AAPS PharmSciTech, Vol. 15, No. 4, August 2014 (© 2014)
DOI: 10.1208/s12249-014-0110-2

Research Article

Experimental and Computational Studies of Physicochemical Properties Influence NSAID-Cyclodextrin Complexation

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Received 17 October 2013; accepted 13 March 2014; published online 10 April 2014

Abstract. The objective of this research was to investigate physicochemical properties of an active pharmaceutical ingredient (API) that influence cyclodextrin complexation through experimental and computational studies. Native β-cyclodextrin (B-CD) and two hydroxypropyl derivatives were first evaluated by conventional phase solubility experiments for their ability to complex four poorly water-soluble nonsteroidal anti-inflammatory drugs (NSAIDs). Differential scanning calorimetry was used to confirm complexation. Secondly, molecular modeling was used to estimate Log *P* and aqueous solubility (S_o) of the NSAIDs. Molecular dynamics simulations (MDS) were used to investigate the thermodynamics and geometry of drug-CD cavity docking. NSAID solubility increased linearly with increasing CD concentration for the two CD derivatives (displaying an A_L profile), whereas increases in drug solubility were low and plateaued in the B-CD solutions (type B profile). The calculated Log *P* and S_o of the NSAIDs were in good concordance with experimental values reported in the literature. Side chain substitutions on the B-CD moiety did not significantly influence complexation. Explicitly, complexation and the associated solubility increase were mainly dependent on the chemical structure of the NSAID. MDS indicated that each NSAID-CD complex had a distinct geometry. Moreover, complexing energy had a large, stabilizing, and fairly constant hydrophobic component for a given CD across the NSAIDs, while electrostatic and solvation interaction complex energies were quite variable but smaller in magnitude.

KEY WORDS: complexation; cyclodextrin; NSAID; simulation modeling; solubility.

INTRODUCTION

The Biopharmaceutics Classification System (BCS) predicts oral drug absorption based on the active pharmaceutical ingredient's aqueous solubility and intrinsic permeability through the gastrointestinal mucosa (1). Drugs that are highly permeable but have limited water solubility are considered BCS class II compounds. To improve bioavailability and the therapeutic effectiveness of such compounds, a number of formulation strategies to increase the dissolution rate of the active and/or enhance drug solubility have been employed. For example, reducing particle size of an active ingredient has been shown to increase the rate of drug dissolution and improve bioavailability (2–5). Examples of techniques to improve drug solubility include the addition of surfactants (6,7), use of co-solvents (8,9), formulating as amorphous solid dispersions (10,11), and cyclodextrin (CD) complexation (12–15).

CDs are cyclic oligosaccharides with a bucket-like structure having a hydrophobic internal cavity and a hydrophilic exterior. This unique structure allows for the formation of inclusion complexes, where lipophilic compounds are non-covalently bound within the cavity. CDs and a number of derivatives have been widely used in oral and parenteral drug delivery systems to improve aqueous solubility and chemical stability of drugs (2,16–19). Topical applications of CDs have also been investigated (20–22). Cyclodextrins differ by the number of glucose units forming the ring structure as well as chemical substitutions on the exterior of the "bucket." Modification through side chain substitutions has overcome the limited aqueous solubility of native β-cyclodextrin (B-CD) (23) and improved complexing capabilities (24).

While a number of papers on CD complexation have been published over the past 20+ years, many of the manuscripts are descriptive studies that characterize the solubility and/or dissolution increase of various poorly soluble compounds (25–28). Several papers have demonstrated CD complexation potential to enhance drug permeability and bioavailability (29,30). Other papers have presented models to predict complexation efficiency with various drugs (31,32,18).

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While these models highlight some of the physicochemical properties important for predicting complexation, such as molecular weight and Log *P* (31), the complexation process is still not well understood. In particular, the role of CD side chain substitutions on the interactions and complexation with ligand molecules is not clear. The current study investigated the complexation of four nonsteroidal anti-inflammatory (NSAID) propionic acid derivatives using β-cyclodextrin and two hydroxypropyl-substituted CD compounds differing in the degree of molar substitution. The goals of the study were to evaluate, characterize, and compare possible molecular mechanisms that might explain the observed differences in complexing for the set of 12 NSAID-CD complexes. Both laboratory experiments and computational modeling were synergistically employed in this study.

MATERIALS AND METHODS

Chemical Structures

The chemical structures of the NSAIDs are shown in Fig. 1. Racemic samples of ibuprofen (IBU), ketoprofen (KET), and naproxen (NAP) were purchased from Spectrum (New Brunswick, NJ), while flurbiprofen (FLU) was obtained from Sigma-Aldrich (St. Louis, MO). In the molecular modeling studies, both the R and S forms of each NSAID were built and individually considered in the modeling studies because racemic structures cannot be simultaneously modeled in a single computational experiment. The model drugs possess carboxylic acid functional groups at one end of the structure, while structural features differ at the other end of each compound.

Figure 2 shows the B-CD structure. The two CD derivatives consist of the same B-CD chemical backbone with varying degrees of hydroxypropyl side chain substitutions: one at a molar substitution of 0.87 (HP-CD) and the other at a molar substitution of 0.62 (HPB-CD). All CDs were provided by Roquette America, Inc. (Geneva, IL). The variable side chain substitutions for HP-CD and HPB-CD were taken into consideration in the molecular modeling studies by randomly placing side chain substituents consistent with the two molar "densities" (0.87 and 0.62) along the B-CD backbone structure for ten samples. The average properties calculated from these ten models were used to represent these two CD derivatives.

Phase Solubility Studies

Conventional phase solubility studies were conducted employing the Higuchi and Connors method (33). Due to its limited aqueous solubility, B-CD was used at a maximum concentration of 14 mM, whereas concentrations up to 200 mM were employed for the hydroxypropyl-substituted CDs. An excess amount of NSAID was added to aqueous solutions of increasing CD concentration. Deionized water alone (in the absence of CD) served as the control. In addition, the influence of pH on complexation was evaluated by phase solubility studies for HPB-CD solutions prepared in phosphate buffer (PBS, pH 7.4 at 24°C according to the USP32/NF method) at the same CD molar concentrations as the deionized water sets. PBS alone (without CD) was the control. The suspensions were mixed for 7 days on a LabLine Instruments table shaker (Melrose Park, IL) at a speed setting of 5½ (on the dial range of 0–10 corresponding to 40–1,100 rpm) and ambient temperature (between 22 and 24°C) to ensure a saturated solution. Aliquots of these solutions were allowed to settle and then filtered (0.45 μm) prior to subsequent use.

