Solubilization and Stabilization of Sodium Dicloxacillin by Cyclodextrin Inclusion

María Magdalena Echezarreta-López[1], Iria Otero-Mazoy[1], Héctor Luís Ramírez[2], Reynaldo Villalonga[2] and Juan José Torres-Labandeira*,[1]

[1]*Departamento de Farmacia e Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Santiago de Compostela, Campus Sur, E-15706 Santiago de Compostela, Spain;* [2]*Center for Enzyme Technology, University of Matanzas, Matanzas 44740, Cuba*

Abstract: The aim of this work is to analyze the effect of cyclodextrin (CD) complexation on the solubilization and stabilization of sodium dicloxacillin in acid aqueous solutions. The effect of four cyclodextrins α-, β-, γ- and hydroxypropyl-β-CD was studied. Phase solubility diagrams obtained are A_L or B_S type, depending on the cyclodextrin used and on the pH of the solution. The highest stability constants of the inclusion complexes are obtained with γ-CD at pH 1 and 2 and HPβ-CD at pH 3. The structure of the inclusion complex in solution is characterized by nuclear magnetic resonance (^{1}H-NMR). This study suggests that the 7-oxo-4-thia-1-azabicyclo group is located in the CD cavity. Nevertheless, molecular modelling calculations predict two different orientations of dicloxacillin in the γ-CD cavity in vacuum and in aqueous solution. In vacuum, the results predict the inclusion of the dichlorophenyl ring of dicloxacillin instead of 7-oxo-4-thia-1-azabicyclo group into the γ-CD cavity. However, the results are different in aqueous solution and this conformation is confirmed by the NMR study. The effect of γ-CD and HPβ-CD in the stability of the drug in solution was studied. The degradation of sodium dicloxacillin in solution follows a pseudo-first-order kinetics and the cyclodextrin do not change this fact. Both cyclodextrins increase the stability of the drug but the efficacy is higher with γ-CD.

Key Words: Benzylpenicillin, cyclodextrin, solubility, drug stability, molecular modeling.

INTRODUCTION

The most significant transformations in the penicillins therapy are those caused by the gastric acidity and by the microbial enzymes, penicillinases. A significant lost in the activity is produced by the hydrolysis of the amide group in the stomach and by the opening of the β-lactam ring [1]. Besides, oral absorption of penicillins is limited by many factors like its low water solubility, its interaction with foods or the formulation used in the administration [2].

The solubility of weak electrolytes (most of the drugs) is strongly influenced by the pH of the solution, but if the pKa value is low, pH adjust is ineffective. Natural or semi-synthetic penicillins are the corresponding salts of N-substituted mono-basics penicillinic acid that contain a carboxylic group with a pKa between 2.5 and 2.8 [2]. When a salt is dissolved in water, the slow initial decomposition provides a gradual fall of the pH (pH 4 or 5) and, after that, the inactivation speed is increased and possibly it can be formed a precipitate. The characteristics of solubility depend, in many cases, on the hydration degree and on ion used for the formation of the salt [3]. Sodium dicloxacillin (DxNa) (Fig. **1**), it is a soluble salt of semi-synthetic penicillin that has a low water solubility and a compromised stability in gastric juice. Its oral absorption is limited by food and for this reason it has been administrated one hour before the meals. Nowadays, it is formulated in hard gelatin capsules and in an oral suspension (USP 24 Ed.).

Multiple technological alternatives have been used to increase water solubility of drugs looking for an improvement in the oral drug bioavailability – modification of drug crystal form, polymer loading, micellar solubilization, cosolvent addition, pH adjustment, inclusion in colloidal systems such as liposomes, preparation of salts, *etc.* [4]. Among all of them, cyclodextrin complexation has shown as the most promising approach [5]. Cyclodextrins (CDs) are cyclic α-1,4-linked oligomers of D-glucopyranose, which have been previously used in drug development to increase the water solubility of lipophilic compounds [6]. These torus shaped oligosaccharides can form inclusion complexes by taking up the guest molecule into their central hydrophobic cavity.

The effect of cyclodextrins on the solubilization and stabilization of penicillins in solutions has been already studied. Szejtli [6] reports the solubilization of ampicillin and meticillin by complexation with β-CD. Concerning stabilization, Mizukami and col. [7] were able to improve the stability of the penicillin G by complexation in γ-CD. The chemical stability of cephalotin was also improved with HPβ-CD [8]. Nevertheless, in spite that in most of the cases CD complexation shows a significant improvement in physical and chemical properties of drug, it have been reported that the inclusion can promote the degradation of the drug [9]. Mentioned paper [9] describes how the rate of degradation of aztreonam and phenoxymethylpenicillin increases when both compounds were included in the cavity of HPβ-CD. The effect was different when the CD used was dimethyl-β-CD [9].

The purpose of this study is to gain an insight into the effect of CDs on the physicochemical properties of sodium dicloxacillin in solution. Four cyclodextrins; α-, β-, γ- and hydroxypropyl-β-cyclodextrin (α-CD, β-CD, γ-CD and HPβ-CD) were used. Phase solubility techniques allowed the calculation of the stability constants of those complexes in media with pH 1, 2 and 3. ^{1}H-NMR was employed to characterize the structure of the complexes formed in aqueous solution. The effect of γ-CD and HPβ-CD on the stability of sodium dicloxacillin was finally analyzed.

