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Correlation between the Stability Constant and pH for β-Cyclodextrin Complexes

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Abstract

In drug formulations, cyclodextrins are used to increase aqueous solubility and chemical stability of drugs via formation of inclusion complexes. For ionizable drug molecules, the complexation strength depends on pH. Increased ionization leads to a more soluble drug, but also results in destabilization of cyclodextrin complexes. Therefore, formulation scientists aim to find a balance between increased drug solubility and high complexation strength. In this work, a theoretical expression for the dependency between the stability constant and pH is presented, allowing the accurate prediction of the stability constant at any pH. The theoretical expression requires three out of four input parameters; the pK_a of the free guest molecule, the pK_a of the complex, and the stability constants for the neutral and fully ionized complex. Stability constants for β-cyclodextrin and ibuprofen complexes were determined by isothermal titration calorimetry at seven pH values (2.5-5.5) and four temperatures (15-55˚C). All these measured stability constants complied with the theoretical expression. Ten additional data sets from the literature comprising eight different drug molecules and three different cyclodextrins confirmed the ability of the theoretical expression to account for the observed pH-dependence of stability constants.

Keywords: pH-dependency; Equilibrium constant; Guest molecule; Hydrophobic interactions; Ionization; Drug formulation;

1. Introduction

Cyclodextrins (CDs) are a widely used excipient in the pharmaceutical and food industries (Jansook et al., 2018; Kurkov et al., 2011). CDs are derived from starch, and consist of several (α-1,4)-linked glucopyranose units (Loftsson et al., 2007; Loftsson and Brewster, 1996). The natural and most common CDs are αCD, βCD and γCD, which consist of 6, 7 or 8 glucopyranose units, respectively. Modification of natural CDs has resulted in pharmaceutically relevant derivatives, including hydroxypropyl-βCD (HP-βCD) and sulfobutylether-βCD (SBE-βCD). CDs have a cone-like structure with a hydrophobic cavity and hydrophilic outer surface (Brewster and Loftsson, 2007). The application of CDs are based on their ability to form inclusion complexes in aqueous solutions (Astray et al., 2009; Jambhekar and Breen, 2016). Through complex formation, CDs can be used to increase solubility, hide unpleasant odor or taste, and improve the chemical stability of active molecules (Astray et al., 2009; Dargó et al., 2018; Junquera et al., 1998; Loftsson and Duchêne, 2007; Perlovich et al., 2003). The ability to increase solubility is particularly important, as many potential drug molecules have low aqueous solubility (Williams et al., 2013).

Interactions between CDs and drug molecules are often accompanied by pH regulation. Altering the pH is often used to increase aqueous solubility of potential drug molecules, because ionization affects the polarity (Williams et al., 2013). Many potential drugs are ionizable compounds, so their solubility depends on pH (Williams et al., 2013). Changes in pH can also affect the chemical stability, because degradation pathways are often affected by pH (Karow et al., 2013). To ensure the high quality of drug and food products, regulation of pH is essential. However, in drug formulations that include CDs, the pH affects the stability constant of the equilibrium between CDs and the guest molecule. The extent of the interaction between CD and guest molecule is governed by the stability constant, and is therefore important for the ability of CDs both to solubilize the guest and the

overall stability of the formulation. The balance between a high stability constant and a suitable solubility for any given formulation is a matter for optimization. Hence, it is important to have a thorough understanding of the relationship between the stability constant and pH.

Several studies empirically describe the effect of pH on complex formation between CDs and ionizable guest molecules (e.g. Al Omari et al., 2006a; Danel et al., 2008; di Cagno, 2016; Jug et al., 2009; Kuwabara et al., 2006; López-Miranda et al., 2018; López-Nicolás et al., 2009; Perlovich et al., 2003). The equilibrium constant (K) for complex formation is affected by pH. The neutral form of a molecule generally forms a stronger complex than the ionized form, because the neutral molecule is more hydrophobic and has a higher affinity for the hydrophobic CD cavity. The relationship between the stability constant and pH has been reported for several guest molecules, including risperidone, pterostilbene, and ibuprofen (Jug et al., 2009; López-Nicolás et al., 2009; Perlovich et al., 2003). However, no quantitative theoretical relation between K and pH is published. A theoretical expression defining how the stability constant depends on pH allows one to predict how changes in pH affects the stability constant, and hence the stability of a formulation.