The filtered samples were lyophilized for differential scanning calorimetry (DSC) evaluation or analyzed for drug content using an Agilent Technologies (Santa Clara, CA) 1260 Infinity series HPLC system with an auto-sampler and a quaternary pump. The experimental conditions, adapted from (34), are shown in Table I. When necessary, samples were diluted with deionized (DI) water or PBS as appropriate.

Stability Constant and Complexation Efficiency Calculation

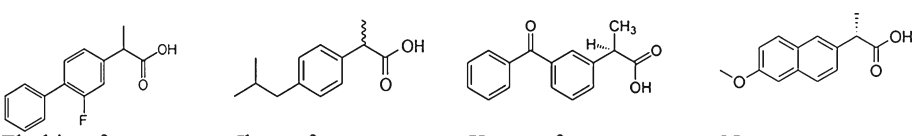
Stability constants ($K_{1:1}$) and complexation efficiencies (CE) of the NSAID-CD complexes were calculated, based on phase solubility experimental profiles, using Eqs. 1 and 2, respectively. In these equations, m is the slope of the NSAID solubility *versus* CD concentration (mM) graph as determined by linear regression. S_0 (mM) is the NSAID solubility in DI water or phosphate buffer as determined after 7 days of mixing. Since $K_{1:1}$ is strongly affected by S_0, CE was also calculated. CE may be considered more precise for evaluating the solubilization effects of cyclodextrins for compounds whose intrinsic solubility (S_0) is less than 0.1 mg/ml (35). An increase in drug solubility, expressed as a ratio of S/S_0 (mM/mM), was also calculated.

$$K_{1:1} = \frac{m}{S_0(1-m)} \tag{1}$$

$$\mathrm{CE} = \frac{m}{(1-m)} \tag{2}$$

Confirmation of Complexation

DSC (TA Instruments MDSC 2920, New Castle, DE) was performed to confirm drug-CD complexation. Drug and CD samples were used as received, whereas filtered samples from the phase solubility studies were lyophilized (FreeZone 4.5L Freeze Dry System, Labconco, Kansas City, MO) prior to



Flurbiprofen Ibuprofen Ketoprofen Naproxen

Fig. 1. Chemical structures of the NSAIDs used in the study

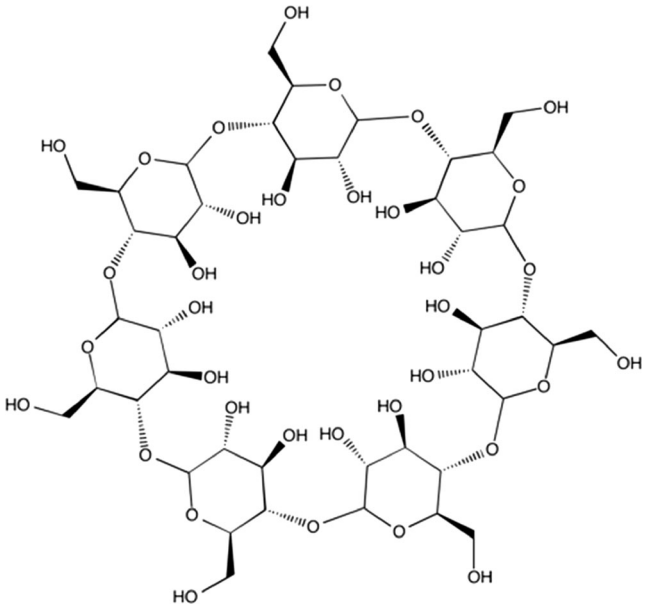


Fig. 2. Chemical structures of B-CD

DSC analysis. In addition, physical blends of drug and CD were prepared at a 1:1 molar ratio and analyzed. Powders were placed in aluminum pans and crimp sealed. Samples were equilibrated for 4 min at 10°C then heated to 225°C at a rate of 10°C/min. Thermograms of NSAIDs, CDs, and physical mixtures were compared to thermograms of lyophilized samples. The absence of a definitive phase 1 melting transition was considered indicative of complexation (20,36,37).

Molecular Dynamics Simulations (MDS) of NSAID-CD Complexation

The NSAIDs, CDs, and the NSAID-CD complexes were individually geometry optimized, and then explored, using the MDS package GROMACS (version 4.5.2 for Linux) with the ffgmx force field (a derivative of the GROMOS87 force field) using the Born solvation estimation (38). The ffgmx force field is advantageous in this application because it is comprised of small molecular functional groups instead of amino acid residues, allowing it to accommodate general organic structures. Steric effects of both NSAID and CD are accounted for with this model. The geometry optimization protocol consisted of two steps: geometry optimization of an initial molecular structure followed by MDS to bring the molecular system to equilibrium. Subsequent 500 ps MDS production runs, at 300 K, were performed starting with the equilibrated molecular system. Properties, including complexing features, were extracted from the MDS production run trajectories. The geometry optimization and all MDS were performed using the Born solvation estimation as part of the overall force field in order to generate relevant and equivalent thermodynamic data in a solvated medium. All complexing MDS were carried out at 1:1 molar concentrations of NSAID to CD. Note that it is not readily possible to perform concentration-dependent MDS of NSAID-CD complexing.

