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Model Predicting Impact of Complexation With Cyclodextrins on Oral Absorption

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ABSTRACT: Significant effort and resource expenditure is dedicated to enabling low-solubility oral drug delivery using solubilization technologies. Cyclodextrins (CD) are cyclic oligosaccharides which form inclusion complexes with many drugs and are often used as solubilizing agents. It is not clear prior to developing a drug delivery device with CD what level of absorption enhancement might be achieved; modeling can provide useful guidance in formulation and minimize resource intensive iterative formulation development. A model was developed to enable quantitative, dynamic prediction of the influence of CD on oral absorption of low solubility drug administered as a pre-formed complex. The predominant effects of CD considered were enhancement of dissolution and slowing of precipitation kinetics, as well as binding of free drug in solution. Simulation results with different parameter values reflective of typical drug and CD properties indicate a potential positive (up to five times increase in drug absorption), negative (up to 50% decrease in absorption) or lack of effect of CD. Comparison of model predictions with in vitro and in vivo experimental results indicate that a systems-based dynamic model incorporating CD complexation and key process kinetics may enable quantitative prediction of impact of CD delivered as a pre-formed complex on drug bioavailability.

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KEYWORDS: cyclodextrins; low-solubility drugs; absorption; bioavailability; modeling

Introduction

Oral drug delivery is preferred due to patient compliance and convenience but is unfortunately not possible for many compounds, in large part due to solubility limitations and associated low oral bioavailability. Natural cyclodextrins (CD) and CD derivatives have been used to aid in dissolution of poorly soluble drugs in the gastrointestinal (GI) tract, thus increasing drug absorption and oral bioavailability. In the literature, there are many studies showing improvement in oral drug bioavailability when drug is dosed in the presence of CD (Carrier et al., 2007; Challa et al., 2005; Choudhury and Nelson, 1992; Evrard et al., 2002; Kim et al., 1998; Loftsson et al., 2004; Miyake et al., 2000; Rajewski and Stella, 1996; Stella and Rajewski, 1997; Strickley, 2004; Thompson, 1997; Uekama, 2004; Uekama et al., 1981). In these studies, a wide range of drug and CD types have been used, and generally mainly positive results are reported, giving little guidance regarding which drug and CD properties (i.e., CD:drug ratio, CD type, and dose) result in improvement of drug absorption and bioavailability upon dosing with CD (Carrier et al., 2007; Davis and Brewster, 2004). Drug, CD, and intestinal properties determine the relative kinetics of key processes in the gastrointestinal tract post-dosing (e.g., dissolution, precipitation, and absorption) and the influence that CD has on these kinetics; however, it is not always obvious a priori what the overall effect of CD on drug absorption and bioavailability, if any, will be. As a result, formulation using CDs can be an iterative, resource intensive process.

In order to address this issue, a neutral compound complex (NCC) Model was developed to predict the effect of CD when drug is dosed as a pre-formed complex of CD. Previously, neutral compound physical mixture (NCPM) model was developed considering the case of drug administration as a physical mixture with CD (Gamsiz et al., 2010a,b). By far, most reports in the literature of the use of CD for enhancing bioavailability have involved a pre-formed complex of drug and CD (Carrier et al., 2007). The focus of NCC model is to understand the effect of CD on dissolution kinetics of drug administered as a pre-formed complex with CD, and thus the change in the free drug concentration in the intestinal lumen, which directly affects drug absorption through the intestinal

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wall (Dressman et al., 1985). In addition to solubilizing effects, CD influences drug precipitation. One of the potential consequences of administering drug as a complex with CD is that high-solubility complex can go into solution quickly in the GI tract, resulting in a supersaturated state and precipitation. The presence of CD can slow drug precipitation by binding some drug in solution (Stella and He, 2008). Here we present the NCC model, describe model predictions for typical drug and CD properties, and compare predictions with in vitro as well as in vivo experimental data to test the ability of a simple model to quantitatively predict the influence of CD on drug bioavailability, and potentially streamline drug development.

Materials and Methods

Development of Neutral Compound Complex (NCC) Model and Simulations

The neutral compound complex model (NCC) predicts dissolution, precipitation, and absorption of a drug delivered in the complex form with CD (Fig. 1). It is assumed that solid complex is introduced to the GI tract at time zero ($t = 0$) and dissolves. Complexation/decomplexation is assumed to be instantaneous relative to other processes and expressed by an equilibrium constant, K (Erden and Celebi, 1988)

$$K = \frac{[\mathrm{com}]}{[D][\mathrm{CD}]} \tag{1}$$

At each point in time, the concentrations of each species (free and complexed) in the intestinal lumen, assumed to be well-mixed, are calculated from mass balances. Assuming a 1:1 molar complex:

$$\left\lfloor D_{\mathrm{solution,total}} \right\rfloor = \lfloor D_{\mathrm{f}} \rfloor + \lfloor \mathrm{com} \rfloor \tag{2}$$

$$\left\lfloor \mathrm{CD}_{\mathrm{solution,total}} \right\rfloor = \lfloor \mathrm{CD}_{\mathrm{f}} \rfloor + \lfloor \mathrm{com} \rfloor \tag{3}$$

Combining Equations 1–3, a quadratic equation is obtained for $[D_{\mathrm{f}}]$:

$$\left[D_f\right]^2 + \left[D_f\right]\left(\left[CD_{solution,total}\right] - \left[D_{solution,totl}\right] + 1/K\right) - \left[D_{solution,total}\right]/K = 0 \tag{4}$$

Concentrations of free CD and complex ($[\mathrm{CD_f}]$ and [com]) are then found by mass balance.

