DISSOLUTION STUDIES OF PHYSICAL MIXTURES OF INDOMETHACIN WITH ALPHA- AND GAMMA-CYCLODEXTRINS

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Abstract

Oral administration of indomethacin has been limited by its poor water solubility. Cyclodextrins have been recognized as potential candidates to overcome the poor solubility of indomethacin through the formation of inclusion complexes.

The aim of our study was to compare the dissolution profiles of pure indomethacin and its mixtures with α- and γ-cyclodextrins The inclusion complexes of indomethacin with α- and γ-cyclodextrins were prepared by direct mixing in dissolution vessel. Fixed volumes of the dissolution medium were withdrawn at 0,5; 1 and 4 hours. Dissolution tests were performed on the USP Apparatus 2, rotating speed 100 rpm at 37±0,5°C, 500 ml, distilled water and 0,1 M HCl solution). Quantification of dissolved indomethacin was performed by UV/VIS spectrophotometric method at the absorption maximum around 320 nm.

The results were expressed as relative dissolution rate (ratio between indomethacin dissolved from its physical mixtures with α- and γ-cyclodextrins and that dissolved the pure drug).

Relative dissolution rates of indomethacin in combination with α- and γ-cyclodextrins at the end of testing were in the range of 91,66 to 337,14 % (for α- cyclodextrin) and in the range of 128,57 to 301,92 % (for γ-cyclodextrin).

The complexation of indomethacin with α- and γ-cyclodextrins resulted in the enhancement of dissolution rate.

KEY WORDS: dissolution, indomethacin, alpha-, gamma-cyclodextrin, physical mixture

Introduction

Cyclodextrins (also known as cycoamyloses, cyclo-maltoses and Schardinger dextrins) are cyclic oli-gosaccharides consisting of six α-, seven-β, eight-γ, nine-δ, ten ε-cyclodextrins or more glycopyranose units linked by α-(1,4) α-D-glucopyranose units (1,2) They are produced as a result of intra-molecular glycolisation, which occurs during starch degra-dation mediated by cyclodextrin glycanotransfer-ase (CGTase) enzyme (3). Due to the chair con-formation of the glucopyranose units, the CDs take the shape of a truncated cone or torus rather than a perfect cylinder (Table 1., Figure 1.) (4,5,6).

The hydroxyl functions are orientated to the cone ex-terior with the primary hydroxyl groups of the sugar residues at the narrow edge of the cone and the sec-ondary hydroxyl groups at the wider edge. The central cavity of the CD molecule is lined with skeletal carbons and ethereal oxygens of the glucose residue, which gives it a relatively lipophilic character (6,8,9,10,11,12).

The polarity of the cavity has been estimated to be similar to that of an aqueous ethanolic so-lution (12). In aqueous solutions, the hydroxy groups form hydrogen bonds with the surround-ing water molecules resulting in a hydration shell around the dissolved CD molecule (13, 14, 15, 16). Cyclodextrins (CD) interact with poorly-water soluble compounds to increase their apparent solubility. The most prominent mechanism by which this solubiliza-tion occur is inclusion complex formation in which the guest and host molecules are in dynamic equi-librium with the complex (6). The increased appar-ent solubility can enable solution-based dosage forms such as parenteral i.v. formulations and oral liquids. In addition, increasing the apparent solubility of a drug can, through the Noyes–Whitney equation, increase drug dissolution rate and for compounds whose bio-availability is limited by solubility or dissolution rate, can act to increase their bioavailability (17,18, 19). Biopharmaceutical Classification System (BCS) is a useful approach in assessing the potential applica-tion of CDs in this context. The BCS divides drugs and drug candidates into 4 classes based on their solubility and permeability characteristics (20,21,22).