Table I. HPLC Experimental Conditions Used to Quantify NSAID Concentrations

Parameter	Condition
Mobile phase	37:15:48 ACN/MeOH/water (pH 3.2 with *ortho*-phosphoric acid)
Flow rate	1.1 mL/min (1.0 mL/min ketoprofen)
UV detector wavelength	230 nm (250 nm naproxen)
Column	Grace Econosphere C_{18} column 5 μm; 4.6 mm×15 cm

UV ultraviolet, *ACN* acetonitrile, *MeOH* methanol

Molecular Modeling Estimates of Log *P* and Log S_0

The Molecular Operating Environment (MOE, Chemical Computing Group, Montreal, Quebec, Canada) suite of molecular modeling software packages was used to estimate Log *P* and Log S_0 of the NSAID drugs and CDs (39,40). The MOE descriptors are a combination of empirically estimated chemical group type based upon the bonding topology and atom-type content of the molecule. The MOE-estimated Log *P* and Log S_0 values are based upon neutral (non-ionized) forms of each of the four NSAIDs. It is not generally possible to compute Log *P* and/or Log S_0 values for ionized/charged species using the group additive computational methodology of the MOE software.

An alternate set of computed Log *P* values reported by Pedraza *et al.* (41) were also considered. All Log *P* values were based upon the neutral (non-ionized) form of each NSAID, and there was no correction for possible ionization of the NSAIDs. It is important to note that current computational methods, including those employed in this study, cannot compute Log *P* and/or Log S_0 as a function of solution concentration.

RESULTS AND DISCUSSION

Solubility

Table II shows NSAID solubility (S_0) as determined experimentally in DI water and in a pH 7.4 aqueous phosphate buffer system. The pH of the saturated solutions in DI water ranged from 4.3 to 4.9. The solubility of all NSAIDs investigated was very low in the unbuffered DI water and increased significantly (approximately 100 times) in the pH 7.4 buffer, presumably due to ionization of the drug. Regardless of pH, ketoprofen exhibited the highest solubility followed by ibuprofen. Naproxen and flurbiprofen solubilities were similar. The calculated molecular modeling (MOE) aqueous solubility (Log S_o), converted to S_o, as well as the aqueous solubility of each NSAID as reported in the literature, is also given in Table II for comparison. For each individual NSAID, the solubility determined experimentally in unbuffered DI water, the MOE estimated solubility values, and the solubilities reported in the literature are generally in good agreement.

Figure 3 shows the drug solubility of the four NSAIDs as a function of B-CD concentration. The addition of B-CD did not significantly increase the solubility of flurbiprofen (maximum solubility increase (ratio of S/S_0) of only 1.79). In contrast, ketoprofen was solubilized to the greatest extent (S/S_0= 7.8), although maximum drug solubility was reached at a concentration of 12 mM B-CD, and higher B-CD concentration

Table II. Solubility (S_0) of NSAIDs (mM)

	DI water	MOE estimated solubility (neutral form)	Literature reported value[a]	PBS pH 7.4
Flurbiprofen (pK_a=4.3)	2.23×10^{-1}	2.9×10^{-1}	3.7×10^{-1}	18.2
Ibuprofen (pK_a=5.2)	3.86×10^{-1}	3.0×10^{-1}	1.0×10^{-1}	24.5
Ketoprofen (pK_a=4.8)	6.73×10^{-1}	4.8×10^{-1}	6.1×10^{-1}	35.1
Naproxen (pK_a=4.2)	2.88×10^{-1}	1.9×10^{-1}	8.7×10^{-2}	18.5

DI deionized, *MOE* Molecular Operating Environment, *PBS* phosphate-buffered saline
[a] From http://www.drugbank.ca (42)

did not further increase drug solubility, indicating type B_s complexation. The solubility of ibuprofen also plateaued, albeit at a lower B-CD concentration (4 mM). These results are in agreement with Salustio *et al.* (43) who reported type B_s complexation for ibuprofen in B-CD solutions. Only naproxen showed a linear increase in drug solubility as a function of B-CD concentration.

In contrast to B-CD, NSAID solubility increased significantly when the side chain-substituted CDs (HP-CD and HPB-CD) were used, as shown in Fig. 4. The increase in drug solubility was generally linear with increased CD concentration, indicating type A_L complexation or a 1:1 molar ratio of NSAID-CD. It is interesting to note that the increase in drug solubility was not significantly influenced by the extent of the hydroxypropyl side chain substitutions on the CD moiety. This can be seen readily in Table III, which shows the calculated solubility increase (S/S_0) at maximum CD concentrations. These results are consistent with the respective polar side chains of the two derivatized CDs adopting collective sets of conformational states which are on the exterior [outside] of each of the two CDs. Consequently, these side chains have little complexing interactions with molecules that enter the interior of the CD. That is, the interiors of the two CD cavities are largely the same for the two substituted CD compounds and thus would be expected to interact with each NSAID in a similar manner. In addition to inclusion complexes, CDs have also been reported to form non-inclusion complexes as well as aggregates of complexes (23,44). and in these cases, differences in solubilization capabilities with side chain substitutions would be expected. Since NSAID solubility increases were independent of the extent of side chain substitution, it is unlikely that such non-inclusion complexes or aggregated complexes were formed.

To further investigate the influence of polar side chain substitutions and the interior of the CD moiety on drug solubility based on experimentally obtained data, Fig. 5 compares drug solubility as a function of CD concentration between B-CD and the lower concentrations of the derivatized CDs. The hydroxypropyl side chains on the CD backbone increased the solubility of flurbiprofen and ibuprofen with no significant change observed in naproxen or ketoprofen solubility.

Log *P* and Lipophilicity

The MOE-based estimates of Log *P* values and values previously reported in the literature of the four studied NSAIDs are given in Table IV. The computed MOE values of Log *P* are in good agreement with reported experimental values, but not as good, overall, as the predicted values reported by Pedraza (41). The Pedraza Log *P* values are based upon a computational model in which the members of the set of chemical groups used to compute Log *P* of a compound were derived from, and unique to, specific classes of chemicals. In contrast, a "universal" set of chemical groups were used to compute Log *P* in the current MOE computational model. Thus, it is not surprising that the Pedraza Log *P* values are somewhat better than the MOE Log *P* values when compared to reported experimental data. However, the MOE computational model is less limited in terms of general use than the Pedraza model and thus potentially more broadly applicable because the chemical group database values required to compute Log *P* are more likely available for an arbitrary compound.

