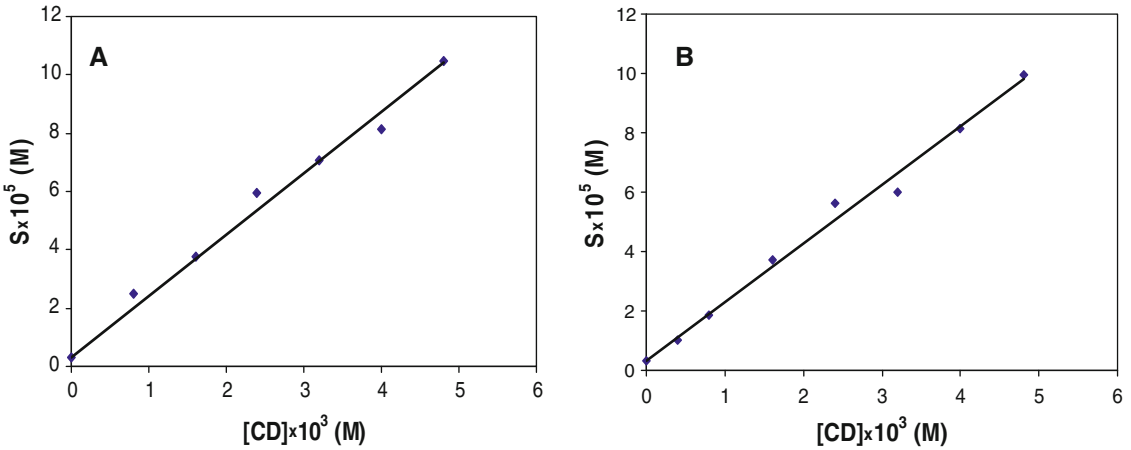

Fig. 2 Solubility isotherms of ebastine with methyl β-CD (**a**) and hydroxypropylβ-CD (**b**)

Solid state complexation

The XRD patterns of the systems prepared with randomly methylated β-CD are shown in Fig. 3. The physical mixture presented, overlapped with the amorphous profile of Me-β-CD, sharp peaks that corresponded to the crystalline structure of EB, among which the most intense were those at diffraction angles (2θ) of 16.2, 17.0, 18.9 and 19.5°. The 1:1 melted system presented a characteristic diffraction pattern with new signals at (2θ) 8.8, 11.6 and 17.6°, this fact, together with the disappearance of EB peaks, suggested the formation of a complex with a new crystalline structure. The 1:1 kneaded system led to a mixture of complex and free drug, as the peaks of pure EB were present together with those of the complex. When the amount of Me-β-CD was increased up to a 1:2 ratio complexation was complete, as there is no evidence of the drug diffraction peaks which are present in the 1:2 physical mixture.

It is worthy of note that the complex characteristic peaks at angles (2θ) of 11.6 and 17.6 correspond to those of the head-to-head channel-type structure of β-CD [11]. The formation of a crystalline complex with randomly methylated β-CD is unusual as it tends to form amorphous systems. Channel structures are usually associated to complexation of the parent β-CD with long chain guest molecules.

Complexation with HP-β-CD in the solid state was not clearly evidenced because only a slight reduction of drug crystallinity was detected. The size of the substituents of HP-β-CD may hinder the possibility of giving rise to an organised crystalline structure.

Sorption in a β-CD polymer

The chemical cross-linking of cyclodextrins with epichlorohydrin results in the formation of polymeric hydrogels

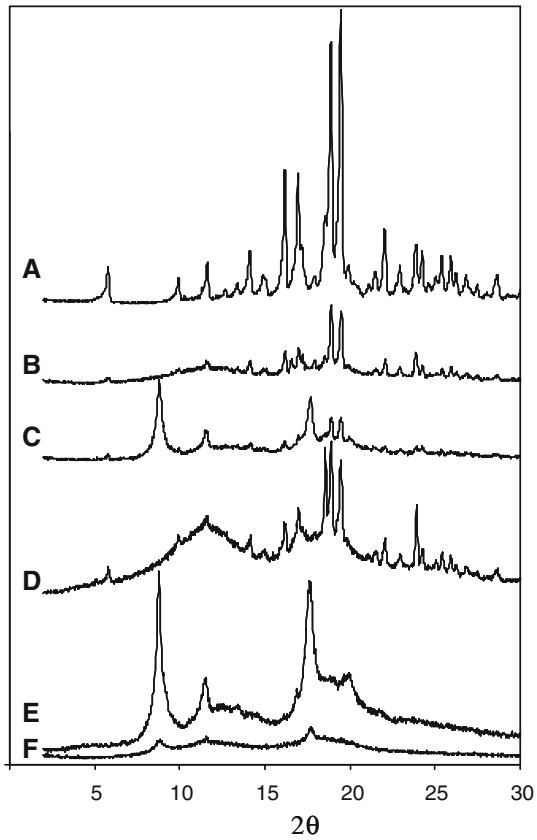

Fig. 3 X ray diffraction patterns of the systems ebastine-methylβ-CD: pure ebastine (**a**) 1:1 physical mixture (**b**), 1:1 kneaded system (**c**), 1:2 physical mixture (**d**), 1:2 kneaded system (**e**), 1:1 melted system (**f**)

with the capability of retaining poorly soluble drugs. Two polymers of similar characteristics have been employed, one was obtained by suspension (commercial product) and the other was synthesised in our laboratory by block polymerisation. The amounts loaded in the commercial and synthesised polymers were similar, 7.3 ± 0.6 and