According to this system, Class I consists of highly *water-soluble* drugs that are well absorbed from the gastrointestinal (GI) tract and, in general have the pre-ferred physicochemical properties. Drugs in Class I have high bioavailability after oral administration (23, 24). Class II consists of *water-insoluble* drugs that, when dis-solved, are well absorbed from the GI tract. The dissolu-tion rate *in vivo* is usually the rate-limiting step in drug

Cyclodextrin	Number of glucose units	Dimensions (nm)			$t_{1/2}$ of ring open-ing (h)	Mean $K_{1:1}$[a] (M^{-1})
		H	OD	ID		
α-Cyclodextrin (αCD)	6	0,78	1,37	0,57	33	130±8
β-Cyclodextrin (βCD)	7	0,78	1,53	0,78	29	490±8
γ-Cyclodextrin (γCD)	8	0,78	1,69	0,95	15	350±9

TABLE 1. Characteristics of the natural cyclodextrins αCD, βCD and γCD

FIGURE 1. Structure of the natural cyclodextrins α-CD, β-CD and γ-CD
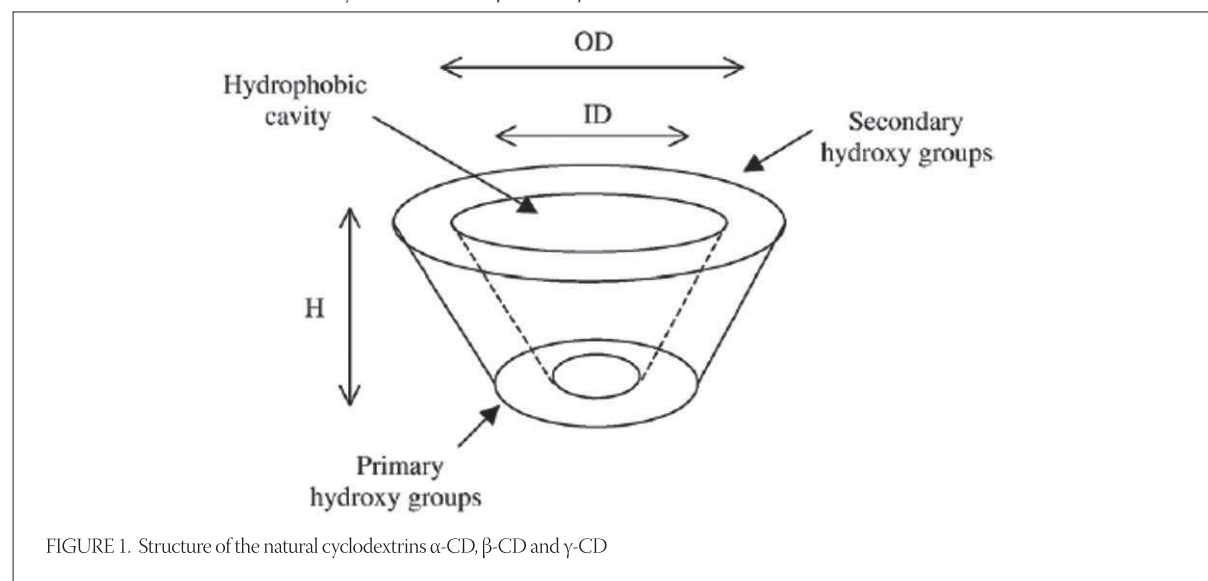
[a] Stability constants (binding constants) of 1:1 guest/CD complexes in aqueous solutions at 25±5°C (7). Population mean±standard deviation.

absorption. Commonly, drugs in this Class have variable absorption due to numerous formulation effects and *in vivo* variables that can affect their dissolution profile. Class III consists of *water-soluble* drugs that do not permeate biomembranes readily; Class IV consists of *water-insoluble* drugs that, when solubilises, do not permeate biomembranes readily. Unfortunately, most chemical entities are water-insoluble lipophylic compounds or, in other words, Class II or even Class IV compounds. It can be quite challenging for a formulation scientists to develop usable pharmaceutical formulation product from such compounds (25, 26, 27).