Dissolution

Dissolution of the solid complex particle is driven by concentration gradients of both complex and free drug between the solid complex surface and the medium:

$$\frac{\mathrm{d}C_{\mathrm{com,diss}}}{\mathrm{d}t} = -\frac{A^{\mathrm{T}}_{\mathrm{com}}}{h_{\mathrm{com}} V_{\mathrm{lumen}}}\left(D_{\mathrm{com}}\left(\mathrm{com}_{\mathrm{solty}} - \mathrm{com}\right)\right) + D_{\mathrm{drug}}\left(\left[D_{\mathrm{surf,com}}\right] - [D_{\mathrm{f}}]\right) \tag{5}$$

$A^{\mathrm{T}}{}_{\mathrm{com}}$ is the total solid complex surface area and h_{com} is the thickness of the unstirred boundary layer surrounding the complex particle, assumed to be equal to the radius of the particle or 30 μm, whichever is lower in value (Dressman and Fleisher, 1986; Higuchi et al., 1958). The surface area of each particle is calculated from the total mass of solid material remaining at a given point in time, density, and particle number (Gamsiz et al., 2010a). To calculate the concentration of drug at the surface of the dissolving complex particle, equality of the net fluxes of drug and CD into the intestinal lumen is assumed, based on the dosing of a 1:1 drug to CD complex and no accumulation of drug or CD in the boundary layer:

$$D_{\mathrm{drug}}\left(\left[D_{\mathrm{surf,com}}\right] - [D_{\mathrm{f}}]\right) = D_{\mathrm{CD}}\left(\left[\mathrm{CD}_{\mathrm{surf,com}}\right] - [\mathrm{CD_f}]\right) \tag{6}$$

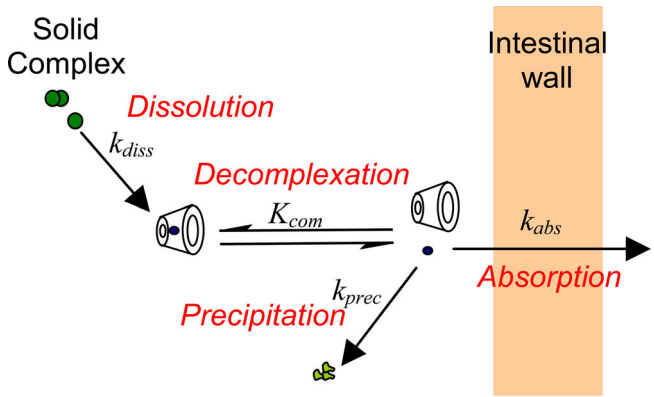

Figure 1. Schematic of model system for a neutral compound delivered as a pre-formed complex.

Equilibrium between complex and free drug is assumed to exist at the surface of the complex, as elsewhere in the intestinal lumen:

$$K = \frac{\mathrm{com}_{\mathrm{solty}}}{\left[D_{\mathrm{surf,com}}\right]\left[\mathrm{CD}_{\mathrm{surf,com}}\right]} \tag{7}$$

Combining Equations 6 and 7, a quadratic equation is obtained for $D_{\mathrm{surf,com}}$:

$$\left[D_{\mathrm{surf,com}}\right]^2 + \left(\frac{D_{\mathrm{CD}}}{D_{\mathrm{drug}}}\left[\mathrm{CD_f}\right] - \left[D_{\mathrm{f}}\right]\right)\left[D_{\mathrm{surf,com}}\right] - \left(\mathrm{com}_{\mathrm{solty}}\frac{1}{K}\frac{D_{\mathrm{CD}}}{D_{\mathrm{drug}}}\right) = 0 \tag{8}$$

As some simulations applied to ionizable drugs, specifically weak acids, the equations above were modified slightly to incorporate the influence of pH and pKa on compound charge and dissolution (Gamsiz et al., 2010a).

Precipitation

Drug precipitation is assumed to be a first order process whose rate is proportional to the difference between free drug concentration and the intrinsic drug solubility (supersaturation)

$$\frac{\mathrm{d}C_{\mathrm{drug,prec}}}{\mathrm{d}t} = k_{\mathrm{prec}}\left(\left[D_{\mathrm{f}}\right] - D_{\mathrm{solty}}\right) \tag{9}$$

precipitated drug re-dissolves; a dissolution equation similar to that explained above was used to consider the influence of CD on precipitated drug dissolution (Gamsiz et al., 2010a).

Absorption

Drug absorption is expressed as first order dependence on free drug concentration, which accurately represents absorption of many drugs (Dressman and Fleisher, 1986). It is generally accepted in the literature that free drug, but not complex, is absorbed across intestinal epithelium

$$\frac{\mathrm{d}C_{\mathrm{drug,abs}}}{\mathrm{d}t} = k_{\mathrm{abs}}\left[D_{\mathrm{f}}\right] \tag{10}$$

Pharmacokinetic Model

A one-compartment pharmacokinetic model was incorporated into the NCC model (NCC/PKmodel) to predict drug blood plasma concentration and oral bioavailability, the percent of the drug delivered present in the blood. Drug absorption was related to blood plasma concentration by volume of distribution, and drug clearance was expressed as:

$$\frac{\mathrm{d}C_{\mathrm{drug,clearance}}}{\mathrm{d}t} = -k_{\mathrm{clearance}}\left[D_{\mathrm{plasma}}\right] \tag{11}$$

Controlled Release

Controlled release (CR) delivery is represented by dividing the dose (drug and CD) by the release time to determine the rate at which solid drug is introduced to the intestinal lumen. Release time was assumed to be the entire simulation duration.

Numerical Solutions

Model expressions were incorporated into MATLAB® code using a Runge-Kutta numerical solution. At each point in time, concentrations of free drug, complexed drug and free CD were calculated as described above. For that same time interval, rates of dissolution, precipitation, re-dissolution, and absorption were calculated. The time interval chosen was a second.

To examine model predictions, simulations were run using different input parameters reflective of typical drug, CD, and intestinal lumen properties (Table I). The densities and particle sizes were chosen to represent those of a typical solid particulate. Drug molecular weight was assumed to be 350 g/mol, which is the molecular weight of a typical small drug molecule (Carrier et al., 2007). Precipitation constants of a variety of compounds were measured in our laboratory (data not shown); the precipitation constant used in the simulations was chosen to be 0.6 min^{-1}, which is in the range of measured first order precipitation constants. The properties of CD were those of β-CD (Cameron and Fielding, 2002); molecular weight, solubility, and density were taken from SciFinder®, and density of complex was assumed to be that of

Table I. Input parameters used in simulations.

Parameter	Drug	CD	Complex
Molecular weight (g/mol)	350	1,135	1,485
Density (g/mL)	1.25		1.5
Diffusivity (cm^2/s)	5e−6	3.2e−6	3.2e−6
Particle diameter (μm)	20		20
Solubility (mgA/mL)	0.01, 0.1	18.5	10
Precipitation constant (min^{-1})	0.6		
Dose (mgA)	25, 300		
First order absorption constant (min^{-1})	0.01, 0.1		
Volume of lumen (mL)	50, 150		
CD:drug ratio	0, 1		
Binding constant (M^{-1})	1,000, 10,000		

β-CD. The solubility of complex was chosen to be 100–1,000 times greater than drug solubility; this was the range of solubility increase determined for specific compounds (Table II). Other input parameters were chosen to represent "low" and "high" values, including drug solubility, absorption constant, dose, binding constant, and volume of the intestinal lumen (Table I).