The aim of the present work is to derive a theoretical expression for the stability constant as a function of pH and test its validity with various drug:CD complexes. The theoretical expression is potentially a valuable tool for formulation scientists who currently rely on general experience. It permits the determination of the stability constant at any degree of ionization of the guest molecule. To validate the theoretical expression, stability constants for a model system of β-CD and ibuprofen were determined at four temperatures and seven pH values. Ibuprofen was chosen as a model drug since it is known to form an inclusion complex with β-CD (Castronuovo and Niccoli, 2013; Waters et al., 2010; Xing et al., 2009). Ibuprofen is a monoprotic acid meaning that a neutral form of

ibuprofen exists at low pH and an ionized form exists at high pH. In addition, the theoretical expression was validated on ten published data sets found for different complexes between drugs and β-CDs. The ten data sets contain a total of eight different guest molecules and three different β-CDs.

2. Material and Methods

2.1 Reagents

Ibuprofen (CAS: 15687-27-1, ≥98%, M_w = 206.28 g/mol) and β-CD (CAS: 7585-39-9, ≥97%, M_w = 1134.98 g/mol) was purchased from Sigma-Aldrich and used as received. All other chemicals used were of analytical grade.

2.2 pK_a determination

Determination of pK_a for ibuprofen and the β-CD:ibuprofen complex was carried out using the Sirius T3 titrator (Sirius Analytical Instruments Ltd, East Sussex, UK), measuring the UV-VIS spectrum during titration. Experiments were conducted at 25˚C. The ionic strength was kept constant by using water containing 0.15 M potassium chloride. The pK_a of the complex was determined in water, and the pK_a of ibuprofen was, due to poor solubility, determined using DMSO as a co-solvent and extrapolated to 0% co-solvent by the Yasuda-Shedlowsky method. All data were processed using the accompanying Sirius software.

2.3 Isothermal Titration Calorimetry

Isothermal Titration Calorimetry (ITC) was used to determine the stability constant for complexation between β-CD and ibuprofen at seven different pH values from 2.5-5.5 at 0.5 pH unit intervals and at four different temperatures: 15, 25, 40, and 55˚C. Both β-CD and ibuprofen were

prepared in 50 mM sodium acetate buffer. The pH of the solution was adjusted at room temperature with either 1 M NaOH or 10% HCl to obtain the desired pH value. The pK_a value of acetate buffer has been shown to change very little within the temperature range used in this study (Fukada and Takahashi, 1998), thus this change was neglected. As ibuprofen has a low solubility in water, concentrations in the range 0.2-0.5 mM were used, depending on the pH of the buffer. The concentration of β-CD in each experiment was 10 times the concentration of ibuprofen. ITC measurements were performed using a MicroCal VP-ITC MicroCalorimeter (Malvern Panalytical, Worcestershire, UK). Ibuprofen solution was loaded into the calorimetric cell, having a total volume of 1.4257 mL. The 250-µL injection syringe was loaded with β-CD. Titrations were carried out by adding 10 µL titrant (except for the first injection which was 4 µL) until the syringe was emptied. The titrant was injected during 20 s with an interval of 300 s between injections to ensure return to baseline. The stirring speed was 310 rpm. The heat of dilution was corrected for by subtracting the heat signal for a blank titration of titrant into buffer without ibuprofen. After collection of a complete data set for a specific pH value, i.e. ITC data for all five temperatures, the data was globally fitted using an in-house MatLab script that assumes that the temperature dependence of the binding constant was governed by the van't Hoff equation (Schönbeck et al., 2012).