Non-steroidal anti-inflammatory drug (NSAID), indomethacin, was chosen as a model substance for our study. It is widely used as analgesics and for the treatment of local and systemic inflammatory pathologies and rheumatoid arthritis as well. Biopharmaceutical Classification System (BCS) lists indomethacin as Class II drug.

The aim of this study was to examine:

◊ the influence of certain cyclodextrins (α- and γ-) and different molar ratios (drug : cyclodextrin) on the solubility and dissolution rate of indomethacin in acidic and neutral dissolution medium.
◊ the level of in-situ complex forming within proposed testing period (4 hours)

Material and Methods

Reagents
The used reagents were all of analytical grade, unless otherwise stated. Indomethacin working standard, α- and γ-cyclodextrin were provided by Merck (Buchs, Switzerland). Hydrochloric acid (37%) was provided by Merck (Darmstadt, Germany).
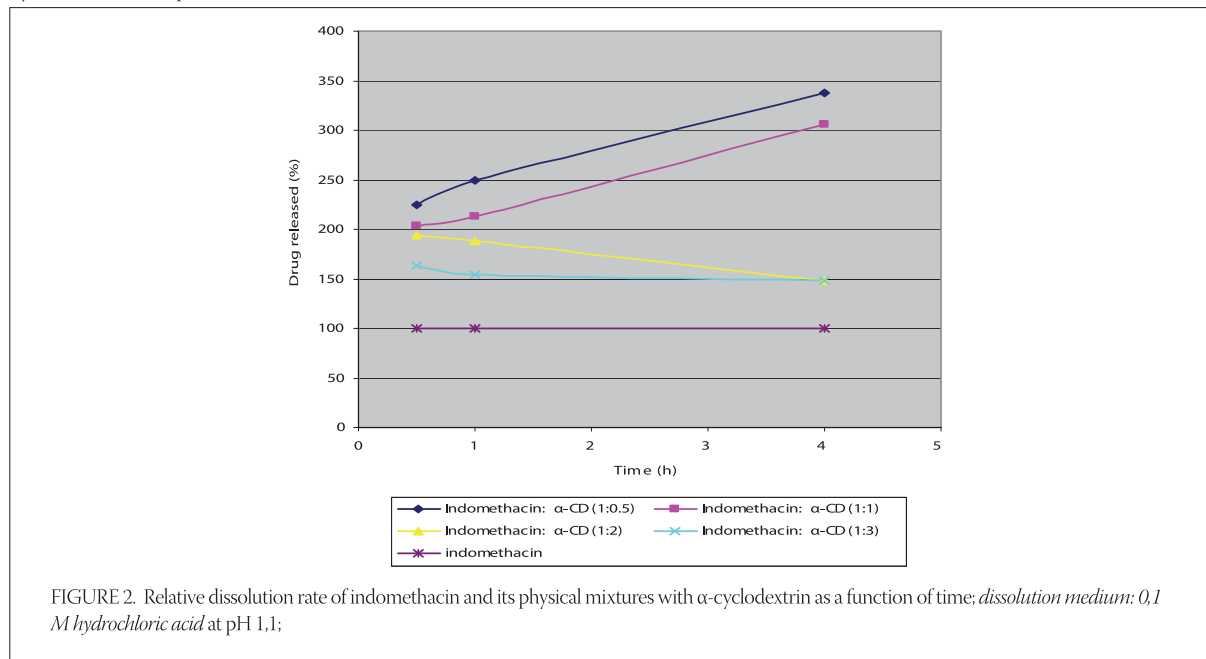
Dissolution profile analysis
The dissolution tests, were performed using USP apparatus 2, Van Kel VK 7010 dissolution tester, at a stirring speed of 100 rpm Van Kel, Cary, NC, USA). The dissolution apparatus was maintained at 37°C throughout the experiment. Prior to use, the dissolution medium was dearated in the ultrasonic bath and heated to 41°C, filtered using a 0,45 μm membrane filter (Sartorious GmbH, Goettingen, Germany) and transferred into dissolution vessel. The analysis was performed immediately after the medium cooled down to 37°C.

