# A heuristic model to quantify the potential impact of excess cyclodextrin on the oral drug absorption from aqueous solutions

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**Graphical abstract:**

**Abstract**

The intestinal drug solubilizing capacity  of a drug formulated as an aqueous cyclodextrin solution is a recently proposed quantity to predict the required cyclodextrin concentration needed to fully solubilize the drug in the intestinal lumen. According to this concept the cyclodextrin concentration in the aqueous formulation must be higher than the amount needed to solubilize the compound while on stock and due to the displacement of drugs from the cyclodextrin cavity by bile salts present in the intestinal lumen. On the other hand, dosing cyclodextrin at a surplus capacity of  is expected to result in decreased free intestinal drug concentrations and thus potentially a lower fraction absorbed. In this study, data from 3 previous in vivo studies in rats with fixed concentration of compounds (respectively danazol, cinnarizine and benzo[A]pyrene) and varying cyclodextrin concentrations in excess of  was analyzed. The model was developed for danazol and applied to the two other compounds. The absorption, as quantified from the area under the plasma concentration-time profile, was predicted to decrease for elevated concentrations of co-administered cyclodextrin in accordance with the in vivo data. In addition, at high cyclodextrin concentrations a delay in *T*max and a decrease in *C*max were predicted, again in accordance with experimental observations. These observations were rationalized in terms of the free intestinal drug concentration by a chemical equilibrium model for . This model depends on the quantity termed the dimensionless dose concentration  given as the fraction of the permeation number (Pn) and dose number (Do) which provides the formulation scientist with a critical quality attribute for assessing the implication of having excess cyclodextrin in an oral solution.

**Keywords:** cyclodextrins, pharmacokinetics, modeling, fraction absorbed, biopharmaceutics classification system, oral absorption, rats

**1. Introduction**

Oral delivery maintains to be the most frequent route of drug administration [1]. However, recent trends in drug discovery have resulted in an increasing number of drug candidates with a poor aqueous solubility [2]. Drugs with a low solubility typically have a low and variable oral absorption, as only drug molecules on free solvated form are available for uptake across the intestinal membrane [3]. Major challenges in the drug development process are thus currently faced by the pharmaceutical industry.

A simple model to predict oral drug absorption is given by the one-compartment mass balance model, which assumes that the absorption rate is proportional to the permeability (*P*eff) and the drug concentration (*C*) in accordance with Fick’s 1st law for flux; [3]. This model structure underlies the scientific rationale behind the biopharmaceutics classification system (BCS) [4], [5], which has been adapted by a number of regulatory agencies. Three different regimens of rate limitations are evident in the mass balance approach: i) if the absorption process mainly is limited by the dissolution of the non-absorbable particles, the absorption process is characterized as dissolution rate-limited, ii) dissolved drug molecules, which have passed the dissolution obstacle, must subsequently pass the intestinal membrane and the membrane’s adjacent unstirred water layer (UWL) to be absorbed, iii) if the intestinal permeation is the only limitation, the absorption process is characterized as permeability-limited. For poorly soluble drugs the free concentration is often limited by solubility and the absorption process is characterized as solubility-permeability limited [3].

In order to improve the oral drug absorption of low soluble compounds, a number of formulation strategies exist [2]. Cyclodextrins are one such strategy, which is frequently applied in development of drugs with insufficient solubility [6]. Cyclodextrins are cyclic macromolecules capable of including a large variety of small hydrophobic molecules into their cavity region by non-covalent interactions. The cyclodextrin molecule is a torus shaped ring and due to the spatial distribution of the hydroxyl groups the cyclodextrin has a polar hydrophilic outside and a non-polar hydrophobic cavity. As a consequence of this particular structure, cyclodextrins have the ability to form aqueous inclusion complexes through molecular encapsulation of a wide range of organic compounds [7], [8].

Cyclodextrins in an aqueous formulation are referred to as drug solubilizers. Another class of methods to address the solubility limitations are solubility enhancing techniques [9]. The mechanism by which these techniques increase the absorption is by producing a transient supersaturated state, thus temporarily bypassing the thermodynamic solubility. This is one of the mechanism of action for a cyclodextrin formulation incorporated into a solid dosage form [10], [11]. However, in the current work the focus is on cyclodextrins as aqueous solutions, where the total aqueous solubility is increased as the concentration of the solubilised drug molecule drug will be given by the sum of the concentrations of drug in the complex and the free form. This is sometimes referred to as enhanced solubility [10], [12], [13], but it is important to realize that an aqueous cyclodextrin solution in equilibrium does not have the potential to increase the free drug concentration above the compounds inherent solubility.