In Vitro Dissolution, Precipitation, and Absorption Experiments

For testing model validity, in vitro experiments designed to simulate the influence of CD complexation on dissolution and precipitation as well as on combined dissolution, precipitation and absorption were conducted. Parameters used in simulations of these in vitro experiments were either measured in our laboratory, obtained from other literature sources, or estimated from theory (Li and Carr, 1997; Table II).

Materials

Naproxen and β-CD (Molecular weights 230.26 and 1,135 g/mol, respectively) were kindly donated by Pfizer. All salts were reagent grade and were purchased from Fisher Scientific, Cambridge, MA. Deionized water was used to prepare all solutions. Caco-2 culture medium and supplements for Caco-2 cells were from American Type Culture Collection (ATCC).

Preparation of Complex

Pre-formed complex of naproxen with β-CD in 1:1 molar ratio was prepared by the freeze-drying method (Erden and Celebi, 1988). Briefly, 1 g of naproxen and 5 g of β-CD were dissolved in 200 mL of deionized water, stirred at room temperature for 2 days, and lyophilized. A Bruken D5005 X-ray diffractometer was used to characterize crystal structure.

Calculation of Complex Solubility and Binding Constant

Complex solubility was calculated from initial dissolution kinetics. Solid complex equivalent to 25 mg active compound was dissolved in 50 mL of simulated gastric fluid (SGF; 37°C, 100 rpm); samples were withdrawn and filtered (0.45 μm pore size PVDF) every second for 5 s and analyzed by UV spectrophotometer. Complex solubility was calculated using Equation 5, assuming negligible concentrations of complex and drug in solution in the receiver fluid, and flux of free drug away from the dissolving complex surface relative to flux of complex. This latter assumption was based on magnitude of free drug concentration at the dissolving surface relative to complex solubility; at the beginning of the dissolution process, the free drug concentration at the particle surface can be calculated from the equilibrium relationship and the fact that molar quantities of free drug and CD at the dissolving particle surface should initially be equal:

$$\left[D_{\text{surf,com}}\right] = \left(\frac{\text{com}_{\text{solty}}}{K}\right)^{1/2} \tag{12}$$

binding constant was measured by phase solubility method (Gamsiz et al., 2010a; Higuchi and Connors, 1965).

Dissolution

SGF without any enzymes was prepared according to US Pharmacopeia for dissolution experiments. Solid drug

Table II. User input parameters used in simulations for comparison with in vitro data.

Parameter	Naproxen	β, CD	Naproxen/β, CD complex
Molecular weight (g/mol)	230[a]	1,135[a]	1,365
Density (g/mL)	1.2[a]	1.5[b]	1.5[b]
Diffusivity (cm^2/s)	3.28e−5[c]	1.44e−5[c]	1.44e−5[c]
Particle size (μm)	35[b]	35[b]	35[b]
Solubility (mgA/mL)	0.027[d] (pH 1.2) 4.6[d] (pH 7.5)	18.5; Loftsson et al. (2004)	3.2[c]
First order precipitation constant (min^{-1})	10.4[d] (pH 1.2)		
First order absorption constant (min^{-1})	0 (dissolution) 4.88e−4[d] (absorption)		
Binding constant, unionized drug (M^{-1})	1,853[d]		
Binding constant, ionized drug (M^{-1})	36[d]		
Clearance constant (s^{-1})	N/A		
Volume of distribution (L)	N/A		
Dose (mgA)	25, 300 (dissolution) 2.5 (absorption)		
CD:drug molar ratio	1		
Volume of intestinal lumen, or, for in vitro experiments, vessel volume (mL)	50 (dissolution) 5 (absorption)		
pKa	4.84[a]		

[a]Parameter value was obtained from SciFinder®.
[b]Parameter value was assumed.
[c]Parameter value was experimentally measured.
[d]Parameter value was calculated using the Wilke-Chang equation (Li and Carr, 1997). Viscosity used was that of water (0.697 cP).

(25 mgA) in the presence (as a pre-formed complex) or the absence of solid CD under sink conditions was added to 50 mL SGF (37°C, 100 rpm). Samples (4 mL)were removed, filtered (0.45 μm PVDF), and analyzed via UV spectrophotometer in triplicate. Drug solubility was measured as previously described (Gamsiz et al., 2010a).

Precipitation

Solution of naproxen as a pre-formed complex (equivalent to 2.5 mgA/mL) was prepared in SIF, in which the drug is highly soluble. A 5 mL aliquot of the solution was added to 45 mL of SGF (37°C, 100 rpm). Samples (1 mL)were analyzed using UV spectrophotometry to assess precipitation kinetics in the presence of CD and measure the first order precipitation constant of naproxen.

Absorption Experiments

A diffusion cell apparatus (Harvard Apparatus, Holliston, MA) with a Caco-2 monolayer separating two chambers was used to measure drug absorption kinetics. Caco-2 cells with passage number 30–35 were cultured ($\leq$10,000 cells/cm^2) on Snap-wells® containing 0.5 and 2.6 mL of medium in the upper and lower compartments, respectively, for 21–30 days as previously described (Gamsiz et al., 2010a). During the experiments, mixing of Kreb's buffer (pH 7.5, 37°C), prepared according to the Harvard Apparatus diffusion chamber system user's manual, was provided by air manifolds. TEER throughout each experiment ranged between 300–1,000 Ω cm^2. Drug, either in solution (0.5 mgA/mL) or solid (0.5 mgA/mL, alone or as a pre-formed complex) was introduced to the donor compartment (total volume of 5 mL). Samples (1 mL)were removed at timed intervals from the acceptor compartment and analyzed via a UV spectrophotometer (Gamsiz et al., 2010a).

Comparison to In Vivo Data

Data from in vivo experiments reported in the literature were used to test the model's ability to predict bioavailability. Most input parameters (Table III; e.g., binding constant and drug solubility) were obtained from the cited study of the influence of CD complexation on bioavailability. Parameters not available from the cited study were measured in our laboratory, obtained from other literature sources, estimated from theory, or, if no other source was available, assumed based on a reasonable value (e.g., volume of the intestinal lumen), as indicated for each parameter.