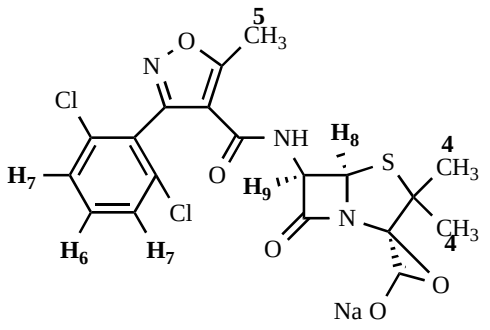

Fig. (1). Chemical structure of sodium dicloxacillin (DxNa). Some hydrogen atoms were omitted for clarity.

*Address correspondence to this author at the Departamento de Farmacia e Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Santiago de Compostela, Campus Sur, E-15706 Santiago de Compostela, Spain; Tel: +34 981 563 100; Fax: +34 981 547 148; E-mail: ffjuant@usc.es

1570-1638/08 $55.00+.00

2. MATERIALS AND METHODS

2.1. Materials

Dicloxacillin sodium monohydrate: (2*S*, 5*R*, 6*R*)-6-[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate, $C_{19}H_{16}Cl_2N_3NaO_5S \cdot H_2O$, abbreviated as DxNa, was obtained from Sigma Chemical (Germany) and utilized without further treatment. The β-cyclodextrin (β-CD), and hydroxypropyl-β -cyclodextrin (HPβ-CD) with 4.6 degree of substitution were a gift of Roquette-Laisa (Spain) and Janssen Pharmaceutica N.V. (Beerse, Belgium), respectively. The α-cyclodextrin (α-CD) was purchased from Cyclolab (Hungary) and γ-cyclodextrin (γ-CD) was purchased from Waker Quimica Ibérica, S.A. (Spain). The acid aqueous solutions were prepared at interval pH 1.0-3.0 adjusting the pH with hydrochloric acid 0.1N [10]. The ionic strength of the buffer solutions employed for the solubility and kinetic studies was not adjusted. All other reagents were of analytical reagent grades.

2.2. Solubility Studies

An excess of DxNa was added to aqueous solutions with a pH value of 1 to 3, adjusting the pH with HCl 0.1N. The suspensions were shaken at 25 ºC until equilibrium was reached (12 h), and then filtered through 0.22 μm Millipore® cellulose acetate membrane filters. The concentration of the dissolved drug was measured by UV spectrophotometry (Shimadzu UV-240-Graphicord) at 227 nm ($E_{1\%1cm}$ = 332.75). All samples were prepared in duplicate.

2.3. Phase Solubility Diagrams

Solubility studies were carried out according to procedure proposed by Higuchi and Connors [11]. An excess of DxNa was added to aqueous solutions with a pH value of 1, 2 or 3 containing various concentration of cyclodextrin (α-CD, 19.0-77.5 mM; β-CD, 2.6-10.5 mM; γ-CD, 19.0-77.3 mM and HPβ-CD, 19.0-77.0 mM). The suspensions were shaken at 25 ºC until equilibrium was reached (12 h), and then filtered as described in point 2.2. The concentration of the dissolved drug was measured by UV spectrophotometry. All samples were prepared in duplicate. The apparent solubility constant of the complex, $K_{1:1}$, was calculated from the initial straight-line portion of the solubility diagram, according to the following expression:

$$K_{1:1} = \text{slope} / S_0 (1 - \text{slope}) \quad (1)$$

where S_0 is the solubility of DxNa without CDs.

2.4. ^{1}H-NMR Studies

Proton - nuclear magnetic resonance spectra were recorded on a Brücker WN 300 spectrometer (Brücker Anal.) at 25 ºC. Samples were dissolved in deuterated water (Sigma Chemical, Spain). The internal reference was a peak due to small amounts of DHO and H_2O presents as impurities (assigned a value of δ = 4.6 ppm).

2.5. Molecular Modeling Studies

Molecular modeling was carried out on an IBM Pentium III 700 MHz personal computer. The molecular mechanics MM2 force field [12] method implemented in Hyperchem software [13] was used for molecular modeling calculations. The geometry parameters of γ-CD [14] was obtained from X-ray diffraction data as deposited in the Cambridge Crystallographic Databank. Modeling was performed by docking the optimized structure of the dicloxacillin into the CD cavity and allowing for full geometry optimization. Different orientations of the dicloxacillin in the CD cavity were tested taking into account the different dicloxacillin groups that could be included in both the narrow and the wide part of the cavity. Modeling was performed both in vacuum and considering the complex to be in a box of water molecules with dimensions x = 20.0, y = 20.0 and z = 20.0 Å. The minimum distance between solvent and solute atoms was fixed at 2.3 Å.