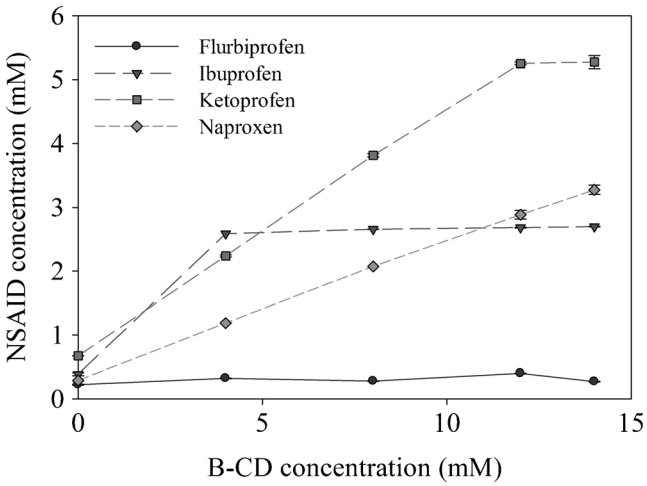


Fig. 3. NSAID solubility in DI water as a function of B-CD concentration

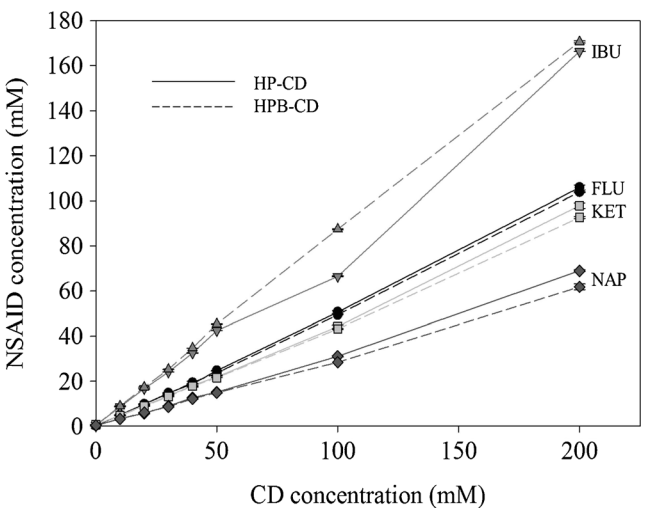


Fig. 4. NSAID solubility as a function of CD

Table III. Drug Solubility Increase Ratio (S/S_0)

	HP-CD in H_2O	HPB-CD in H_2O	HPB-CD in PBS
Flurbiprofen	110.41	105.83	2.45
Ibuprofen	110.02	117.36	2.08
Ketoprofen	32.49	31.97	1.34
Naproxen	52.39	51.39	2.04

HP-CD hydroxypropyl-β-cyclodextrin, 0.87 molar substitution, *HPB-CD*, hydroxypropyl-β-cyclodextrin, 0.62 molar substitution, *PBS* phosphate-buffered saline

Overall, the two types of computed Log *P* values and the reported experimental Log *P* values (see Table IV) are all in good agreement with one another for each of the four NSAIDs, respectively. Moreover, there is also good agreement between the respective computed and experimental S_o values (see Table II) of the four NSAIDs. Thus, the overall concurrence of the NSAID experimental and computed Log *P* and S_o values adds confidence in the reliability of discussing the roles of these properties in both the solubility and complexation behavior of the NSAID-CD systems of this study.

While a specific molecular mechanism cannot explain the observed differences in NSAIDs solubility, the overall behavior can be related to their relative Log *P* values. Flurbiprofen and ibuprofen are more lipophilic than naproxen and ketoprofen, having Log *P* values around 4, which are about one log unit (a factor of 10 on an absolute scale) greater than the Log *P* of the other two NSAIDs (around 3). The polar side chains of the derivatized CDs (HP and HPB) may provide an overall solvent environment which entropically drives the more lipophilic NSAIDS (flurbiprofen and ibuprofen) to preferentially initially enter into the CD cavity as compared to B-CD. The exterior surface of B-CD is more hydrophobic compared to both side chain substituted CD surfaces. Thus, the more lipophilic NSAIDs will find better, and more, relatively nonspecific binding sites on the exterior surface of B-CD than is thermodynamically available for the CDs with polar side chains. These many favorable exterior binding modes for B-CD then compete with entry of the NSAID into the B-CD cavity. In contrast, the polar side chains of HP- and HPB-CD decrease nonspecific, but favorable, surface binding of lipophilic NSAIDs as compared to B-CD and thereby make entry into their CD cavities a more thermodynamically preferred event. Thus, the greater the lipophilicity of the NSAID is, the greater is this overall differential thermodynamic binding behavior between B-CD and the CDs having polar side chains. Further, the favorable enthalpy associated with the hydrophobic interaction of the inserted NSAID with the interior of the CD cavity should be greater for the more lipophilic flurbiprofen and ibuprofen NSAIDs in comparison to less lipophilic naproxen and ketoprofen.

Stability Constants, Complexation Efficiencies, Binding Constants, and Complex Geometries

Experimental Stability Constants and Complexation Efficiencies

The calculated stability constants and complexation efficiencies (calculated from solubility experimental curves) of NSAID-derivatized CD complexes are summarized in