These samples were also filtered using a 0,45 μm membrane filter (Sartorious GmbH, Goettingen, Germany). The dissolution apparatus was connected with UV/VIS spectrophotometer Shimadzu 1601 (Shimadzu, Kyoto, Japan). Determination of dissolution rates for the active ingredient was carried out by the UV/VIS spectrophotometric method at 320 nm. All dissolution tests were performed in triplicate. The dissolution profiles of indomethacin (pure drug and in combination with α- and γ-cyclodextrin) were determined in two dissolution media: distilled water at pH 6,2 and 0,1 M hydrochloric acid at pH 1,1. Dissolution media were selected based on data on indomethacin solubility in these media (28). The solubility of indomethacin at pH 6,2 was >500 μg/ml; solubility of indomethacin at pH 1,1 was 1 μg/ml. Samples in the amount of 10 ml were withdrawn at the following intervals (after 0,5; 1 and 4 hours) and collected for the analysis. Correction for volume was calculated mathematically, considering that withdrawn samples were not supplemented with an equal volume of fresh dissolution fluid to maintain a constant total volume. The dissolution profile of indomethacin, pure or mixed with α- and γ-cyclodextrin at various molar ratios (indomethacin: cyclodextrin =1:0,5; 1:1; 1:2 and 1:3) was determined at pH 1,1 and at pH 6,2 (distilled water). In addition, at pH 1,1 quantity of 20 μg of indomethacin was dispersed in 500 ml of dissolution medium while at pH 6,2 we used 50 μg of drug in the same volume. The inclusion complexes of indomethacin with α- and γ-cyclodextrins were prepared by direct mixing in dissolution vessel. The results were expressed as relative dissolution rate (ratio between quantity of indomethacin dissolved from its physical mixtures with α- and γ-cyclodextrins and that dissolved from pure drug), due to extremely low solubility of indomethacin (almost insoluble in aqueous medium: pH 1,1: 0,001 mg/ ml; pH 5,0: 0,01 mg/ ml).

Results and Discussion

The results of dissolution studies of pure indomethacin and its physical mixtures with α-cyclodextrin in 0,1 M hydrochloric acid at pH 1,1 and distilled water at pH 6,2 are summarized in Table 2., and Figure 2. and Table 3., and Figure 3, respectively. The results of dissolution studies of pure indomethacin and its physical mixtures with γ-cyclodextrin in 0,1 M hydrochloric acid at pH 1,1 and distilled water at pH 6,2 are summarized in Table 4., and Figure 4. and Table 5., and Figure 5, respectively.

Time (hours)	Molar ratio				Indomethacin
	(indomethacin : α-CD)				
	1:0,5	1:1	1:2	1:3	
	Relative dissolution rate (%)				
0,5	224,5± 11,6 %	204,1± 9,3 %	193,9± 7,0 %	163,3± 8,1 %	100,0± 8,0 %
1	250,0± 10,1 %	213,5± 14,2 %	188,5± 10,9 %	153,9± 13,7 %	100,0± 7,6 %
4	337,1± 13,4 %	305,7± 12,8 %	148,5± 11,1 %	148,6± 12,5 %	100,0± 6,5 %

TABLE 2. Relative dissolution rate of indomethacin and its physical mixtures with α-cyclodextrin as a function of time; dissolution medium: 0,1 M hydrochloric acid at pH 1,1;

FIGURE 2. Relative dissolution rate of indomethacin and its physical mixtures with α-cyclodextrin as a function of time; *dissolution medium: 0,1 M hydrochloric acid* at pH 1,1;