3. Results

3.1 ITC determination of stability constant for the ibuprofen:β-CD complex

Data from several ITC experiments are presented in Figure 1. Using the MatLab script, the data was curve-fitted to yield the stability constant and the reaction enthalpy. The experiments were performed at several pH values and at 15, 25, 40, and 55°C, which means that 35 titrations were performed. The stability constant depended on both temperature and pH (Fig. 2). The van't Hoff

equation describes the temperature-dependency of the stability constant, which decreased with increasing temperature. There was a good fit between theoretical van't Hoff calculations and data obtained by ITC, which indicated reliable data since the van't Hoff expression can be used as an internal check of calorimetric data (Kantonen et al., 2018). The stability constant was 1.06×10^4 M^{-1} at 25˚C and pH 5.5. This value corresponds to published values which range from 0.7 to 1.9×10^4 M^{-1} at values of pH where ibuprofen was un-ionized (Bertaut and Landy, 2014; Cirri et al., 2006; di Cagno et al., 2011; Hergert and Escandar, 2003; Manzoori and Amjadi, 2003; Mura et al., 1998; Waters et al., 2010; Xing et al., 2009). One study reported a higher value of 5.0×10^4 M^{-1} at pH 7.4 (Castronuovo and Niccoli, 2013). The binding enthalpy was determined to be -16.5 kJ/mol, which was consistent with the published value of -14.0 kJ/mol for the same system (Bertaut and Landy, 2014). The heat capacity was -402 J/mol/K in the present study, which is consistent with the -394 J/mol/K previously reported (Bertaut and Landy, 2014).

3.2 pK_a determination of ibuprofen and ibuprofen:β-CD complex

The pK_a values for ibuprofen and the ibuprofen:β-CD complex were determined from changes in the UV spectrum during a titration (Fig. 3). As a compound becomes charged, the UV absorbance changes, which allows the determination of pK_a. The measured pK_a for ibuprofen was 4.53, which was consistent with the pK_a range of 4.4-5.2 reported in the literature (Avdeef, 2007; Avdeef et al., 2000; Loftsson and Brewster, 1996; Perlovich et al., 2003). The measured pKa for the ibuprofen:β-CD complex was 4.70. This was expected to differ from the pK_a value for free ibuprofen due to its interaction with CD.

3.3 Theoretical expression for the stability constant as function of pH

It is essential to understand the effect of pH on CD:drug complexation, as many drugs are ionizable, are thus sensitive to changes in pH. A theoretical expression will allow scientists to predict this sensitivity. A theoretical expression for the stability constant, K_B, as a function of pH can be derived from physical chemical principles. Reactions between drugs and CDs are equilibrium reactions.

$$D + CD \rightleftharpoons D{:}CD \tag{1}$$

The equilibrium constant, often referred to as the stability constant, for CD complexes, can be expressed in terms of the concentrations of the reacting species.

$$K_B = \frac{[D{:}CD]}{[D][CD]} \tag{2}$$

For ionizable compounds with one pK_a value, equilibria can be written for both neutral and ionized species.

$$HD + CD \rightleftharpoons HD{:}CD \tag{3}$$

$$D^- + CD \rightleftharpoons CD{:}D^- \tag{4}$$

Where HD represents the neutral form of the drug, and D^- represents the charged form. Correspondingly, also two equilibrium constants also exist.

$$K_{neu} = \frac{[HD{:}CD]}{[HD][CD]} \tag{5}$$

$$K_{ion} = \frac{[CD{:}D^-]}{[D^-][CD]} \tag{6}$$

Thus, the overall stability constant for the drug can be expressed as follows.