Table 1. Solubility of Sodium Dicloxacillin at Acidic pH

pH	DxNa (mM)
pH 1	$0.63 \pm 2.24\ 10^{-5}$
pH 1.5	$0.25 \pm 1.68\ 10^{-5}$
pH 2	$0.17 \pm 5.81\ 10^{-6}$
pH 2.5	$1.47 \pm 3.96\ 10^{-5}$
pH 3	3.98 ± 0.00

All molecular graphics were constructed using RasWin visualization software (http://www.OpenRasMol.org).

2.6. Kinetic Studies

Kinetic studies carried out by adding stock solution of DxNa (19.5 mM), previously equilibrated at desired temperature in a water bath, in 50 ml of an aqueous CD solution. Two CDs were used, HPβ-CD and γ-CD. The pH of the solutions was 1, 2 and 3. The final DxNa concentration was 0.039 mM, and the CD concentration was 0, 0.5% and 2% w/v. The conditions of the study to guarantee a minimum degradation of the drug were determined in preliminary studies in agreement with the methodology described by Doyle and col. [15]. All reactions were run under a pseudo-first-order conditions. Aliquots, at various intervals, were measured by spectrophotometry and the pseudo-first order rate constant was determined from the disappearance of the drug by linear regression of natural logarithm of the concentration.

3. RESULTS AND DISCUSSION

3.1. Solubility Studies

For many penicillins drugs, the reduced water solubility produces an erratic and incomplete absorption in the low part of the gastrointestinal tract. This can alter the intestinal flora and cause vomits and diarrheas [16]. Table **1** shows the solubility of DxNa in the pH range 1 to 3. The minimum value was found at pH 2. DxNa has two ionizables groups in the structure: the carboxyl group of the β-lactamic ring and an amide group in the lateral chain. This fact can give an amphoteric character to the structure. The pKa is 2.6 from the solubility data at the pH range studies indicates that the ionization of both groups is different. In fact, the lowest solubility value could correspond to the zwitterion form of the molecule because in this condition the molecule is uncharged.

The pH of the dissolution medium modified the interaction of the drug with the cyclodextrins as can be concluded from the phase solubility diagrams (Fig. **2**). At pH 1 and pH 2, phase solubility diagrams of DxNa for α-CD, β-CD and HPβ-CD can be classified as A_L type (linear diagram) on the classification of Higuchi and Connors [11]. On the contrary, γ-CD shows B_S type diagram. At pH 3 only α-CD shows an A_L type, either β-CD, γ-CD or HPβ-CD shows a B_S type diagram. The slope of the initial portion of the curve was less than one in all cases and, therefore, it is possible to assume a 1:1 molar ratio for all the inclusion complexes studied here.

The apparent stability constant of the complexes, $K_{1:1}$, were estimated from the initial straight-line portion of the phase solubility diagram (Table **2**). Highest values of the stability constant were obtained for all cyclodextrins at pH 2, which supports the hypothesis that at this pH the molecule is non-ionized (zwitterions form). In fact, the interaction between a cyclodextrin and a drug molecule is better when this is not ionized. In fact inside the cavity of the CD there are a variable number of water molecules in a hydrophobic environment with a high energy level that can be substituted by any hydrophobic compound. This substitution, and therefore, the inclusion will be

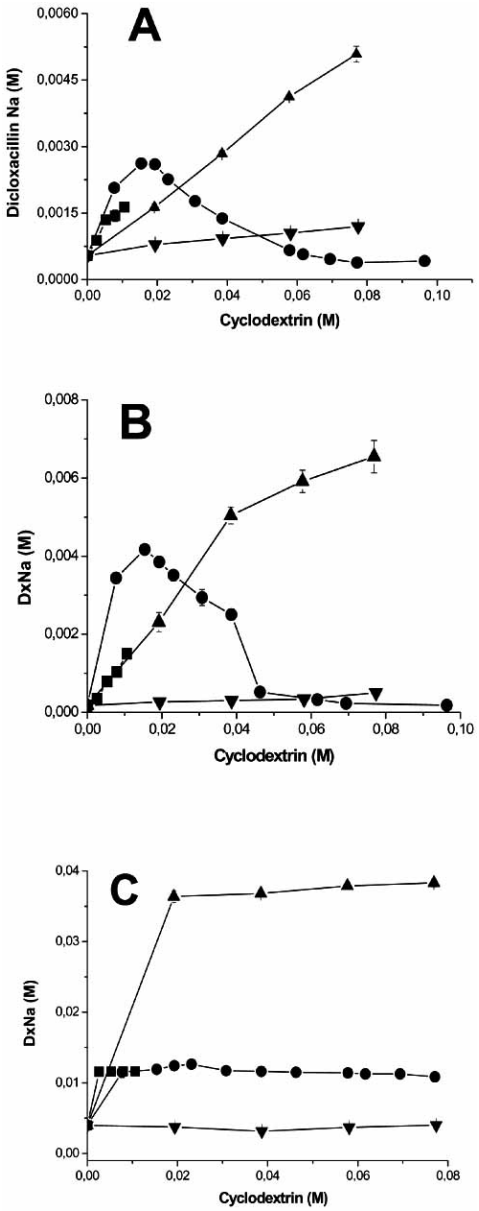

Fig. (2). Diagrams of solubility of DxNa in presence of CD at (a) pH 1, (b) pH 2, (c) pH 3. ▼α-CD; ■ β-CD; ● γ-CD; ▲ HPβ-CD.

more favorable with a non ionic compound that with an ionic one [17].