Results and Discussion

Model predictions of the impact on absorption of dosing drug as a pre-formed complex compared to drug alone were assessed using typical drug and intestinal lumen properties as input (Table I), with CD properties reflective of β-CD (Fig. 2). One interesting prediction is the variable effect of CD on overall absorption. Most literature reports describe a positive influence of CD on bioavailability, while model predictions indicate a positive, negative, or no effect on overall drug absorption, depending on drug, CD, and intestinal properties. These effects are due to the relative kinetics of processes occurring in the GI tract (e.g., dissolution vs. absorption) and the competing positive and negative effects of dosing with CD. Dosing a pre-formed complex results in increased dissolution kinetics (due to high solubility of the complex), and solubilized CD has a preventative effect on drug precipitation, as it binds some free drug in solution, reducing the driving force for precipitation. The pre-formed complex of the drug goes into solution very rapidly and, if supersaturation is achieved, precipitates to a degree depending on level of supersaturation and precipitation kinetics (Stella and He, 2008). Binding of free drug in solution also slows drug absorption, however. As anticipated, cases where a positive effect of CD is predicted are generally associated with properties consistent with dissolution being the rate-limiting step in overall absorption. However, although a predicted effect on overall absorption can often be reasoned, it is not always obvious a priori due to simultaneous competing effects of properties on process kinetics.

Model Predictions

Low k_{abs}, 2 h Post-Dosing

Simulations for low absorption constant ($k_{abs} = 0.01\ min^{-1}$) drug at different doses (25 and 300 mgA) predict positive, negative or no effect of CD on drug absorption, depending on parameter values (Fig. 2a). When drug solubility is relatively low (0.01 mgA/mL), minimal effect of CD is predicted by the model, regardless of whether the binding constant is relatively low (1,000 M^{-1}) or high (10,000 M^{-1}), for both IR and CR formulations. In these cases, although drug goes into solution quickly when delivered as a pre-formed complex, driving force for precipitation (due to the low solubility) is high, and precipitation occurs before appreciable absorption can take place. As a result, overall percent absorption is predicted to be very low after 2 h (<10%).

For relatively high solubility drug (0.1 mgA/mL), a more significant positive or negative effect of CD on drug absorption is predicted. This is somewhat counterintuitive in the positive effect case, as enhancement in absorption is generally associated with the solubilizing properties of CD, and would therefore be expected to be more significant for a lower solubility drug. However, the driving force for precipitation is also more significant for lower solubility drug; this highlights the utility of modeling for enabling prediction of the overall impact of these competing effects on absorption. For a lower dose (25 mgA) of higher solubility drug, it is predicted that CD has a slight positive effect for the binding constant of 1,000 M^{-1}, but hinders absorption at a binding constant of 10,000 M^{-1}, where benefits of aiding in dissolution are offset by hindrance to absorption due to binding of free drug in solution.

Table III. User input parameters used in simulations for comparison with in vivo data.

Parameter	Naproxen[25]	Carbamazepine[3]	Prednisolone[26]	Tacrolimus[27]	Glibenclamide[28]
Molecular weight (g/mol)	230[a]	236[a]	360[a]	822[a]	494[a]
Density (g/mL)	1.2[a]	1.3[a]	1.3[a]	1.2[a]	1.4[a]
Diffusivity (cm^2/s)	1.59e−5[b]	1.64e−5[b]	1.28e−5[b]	7.50e−6[b]	1.09e−5[b]
Particle size (μm)	35[c]	35[c]	75	75	35[c]
Solubility (mgA/mL)	0.027[d] (pH 1.2) 4.6[d] (pH 7.5)	0.078 (pH 7.4)	0.25[29]	0.0035	0.00618 (pH 7.4)
pKa	4.84 (a)[a]	13.94 (a)[a]	12.5 (a)[a]	10 (a)[a]	5.3 (a)[a]
First order precipitation constant (min^{-1})	10.4[d] (pH 1.2)	N/A	N/A	10[e]	10[e]
Dose (mgA)	200	6.25	10	1.125	3
Volume of intestinal lumen, or, for in vitro experiments, vessel volume (mL)	150[c](Human)	5[c] (Rat)	150[c] (Human)	5[c] (Rat)	100[c] (Dog)
First order absorption constant (min^{-1})	0.1[f]	0.09[30]	0.1[31]	0.075[32]	0.0248[33]
Clearance constant (s^{-1})	1.3	1.0e−4[3]	1.1e−4[31]	4.7e−5	1.1e−4
Volume of distribution (L)	9.6	0.275[34]	16.7[31]	20[g]	1.9
CD type, MW	β-CD, 1,135	HP-β-CD, 1,460	β-CD, 1,135	HP-β-CD, 1,413 DM-β-CD, 1,345 SBE-CD, 1,756	HP-β-CD, 1,460
CD:drug molar ratio	1	1[3]	2	50	55
CD, diffusivity	6.98e−6[b]	6.00e−6[b]	6.98e−6[b]	6.12e−6[b]	6.00e−6[b]
CD, solubility	18.5[1]	600[16]	18.5[1]	500[16]	500[16]
CD, particle size	35[c]	35[c]	35[c]	35[c]	35[c]
CD, density	1.5[c]	1.5[c]	1.5[c]	1.5[c]	1.5[c]
Binding constant, unionized drug (M^{-1})	1853[d]	665[3]	3,600	HP-β-CD, 420 DM-β-CD, 6,060 SBE-CD, 360	1,471
Binding constant, ionized drug (M^{-1})	36[d]	N/A[h]	N/A[h]	N/A	322
Complex solubility (mgA/mL)	3.2[d]	8[i]	47.7[i]	HP-β-CD, 0.012 DM-β-CD, 0.044 SBE-CD, 0.018	0.6[j]

[a]Parameter value was obtained from SciFinder®. (a), acidic.

[b]Parameter value was calculated using the Wilke-Chang equation[24]. The viscosity used in the calculations was that of intestinal contents (6 cP)[35].

[c]Parameter value was assumed.

[d]Parameter value was experimentally measured.

[e]This value was selected as a representative first order precipitation constant in the range of values measured for various pharmaceutical compounds in our lab (data not shown). A value of 1 min^{-1} was also tested; results were similar.

[f]The absorption constant for Naproxen measured in our lab in a diffusion chamber was 4.88e−4 min^{-1}. Scaling this value to an in vivo value by the ratio of surface area to volume would result in an increase of about 1,000-fold. However, as most absorption constants for drugs are 0.1 min^{-1} or lower, we assumed a value of 0.1 min^{-1}.

[g]This value was selected for correct magnitude of plasma concentrations for the "no CD" case. It is higher than a value of approximately 3 L reported for a model-independent analysis in this study.