The prediction of drug permeation from aqueous cyclodextrin solutions over different types of *in vitro* membranes have been described with a relative high degree of success [12], [14]–[16]. However, experiments are easier to predict *in vitro* than *in vivo* and Carrier *et al.*[10] concluded that no dosing guidelines for cyclodextrin formulations currently exist for the *in vivo* situation. Nonetheless, an integration of the mechanisms described for *in vitro* assays may reveal some of the critical quality attributes (CQA) towards suboptimal absorption *in vivo* by overdosing the formulation with cyclodextrins. This may be of particular interest in situations where the formulations scientist is forced to take fast decisions and act under incomplete knowledge.

The influence of cyclodextrins on oral absorption has previously been modelled from a solid dosage form by Gamsiz *et al.* [11], [17]–[19]. This work especially focused on capturing the effect of supersaturation. In the present study, the focus is on deriving dosing guidelines for an aqueous cyclodextrin solution where all concentrations are assumed to be in an instantaneous equilibrium [20] and supersaturation therefore does not occur. The free drug concentration is therefore limited by the drugs intrinsic solubility while the kinetics of the drug release from the cyclodextrin cavity is considered fast and therefore not rate-limiting for the absorption.

Previously published *in vivo* bioavailability studies in rats using aqueous cyclodextrin solutions have investigated the *in vivo* potential for cyclodextrin overdosage using three different model compounds i.e. danazol [21], cinnarizine [21] and benzo[A]pyrene [22]. These studies were performed with cyclodextrin dosing up to the highest experimentally obtainable concentration and formulations were therefore severely overdosed with cyclodextrins relative to the amount of cyclodextrin needed to solubilize the compounds. Danazol was reported to be the compound with the largest decrease in absorption as a result of escalating cyclodextrin doses [21]. Therefore this compound is especially interesting when developing a predictive model to evaluate the risk of cyclodextrin overdosage.

The purpose of the present study was to provide a theoretical framework to define the critical quality attributes for overdosing cyclodextrins in the formulation based on a chemical equilibrium model previously derived by Olesen *et al.* [23]. This was used to predict the potential decrease in drug fraction absorbed (Fa) based upon the amount of cyclodextrin dosed, thus rationalizing the previously conducted experiments in terms of a mass balance approach.

**2. Theory**

*2.1 Optimal dosing criterion of a cyclodextrin formulated drug solution*

*In vitro* it has been observed that maximum absorption enhancement is obtained when just enough cyclodextrin is used to solubilize all the compound [24]–[26]. This total cyclodextrin concentration is denoted  and is shown in Fig. 1 (an overview of the terms used in this work can be found in the supporting information S.1). Below  a suspension is obtained, which is undesirable from an absorption perspective because some of the drug molecules will be found as unabsorbable particles. Above  all drug molecules are in solution either on free or complexed form, however, when more cyclodextrin are added than required to fully solubilize the drug (that is more than) a decrease in the free drug concentration and thereby the absorption is expected.

*In vivo* the situation is more complicated. One complicating factor is that bile salts present in the intestinal environment compete with the drug molecule for the cyclodextrin cavity. Consequently, the required total cyclodextrin concentration to achieve full drug solubilisation, , is higher *in vivo*. Further, the decrease in absorption above  is influenced by the interaction between cyclodextrins and bile salts. Ono *et al.* [15]investigated the fraction of phenacetin remaining in the intestinal lumen of rats when phenacetin was dosed as a solution at a concentration of 0.01 mM, i.e. well below its solubility of ~4.3 mM [27]. The highest uptake of phenacetin was repoted when the drug was administered alone, while a decline in the flux was observed when cyclodextrin was coadministered. Adding increasing amounts of taurocholate together with the the cyclodextrin almost reversed the influence of cyclodextrin and the absorption increased. Ono and coworkers therefore concluded that an increase in the concentration of bile salt displaces the equilibrium in accordance with Chaterlier’s principle and more drug is found on the free form.