At pH 1 and 2, the highest stability constants were found for γ-CD. This cyclodextrin might fix the drug better than the others because of its bigger cavity size. Nevertheless, at pH 3, HPβ-CD shows the highest value of $K_{1:1}$. At this pH value, DxNa is ionized and it has been found that this derivative might interact well with this kind of molecules. Wouessidjewe and col. [18] found that the ionized indometacin salt forms inclusion complexes with β-CD and HPβ-CD but do not interact with the no-ionized form (acid). Therefore, from the values calculated of the stability constants, γ-CD and HPβ-CD were selected to be used in the stability studies.

3.2. NMR Studies

NMR is a technique commonly used to characterize drug-CD interaction either in solution or in solid state [19, 20]. This information may be obtained mainly based on the chemical shifts that show the protons of the drug and the cyclodextrin when the inclusion occurs. The protons of the guest - drug molecule - will be strongly shielded when the inclusion takes place. On the other hand, the protons of the host – cyclodextrin – show changes toward high field [21-23]. In this paper, ^{1}H-NMR was use to characterize the interaction in water of DxNa with four cyclodextrin derivatives: α-CD, β-CD, γ-CD and HPβ-CD. Chemical shifts changes of the protons of DxNa in increasing concentrations (1:0 to 1:2 mole:mole DxNa:CD) of four cyclodextrin derivatives were analyzed.

Fig. **3** show the induced chemical shift changes for the hydrogen atoms of DxNa whose signals were not masked by the CD signals as a function of the CD-DxNa molar ratio. Downfield shifts of the protons of DxNa are caused by variations of the local polarity due to the inclusion inside the CD cavity. These chemical shifts are dissimilar in the presence of the four derivatives. In fact, the chemical shifts of DxNa protons in the presence of α-CD can be consider not significant, and therefore, indicates that the inclusion do not takes place. This confirms the low effect of this CD on the solubility of the drug.

In the presence of β-CD, γ-CD and HPβ-CD protons H-8, H-9, H-6 and H-7 suffer the most significant chemical shifts, This indicated that the phenyl ring (H-6 and H-7) and a part of the β-lactamic group are included in the cavity. The intensity of the changes is different depending on the CD considered: the highest are those obtained with HPβ-CD and with γ-CD. It is important to point out that with γ-CD were obtained the most significant changes for the protons of the methyl group of the isoxazol ring (H-5) and for those of the methyl group of the β-lactamic ring. The reason for this particular interaction could be the big cavity that has this last CD.

The inclusion of the drug inside the CD could occur either through the wide or narrow edge of the cavity. Protons H-3 and H-5 of the CD are oriented to the wide and the narrow side respectively, and the chemical shift are bigger for the proton on the side in which the complexation takes place. These studies were carried out to analyse DxNa-CD inclusion complexes. Fig. **4** shows the effect of in-

Table 2. Stability Constants Calculated from the Phase Solubility Diagrams for the Complexes DxNa:CD at Different pHs

pH	Complex Constant ($K_{1:1}$)			
	α-CD	β-CD	γ-CD	HPβ-CD
1	A_L	A_L	B_S	A_L
	16,64	214,48	285,46	122,97
2	A_L	A_L	B_S	A_L
	21,64	854,65	2044,55	655,70
3	A_L	A_L	B_S	A_L
	1,08	343,65	134,17	1793,39