[h]It was determined that precipitation did not occur as there was no supersaturated drug in these simulations when the precipitation constant was set to zero.

[i]Calculated from reported dissolution kinetics.

[j]This value was selected as 100× the solubility considering enhancement in solubility by complexation for other compounds tested (factors of approximately 20–1,600). Complex solubilities of 0.06 and 6 mgA/mL (approximately 10 and 1,000-fold increases in solubility) were also tested with very similar results.

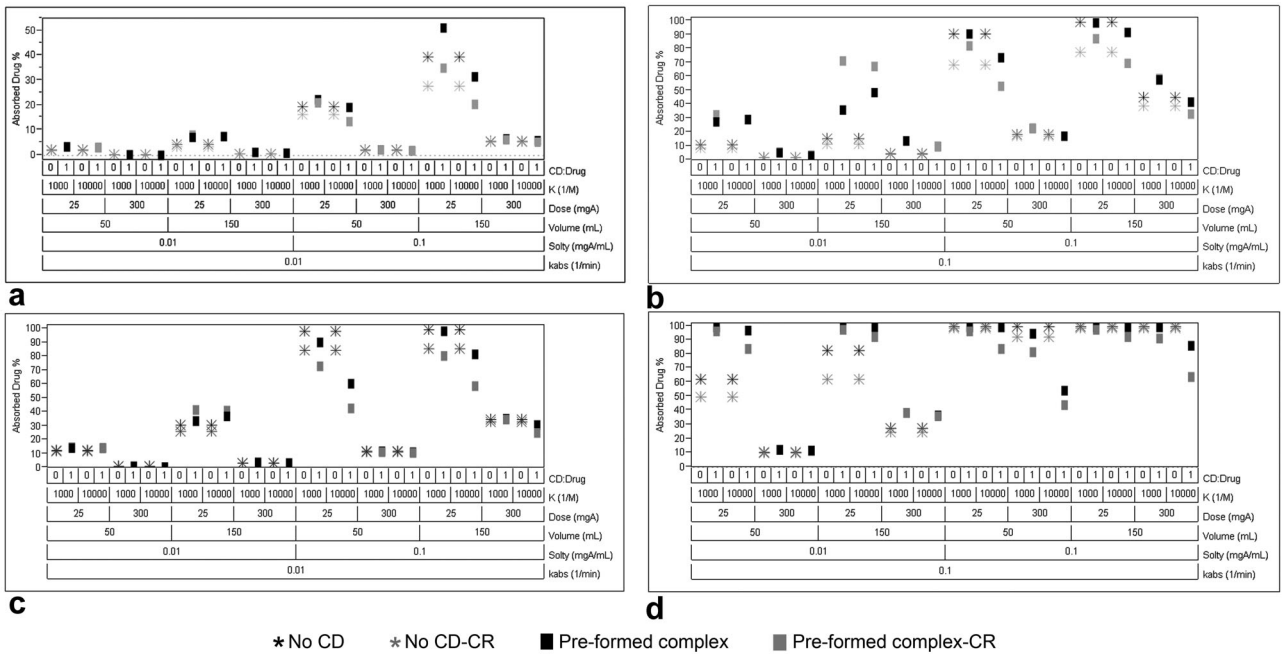

Figure 2. Variability charts showing the predicted influence of dosing drug as a pre-formed complex on percent drug absorption 2 (**a and b**) or 12 (**c and d**) h post-dosing for different values of binding constant (*K*), dose, and intestinal volume. A relatively low (a and c) or high (b and d) absorption constant is assumed.

High k_{abs}, 2 h Post-Dosing

For relatively high absorption constant (0.1 min^{-1}), absorption is less likely while dissolution is more likely to be the rate-limiting step, especially for lower solubility (0.01 mgA/mL) drug. The NCC model predicts CD will generally have a positive effect on drug absorption of lower solubility drug, with the degree of increase in absorption, depending on parameter values used, ranging from minimal (a delta of <5%) to substantial (a delta of approximately 60%; Fig. 2b). The positive effect of CD on drug absorption for both IR and CR formulations is a result of aiding in drug dissolution and hindering drug precipitation. The effect is more significant than for the case of the relatively low absorption constant, since drug is able to be absorbed before it precipitates.

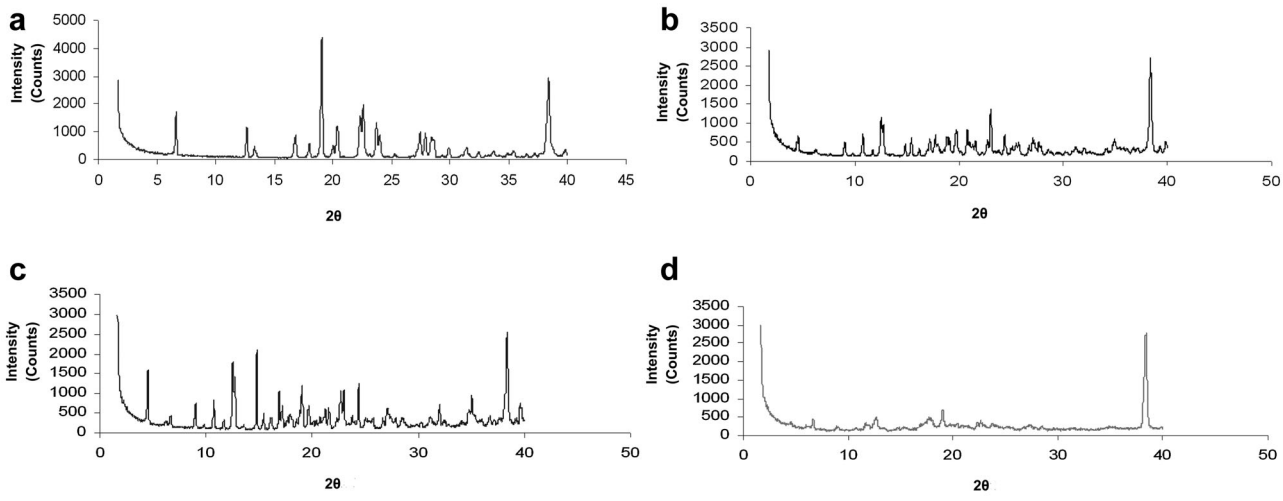

Figure 3. X-ray diffractometer analysis of (**a**) Naproxen, (**b**) β-CD, (**c**) physical mixture of Naproxen/β-CD, and (**d**) pre-formed complex of Naproxen/β-CD.