**Figure 1:** *The relationship between the HPβCD concentration and the flux of acetazolamide from an aqueous solution. The concentration of acetazolamide was kept constant at 1.0% (w/v) while the cyclodextrin concentration ranged from 12% to 40% (w/v). An optimum is seen to occur in the middle of the range for cyclodextrin. Reproduced from Loftsson et al. 2011* [25] *with permission.*

Although, *in vitro* and *in situ* data has been well described, the translation of this knowledge into a predictive *in vivo* model is still missing. From the mechanisms described above a necessary prerequisite to derive an optimal dosing guideline is to define the drug concentration, which can be solubilized by a given amount of cyclodextrin in the intestinal tract where bile salts are present. This quantity was denoted the intestinal drug solubilizing capacity, , in a recent work published by Olesen *et al.* [23] and its value was predicted taking the effect of bile salts displacement of the drug from the cyclodextrin cavity into account:

 Eq. 1

where  is the formulations intestinal solubilization capacity (SC) of the drug, *CD*tot and *BS*tot are the total cyclodextrin concentration and total bile salt concentration, repectively, *K*D:CD is the drug-cyclodextrin complexation constant, and *D*sol is the intrinsic aqueous solubility of the drug. If cyclodextrins are added above this limit a decrease in the fraction absorbed is expected [25], [26], [28]. Optimal dosing is therefore achieved when the cyclodextrin solution is saturated with drug molecules, i.e. when the intestinal drug solubilizing capacity  equals the total concentration of drug in the gastrointestinal tract.

A potential complication of this concept is that bile salts are found on a micellar form in the intestinal tract. These micelles have the ability to solubilize drug molecules thus increasing the amount of drug dissolved in the intestinal environment. The solubility to predict the intestinal absorption is therefore often measured in a biorelevant medium where micelle solubilisation of the drug occurs. However, when dosing a cyclodextrin solution the free bile salt concentration will in many cases be depleted due to the complexation with cyclodextrins when dosed in a concentration equal to the drug solubilizing capacity, , as derived in Olesen *et al.* [23]. Micellar solubilization is therefore not relevant in this case and the solubility should instead be measured in an aqueous buffer representing the pH of the intestinal tract.

*2.2 Equilibrium concentrations for an overdosed aqueous solution*

As previously described, at least two factors needs to be taken into account to calculate the free intestinal drug concentration of a drug administered in a cyclodextrin solution; i) the complexation between cyclodextrin and drug, and ii) the displacement of drugs from the cyclodextrin cavity by bile salts [23]. These mechanisms can be formulated mathematically by the law of mass-action and from conservation of mass from the equilibrium model in equilibrium system 1.

C

D

+

D:CD

+

BS

D

BS:CD

**Equilibrium system 1:** Equilibrium system modelled, where the molecular entities refers to the bile salt (BS), cyclodextrin (CD), bile salt-cyclodextrin complex (BS:CD), drug (D) and drug-cyclodextrin complex (D:CD).

Besides the equilibriums given in equilibrium system 1, an additional complication is that bile salts in surplus of the critical micelle concentration will be present as micelles, but as previously demonstrated by Olesen [23], the bile salts will have a higher affinity for the cyclodextrin than the bile salt micelle. The reactions in equilibrium system 1 are all assumed to be faster than the absorption process and therefore in instantaneous equilibrium [7], [20]. In the design of a formulation, both the total cyclodextrin and drug concentration must be chosen as both of these quantities will affect the free drug concentration. It can be shown that for certain values of the dimensionless variables characterizing the system (that is when the so-called complexation efficiency given by CE=*K*D:CD·*D*sol is much smaller than 1 and the dose number  is much larger than 1), the free drug concentration is well-approximated by [23]:

 Eq. 2

where  is the total cyclodextrin concentration required to solubilize all drug molecules, i.e. Eq. 1 solved for *CD*tot. Notice that Eq. 2 is a piecewise function and that the drug solubilizing capacity  depends on the total cyclodextrin concentration as described by Eq. 1.

*2.3 Predictions of danazol concentrations during intestinal transit*

To illustrate the framework in Eq. 1 and 2 above, the data from the in vivo studies conducted by Holm *et al.* (2015) was adapted. The magnitudes of the dimensionless variables for danazol must be examined in order to apply the equilibrium condition in Eq. 2. Danazol is a BCS class II compound with a molecular weight of 337.46 g/mol, an aqueous solubility of *D*sol =0.0018 mM and a binding constant to hydroxypropyl-*β*-cyclodextrin of *K*D:CD = 61.9 mM-1 [29]. The complexation efficiency (CE) of danazol is thus CE=*K*D:CD·*D*sol=0.11 and the approximations in Eq. 2 can therefore be adapted in the present work given that CE is an order of magnitude lower than 1.