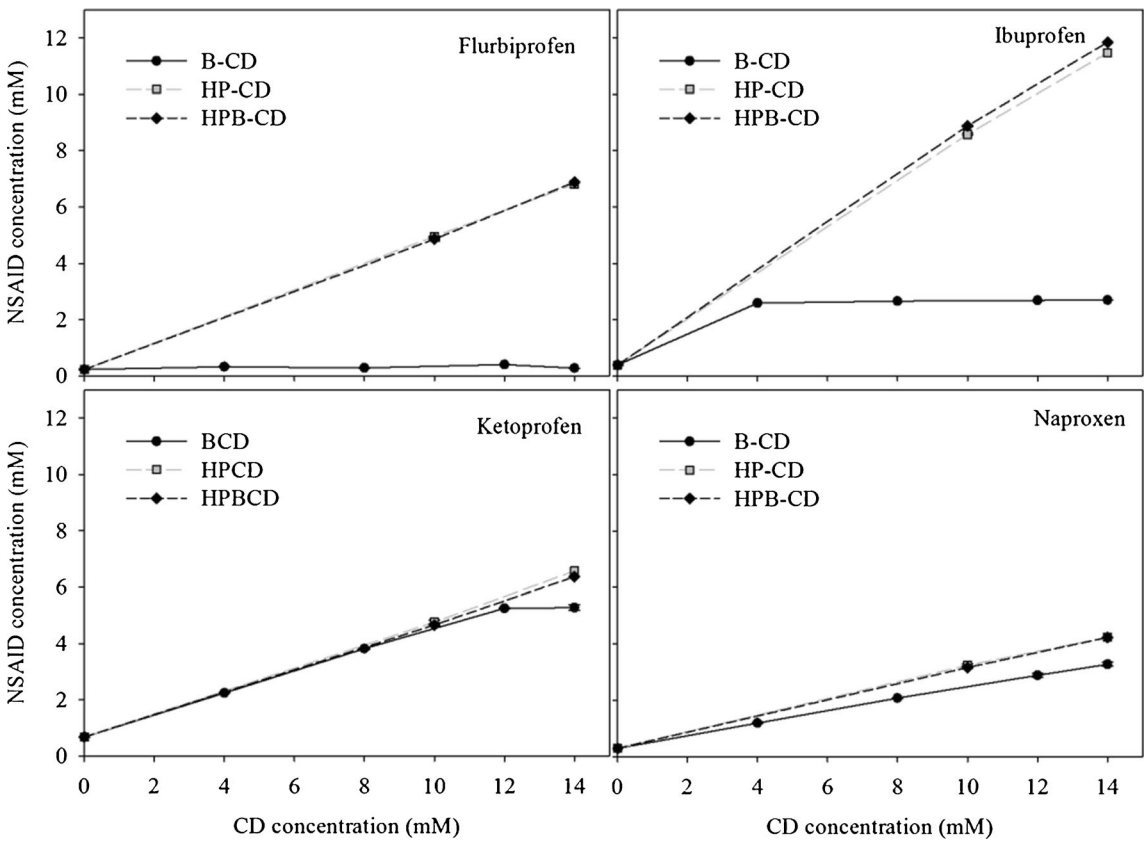


Fig. 5. Solubility increase profile for side chain-substituted CDs in comparison with B-CD

Table IV. Observed and Computed Log *P* Values for the Four NSAIDs of This Study

NSAID	Calculated Log *P* reported by (31)[a]	Log *P* predicted by the MOE Software	Log *P* experimental[b]
Flurbiprofen	4.2	4.2	4.16
Ibuprofen	4.2	3.6	3.97
Ketoprofen	3.1	3.6	3.12
Naproxen	3.2	3.3	3.18

MOE Molecular Operating Environment
[a] Theoretical octanol/water partition coefficient values obtained from the Virtual Computational Chemistry Laboratory and reported by Pedraza *et al.* (41)
[b] From http://www.drugbank.ca (5)

Tables V and VI, respectively. The calculated complexation efficiency values correlated well with the solubility data presented in Fig. 4, with the highest complexation efficiency of all NSAIDs noted with ibuprofen. It is interesting to note that similar complexation efficiencies were generally obtained for each NSAID, irrespective of the degree of hydroxypropyl side chain densities on the CD moiety.

The stability constant $K_{1:1}$ (M^{-1}) calculated from the experimental data for each of the complexes was converted to its corresponding free energy of complexation, ΔG, using the relationship

$$\Delta G = -RT \ln K_{1:1} \tag{3}$$

and is reported as part of Table VI. Overall, $K_{1:1}$ and ΔG indicate little difference in the complexation strength between HP-CD and HPB-CD for a given NSAID in the non-buffered solutions. In addition, similar complexation strengths between all NSAID-CD complexes in the non-buffered solutions were noted. The strength of complex binding is on the order of a strong hydrogen bond and about the same as found for ligands binding to protein drug carriers like human serum albumin (45).

Figure 6 shows the NSAID solubility *versus* HPB-CD concentration in DI water and in a phosphate buffer system. Clearly, the increase in solubility is dependent on the NSAID moiety. The solubility increase (S/S_0, Table VI) and the stability constants ($K_{1:1}$, Table VI) were significantly lower in the buffered system, irrespective of NSAID. At pH 7.4, all four NSAIDs (with pK_a values between 4.2 and 5.2) would be highly ionized. NSAID solubility in the absence of CD was approximately 100 times higher in the phosphate buffer than in DI water alone, as

Table VI. Complexation Efficiencies Calculated from Experimental Data

	B-CD	HP-CD	HPB-CD	HPB-CD in PBS[a]
Flurbiprofen	0.01	1.13	1.08	1.09
Ibuprofen	1.22	4.18	5.85	1.37
Ketoprofen	0.62	0.94	0.85	0.39
Naproxen	0.27	0.52	0.44	0.64

B-CD β-cyclodextrin, *PBS* phosphate-buffered saline, *HP-CD* hydroxypropyl-β-cyclodextrin, 0.87 molar substitution, *HPB-CD* hydroxypropyl-β-cyclodextrin, 0.62 molar substitution
[a] Calculated based on linear regression from 10 to 50 mM

shown in Table VI, and this could contribute to the lower solubility increase ratio and stability constants, since S_o appears in the denominator of both terms (i.e., S/S_o and Eq. 1). In addition, because of ionization and thus positive interactions with water through hydrogen bond formation, there is a decreased thermodynamic driving force for complexation. It should also be noted that the MOE-computed solubility of each of the four NSAIDs in Table VI is for the neutral form of the compound, and these values track with our experimentally reported solubilities in DI water and data reported in the literature. These observations would suggest that the NSAID solubility increase ratio and the stability constants are significantly higher when the NSAID is effectively in a neutral, unionized state. These results are in agreement with Perlovich and coworkers who found weaker interactions between drug and CD when the drug was ionized (46).