$$K_B = \frac{[D{:}CD]}{[D][CD]} = \frac{[HD{:}CD]+[CD{:}D^-]}{([HD]+[D^-])[CD]} = \frac{[HD{:}CD]}{([HD]+[D^-])[CD]} + \frac{[CD{:}D^-]}{([HD]+[D^-])[CD]}$$

$$= \frac{[HD]}{[HD]+[D^-]}\frac{[HD{:}CD]}{[HD][CD]} + \frac{[D^-]}{[HD]+[D^-]}\frac{[CD{:}D^-]}{[D^-][CD]} \tag{7}$$

Parts of the equation can be substituted with the terms K_{neu} and K_{ion}.

$$K_B = \frac{[HD]}{[HD]+[D^-]}K_{neu} + \frac{[D^-]}{[HD]+[D^-]}K_{ion} \tag{8}$$

The concentrations of the neutral and charged drug are equal to the total concentration of the drug, also referred to as the formal concentration of the drug.

$$C_D = [HD] + [D^-] \tag{9}$$

Thus, the fractions in equation 8 can be expressed as the dissociation degree α:

$$\alpha = \frac{[D^-]}{C_D} \tag{10}$$

This allows the theoretical expression to be further simplified.

$$K_B = (1 - \alpha)K_{neu} + \alpha K_{ion} \tag{11}$$

This demonstrates how the equilibrium constant of the drug:CD complex can be expressed as a function of the dissociation degree and the equilibrium constants for the neutral and charged drug:CD complex. The dissociation degree of the drug depends on the pH. The dissociation degree can be expressed in terms of the ionized and neutral form of the drug.

$$1/\alpha = 1/\frac{[D^-]}{C_D} = 1/\frac{[D^-]}{[HD]+[D^-]} = \frac{[HD]}{[D^-]} + 1 \tag{12}$$

The ionized and neutral form of the drug represents the base and acid, and the Henderson-Hasselbalch equation describes how the pH is correlated with the concentrations of the acid and base forms of the drug.

$$pH = pK_a + log\frac{[base]}{[acid]} = pK_a + log\frac{[D^-]}{[HD]} \tag{13}$$

$$\frac{[D^-]}{[HD]} = 10^{pH-pK_a} \tag{14}$$

$$\frac{[HD]}{[D^-]} = 10^{pK_a-pH}$$

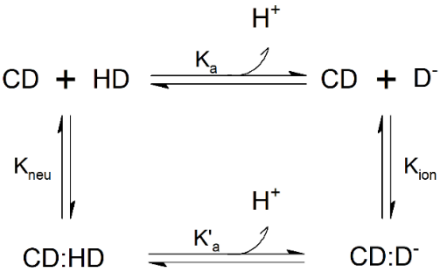
By combining these expressions, it is possible to express K_B as a function of pH with the pK_a, K_{ion} og K_{neu}.

$$K_B = \left(1 - \left(1/(10^{pK_a-pH} + 1)\right)\right)K_{neu} + \left(1/(10^{pK_a-pH} + 1)\right)K_{ion} \tag{15}$$

This expression can predict how K_B depends on pH for a monoprotic acid with a 1:1 binding mechanism between CD and drug. A similar expression exists for a monoprotic base.

$$K_B = \left(1 - \left({}^{1}/_{(10^{pH-pK_a}+1)}\right)\right)K_{neu} + \left({}^{1}/_{(10^{pH-pK_a}+1)}\right)K_{ion} \quad (16)$$

It is necessary to experimentally determine pK_a, K_{ion} and K_{neu} according to the theoretical expression. The binding equilibria for the ionized and the neutral species are linked by protonation reactions, and this equilibria diagram shows the relationship between K_{neu}, K_{ion}, K_a and K'_a, and it can be expressed mathematically.



$$K_{neu}K'_a = K_{ion}K_a \quad (17)$$

The relationship between K_{neu}, K_{ion} and the two pK_a terms has been published (Connors and Lipari, 1976). This link is important as it demonstrates that the pH dependence of K_B can also be expressed in terms of the two pK_a values and either K_{ion} or K_{neu}.