To predict the intestinal absorption the proper quantities to consider is the intestinal molar concentration, however, for convenience dosing units are often stated in gram of drug per kilogram of subject. To estimate the initial intestinal concentration the moles of drug and the volume of the gastrointestinal fluid must be known. The average weight of the rats used in the study by Holm *et al.* was approximately 300 g. Moles of danazol dosed was therefore and the injection volume was . The steady-state fluid volume of 3 mL in rats [30], [31] is rapidly reestablished after ingestion of water, and the administered fluid was thus neglected in the calculation and the intestinal danazol concentration therefore assumed to be 4.1 mM.

As discussed above, the amount of cyclodextrin required to solubilize the initial dose in the small intestine is affected by the displacement of the drug from the cyclodextrin cavity by bile salt. The minimum cyclodextrin concentration required to solubilize 4.1 mM danazol in the small intestine in presence of 20 mM bile [3] is therefore:

 Eq. 3

The dosing scheme used by Holm *et al.* [21] is outlined in Table 1 with concentrations stated in both traditional dosing units and in molar concentrations along with the drug solubilizing quantity  of each dosing.

Table 1: Dose of danazol and HP*β*CD used in the rat experiments published by Holm *et al.* (2015) in terms of traditional nonclinical units and units appropriate for the equilibrium model and characterization of each dose in terms of the drug solubilising capacity.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group no. | Danazol | Danazol | HP*β*CD | HP*β*CD |  |
|  |  | mM | g/L | mM | mM |
| 1 | 14 | 4.1 | 86 | 61 | 4.1 |
| 2 | 14 | 4.1 | 100 | 71 | 5.1 |
| 3 | 14 | 4.1 | 200 | 142 | 12.2 |
| 4 | 14 | 4.1 | 300 | 213 | 19.4 |

The influence of cyclodextrin doses in Table 1, as given by Eq. 2, on the free drug concentration is shown in Fig. 2. The initial free drug concentration is shown in Fig. 2A (black line). The theoretical decrease in the free drug concentration when cyclodextrin is overdosed is clearly seen as indicated by the dashed lines representing the four different doses of cyclodextrin in Table 1. Fig. 2B represents the free drug concentration as a function of the total drug concentration remaining in the intestinal tract, it is seen that the free drug concentration declines as the total drug concentration decreases due to absorption.



B

A

**Figure 2:** *(A) The initial free danazol concentration* [*D*GI(0)] *as a function of the total cyclodextrin concentration for the dosing shown in Table 1 as given by Eq. 2. (B) Free danazol concentration as a function of total danazol concentration given by Eq. 2 for the dosing shown in Table 1 illustrating the drug concentration* [*D*GI(*t*)] *during the time course of the absorption process.*

Having established a framework to calculate the free drug concentration, the following section will couple these concentrations to the kinetics of the absorption process.

*2.4 General permeation equation*

The flux over the epithelial membrane is described by:

 Eq. 4

where is the flux, is the epithelial membrane permeability and  is the concentration of free drug at the epithelial membrane.

If drug diffusivity within the membrane is very high, the drug transport becomes limited by the unstirred water layer present on the inside of the epithelial membrane [32]. As shown in supporting information S.2 the concentration of the drug in the presence of an unstirred water layer at the epithelial border is given by:

 Eq. 5

where *P*UWL is the permeability of the unstirred water layer.

Recently, Sugano proposed a gastrointestinal unified theory (GUT) framework with an overall equation to calculate the permeation rate [3]. This equation is adapted here in a slightly revised form to describe the plasma concentration:

 Eq. 6

where *R*GI=0.2 cm is the radius of the small intestine of the rat, DF=1.7 is the degree of flatness of the intestinal tube (value adapted from humans), *V*GI and *V*plasma are the distribution volumes of the plasma and GI compartment, respectively, and Fg and Fh are respectively the fraction not metabolized in the intestinal epithelial cells and in the hepatic first pass metabolism.

Substituting Eq. 4 into Eq. 6 yields:

 where  Eq. 7

where the permeation rate constant  is defined as:

 Eq. 8

In the GUT framework an accessibility parameter to the epithelial membrane and surface area expansion coefficients of the villi and plicate structures are described, however, due to simplicity it has been omitted in the present work. The epithelial membrane permeability  was estimated from the octanol-water partition coefficient [3]:

 Eq. 9

where log *P*oct is the octanol-water partition coefficient and *MW* is the molecular weight of the drug in gram per mole. This formula for the permeability is valid for  and in case  was larger than 0.1 cm/s based on Eq. 9 then  was set to 0.1 cm/s.