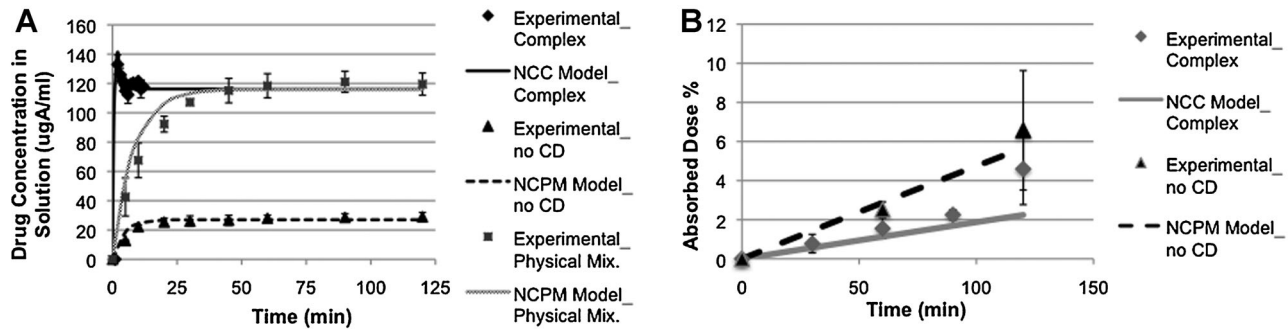

Figure 4. A: Experimental and simulation results for dissolution of Naproxen, Naproxen/β-CD physical mixture and Naproxen/β-CD pre-formed complex in SGF (volume = 50 mL, dose = 25 mgA, 2 h duration post-dosing). B: Experimental and simulation results for absorption of Naproxen through Caco-2 cell monolayer dosed as a pre-formed complex of β-CD and in the absence of CD (volume = 5 mL in apical and basolateral compartments, dose = 2.5 mgA).

For a higher solubility drug (0.1 mgA/mL), improvement in dissolution kinetics by dosing with CD does not have as relatively significant of an effect as it does for a lower solubility drug. As a result, a less significant positive, and sometimes negative, effect of dosing with CD is predicted. The trends predicted for IR and CR are generally similar, although it is interesting to note that the effect of CD on CR can be greater or less than the predicted effect on IR, depending on parameter values.

Low k_{abs}, 12 h Post-Dosing

For a relatively low absorption constant ($0.01\,min^{-1}$) and higher dose (300 mgA) of lower solubility (0.01 mgA/mL) drug, predictions for 12 h post-dosing are similar to the 2-h post-dosing predictions (Fig. 2c); minimal effect of CD is observed, again due to drug going into solution and then precipitating quickly due to high driving force for precipitation associated with the relatively high concentration of drug in solution. For a higher solubility compound (0.1 mgA/mL), when the drug is dosed as 25 mgA in both IR and CR formulations, CD hinders overall absorption by holding the drug as complex form. This effect is more significant for the moderate binding constant at 12 h than it was at 2 h (when a slight positive effect was predicted). For these cases, when drug is dosed without CD in an IR formulation, percent absorption is predicted to be around 100%. This highlights the importance of considering the time over which absorption is expected to occur in assessing overall influence of CD on absorption.

High k_{abs}, 12 h Post-Dosing

Simulation results for relatively high absorption constant ($0.1\,min^{-1}$) 12 h post-dosing again show either positive, negative or no effect of CD on drug absorption (Fig. 2d), in contrast to 2 h post-dosing, when CD effects were generally positive. For low solubility drug, CD has a positive effect on absorption; trends are similar to those observed for 2-h simulations, except that overall percent absorption is of course higher. For high solubility drug, percent absorbed predicted is almost 100% for all cases without CD. If the simulation were run for a longer period of time, eventually absorption from CD containing formulations would be essentially complete.

In Vitro Experiments

Characterization of Complex

Naproxen, β-CD, physical mixture and naproxen/β-CD complex were analyzed by X-ray powder diffractometry (Fig. 3). The diffraction pattern of the physical mixture was a combination of naproxen and β-CD patterns, with lower intensity peaks. The diffraction pattern of the complex showed only very few peaks with low intensity indicating that inclusion complex is more amorphous than the physical mixture and pure naproxen, and that complex was indeed formed.

Model Prediction of Influence of CD on Dissolution

In vitro experiments were run to test the ability of NCC model to predict the influence of CD on key processes involved in drug absorption in the GI tract. Dissolution of low solubility compoundnaproxen as a pre-formed complex with β-CD was tested in a simulated gastric environment. To simulate dissolution in the absence of absorption, the absorption constant in the model was set to zero, and the volume was set equal to the medium volume used in the dissolution test. As a parameter input to NCC model, the first order precipitation constant was measured as described above to be $10.4\,min^{-1}$ (data not shown). NCC model predicted the dissolution kinetics of naproxen dosed as a pre-formed complex with CD in SGF with reasonable accuracy (Fig. 4a). The dissolution kinetics of naproxen