Time (hours)	Molar ratio				Indomethacin
	(indomethacin : α-CD)				
	1:0,5	1:1	1:2	1:3	
	Relative dissolution rate (%)				
0,5	134,8± 8,9 %	113,8± 8,1 %	144,3± 8,8 %	172,0± 9,6 %	100,0± 6,8 %
1	91,7± 7,4 %	99,6± 10,5 %	109,9± 8,2 %	119,7± 8,8 %	100,0± 6,3 %
4	104,5± 9,1 %	103,4± 9,9 %	110,8± 7,3 %	107,5± 11,7 %	100,0± 6,2 %

TABLE 3. Relative dissolution rate of indomethacin and its physical mixtures with α-cyclodextrin as a function of time; *dissolution medium: distilled water* at pH 6,2;
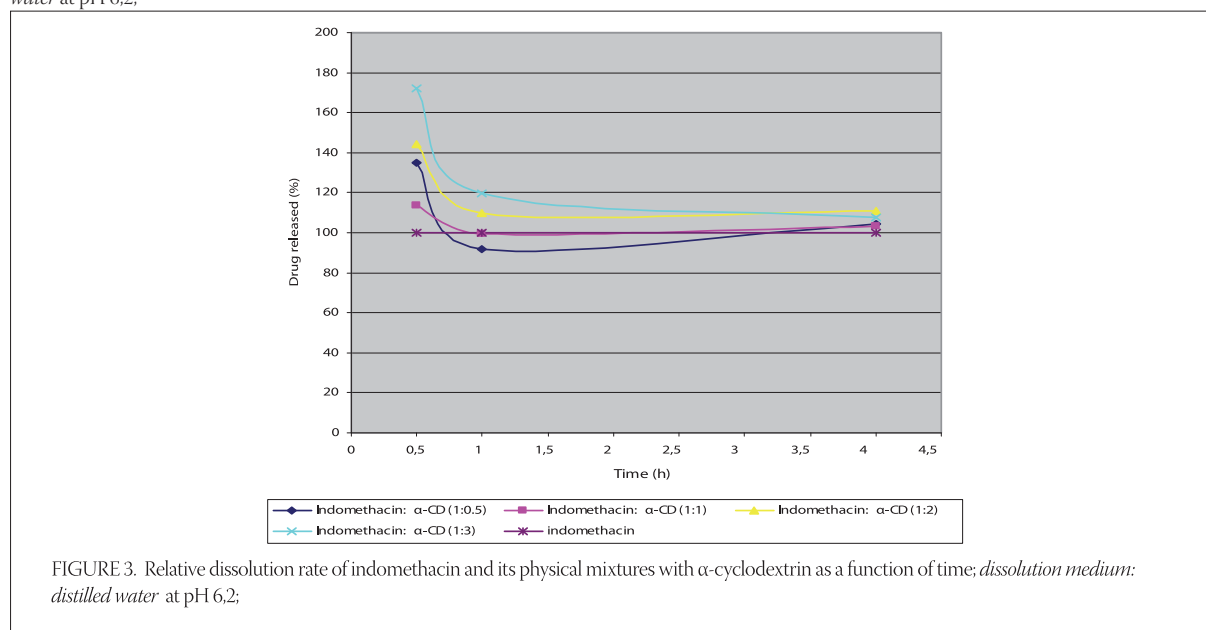
FIGURE 3. Relative dissolution rate of indomethacin and its physical mixtures with α-cyclodextrin as a function of time; *dissolution medium: distilled water* at pH 6,2;

Time (hours)	Molar ratio (indomethacin : γ-CD)				Indomethacin
	1:0,5	1:1	1:2	1:3	
	Relative dissolution rate (%)				
0,5	163,3± 9,4 %	244,9± 7,9 %	285,7± 10,2 %	285,7± 16,5 %	100,0± 8,0 %
1	166,2± 8,7 %	246,2± 12,6 %	301,9± 14,0 %	282,7± 18,9 %	100,0± 7,6 %
4	128,6± 8,1 %	184,3± 11,8 %	247,1± 12,2 %	240,0± 16,4 %	100,0± 6,5 %