*2.3 Amount absorbed*

To provide a simple heuristic model, it is in this work assumed that the permeation only is limited by the epithelial membrane and not by the unstirred water layer. Therefore, the effect of cyclodextrin overdosing directly translates into a decreased absorption. To simplify the notation we introduce an absorption rate constant , which assumes that the barrier in the unstirred water layer is negligible:

 Eq. 10

In addition, it is assumed that drug absorption only takes place in the small intestine. The model of absorption is based on the mass balance approach [4], [33] and the absorption process is modeled as first-order kinetics. Based on the equilibrium condition in Eq. 2 it can be shown that the fraction of drug absorbed is given by (see supporting information S.3):

 Eq. 11

where *T*si is the small intestinal transit time. The time for chyme to transverse the small intestine of both rats and humans is approximately 3-4 h [34]. The commonly used estimate for the mean small intestinal transit time in humans *T*si = 3.5 h is therefore used in this work [3].

*2.4 Plasma concentration-time profile*

A first-order one-compartment model is assumed with first-order absorption and elimination constants to simulate a plasma concentration-time profile. To account for the small intestinal transit time, the absorption process is stopped at *t* = *T*si in accordance with the so-called *T*si model [3]. Based on the equilibrium condition in Eq. 2 it can be shown that the plasma concentration-time profile is given by (see supporting information S.4):

 Eq. 12

Overdosing cyclodextrins therefore decreases the apparent absorption rate constant given by  by increasing the drug solubilizing capacity, . An overdose of  is therefore expected to result in a delay in *T*max and a decrease in *C*max in the plasma concentration-time profile.

**3. Results**

Danazol data and cinnarizine data was adapted from Holm *et al.* [21], benzo[a]pyrene data was adapted from Olesen *et al.* [22].

Initially, it was investigated whether the plasma concentration-time profiles could be rationalized in terms of the drug solubilizing capacity ** and Eq. 12. Of special interest in this context was danazol as this was the compound, which had the largest reduction in fraction absorbed when the cyclodextrin concentration was increased. For the lowest dosing of cyclodextrin the apparent absorption rate was simulated with an apparent absorption rate given by . For the three subsequent doses the absorption rate constant was found by extrapolating this value in accordance with the intestinal drug solubilizing capacities  given in table 1. The apparent absorption rate constants were thus given by 2.2 h-1, 1.8 h-1, 0.74 h-1 and 0.47 h-1. The elimination rate constant was in all cases fixed at . Simulations of Eq. 12 are presented in Fig 3A. In Fig. 3B the experimental data presented by Holm *et al.* are shown for comparison.

 

A

B

**Figure 3:** *A) Graphical illustration of Eq. 12 for k*e*=0.7 h-1 and various values of the apparent absorption rate constant  as indicated in the figure legend, the delay in T*max *and decrease in C*max *are seen to increase as a function of overdosing, i.e. increasing values of . B) Rats dosed with 4.1 mM danazol and various doses of HPβCD as given in table 1, data from Holm et al. (2015)* [21]*.*

**4. Discussion**

From Fig. 3B it is seen that overdosing cyclodextrin affects the plasma concentration-time profiles of danazol and this effect is well reproduced by the simulation conducted in Fig 3A. The dynamics of the decrease is a delayed *T*max and a decreased *C*max, which is explained by the model from a decrease in the apparent absorption rate constant ** due to an increasing drug solubilizing capacity, , with increasing cyclodextrin doses. However, it would be unwise to expect a complete accordance between model simulations and experimental results as the fraction absorbed in general is higher and nonlinearly related to the bioavailability due to metabolism, which is not included in the present model. In addition, several parameters included in the model could only be roughly estimated based on existing knowledge on the intestinal physiology.

Aqueous cyclodextrin solutions are especially applied in the early drug development process, but currently there are no guidelines to avoid overdosing. Currently there are a number of situations where the formulation scientist is at risk of overdosing with cyclodextrins. These include cases where it is desired to keep a fixed concentration of the vehicle while the concentration of the drug are changed, which could occur in pharmacological and toxicological studies of a compound. In addition, the underlying physical parameters are often not known and the formulator therefore might decide to coadminister excess cyclodextrin to make sure that all drug molecules are solubilized, i.e. to account for variations in storage temperature or batch to batch variations of degree of substitution of the cyclodextrin etc.

*4.1 Cyclodextrin solution saturated with drug molecules*

The BCS system, derived by Amidon and coworkers (1993), has a scientific origin in a one-compartment mass balance model, similar to the model applied in this work. The main outcome of the BCS model was the establishment of dissolution, permeability and solubility as the three main factors underlying oral drug absorption in terms of three dimensionless numbers; i) the dissolution number Dn=*k*diss·*T*si, ii) the dose number , iii) and the permeation number Pn=*k*abs·*T*si (in the BCS this number was defined similarly with An=2·Pn, but is here denoted Pn to emphasize the foundation in the GUT framework [3]). Drugs are classified in the BCS system from I-IV according to the magnitude of the last two dimensionless parameters often plotted in a plane as a (Do,Pn)-parameterization.