Theoretically, it is possible to predict the stability constant at any pH by using Eq. 15 or 16. To use the theoretical expression, three out of the four parameters in Eq. 17 must be known; i.e., the pK_a values of the free and bound guest molecule, and the equilibrium constants for the binding of the neutral and ionized guest.

4. Discussion

4.1 Empirical confirmation of theoretical expression based on ibuprofen:β-CD

The stability constant for ibuprofen:β-CD was used to confirm the theoretical expression derived above (Eq. 15). The results showed a nice correlation between the theoretical expression and the empirical data (Fig. 4). The three input parameters in the theoretical expression are K_{neu}, K_{ion} and pK_a for free ibuprofen. K_{neu} and K_{ion} were estimated based on the relationship between K and the ionization degree at different pH values (Eq. 11). The pK_a value of ibuprofen was 4.53. The results showed that at higher pH, where ibuprofen was ionized, the tendency to form complexes was lower. This has also been reported for other acidic compounds (Al Omari et al., 2006).

As described in section 3.2, the theoretical expression requires three input parameters. In this study, we have mainly used K_{neu}, K_{ion} and pK_a for the free molecule. In theory (Eq. 17), the theoretical expression will yield the same result no matter which three out of the possible four input parameters are chosen. In the example with ibuprofen:β-CD, the measured stability constants show a difference of 0.3 pH units between the pK_a value of the free guest molecule and the pK_a value of the complex (Eq. 17). However, the pK_a value of ibuprofen was determined to be 4.53, and the pK_a value of the complex was determined to be 4.70, resulting in a difference of only 0.17 pH units. A small change in either pK_a value will affect the theoretical expression. It is common that pK_a values reported in the literature differ by up to 0.8 pH units, thus using these value will yield uncertain predictions of the stability constant as function of pH. Therefore, we suggest using K_{neu}, K_{ion} and pK_a of the free molecule as input parameters.

4.2 Comparison of theoretical expression with empirical data sets from the literature

The theoretical expression relies on physical chemical principles, and should be valid for all CD complexes. In this work, the theoretical expression was verified for complexes with β-CD, HP-β-CD and SBE-β-CD (shown in Figs. S1-S4). Several studies were found in the literature where the

stability constant for the neutral and ionized drug:CD complex was presented along with the pK_a value of the drug. Four complexes are presented (Table 1) fully in the main text, whereas additional six examples can be found in the supplementary material (Figs. S1-S4). The examples comprise drug:CD complexes with significant difference in the chemical structure and physicochemical properties of the drug molecules.

Table 1. Published stability constants at specific pH values and the corresponding ionization degree of the molecules for three CD complexes. The ionization degree was calculated from the reported pK_a values.

Complex	pH	Stability constant	Ionization degree (%)
Pizotifen:β-CD (pK_a = 8.9)	3.4	460	100.0
	6.8	1720	99.2
(Al Omari et al., 2008)	7.2	2300	98.0
	8.7	32000	61.3
	11.7	72000	0.2
Ketotifen:β-CD (pK_a = 8.5)	5.9	1.5	99.7
	6.8	470	98.0
(Al Omari et al., 2008)	7.2	870	85.2
	8.7	3800	38.7
	12.1	10000	0.0
Ibuprofen:HP-β-CD (pK_a = 4.41)	3.2	11600	5.8
	4.0	11000	25.7
(Perlovich et al., 2003)	5.0	7300	79.6
	6.2	4500	98.4
	7.0	4000	99.7
	8.0	4000	100.0

Based on the data published in these studies, the theoretical dependence of the stability constant as a function of pH for these complexes was successfully predicted.