TABLE 4. Relative dissolution rate of indomethacin and its physical mixtures with γ-cyclodextrin as a function of time; *dissolution medium: 0,1 M hydrochloric acid* at pH 1,1;
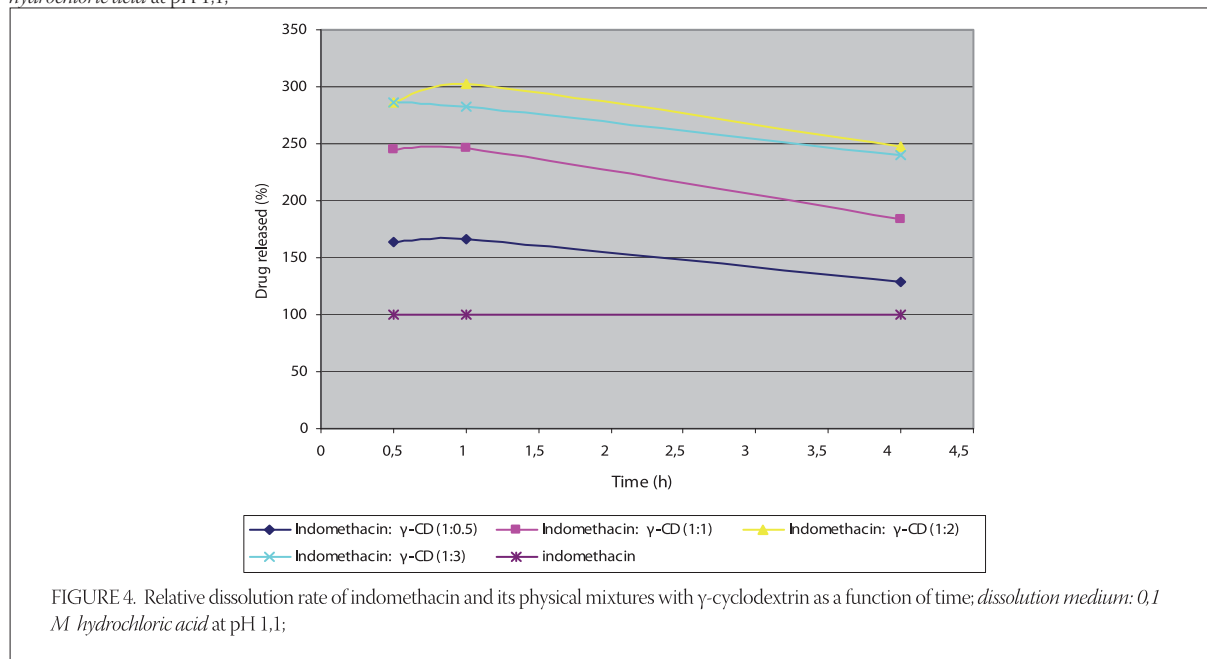

FIGURE 4. Relative dissolution rate of indomethacin and its physical mixtures with γ-cyclodextrin as a function of time; *dissolution medium: 0,1 M hydrochloric acid* at pH 1,1;

Time (hours)	Molar ratio (indomethacin : γ-CD)				Indomethacin
	1:0,5	1:1	1:2	1:3	
	Relative dissolution rate (%)				
0,5	190,1± 8,4 %	218,2± 9,0 %	345,2± 16,1 %	155,0± 8,1 %	100,0± 6,8 %
1	181,5± 9,7 %	190,9± 9,8 %	261,0± 14,9 %	167,0± 10,7 %	100,0± 6,3 %
4	166,3± 10,1 %	175,6± 9,2 %	212,4± 14,2 %	180,8± 11,2 %	100,0± 6,2 %