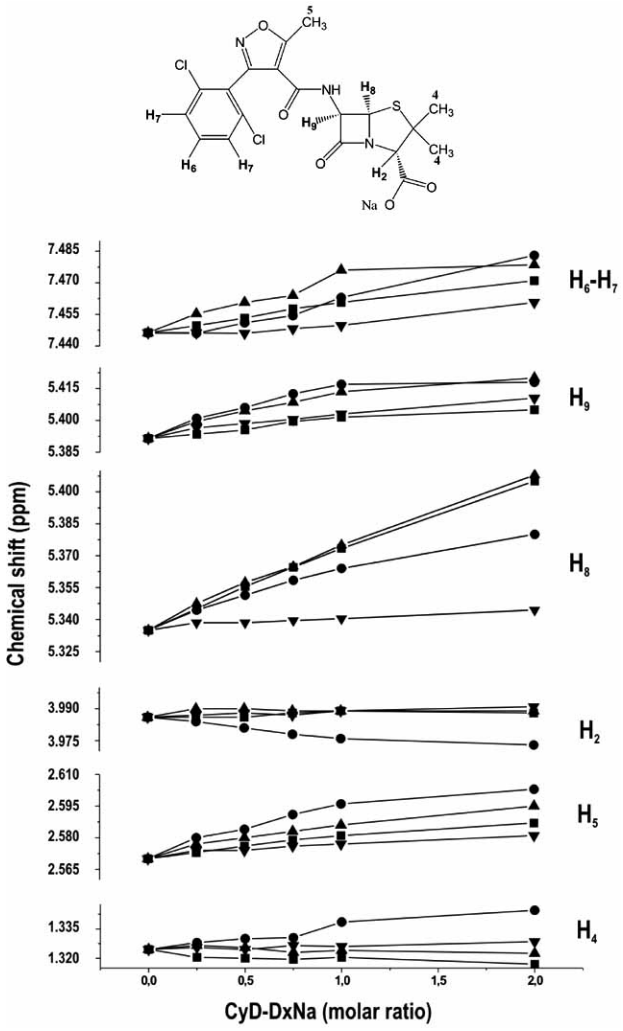

Fig. (3). Chemical shifts of protons of DxNa (panel **A**) in solutions varying in CD concentration. ▼α-CD; ■ β-CD; ● γ-CD; ▲ HPβ-CD (panel **B**).

crease concentrations of DxNa on the β-CD protons. It is clear that both protons change toward high field and the effect is higher for H-3. Similar effect was found for γ-CD and HPβ-CD (figures not show). It can be conclude that the DxNa is included through the wide side of the cyclodextrin.

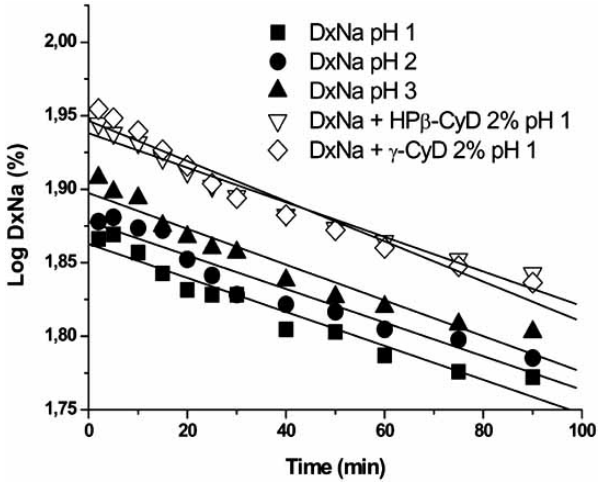

Fig. (6). Typical first-order plots for the hydrolysis of DxNa in aqueous solutions at pH 1, 2 and 3, alone and in the presence of γ-CD and HPβ-CD.

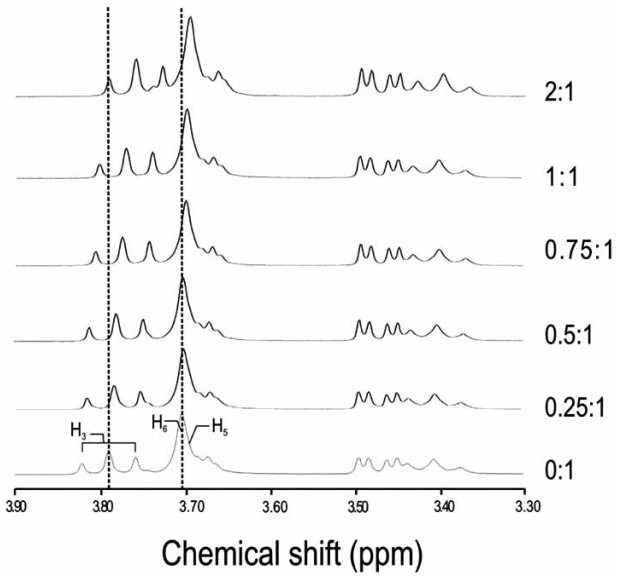

Fig. (4). Chemical shifts of protons of β-CD in solutions varying in DxNa concentration.

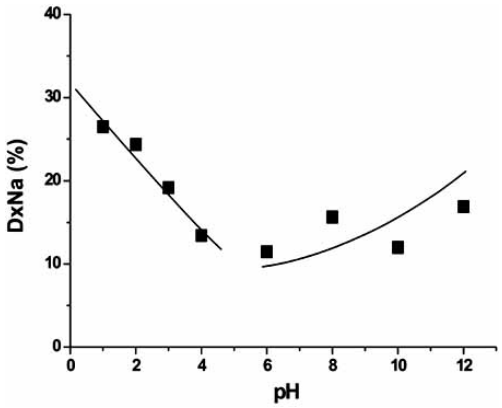

Fig. (5). pH-stability profile of DxNa.

3.3. Kinetic Studies

The hydrolysis of DxNa in acidic media of pH 1, 2 and 3 was followed by UV spectrophotometry. The drug shows a maximum at 227 nm. When the degradation takes place, the absorption decreases and a new peak appears at 350 nm corresponding to the dicloxacillenic acids produced [24]. These acids have been identified as intermediates in the hydrolysis reaction, formed as a consequence of an intramolecular reorganization [25]. The stability of DxNa is pH dependent and, as shown in Fig. **5**, the pH of maximum stability is in the range 4 to 6. Similar results were described by Pawelczyk *et al* [26].