From Eq. 11 it is seen that the (Do,Pn) parameters from the BCS system are present in the solution for the fraction absorbed, for an initially exactly fully solubilized cyclodextrin formulation, that is when the total drug concentration equals the drug solubilizing capacity :

 Eq. 13

However, drugs with different permeation numbers can have a similar fraction absorbed according to Eq. 13 if the permeation number is compensated by the dose number. For a cyclodextrin formulation the independence in the two BCS parameters, that is Do and Pn, is therefore not an optimal way to address the absorption problem, rather the amount absorbed depends on the ratio of the permeation number to the dose number. A better parameterization is therefore given in terms of the ratio of the original parameterization of the BCS plane, which here is denoted the dimensionless dose concentration:

 Eq. 14

The dimensionless dose concentration expresses how large the dose is compared to the maximum absorbable dose (MAD=*k*abs·*D*sol·*T*si) that can be absorbed when the free drug concentration is at the saturated solubility. It follows from Eq. 13 and 14 that the fraction absorbed in terms of the dimensionless dose concentration is given by:

 Eq. 15

In Fig. 4 these two types of parameterizations are illustrated for contour levels of the fraction absorbed as given by Eq. 15. Fig. 4A shows the BCS plane for class II drugs with the dose number on the horizontal axis and permeation numbers on the vertical axis. It is seen that the contour levels falls over different values of the permeation number and dose number due to the compensation of these two parameters. Fig. 4B shows a double logarithmic plot of the BCS plane for class II drugs, whereby the contour lines becomes parallel and the parameterization in terms of the dimensionless dose number is shown with the red axis. It is seen that the dimensionless dose concentration is an optimal parameterization in the sense that it is perpendicular to the level curves.

  

B

A

**Figure 4:** *Illustration of the relation between the (*Do*,*Pn*)-parameterization of the BCS system and the -parameterization relevant for cyclodextrin solutions. Contour lines shows levels of the fraction absorbed given by Eq. 13. Fig. 4A shows the original BCS plane and Fig. 4B shows a double logaritmic plot of the BCS plane for class II drugs, the values of  is shown along the red axis.*

The BCS system was originally developed for solid oral dosage forms with the purpose of establishing bioequivalence from *in vitro* data of low risk (class I) compounds [35], however, recent requirements in drug development have increased the focus on BCS class II drugs. Cyclodextrins are normally stated to be most relevant in the formulation of class II drugs [36]–[38], but in lights of the development classification system (DCS) suggested by Butler and Dressman [35], this can be limited to class IIb drugs as class IIa drugs usually can be designed to achieve complete oral absorption without resorting to solubilization technologies such as cyclodextrins.

The implication of Eq. 15 is therefore that the BCS system will make a redundant classification of the formulation potential of an aqueous cyclodextrin formulation and such a classification will therefore hardly be able to reflect the fraction absorbed precisely. Rather the fraction and amount absorbed is better characterized in terms of the dimensionless dose concentration, because a simple one-dimensional parameter  is sufficient, as seen in the dose-absorption plot in Fig. 5. These plots show a simulation of the fraction absorbed and amount absorbed similar to Fig. 4, but now shown as a function of the  parameter and for a dosing of 1 mol.



B

A

**Figure 5:** *Dose-absorption plot illustrating the contradictory objectives for dose optimization for a drug molecule exactly fully solubilized by a cyclodextrin solution for a maximum absorbable dose of MAD=1 on a logarithmic scale A) and a linear scale B). The fraction absorbed is shown on the left vertical axis (blue line) and the amount absorbed is shown on the right vertical axis (red line). In both cases the dimensionless dose concentration is varied between 0 and 10. The dashed line indicates the border between class IIa and IIb compounds in the DCS system.*

From the plots in Fig. 5, the contradictory objectives for dose optimization, in terms of the fraction absorbed and amount absorbed is observed. It is seen that increasing the dose - or equivalently ** - increases the amount absorbed, but this is on the expense of decreasing the fraction absorbed. For very small dimensionless dose numbers, the fraction absorbed approaches one and the amount absorbed approaches zero whereas for large dimensionless dose numbers the amount absorbed approaches the maximum absorbable dose and the fraction absorbed approaches zero.