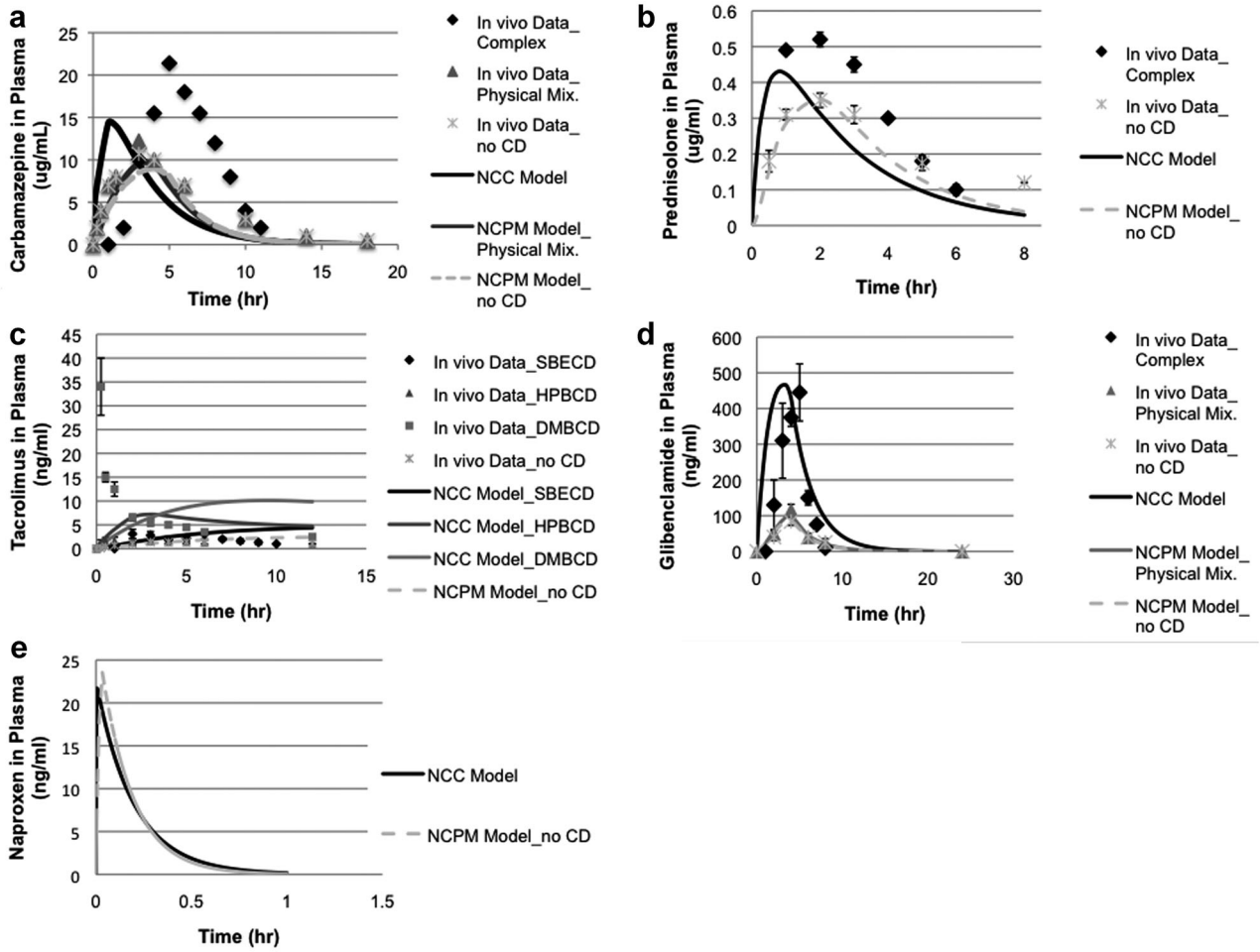

Figure 5. Comparison of NCC/PK and NCPM/PK model predictions with in vivo experimental results for Carbamazepine (**a**), Prednisolone (**b**), Tacrolimus (**c**), Glibenclimade (**d**), and Naproxen (**e**) delivered as a pre-formed complex compared to drug alone (no CD). For Carbamazepine and Glibenclamide, NCPM/PK model predictions and experimental results for a physical mixture with CD are also shown.

dosed as a pre-formed complex was increased significantly compared to those of pure naproxen and physical mixture (Fig. 4a). In naproxen complex dissolution, drug goes into solution very fast, supersaturates, and precipitates until the concentration is equal to the total concentration of drug and CD in equilibrium.

Model Prediction of Influence of CD on Absorption

To test the ability of NCC model to predict influence of CD on combined dissolution and absorption, drug absorption through a Caco-2 cell monolayer in a diffusion cell system was performed after dosing complex and naproxen in the absence of CD. In contrast to the observed positive effect of β-CD on naproxen dissolution kinetics, β-CD slightly hurts overall drug absorption (Fig. 4b). Kreb's buffer used in absorption studies has a pH of 7.5, and naproxen, which is a weak acid with a pKa of 4.84, becomes ionized in Krebs buffer and has a solubility of 4.6 mg/mL. The negative effect of β-CD on absorption of naproxen through a Caco-2 cell monolayer was predicted by the modified NCC model considering ionization of acidic species in solution. Due to the high solubility of naproxen, the limiting step of the overall absorption process in this case is not dissolution, and CD hinders absorption by holding the drug as a complex in solution. A negative effect of β-CD on absorption of naproxen was also observed when it was dosed as a physical mixture and predicted by the previously reported NCPM model (Gamsiz et al., 2010a).

Comparison of Model Predictions With In Vivo Bioavailability Data

To test the ability of the developed model to predict in vivo data, literature data from in vivo experiments with carbamazepine, glibenclamide, naproxen, prednisolone, and tacrolimus (Arima et al., 2001; Choudhury and Nelson, 1992; Otero-Espinar et al., 1991; Savolainen et al., 1998; Uekama

et al., 1983) were compared with NCC/Pharmacokinetic (PK) model predictions (Fig. 5a–e, respectively). In these experiments, drug was orally administered as a pre-formed complex with various forms of CD to various species. Carbamazepine, glibenclamide, and predinisolone all demonstrated enhanced bioavailability when dosed as a pre-formed complex with CD, and these effects were predicted reasonably well by the developed NCC model. However, CD didn't have a significant effect on carbamazepine or glibenclamide bioavailability when administered as a physical mixture, as was predicted by the NCPM/PK model. Concentration of drug excreted in urine, rather than actual blood plasma concentration, was reported for naproxen (Otero-Espinar et al., 1991). However, it was stated that the values in urine were directly proportional to blood plasma levels. In simulations of these studies, it was predicted that formation of a pre-formed complex would not significantly influence naproxen pharmacokinetics, and this was observed in experimental results (Fig. 5). Dosing as a pre-formed complex with DM-β-CD was shown to have a significant effect on bioavailability of glibenclamide, while dosing as a pre-formed complex with SBECD and HPBCD had much less significant effects. While the general trends in enhancement in glibenclamide blood plasma concentrations were concurrent with in vivo results, the kinetics and extent of enhancement are not well predicted by the developed model. This could potentially be due to physiological effects of the very high dose of CD (50:1 CD:drug ratio).

The presented model considers the theoretical influence of complexation with CD on drug absorption and bioavailability. CD can be dosed either as a physical mixture or a pre-formed complex with a given drug (Carrier et al., 2007). The positive influence of CD achieved by dosing as a physical mixture is due to the increase in dissolution kinetics (Gamsiz et al., 2010b); when drug is delivered as a pre-formed complex, the presence of CD in the form of a high-solubility solid complex aids in drug dissolution kinetics and prevents possible drug precipitation, as described above. In the literature, there are many studies reporting an increase in drug bioavailability when it is delivered as a pre-formed complex as opposed to a physical mixture (Carrier et al., 2007). This observation supports the general overall prediction of a more significant affect on drug absorption when drug is delivered as a pre-formed complex with CD relative to a physical mixture (Gamsiz et al., 2010a,b).