Both pizotifen and ketotifen can both form complexes with β-CD (Al Omari et al., 2008). Complex formation was investigated at pH; 3.4, 5.9, 6.8, 7.2, 8.7, 11.7 and 12.1 covering the neutral and fully ionized form of the compounds with pK_as of 8.9 and 8.5, respectively (Al Omari et al., 2008). The pK_a values as well as K_{neu} and K_{ion} for pizotifen and ketotifen (values presented in Table 1) were used to plot the theoretical expression (Eq. 16). The measured stability constants for pizotifen:β-CD were in agreement with the plotted theoretical expression, while the stability constants for ketotifen:β-CD showed small deviations (Fig. 5). It is common for reported stability constants to vary for the same complex, particularly when different experimental methods are used in the

determination. Thus the small deviations from the theoretical expression are well within an acceptable variation. Other examples of pH-dependent complexation for similar complexes can be found in the supplementary material (Figs. S1-S4). The theoretical expression (Eq. 15 and 16) was well fitted by all presented empirical data sets.

The theoretical expression assumes a simple equilibrium reaction between CD and the guest molecule. The derivatives of the natural CDs, e.g. HP-β-CD, have a more complicated structure because the substitution of the hydroxyl groups on β-CD is random (Jansook et al., 2018). Thus, a solution of HP-β-CD consists of an isomeric mixture of different HP-β-CDs. The same principle holds for other derivatives of the natural CDs, e.g., SBE-β-CD. However, the stability constant of complexes with HP-β-CD is measured as a bulk stability constant and represents an average stability constant, thus the theoretical expression is still valid. The published stability constant for risperidone:HP-β-CD is measured at five different pH-values (Jug et al., 2009), and the theoretical expression was in agreement with the empirical data (Fig. S5). The same conclusions are valid for 7-hydroxy-4-methylcoumarin:SBE-β-CD, where the theoretical expression was consistent with the empirical data (Fig. S4). According to these data, the theoretical expression can also be verified for derivatives of β-CD complexes. In principle, the theoretical expression should apply to complexes with α-CDs and γ-CDs as well, but the present study is limited to β-CDs.

The stability constant for ibuprofen:HP-β-CD is described in the literature (Perlovich et al., 2003). The stability constant was reported for the pH range 3.2-8.0, and the empirical data were used to find the theoretical dependency (Eq. 15) for the complex as a function of pH (Fig. 6). Input parameters used were K_{ion}, K_{neu} and pK_a. K_{neu} was not reported, because at pH 3.2, only 94.2 % of ibuprofen will be neutral. This will cause some error resulting in a discrepancy between the

empirical data and the theoretical expression. All data points were systematically to the right of the theoretical expression line when using the reported pK_a value of 4.41 (Fig. 6, solid line). This indicated that the pK_a value used as input in the theoretical expression was lower than the actual pK_a value in the experiment. The reported pK_a value for ibuprofen varies in the range 4.4-5.2 (Avdeef, 2007; Avdeef et al., 2000; Loftsson and Brewster, 1996; Perlovich et al., 2003). Variation in pK_a values for a compound is common, and may result from differences in the experimental method used (Mioduszewska et al., 2017). Variation can also be due to difference in the chemical environment, e.g. tonicity of the solution. By changing the pK_a value used in the theoretical expression from 4.41 to 4.90 (Fig. 6, dashed line), a better agreement with the published data was obtained. Uncertainties in pK_a values and stability constants will significantly affect results from the theoretical expression, as these constants are used as input parameters in the expression (Eq. 15 and 16). Accurate estimates of these constants must be known to receive an accurate prediction using the theoretical expression. Published values for the pK_a value often vary, and can be influenced by factors such as the presence of background electrolytes or co-solvents (Samuelsen et al., 2019). Thus, it is important to recognize and match all experimental conditions to improve the accuracy of the predicted values.

All compounds investigated above showed a good agreement between the theoretical expression and the published empirical data. In total, ten complexes were investigated consisting of eight compounds bound to one of three different β-CDs. The eight compounds have different molecular weights and physicochemical properties. Results with all compounds showed that the neutral form of a molecule has a higher stability constant than the ionized form. Ionized molecules have a higher solubility in water due to their hydrophilic nature, and thus stability constants for complexation between ionized guests and CDs are expected to be lower as the cavities of CDs are hydrophobic. A

few studies have reported results contradicting this behavior, i.e. where there is no clear relationship between the ionization degree and the stability constant (Al Omari et al., 2009, 2007, 2006). When investigating these examples closer, it is seen that this contradictory behavior seems to be connected to the use of citrate buffer. However, there might be other reasons for why these few examples differ. The general trends from the literature are in agreement with the theoretical expression presented in this work.