For the formulation scientist it is important to realize that a cyclodextrin concentration equal to exactly the amount required to solubilize the drug, as assumed in Fig. 5, is not unique; rather many different choices of the drug and cyclodextrin dose will achieve this criterion and thus locate the formulation on the **-axis in Fig. 5. However, the formulation scientist is forced to make a specific choice of dose satisfying the criterion for optimal dosing, but this choice of dose will affect both the fraction absorbed and amount absorbed and thereby the performance of the drug product. It is though important to bear in mind that this conclusion is based solely on considerations of the free drug concentration and does not take the effect of the unstirred water layer into account.

*4.2 Quantification of overdosing*

Finally, the question of when the formulation scientist is at risk of overdosing the formulation with cyclodextrins is addressed. This problem can be quantified by inspecting the reduction in fraction absorbed relative to an optimal dose defined as , where  is the amount absorbed for a certain amount of overdosing and  is the amount absorbed when cyclodextrins are dosed optimal (see supporting information S.5). In Fig. 6 the reduction in fraction absorbed Farel is plotted as a function of the dimensionless dose concentration  and the fractional overdose defined as . Ideally, the formulation scientist will add sufficient cyclodextrin to ensure that the drug is exactly fully solubilized corresponding to a fractional overdose  on the vertical axis in Fig. 6. For  the fraction absorbed is as shown in Fig. 5. However, the drug solubilizing capacity  is in practice only known with a large uncertainty and therefore the formulation can easily possess excess cyclodextrin i.e. . From Fig. 6 it is seen that DCS class IIa with  (left of the vertical black line) is relative robust towards overdosing whereas DCS class IIb with  (right of the vertical black line) is much more sensitive towards adding too much cyclodextrin. In this light it must also be stated that DCS class IIa usually can be designed to achieve complete absorption without cyclodextrin.

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**Fig. 6:** *The reduction in fraction absorbed* *due to overdosing as a function of the dimensionless dose concentration  and fractional overdose . The vertical black line indicates the border between class IIa and IIb compounds in the DCS system. The 4 red dots indicate the model predictions of the reduction in fraction absorbed for danazol described above.*

*4.3 Evaluation of the model relative to in vivo data*

Three sets of *in vivo* data were adapted from the literature with compounds overdosed with hydroxypropyl-β-cyclodextrins, these include cinnarizine [21], danazol[21], and benzo[a]pyrene [22]. These compounds will be evaluated based upon the model described above. Their physical chemical parameters are defined in Table 2.

Table 2: Parameter values

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Danazol** | **Cinnarizine** | **Benzo[A]pyrene** |
| Intestinal drug concentration  (mM) | 4.1 [21] | 2.7 [21] | 4×10-4 [22] |
| Molecular weight, *MW* (g/mol) | 337.46 | 368.514 | 252.32 |
| Solubility, *D*sol (mM) | 1.8×10-3 [29] | 4.07×10-5 [39] | 4.4×10-6 [40] |
| log *P*oct, log *K*o/w | 4.53±0.32 [41] | 5.7 [42] | 6.35 [43] |
| Drug-cyclodextrin complexation constant, *K*D:CD (mM-1) | 61.9 [29] | K11 = 22.500 and K21 = 4.000 [39] | 25.9 [44] |
| Complexation efficiency CE | 0.11 | Not defined | 1.1×10-4 |

The first molecule, cinnarizine, is a diprotic base with p*K*a=2.64 and p*K*a=7.78 [45]. The model derived in this work is strictly only valid for neutral compounds participating in a single equilibrium with the cyclodextrin. However, some insights from modeling regarding cyclodextrin overdosing can still be obtained for compounds that can be protonated. In the data adapted from Holm *et al.*[21] the lowest concentration of cyclodextrin was the minimum amount to solubilize the dose of 1 mg/mL cinnarizine at pH 4.5. In the small intestine the pH is rapidly increased to a value of approximately 6.5, which changes the equilibrium system. To quantify the free cinnarizine concentration the complexation constant for both the protonated and uncharged cinnarizine combined with the pH equilibrium must be taken into account as shown by Okimoto *et al.* [39]. In addition bile salt present in the small intestine competes with cinnarizine about occupying the cyclodextrin cavity. As shown in supporting information S.6 the parameter values predicted that the cinnarizine solution is not overdosed with cyclodextrin at pH 6.5. This is in accordance with the experimental observations for cinnarizine were the absorption was only reduced to a very small extend even though the cyclodextrin was added in very high amounts[21].