Complexation Confirmation by DSC

Thermal analysis was carried out to confirm drug-CD complexation. Figure 7 shows an example set of thermograms for ibuprofen and HPB-CD. Melt transitions were readily apparent for each NSAID alone, and the melting points were in good agreement with those reported in the literature. Melt transitions were also observed in the NSAID/CD physical blends, suggesting incomplete complexation by the CDs in this case. In contrast, the lack of a melt transition in the lyophilized samples is indicative of NSAID/CD complexation. Note that drug in water without CD present was subjected to the same lyophilization followed by DSC analysis and demonstrated that processing did not alter the physical state of the drug. The exception to these general observations was seen for ketoprofen and naproxen in B-CD, as very broad transitions in the lyophilized samples were detected (data not shown).

Table V. Experimental Stability Constants $K_{1:1}$ (M^{-1}) and the Calculated Corresponding Free Energy of Complexing (ΔG, kcal/mol)

	B-CD	HP-CD	HPB-CD	HPB-CD in PBS[a]
Flurbiprofen	5.48×10^{1} (−2.38)	5.05×10^{3} (−5.01)	4.83×10^{3} (−4.97)	5.97×10^{1} (−2.45)
Ibuprofen	3.17×10^{3} (−4.84)	1.08×10^{4} (−5.70)	1.51×10^{4} (−6.00)	5.58×10^{1} (−2.41)
Ketoprofen	9.22×10^{2} (−4.09)	1.40×10^{3} (−4.22)	1.26×10^{3} (−4.21)	1.13×10^{1} (−1.45)
Naproxen	9.44×10^{2} (−4.12)	1.81×10^{3} (−4.40)	1.53×10^{3} (−4.37)	3.45×10^{1} (−2.12)

B-CD β-cyclodextrin, *PBS* phosphate-buffered saline, *HP-CD* hydroxypropyl-β-cyclodextrin, 0.87 molar substitution, *HPB-CD* hydroxypropyl-β-cyclodextrin, 0.62 molar substitution
[a] Calculated based on linear regression from 10 to 50 mM

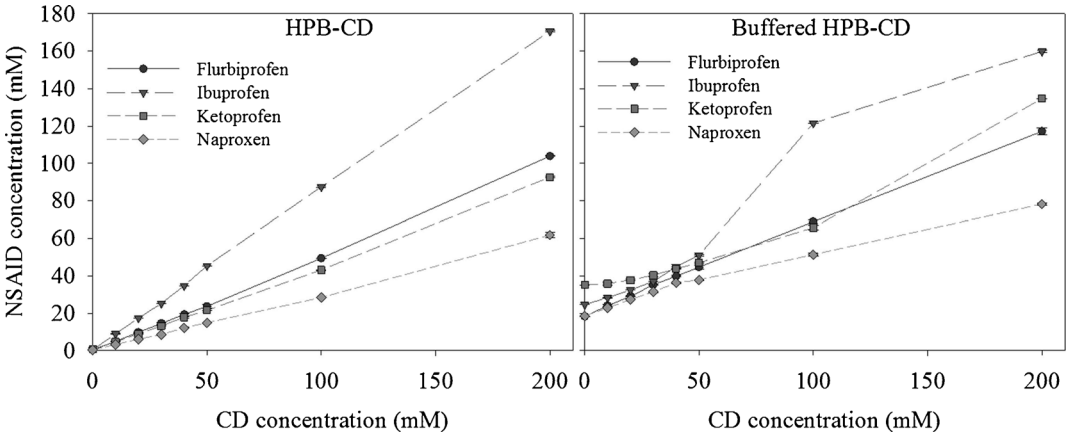


Fig. 6. Comparison of NSAID solubility increase in HPB-CD solutions of **a** DI water and **b** 7.4 phosphate buffer

MDS Calculations of Complex Binding Constants and Geometries

The lowest energy complex structures extracted from the MDS trajectories for the four NSAIDs in the B-CD bucket are shown in Fig. 8. Only the B-CD-NSAID complexes are shown because the side chains of HP-CD and HPB-CD interfere in visualizing the NSAID inside the CD. In each of the complexes, the B-CD is indicated by chemical bonds in yellow while the NSAID bonds are colored green. The structures on the left in each pair of images for a given NSAID are views looking down into the B-CD bucket from the "top" of the NSAID insertion point while those on the right are corresponding "side" views of the complexes.

An inspection of the lowest-energy geometries in Fig. 8 leads to three general observations across this set of complexes:

1. The carboxyl group, common to all four NSAIDS, protrudes out into the aqueous media from its position in the B-CD bucket. This geometry of complexing permits maximum favorable aqueous solvation of the carboxyl while still realizing substantial hydrophobic interactions between the interior of the B-CD and the non-polar portions of the NSAIDs.
2. The B-CD is flexible and adjusts its conformation to best accommodate the NSAID.
3. There does not appear to be any explicit pair-wise atomic interaction between a chemical group of the NSAID and a chemical group of the B-CD that overtly constrains the geometry of any of the complexes, that is, there is no specific interaction, such as a hydrogen bond between the NSAID and CD that drives the complexing process.

The computed binding free energies of CD-NSAID complexes (ΔG) which by definition are proportional to the experimental stability constants (Table VI) are given in Table VII. The computed binding free energies reported are in kilocalorie per mole, while the stability constants represent the ratio of bound to unbound NSAID to CD. These two measures are mutually complementary indicators of NSAID-CD complexing. The experimentally calculated $K_{1:1}$ values, however, are determined from a racemic mixture whereas