TABLE 5. Relative dissolution rate of indomethacin and its physical mixtures with γ-cyclodextrin as a function of time; *dissolution medium: distilled water* at pH 6,2;
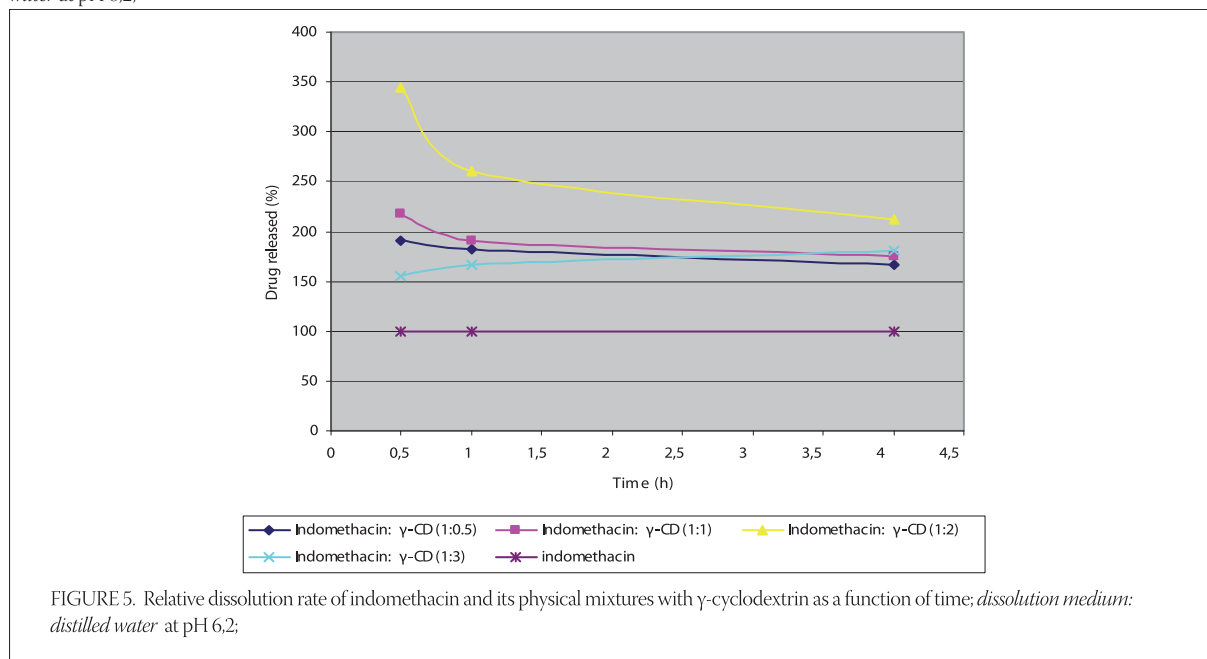

FIGURE 5. Relative dissolution rate of indomethacin and its physical mixtures with γ-cyclodextrin as a function of time; *dissolution medium: distilled water* at pH 6,2;