How the stability constant varies with pH for CD complexes is of interest for pharmaceutical scientists, as this knowledge can be used during drug formulation with CDs and helps to ensure that a robust formulation is defined. Regulation of pH and the addition of CDs are two strategies used to increase solubility of poorly soluble compounds. Ionizing the drug molecule by changing pH will increase its solubility. The use of CDs to increase solubility is another strategy. Often a combination of these strategies is used. However, the pH of a solution will affect the stability constants, i.e. the strength of the interaction between CD and guest molecule, as described above. An increase in ionization by changing the pH, leads to an improved aqueous solubility, but also to the destabilization of CD complexes. During drug formulation, it is necessary to find the optimal pH of a solution, so there is a balance between the aqueous solubility and the stability constant. By applying the theoretical expression, it is possible to identify the stability constant at any pH value. This will make it easier to find the optimal pH of a solution as the pH can be manipulated during formulation. The theoretical expression can also be applied during processing of potential drug formulations. During processing, products are often exposed to varying temperatures, which can affect the buffer pH (Samuelsen et al., 2019). An example is Tris buffer, which is commonly used in the pharmaceutical industry, although it is very sensitive to changes in temperature. It has a pK_a of 8.06, and when temperature increases (e.g. during autoclaving), the pK_a value decreases,

resulting in a decrease in solution pH. As shown in the present work, a change in pH will affect the stability constant of CD complexes.

5. Conclusions

The stability constants for CD complexes with ionizable guest molecules depend on pH. Based on chemical equilibrium principles, a theoretical expression for the pH dependence of the stability constant was derived. To use the theoretical expression for a specific CD complex, three of the four following parameters must be experimentally determined; the pK_a values of the free and bound guest molecule, and the equilibrium constants for the binding of the neutral and ionized guests, respectively. Knowing all these inputs allows for accurate prediction of the binding constant at all pH values.

ITC data for complex formation between ibuprofen and β-CD confirms the accuracy of the derived theoretical expression. Furthermore, the use of ten data sets from independent published studies with various drug molecules and three types of β-CDs confirm the accuracy of the theoretical expression.

The theoretical expression has the potential to aid formulation scientists when finding the optimal pH value for a formulation containing CDs.

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Figure legends

Figure 1. ITC data for a titration of β-CD into ibuprofen at pH 5.5 and 25°C. The x-axis represents the molar ratio of the formed complex, and the y-axis shows the heat. This a representative example of the ITC data generated.

Figure 2. Measured stability constants as a function of pH at 15, 25, 40, and 55˚C for complex formation between ibuprofen and β-CD.

Figure 3. UV absorbance of ibuprofen:β-CD complex during titration. This is a representative example of pK_a data obtained from Sirius T3 titrator.

Figure 4. Stability constants at several pH values for complex formation between ibuprofen and β-CD determined by ITC (diamonds). The theoretical expression (solid line) is based on the stability constant of the neutral and ionized complex as well as the pK_a value of ibuprofen (4.53).

Figure 5. Complex formation between pizotifen (A) and ketotifen (B) with β-CD, respectively, at various pH values as reported by Al Omari et al. (2008a). The stability constants for the neutral and ionized species as well as the pK_a values are used to calculate the theoretical expression (solid line) for the specific complexes.

Figure 6. Complex formation between Ibuprofen and HP-β-CD at several pH values published by Perlovich et al. (2003). The theoretical expression (solid line) is based on the stability constants for the neutral and ionized form of the complex and the pK_a value of 4.41 reported by Perlovich et al. (2003) and a pK_a of 4.90 (dashed line).