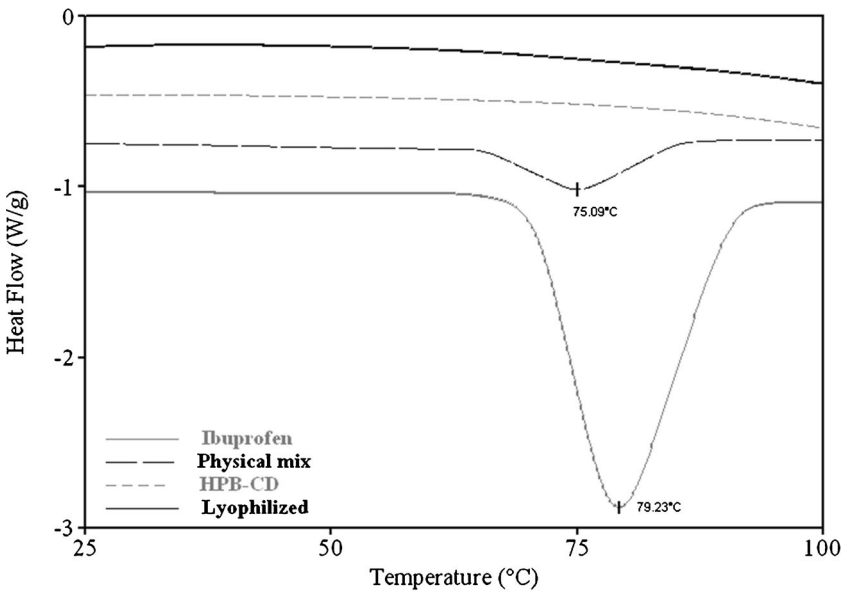


Fig. 7. DSC thermograms for ibuprofen/HPB-CD

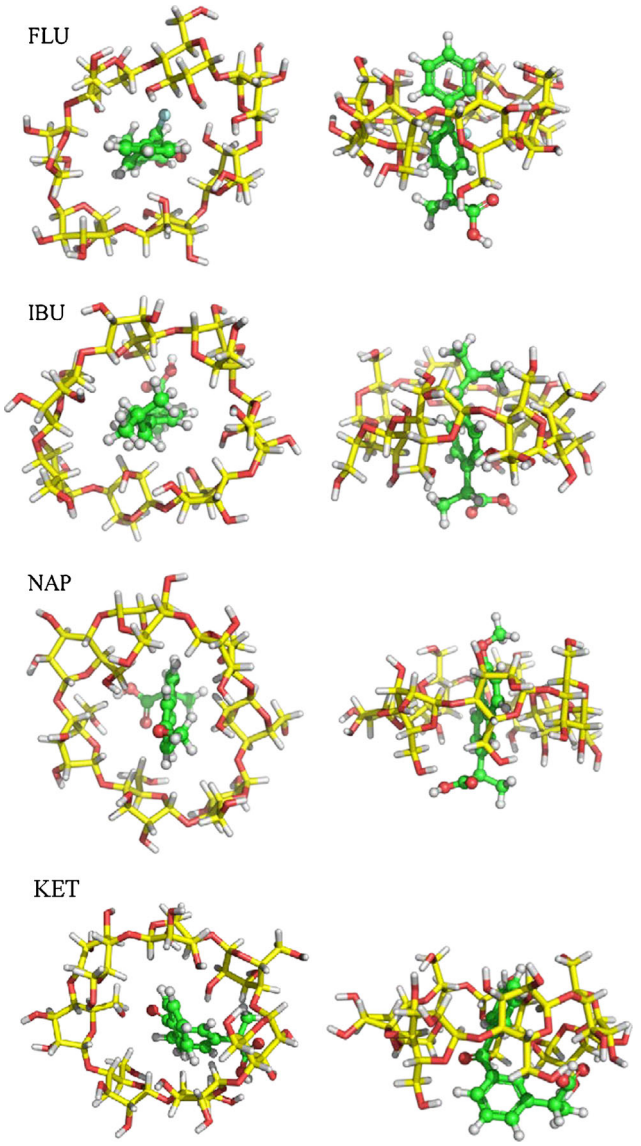


Fig. 8. Top (*left*) and side (*right*) structural representations of the complexes of R-NSAID-B-CD complexes. B-CD is delineated with *yellow* chemical bonds and the NSAIDs are denoted with *green* chemical bonds

Table VII. The Computed Binding Free Energies, ΔG, in kcal/mol, of the CD-NSAID Complexes

	B-CD	HP-CD*	HPB-CD[a]
Flurbiprofen	[R] −4.58	[R] −4.80	[R] −4.31
	[S] −3.46	[S] −4.04	[S] −3.68
Ibuprofen	[R] −4.19	[R] −4.59	[R] −4.73
	[S] −4.04	[S] −3.37	[S] −4.31
Ketoprofen	[R] −2.97	[R] −3.16	[R] −3.25
	[S] −3.51	[S] −3.00	[S] −3.43
Naproxen	[R] −4.14	[R] −4.43	[R] −4.02
	[S] −3.73	[S] −3.51	[S] −3.19

B-CD β-cyclodextrin, *HP-CD* hydroxypropyl-β-cyclodextrin, 0.87 molar substitution, *HPB-CD* hydroxypropyl-β-cyclodextrin, 0.62 molar substitution

[a] Statistical average ΔG estimates for ten diversely side chain-substituted B-CD backbone structures

the binding free energies (ΔG) obtained by MDS must be done explicitly for individual R or S isomers. In addition, the MDS-predicted ΔG are for a 1:1 molar ratio of NSAID-CD in a pure aqueous solution. In so far as these conditions were not met experimentally, the experimental $K_{1:1}$ values may be less in agreement with the predicted ΔG values. Nevertheless, there is a good correspondence between the data in Table V and in Table VII, demonstrating increasing $K_{1:1}$ values (increasing strength of complexing) and decreasing ΔG values (also a measure of increasing complexing propensity). Moreover, this correlative correspondence between $K_{1:1}$ and ΔG is also realized for the HP-CD-NSAID and HPB-CD-NSAID complexes in spite of the fact that the locations of the hydroxypropyl substituents are unspecified, and presumably random, for each stated densities of side chain substitution.