Only the effect of two CD, namely HPβ-CD and γ-CD, were used because show the best interaction with DxNa according with the values of the stability constant (Table **2**). In this study two concentrations of both mentioned above CDs were used: 0.5% and 2%. The hydrolysis of DxNa followed first-order kinetics in the pH range used. Introduction of CDs do not affect the observed kinetic order as shown in Fig. **6**.

The influence of CDs is usually evaluated comparing the values of the stability constants (k_{obs}) determined in the presence of the CD with that in its absence [26, 28]. Table **3** shows the calculated values of shelf-life (t_{90}, time for 10% decomposition) of DxNa in the presence of the CDs. These results confirm the stabilizing effect of both used CD and the effect of the concentration. The effect is higher in

Table 3. Calculated Values of $t_{90\%}$ for the Hydrolysis of DxNa in Media of pH 1, 2 and 3 in the Absence and Presence of HPβ-CD and γ-CD at a Concentration of 0.5% and 2% (w/v)

pH	$t_{90\%}$ (min)				
	Free DxNa	HPβ-CD		γ-CD	
		0.5 %	2 %	0.5 %	2 %
pH 1	0.75	1.20	1.66	0.90	2.28
pH 2	0.82	1.02	5.09	1.20	12.54
pH 3	1.04	2.41	1.90	3.38	23.70

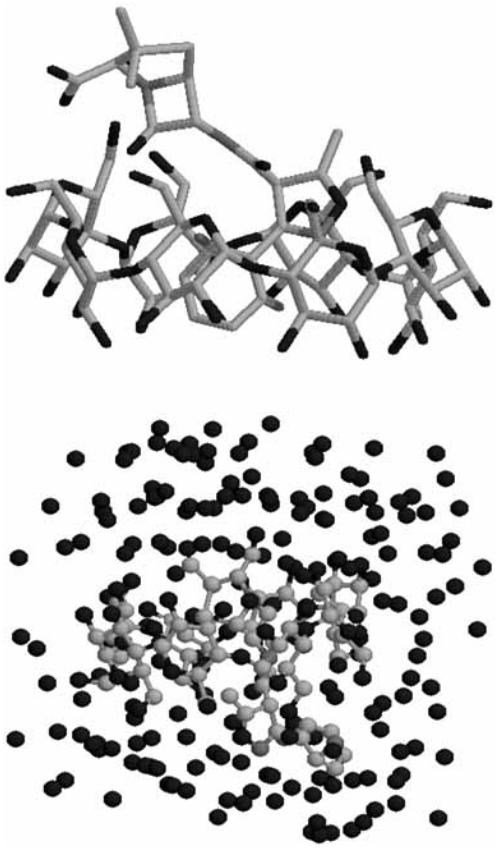

Fig. (7). Energy-minimized predicted structure of the DxNa-γ-CD complex in vacuum (panel **A**) and in aqueous solvent (panel **B**).

the presence of γ-CD which confirms that is, as consequence of their biggest cavity, the γ-CD shows bigger effect stabilizer to same concentration [27, 28]. On the other hand, a direct relation between concentration and stability effect can be established and in both cases, the best results were obtained at the highest concentration of CD, i.e. 2%. This ratio represents a ratio DxNa: CD of 1:400 (mol:mol), and therefore, in this conditions, the drug is mainly in complexed form and highly protected form the hydrolysis.

3.4. Computational Studies

Computational studies on drug-CD interactions are carried out, in general, to find the most probable conformation of the complex [29, 30]. In this paper, we conduct a computational molecular modeling study on the inclusion of dicloxacillin into the γ-CD cavity because it was used in the kinetic studies. HPβ-CD was not analyzed because this derivative is a complex mixture of different structures. The main objective is to gain insights about the role that the geometry of the complex plays on the enhancement of stability of the drug in aqueous media. A computational analysis of the different orientations of dicloxacillin into the CD was carried out.

Table 4. Energies of the Possible Conformations of the Complexes of DxNa and γ-CD as Estimated by MM2

Conformer	γ-CD in vacuum	γ-CD with aqueous solvent
"2,6-dichlorophenyl" ring inside the cavity in the broad end	0.03	15.15
"2,6-dichlorophenyl" ring inside the cavity in the la narrow end	0.00	6.35
"7-oxo-4-thia-1-azabicyclo" rings inside the cavity in the broad end	1.96	0.00
"7-oxo-4-thia-1-azabicyclo" rings inside the cavity in the narrow end	2.14	2.86

The complexation of dicloxacillin could occur either including the 2, 6-dichlorophenyl ring or the 7-oxo-4-thia-1-azabicyclo ring into the cavity. Specifically, complexes in which both groups are included into either the narrower or the wider side of the CD cavity, were modelled. The full-geometry optimization of such complexes (MM2 force field) showed that the inclusion of the 2, 6-dichlorophenyl group in the narrow side of the cavity is the most energetically favorable orientation (Table **4**, Fig. **7**-**A**). However, it is worth to note that the energy difference between this conformer and the inclusion of the 2, 6-dichlorophenyl group into the wider side of the cavity is very small (less than 0.03 kcal/mol), suggesting that both orientations may occur simultaneously in vacuum (absence of solvent). Similar conclusions were recently reached by Piel *et al*. [30] in an analysis of the complexation of miconazole with β- and γ-CD in the absence of solvent.