For danazol and benzo[A]pyrene the dimensionless dose concentration  and the fractional overdose  was estimated as derived in supporting information S.7. Both compounds had complexation efficiencies well below 1 and the model derived here should therefore be applicable [23]. For danazol the nondimensional dose concentration was estimated to  and the fractional overdosing for the four doses were . The absorption of danazol was relative to initial dosing given by 1, 1.02, 0.55 and 0.55 [21] for the four doses of cyclodextrin, which was in close accordance with the predictions from the model 1, 0.99, 0.88 and 0.73 as indicated by the red dots in Fig. 6.

For benzo[A]pyrene co-administered with hydroxypropyl-*β*-cyclodextrin the nondimensional dose concentration was estimated to . This quite low value is located outside to the left of the parameter space in Fig. 6 where the sensitivity towards overdosing with cyclodextrins is extremely small. However, as the dosing with cyclodextrins was done up to the highest experimentally achievable level the fractional overdose with cyclodextrins was also extremely large. For the 8 highest doses of hydroxypropyl-*β*-cyclodextrin where more cyclodextrin was added than required to solubilize the drug the fractional overdose was given by . However, the absorption of benzo[A]pyrene was not reduced dramatically relative to initial dosing as given by 1.0, 1.1, 1.1, 1.1, 1.1, 1.1, 1.0, 0.8. This is in line with the model predicting that the reduction in the fraction absorbed relative to the initial dosing was .

In summary, it was predicted that cinnarizine was not overdosed by cyclodextrins due to the shift in pH to the physiological level of 6.5. For danazol and benzo[A]pyrene the developed model was able to predict the sensitivity towards cyclodextrin overdosing at least on a qualitative level. The present work can therefore be used to identify compounds which are vulnerable towards this overdosing phenomenon before any *in vivo* experiments are performed.

*Future perspectives*

For the sake of simplicity, the predictions performed in the present study have not taken the effect of the unstirred water layer into account. However, it has previously been reported that cyclodextrin complexation of drugs enhance diffusion through the unstirred water layer of the intestinal membrane [14], [16], [32], [46]–[48] and according to the equations from the GUT framework presented in the theory section the main diffusion barrier is expected to be in the unstirred water layer for the lipophilic compounds considered in this work with log *P*oct > 2 [3].

Modeling the effect of the unstirred water layer on the absorption has previously been done by Másson *et al.* [14]. This model did, however, not allow predictions from first principles as it included two empirical constants. In principle, such bottom-up estimation including the effect of the unstirred water layer is possible from the framework presented in the theory section. However, accurate predictions require more detailed knowledge about the width and diffusion coefficients in the unstirred water layer and will have to be exploited in the future.

The present work has addressed an absorption model for rats, but most pharmaceuticals are in reality developed to be applied in humans and it is therefore of interest to have a model to quantify the absorption also in this case. A model for humans will have similar nature, but due to differences in the physiology of gastrointestinal tract several parameter values must be adjusted including the total bile salt concentration, the intestinal fluid volume and parameters describing the geometry of the small intestine [3]. Some general remarks about this task are in place. Normally, it is observed that the *P*eff in rats are 6- to 15-fold [49] lower than in humans, however, as the radius of the rat intestine is smaller than that of the human intestine, the permeation rate and Fa becomes similar [3]. The metabolism between rats and humans are significantly different and the bioavailability does therefore not in general correlate [50].

**5. Conclusion**

In the present study, a biopharmaceutical model was derived to examine the effect of the free drug concentration on the absorption from an aqueous cyclodextrin solution. In our previous work a chemical equilibrium model for the free drug concentration was derived. Optimal dosing of an aqueous cyclodextrin solution is achieved when an aqueous cyclodextrin solution is saturated with drug molecules [51] corresponding to a drug dosing at the intestinal drug solubilizing capacity . If the solution is prepared with a higher intestinal drug solubilizing capacity, , than required, the free drug concentration declines compared to the optimal dosing. From these conclusions, the model derived in this work predicted a lower apparent absorption rate constant  resulting in a delay in *T*max and a decrease in *C*max for the plasma concentration-time profile. These predictions were in agreement with *in vivo* experimental observations previously published.

In addition the current work provides a framework for assessing the critical quality attributes to avoid overdosing the formulation with cyclodextrins in terms of a quantity denoted the dimensionless dose concentration** which is equivalent to the ideas behind the division in the developability classification system (DCS) between class IIa and class IIb. For very small values of ** (DCS class IIa) the formulation is insensitive towards overdosing while for large values of ** (DCS class IIb) only a small surplus of cyclodextrin compared to the optimal value will result in a decrease in absorption.

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