Overall, the NSAID isomers have been explicitly considered in the modeling, at least to the extent that each isomer was individually complexed with a CD. Moreover, the HP- and HPB-CD geometries arising from the different sampled distributions of their side chain substituents consistent with the reported substitution densities on the two CDs were also explicitly modeled. Some general features of CD-NSAID complexing were extracted from the MDS including, most notably, the binding thermodynamics reported in Table VII. The calculated ΔG of complexing (using the range defined by the two isomers of each NSAID) are in good agreement both in magnitude and in ranking to the values for the experimental racemic NSAID ΔG values given in Table VII as derived from the stability constants. Once again, the predicted composite magnitudes of the complexing energies (binding affinities) are in the low to mid-level range observed for ligands bound to protein drug carriers such as human serum albumin (13), that is, the MDS studies suggest that NSAID-CD complexing appears to be similar in thermodynamics to protein drug carriers. Each of the two isomers (R- and S-) of each of the NSAIDs exhibit moderately different binding affinities to a given CD. Moreover, the R-isomer is slightly preferred, as evidenced by a lower ΔG of complexing than the S-isomer, for 10 of the 12 complexes (see Table VII). Only ketoprofen showed a mixed isomer preference, as measured by its ΔG of complexing, across the three CDs. The two side chain-substituted CDs investigated in this study, expressed in terms of the statistical choices and extent of side chain substitution onto the B-CD backbone, have minimal impact on the free energy of complexing with a given NSAID. These findings are in agreement with the experimental phase solubility data presented in Fig. 4

The images in Fig. 8, as well as the free energy binding energies (ΔG) reported in Table VII, do not reveal the distribution of the internal energy contributions to the overall complexing energy of the NSAIDs with the CDs. These energy contributions are given in Table VIII for only the R-isomers of the NSAIDs with B-CD. The R- and S-isomer internal energy contributions are largely, on a relative basis, the same but not equivalent. We have not attempted to parse out the individual internal energy contributions to the overall complexing energies of the NSAIDs with HP- and HPB-CD, the two CDs having substituted side chains. The calculated thermodynamic properties for each of these two side chain-substituted CDs, as stated in the "MATERIALS AND METHODS" section, represent the Boltzmann ensemble-averaged thermodynamic behavior from ten CDs each having chemically different side chain-substituted distributions

Table VIII. Computed Complex Interaction (Internal) Energy Contributions (in kcal/mol) of B-CD with the R-Isomers of the NSAIDs

Energy contribution	Flurbiprofen	Ibuprofen	Ketoprofen	Naproxen
Dispersion/hydrophobic	−27.2	−23.4	−26.4	−32.2
Electrostatic + hydrogen bond	−25.5	−11.1	−9.3	4.6
Solvation	26.9	11.7	12.5	−2.7
Total	−25.8	−22.8	−23.2	−30.3

subject to the constraint of a fixed overall percentage. It is not meaningful to select any individual member of these sets as having internal energy contributions representative of the entire set of ten. It is important to note that the complexing energy contributions presented in Table VIII involve only the direct interactions between B-CD and each of the four NSAIDs in an aqueous solvated medium. In contrast, the binding free energies in Table VII are the differences in the free energies (which include the entropy extracted from the MDS trajectories) between the 12 possible solvated NSAID-CD complexes as compared to the sum of the individual isolated solvated CD and isolated individual solvated NSAID internal energies.

An inspection of Table VIII indicates that the overall interaction complexing internal energy between the CD and NSAID has a large, stabilizing dispersion/hydrophobic component for B-CD across all four NSAIDs. Electrostatic energies, including hydrogen bonding and solvation interaction complex energies, between B-CD and each of the NSAIDs are quite variable. However, the sum of the electrostatic and solvation energies is a small, near constant (1 to 2 kcal/mol) value for the four NSAIDs complexing with B-CD. Thus, there appears to be a trade-off between NSAID-CD electrostatic and hydrogen bonding interactions and loss in aqueous solvation energy upon complexing. Ultimately, this trade-off yields about the same net interaction complexing energy for all four NSAIDs with B-CD. Still, while there is this extreme sensitivity between solvation and electrostatic interaction energy of the complexes, it is the hydrophobic energy of complexing which dominates in complex formation.

In terms of the total internal interaction energy, given in the last row of Table VIII, naproxen has the strongest interaction energy with B-CD when complexed. The superior interaction between naproxen and B-CD, as compared to the three NSAIDs, may be the reason why only naproxen showed a linear increase in drug solubility as a function of B-CD concentration in Fig. 8.

Two avenues that might be exploited in designing new CD systems for new drugs are suggested from the data in Table VIII. First, how can the overall hydrophobic energy of complexing be enhanced without decreasing drug solubility? Secondly, how can the trade-off in electrostatic and hydrogen bonding energy be maximized in favor of overall stronger complexing?

CONCLUSIONS

Experimental and computational studies were conducted to characterize the complexing of four NSAIDs with three different CDs to identify physicochemical properties that influence complexation with cyclodextrins. The computed Log P and S_o values determined using the MOE computational model were in good agreement with experimental values. This agreement between computation and experiment results lends support to the reliability and accuracy to results achieved in this study. Drug solubility was found to increase significantly when the hydroxypropyl side chain-substituted CDs were used in comparison to the native B-CD, for all four drugs investigated. These results were attributed to the higher water solubility of the hydroxypropyl derivatives as well as the more hydrophilic exterior that promotes entry of the hydrophobic portion of drug into the interior cavity.

Overall, each NSAID-CD complex has a distinct molecular geometry which is primarily attributed to the extent to which the NSAID is inserted into the CD interior. Although NSAID-CD binding thermodynamics were moderately sensitive to the NSAID isomer, the NSAID-CD geometry, including the extent of NSAID insertion, was not particularly dependent on the isomer but rather on its chemical structure. The CD appears to be able to adjust its conformation to best accommodate the shape of the NSAID in order to minimize the free energy of the complex. MDS indicated that hydrophobic and electrostatic interactions between drug and CD are primarily responsible for the experimentally observed solubility and stability constants. Interaction energy contributions from dispersion/hydrophobic forces appear similar for all NSAIDs but also are the dominant interactions in the complexing process. The electrostatic (including hydrogen bonding) and solvation contributions vary for individual drugs but to a reasonable degree also tend to cancel one another in the complexed state.

ACKNOWLEDGMENTS

Funding for this work was supported in part by Roquette Freres. Computational facilities from both Exe Research LLC and The Chem21 Group, Inc. were made available in order to perform the modeling and molecular dynamics simulation studies reported in this paper.

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