Giving that the main objective concerns the explanation of stabilization of dicloxacillin in water solvent, we will consider that inclusion occurs not under vacuum but in a box of water molecules. We have previously shown that more realistic modeling of the drug-CD complexes are obtained by considering water molecules as solvent instead of calculations simulating vacuum condition [29]. The results obtained by this approach are likewise listed in Table **4**.

Water boxes containing 176 water molecules around complexes of the dicloxacillin and γ-CD were again optimized by the MM2 force field method. Inclusion of the 7-oxo-4-thia-1-azabicyclo group in the wider side of the cavity is, under these conditions, much more favorable (differences from 2.8 to 15.1 kcal/mol) than the other possible orientations. The predicted molecular structure of the MM2-optimized dicloxacillin-γ-CD complex in orientation B is illustrated in Fig. **7**. This predicted orientation differs to that obtained in vacuum media. In the preferred B orientation of dicloxacillin inside of the γ-CD cavity in aqueous solvent, the group involved in the hydrolysis process is included into the cavity and it can be protected from water. These theoretical results fully agree with the ^{1}H-NMR experiments, discussed before and with the experimental observation of the incre-

ment of the stability in water. Also they reaffirm the previous studies suggesting the importance of explicitly considering the solvent in molecular modeling of drug-cyclodextrin inclusion complexes [28].

CONCLUSION

Cyclodextrins can increase the solubility and stability of DxNa in aqueous solutions of pH 1, 2 and 3. The observed order of solubility increasing effect was: γ-CD > HPβ-CD > β-CD > α-CD. The complexation of the drug in γ-CD or HPβ-CD also increase the stability in water solutions. This effect depends on the CD used and on its concentration, γ-CD has been found more effective than the hydroxypropyl-β derivative. The protective effect of the cyclodextrin has been found in the pH values studies and it can be explained because the part of the molecule included in the cavity is the β-lactamic group, as concluded from the NMR analysis and by the computational study performed with γ-CD in aqueous solvent.

ACKNOWLEDGEMENTS

"This work was financed by the Xunta de Galicia (PGIDT07CSA002203PR) AND MEC-FEDER (SAF 2005-01930. R.V. thanks the Consellería de Consellería de Educatión e Ordenacion Universitaria-Zunta de Galicia for supporting his stage in Santiago de Compostela (DOG N° 105. June 1st, 2007, pp 9160). The authors thank Roquette-Laisa España and Janssen Pharmaceutiche for generous donation of β-CD and HPβ-CD, respectively. Special thanks to Dr. Grzegorz Bazylak for his valuable critical remarks".

ABBREVIATIONS

CD	=	Cyclodextrin
DHO	=	Mono-deuterated water
DxNa	=	Dicloxacillin sodium
HPβ-CD	=	Hydroxypropyl-β-cyclodextrin
β-CD	=	β-cyclodextrin

REFERENCES

[1] Perlam D.: *Antibióticos.* In: W. O. Foye: *Principios de química farmacéutica*. Editorial Reverte SA. pp. 794-807, (1991).

[2] Hou J.P., Poole J.W: *β-Lactam antibiotics: Their physicochemical properties and biological activities in relation to structure.* J. Pharm. Sci. 60, 503-532, (1971).

[3] Hou J.P., Poole J.W.: *The amino acid nature of ampicillin and related penicillins.* J. Pharm. Sci. 58, 1510-1515, (1969).

[4] Loftsson T., Brewster, M.E.: *Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization*. J. Pharm. Sci. 85, 1017-1025, (1996).

[5] Mallick S., Pattnaik S., Swain K.: *Current perspectives of solubilization: Potential for improved bioavailability.* Drug Dev. Ind. Pharm. 33, 865-873, (2007).

[6] Szejtli J.: *Cyclodextrin technology*. Kluwer Academic, Doordrecht, p. 256, (1988).

[7] Mizukami Y., Ichimura F., Yamana T.: *Study on the stability of drugs. Part 26. Effect of β-cyclodextrin on degradation of penicillins and cephalotin in acidic and weak alkaline solutions.* Yakuzaigaku 38, 45-50, (1978).

[8] Loftsson T., Johannesson H.R.: *The influence of cyclodextrins on the stability of cephalotin and aztreonam in aqueous solutions*. Pharmazie 49, 292-293, (1994).

[9] Loftsson T., Ólafsdóttir B.J.: *Cyclodextrin-accelerated degradation of β-lactam antibiotics.* Int. J. Pharm. 67, R5-R7, (1991).

[10] Hagga M.E.M., Abounassif M.A., Al-Awady M.I., Gad-Kariem E.A.: *Kinetic study of the degradation of dicloxacillin in aqueous media*. Bull. Chim. Farm. 136, 12-16, (1996).

[11] Higuchi T., Connors K.A.: *Phase solubility techniques.* Adv. Anal. Chem. Instr. 4, 117-212, (1965).