7.7 ± 0.7 mg of EB/g polymer, respectively. The values obtained evidence the reproducibility of the sorption properties of similar polymers synthesised by different polymerization procedures. The mechanism of sorption in CD polymers comprises the inclusion in the cavities and the interaction with the polymeric network [12]. Polymeric systems based on cyclodextrins present good characteristics in relation to mechanical properties, stimuli responsiveness and drug release characteristics [7]. The applications of these polymers in the field of pharmaceuticals are mainly focussed on controlled drug release but there is also a possible application on the treatment of waste water contaminated with drugs. The amounts of EB sorbed in the CD polymers suggested their potential as carrier systems for pharmaceutical applications because the amount of polymer required to load a therapeutic dose of 10 mg is not very high. Some work needs to be done to improve the loading characteristics of these systems.

Conclusion

The solubility isotherms become an important tool in the analysis of complexation of non fluorescent poorly soluble drugs. When the solubility studies were focussed on quantifying inclusion complexation, the use of low cyclodextrin concentrations was a good approach to minimise problems such as CD aggregation, formation of non inclusion compounds, complex stoichiometries, etc. The analysis of the initial portion of the isotherm enables a way to standardise the comparison of inclusion complexation data arising from different host–guest systems. However, when the aim was obtaining a maximum solubilisation for practical purposes regardless non inclusion effects, high enough CD concentrations were employed and EB behaviour resembled that of class I drugs of the BCS.

Finally, randomly methylated β-CD was able to form an inclusion complex with a characteristic structure similar to that of the channel structure of β-CD.

Acknowledgment The authors thank the Ministerio de Ciencia e Innovación (MAT2007-65752) for financial support.

References

1. Dahan, A., Miller, J.M., Amidon, L.: Prediction of solubility and permeability class membership: provisional BCS classification of the world's top oral drugs. AAPS. J. **11**, 740–746 (2009)
2. Gerebtzoff, G., Seelig, A.: In silico prediction of blood-brain barrier permeation using the calculated molecular cross-sectional area as main parameter. J. Chem. Inf. Model **46**, 2638–2650 (2006)
3. Loftsson, T., Brewster, M.E., Másson, M.: Role of cyclodextrins in improving oral drug delivery. Am. J. Drug Deliv. **2**, 261–275 (2004)
4. Brewster, M.E., Loftsson, T.: Cyclodextrins as pharmaceutical solubilizers. Adv. Drug Deliv. Rev. **59**, 645–666 (2007)
5. Szejtli, J., Osa, T. (eds.): Comprehensive Supramolecular Chemistry: Cyclodextrins, vol. 3. Pergamon, Oxford (1996)
6. Uekama, K.: Design and evaluation of cyclodextrin-based drug formulation. Chem. Pharm. Bull. **52**, 900–915 (2004)
7. van de Manakker, F., Vermonden, T., van Nostrum, C.F., Hennink, W.E.: Cyclodextrin-based polymeric materials: synthesis, properties and pharmaceutical/biomedical applications. Biomacromolecules **10**, 3157–3175 (2009)
8. García-Zubiri, I.X., González-Gaitano, G., Isasi, J.R.: Isosteric heats of sorption of 1-naphthol and phenol from aqueous solutions by β-cyclodextrin polymers. J. Colloid Interface Sci. **307**, 64–70 (2007)
9. Connors, K.A.: Binding Constants: the measurement of molecular complex stability, Chapter 8. Wiley, USA (1987)
10. Loftsson, T., Hreinsdóttir, D., Másson, M.: The complexation efficiency. J. Incl. Phenom. Macrocycl. Chem. **57**, 545–552 (2007)
11. Gao, Y., Zhao, X., Dong, B., Zheng, L., Li, N., Zhang, S.: Inclusion complexes of β-cyclodextrin with ionic liquid surfactants. J. Phys. Chem. B **110**, 8576–8581 (2006)
12. García-Zubiri, I.X., González-Gaitano, G., Isasi, J.R.: Sorption models in cyclodextrin polymers: Langmuir, Freundlich and a dual-mode approach. J. Colloid Interface Sci. **337**, 11–18 (2009)