As stated above, we previously developed a model (NCPM model) to predict the impact of CD delivered as a physical mixture with drug on overall drug absorption. The main differences between the mechanics of the NCPM and NCC models are in the consideration of dissolution and precipitation. Dissolution expressions for pure drug and pure CD are present in the NCPM model (Gamsiz et al., 2010a,b). According to these expressions, CD in solution impacts drug dissolution kinetics by contributing an additional flux of drug in complex form away from the dissolving drug surface. In contrast, a dissolution expression for pre-formed complex is included in the NCC model, as described above, in which the high solubility of the complex creates a significant flux of complexed drug away from the dissolving particle surface, and this is the main driving force for fast dissolution kinetics. In the NCPM model, there is no precipitation, as there is not a driving force to create a supersaturated state. However, in the NCC model, there is a first order precipitation expression, since supersaturation can occur as a result of the high-solubility complex dissolving quickly and creating transient free (non-complexed) drug concentrations above equilibrium solubility values.

Discrepancies between predicted and experimental pharmacokinetic profiles are likely due in part to the different literature sources for some parameter values (Table III). For example, carbamazepine's absorption constant and volume of distribution were obtained from different studies (both in rats), as they were not provided in the report of the pharmacokinetic profiles in the presence and absence of CD. The absorption constant was estimated using the Caco-2 model rather than rat tissue, and thus might be lower than the actual value (Gamsiz et al., 2010a,b).

It should be noted that there are multiple effects of CD that are not taken into account in the developed model. CD can have P-glycoprotein (P-gP) inhibition effects that aid in drug absorption, thus increasing bioavailability (Challa et al., 2005; Golstein et al., 1999; Loftsson et al., 2004). CD can modulate the integrity of the cell membrane, thus influencing drug absorption (Nakanishi et al., 1992). CD can also affect drug stability (Arima et al., 2002; Carrier et al., 2007; Loftsson and Brewster, 1996). Bile salts secreted in GI tract may displace drug from the CD cavity, and this may affect dissolution as well as absorption (Holm et al., 2008). The utility of the model in its current form for predicting the influence of CD on drug absorption is thus limited to cases where certain effects are dominant. Therefore, comparisons of model predictions with in vivo data here focused on cases in which the major effect of CD on bioavailability enhancement is believed to be its solubilization effect. The purpose of developing this highly simplified model was to test its ability to predict impact of CD in these cases; future extensions of the model to enable general applicability and accurate prediction for broad classes of drugs should incorporate more complex effects.

It should also be emphasized that the simulation results presented in this study are for a certain set of input parameter values reflective of drug, CD, and intestinal lumen properties. For example, the properties, most notably solubility, of CD used to examine model predictions for ranges of drug and intestinal lumen properties (Table I; Fig. 2) were those of β-CD, rather than a more soluble CD derivative. Thus, of course, quite different effects of CD could be predicted for different values. Slightly different values of assumed particle size (e.g., 35 μm vs. 20 μm) or diffusion coefficient (e.g., $2.5e{-}6\ cm^2/s$ vs. $5e{-}6\ cm^2/s$ for drug) result in significantly different model predictions, and generally a more positive predicted influence of dosing with CD (data not shown). For these different parameters, the predicted improvement in dissolution kinetics when dosing with CD would be more significant. Indeed, given the number of parameters involved

in the model, and potential variability in parameter values due to both inherent system properties and experimental measurement error, it will be extremely important to consider the sensitivity of model predictions to parameter values to maximize utility and strengthen confidence in predictions. Efforts are currently underway to conduct a statistically designed parameter sensitivity analysis to quantify the significance of parameter variation to model predictions.

Conclusion

It is not always easy to predict how complexation with CD will affect drug absorption because of the large range of interacting parameters and their influence on free drug concentration; modeling can aid in making predictions and potentially provide rational guidance for formulation development. NCC model demonstrates the mechanism behind a possible increase, decrease or no effect in percent absorption when CD is dosed as a pre-formed complex with drug. It should be emphasized that the developed model considers the influence of CD on drug dissolution and precipitation due to drug interactions with CD but not other (e.g., biological) effects of CD. In addition, it is important to consider the dependence of model predictions on the particular parameter values, and comparisons of model predictions with much broader experimental sets would be required to thoroughly validate the model. In spite of these limitations, the model captures factors important for considering the influence of CD on many drugs, and predictions of in vitro dissolution/absorption and in vivo bioavailability data generally agreed favorably with experimental results. Overall, a much more significant potential positive effect of dosing drug with CD as a pre-formed complex was predicted compared to dosing as a physical mixture, which agrees with the abundance of literature reports indicating a significant effect on drug bioavailability when dosing drug as a pre-formed complex with CD. Predictions obtained from the model could be a helpful guide in determining if CD is a useful technology to use in an oral formulation, minimizing resource intensive formulation development and testing, and streamlining the drug development process.

Nomenclature

$[CD_f]$	free CD concentration (M)
$[CD_{solution,total}]$	total CD (free and bound) concentration in solution (M)
$[CD_{surf,com}]$	concentration of CD at the surface of dissolving complex particle (M)
[com]	complex concentration (M)
$[com_{solty}]$	concentration of complex at the surface of dissolving complex particle (M)
$[D_f]$	free drug concentration (M)
$[D_{plasma}]$	drug plasma concentration (M)
$[D_{solution,total}]$	total drug (free and bound) concentration in solution (M)
$[D_{surf,com}]$	concentration of drug at the surface of dissolving complex particle (M)
A^T_{com}	total complex surface area (cm^2)
CD	cyclodextrin
$dC_{com,diss}/dt$	dissolution rate of solid complex (M/s)
D_{CD},D_{com},D_{drug}	diffusivity of CD, complex, and drug, respectively (cm^2/s)
$dC_{drug,abs}/dt$	absorption rate (M/s)
$dC_{drug,clearance}/dt$	drug clearance rate (M/s)
$dC_{drug,prec}/dt$	drug precipitation rate (M/s)
D_{solty}	drug solubility (mol/mL)
$[D_{surf,com}]$	concentration of drug at the surface of dissolving complex particle (M)
h_{com}	thickness of unstirred boundary layer (cm)
K	drug-CD binding constant (M^{-1})
k_{abs}	first order absorption constant (s^{-1})
$k_{clearance}$	first order clearance constant (s^{-1})
k_{prec}	first order precipitation constant (s^{-1})
mgA	mg active of drug
V_{lumen}	volume of intestinal lumen (mL)

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