[12] Allinger N.L.: Conformational *analysis. 130. MM2. A hydrocarbon force field utilizing V1 and V2 torsional terms.* J. Am. Chem. Soc. 99, 8127-8134, (1977).

[13] Hyperchem™ 6.03 for Windows. Hypercube, Inc., Waterloo, Ontario, Canada, (2000).

[14] Harata K.: *The structure of the cyclodextrin complex. XX. Crystal structure of uncomplexed hydrated γ-cyclodextrin*. Bull. Chem. Soc. Jpn. 60, 2763-2767, (1987).

[15] Doyle F.P., Nayler J.H.C.: *Penicillins and related substances*. In: Harper N.J., Simmonds A.B. (Eds.): *Advances in Drug Research*.Academic Press, New York, Vol.1., pp.1-69, (1964).

[16] Gennaro A.R., *Remington Farmacia*. Medica Panamericana, Buenos Aires, 17th Ed., Vol.2, pp.1617-1623, (1998).

[17] Redenti E., Szente L., Szejtli J.: *Cyclodextrin complexes of salts of acidic drugs. Thermodynamic properties, structural features, and pharmaceutical applications.* J. Pharm. Sci. 90, 979-986, (2001).

[18] Wouessidjewe D., Lin S.Z., Djedani F., Perly B., Duchene D.: *Investigation of indomethacin/β-cyclodextrin and indomethacin/hydroxypropyl β-cyclodextrin inclusion complexes: NMR contribution to probe the inclusion*. In: Duchene D. (Ed.): Minutes of the 5th International Symposium of Cyclodextrins, Paris, 28-30 March 1990. Editions de Sante, Paris, pp. 332-338, (1990).

[19] Chankvetadze B., Blaschke G., Pintore G.: *Ligand-cyclodextrin complexes. In: U. Holzgrabe, I. Wawer, B. Diehl (eds.), NMR spectroscopy in drug development and analysis.* Wiley-VCH, Weinheim, Germany, pp.155-174, (1999).

[20] Echezarreta-Lopez M.M., Perdomo-Lopez I., Estrada E., Vila-Jato J.L., Torres-Labandeira J.J.: *Utility of nuclear magnetic resonance spectroscopy to characterize the structure of dexamethasone sodium phosphate inclusion complexes with cyclodextrins in solution and to analyze potential competitive effects.* J. Pharm. Sci. 91**,** 1536-1547, (2002).

[21] Perdomo-Lopez I., Rodriguez-Perez A., Yzquierdo-Peiro J.M., White A., Estrada E., Villa T.G., Torres-Labandeira J.J.: *Effect of cyclodextrins on the solubility and antimycotic activity of sertaconazole: experimental and computational studies*. J. Pharm. Sci. 91, 2408-2415, (2002).

[22] Nishijo J., Tsuchitani M.: *Interaction of L-tryptophan with alpha-cyclodextrin: studies with calorimetry and proton nuclear magnetic resonance spectroscopy*. J. Pharm. Sci. 90, 134-140, (2001).

[23] Djedaïni F., Lin S.Z, Perly B., Wouessidjewe D.: *High-field nuclear magnetic resonance techniques for the investigation of a a-cyclodextrin:indomethacin inclusion complex.* J. Pharm. Sci. 79, 643-646, (1990).

[24] Schwartz M.A., Buckwalter F.H.: *Pharmaceutics of penicillin*. J. Pharm. Sci. 51, 1119-1129, (1962).

[25] Yamana T., Tsuji A., Mizukami Y.: *Kinetic approach to the development in beta-lactam antibiotics. I. Comparative stability of semisynthetic penicillins and 6-aminopenicillanic acid in aqueous solution*. Chem. Pharm. Bull. 22, 1186-1197, (1974).

[26] Pawelczyk E., Zajac M., Knitter B.: *Kinetic of drug decomposition. Part 67. Kinetics of degradation of dicloxacilin in aqueous solution.* Pol. J. Pharmacol. Pharm. 33, 241-249, (1981).

[27] Ong J.K., Suderland V.B., McDonald C.: *Influence of hydroxypropyl-β-cyclodextrin on the stability of benzylpenicillin in chloroacetate buffer*. J. Pharm. Pharmacol. 49, 617-621, (1997).

[28] Hada S., Neya S., Funasaki N.: *Aceleration and inhibition of the hydrolysis of penicillin G by dimerization and cyclodextrin inclusion*. Chem. Pharm. Bull. 45, 577-583, (1997).

[29] Estrada E., Perdomo-Lopez I., Torres-Labandeira J.J.: *Molecular modelling (MM2 and PM3) and experimental (NMR and thermal analysis) studies on the inclusion complex of salbutamol and β-cyclodextrin*. J. Org. Chem. 65, 8510-8517, (2000).

[30] Piel G., Dive G., Evrard B.,Van Hess T., Henry D.E., Hassonville S., Delattre L.: *Molecular modelling study of β- and γ-cyclodextrin complexes with miconazole.* Eur. J. Pharm. Sci. 13, 271-279, (2001).

Received: 16 November, 2007 Revised: 03 December, 2007 Accepted: 10 December, 2007