Discussion

It has been widely believed that drug availability in cyclodextrin-containing formulations will be hampered by the slow release of drug molecules from the cyclodextrin cavities. However, it has been shown that the rates for formation and dissociation of drug/cyclodextrin complexes are very close to diffusion controlled limits with complexes being continually formed and broken down (29). Consequently, presence of water-soluble drug/cyclodextrin complexes right at the hydrated epithelial surface will frequently increase the availability of dissolved drug molecules, especially of lipophilic drugs with poor aqueous solubility (30). Studies have shown that cyclodextrin enhance oral bioavailability of FDA's Class II (poor aqueous solubility, high permeability) drugs but they can hamper bioavailability of Class I (high solubility, high permeability) and Class III (high solubility, poor permeability) drugs (31) For a variety of reasons, including toxicological considerations, formulation bulk, production cost, drug bioavailability and isotonicity, it is important to use as small amount of cyclodextrin as possible in pharmaceutical formulations (32). Cyclodextrins (α- and γ-) can significantly increase the dissolution rate of low solubility substances, which on the other hand, can be reduced if the substance in ionized form. Unionized drugs do usually form more stable complexes than their ionic counterparts. However, ionization of a drug increases its apparent intrinsic solubility resulting in enhanced complexation (33). Indomethacin is a weak acid (pKa= 4,2) whose solubility is greatly dependent on the pH value of the dissolution medium (28) (pH 1,1: 0,001 mg/ ml; pH 5,0: 0,01 mg/ ml; pH 6,0: > 0,5 mg/ ml). This study was conducted to prepare inclusion compounds (complexes) with α- and γ -cyclodextrins and the insoluble drug indomethacin, and, to characterize the influence of pH on dissolution and solubility characteristics, among pure indomethacin and the *in situ* prepared complexes . As it was mentioned previously, α- i γ-cyclodextrins are cyclic oligomers composed of six and eight -(1,4)-linked glycosyl units, respectively. They form inclusion cavities (different dimensions) where complexes with insoluble drug indomethacin are formed (cavity volume (nm³): α-CD: 0,174, and γ-CD: 0,472). The central CD cavity provides a lipophilic microenvironment into which suitably sized drug molecules may enter and include. No covalent bonds are formed or broken during the drug/CD complex formation and in aqueous solutions, the complexes are readily dissociated. The rates for formation and dissociation of drug/CD complexes are very close to the diffusion controlled limits and drug/CD complexes are continuously being formed and broken apart (34). The percentage of indomethacin dissolved in 0,1 M hydrochloric acid *at pH 1,1* at the end of 4 hours was 100,0± 6,5 % (Table 2 and 4; Figure 2 and 4). Compared to pure indomethacin, indomethacin- α-CD physical mixture indicated an increase in the dissolution rate of indomethacin in the range of 148,5± 11,1 % to 337,1± 13,4 % (Table 2, Figure 2); indomethacin- γ -CD physical mixture indicated an increase in the dissolution rate of indomethacin in the range of 128,6± 8,1 % to 247,1± 12,2 % (Table 4, Figure 4). The percentage of indomethacin dissolved in *distilled water at pH 6,2* at the end of 4 hours was 100,0± 6,2 % (Table 3 and 5; Figure 3 and 5). Compared to pure indomethacin, indomethacin- α -CD physical mixture indicated an increase in the dissolution rate of indomethacin in the range of 103,4± 9,9 % to 110,8± 7,3 % (Table 3, Figure 3); indomethacin- γ-CD physical mixture indicated an increase in the dissolution rate of indomethacin in the range of 166,3± 10,1 % to 212,4± 14,2 % (Table 5., Figure 5.). Formation of cyclodextrin complexes is an equilibrium process where free guest molecules are in equilibrium with molecules in the complex. For this purpose, different technological processes can be used such as: kneading method, freeze-drying method or evaporation method, what, additionally made production procedure complicated. Preparation of physical mixtures of slightly soluble substances with cyclodextrins is more eligible in order to avoid additional, expensive, time consuming production procedures.

Conclusion

Indomethacin is poorly soluble in acidic medium (pH 1,1) and distilled water (pH 6,2). Solubility of indomethacin can be improved by formation of inclusion complexes with α-CD or γ-CD, regardless of molar ratio of the prepared physical mixture.

Increasing the molar ratio of certain cyclodextrins in physical mixture does not result in proportional increasing in solubility, probably due to the hydrophobicity of indomethacin, which, in aqueous dissolution medium leads to uneven level of *in-situ* complex formation

According to the results obtained in this study, the most optimal molar ratio(s) of:
◊ indomethacin and α-cyclodextrin at pH 1,1 were 1:0,5 and 1:1.
◊ indomethacin and γ-cyclodextrin at pH 1,1 were 1:2 and 1:3.
◊ indomethacin and γ-cyclodextrin at pH 6,2 was 1:2

Addition of α-cyclodextrin to indomethacin at pH 6,2, did not significantly influence its solubility. Proposed molar ratios (1:0,5; 1:1; 1:2 and 1:3) resulted in the similar enhancement of dissolution rate.

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