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Research Paper

Reversible and pH-dependent weak drug-excipient binding does not affect oral bioavailability of high dose drugs

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Abstract

Objectives Drug-excipient binding can affect in-vitro drug release. Literature suggests that drug-excipient ionic binding interaction that is not disrupted by physiological salt concentration in the dissolution medium can impact a drug's oral bioavailability. We investigated whether nondisruption of interaction by physiological salt concentration was an adequate predictor of its biorelevance using the binding of a model amine high dose drug brivanib alaninate (BA) to croscarmellose sodium (CCS) as an example.
Methods BA was formulated into an immediate release tablet using CCS as disintegrant by a wet granulation process. In-vitro drug release was carried out as a function of pH and buffer concentration of the medium. BA-CCS binding was studied in buffer solution and data fitted to a Langmuir isotherm. A simulation model and an isothermal titration calorimetry method were developed to assess the bioavailability risk and strength of drug-excipient binding interaction, independent of physiological salt concentration consideration.
Key findings BA-CCS binding was pH-dependent, reversible, ionic, and not disrupted by increasing the buffer concentration in the dissolution medium. Absorption simulation predictions of no effect of CCS binding on BA's bioavailability were confirmed by a monkey pharmacokinetic study.
Conclusions A pH-dependent and reversible weak drug-excipient binding interaction is unlikely to affect the oral bioavailability of high dose drugs.

Introduction

Unintended physicochemical interaction of an excipient with a drug substance in a dosage form can result in complexation or binding of the drug, resulting in slow and/or incomplete drug release in a dissolution medium. It is important to assess the risk whether such interactions would reduce the bioavailability of a drug from its dosage form.

Drug-excipient binding interaction in the dosage form can affect in-vitro drug release.[1,2] For example, Huang *et al.*[2] reported a strong ionic interaction between metformin and croscarmellose sodium (CCS). This interaction caused the entrapment of metformin in the CCS matrix, leading to low analytical recovery. It was overcome by the use of arginine, a stronger binding competitor, indicating that the interaction was ionic and reversible. Chien *et al.*[3] reported the binding of oxymorphone derivatives with CCS and sodium starch glycolate in water as a function of solution pH and analysed the data using Freundlich adsorption principles. Hollenbeck *et al.*[4] reported the pH-dependence of chlorpheniramine interaction with CCS. Hollenbeck[5] later reported the effect of phenylpropanolamine hydrochloride-CCS interaction on the drug's in-vitro dissolution and in-vivo exposure. In that study, three lactose-based formulations were tested, containing either no disintegrant, 10% w/w starch or CCS as a disintegrant. Although an in-vitro dissolution test in water showed significant (40%) drug binding to CCS, no significant differences were observed among the three formulations in the cumulative amount of drug excreted in the urine of six healthy human subjects over a period of 24 h after a single oral dose. The author, however, did not discuss the reason for lack of in-vivo effect of drug-excipient binding interaction observed in-vitro.

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Literature suggests that drug-excipient binding interactions in the dosage form that affect a drug's in-vitro release may or may not affect a drug's oral bioavailability. For example, increase in drug dissolution by complexation with cyclodextrin corresponded with increased oral bioavailability of griseofulvin and spironolactone; but not of naproxen and tolbutamide.[6–9] Also, reduction in dissolution by complexation of halofantrine with magnesium carbonate and of tetracycline with magnesium aluminum silicate (Veegum) corresponded with their reduced oral bioavailability; but not for the complexation of phenylpropanolamine with CCS.[5,10,11]

Ionic drug-excipient binding interactions are most commonly encountered in the use of ion exchange resins, such as sulfonated and/or carboxylated polystyrene backbone for binding basic drugs, for controlled/sustained drug delivery.[12] For example, complexation of dextromethorphan and phenylpropanolamine with ion exchange resins reduced drug release that corresponded with altered oral bioavailability.[13,14] Whether a release-modifying drug-excipient interaction results in altered oral bioavailability of a drug is conventionally determined on a case-by-case basis.

Guidance on whether drug-excipient binding interaction in an oral dosage form poses risk of low bioavailability of the drug is generally lacking in literature. Fransen *et al.*[15] studied the interaction between three commonly used superdisintegrants and several drugs with different physicochemical properties. In addition to the ionic interactions between cationic drugs and anionic polyelectrolyte disintegrants, such as CCS and sodium starch glycollate, the authors postulated that amphiphilic drugs could interact with superdisintegrants to a greater extent than simply by ion exchange due to greater entropic gain caused by the aggregation of surfactant inside the polyelectrolyte.[15] In such cases, the interaction may not be overcome by increasing the ionic strength of the dissolution medium. Nevertheless, ionic interactions could be disrupted in the presence of physiological salt concentration in the dissolution medium, which was considered as an indication of potential lack of biorelevance of such interactions.[15] Thus, the authors emphasized the importance of the strength of interaction, which was assessed by the reversibility of interaction at physiologically relevant salt concentration – a criterion that was also considered an indication of biorelevance of the interaction.

We addressed the question whether a drug-excipient binding interaction in the dosage form would affect a drug's oral bioavailability. Most basic amine drugs that are substantially ionized and soluble at the same pH as an insoluble excipient of opposite charge are likely to undergo such interactions. Previous literature has suggested that an ionic binding interaction that is overcome with the use of physiological salt concentration is unlikely to reduce a drug's oral bioavailability. However, there is no guidance in literature regarding the drugs whose interaction may not be overcome by physiological salt concentration. Using an example of one such drug-excipient interaction, we probed whether in-vitro techniques such as Langmuir binding isotherm and isothermal titration calorimetry (ITC) to assess the extent and strength of an interaction could indicate in-vivo relevance of such an interaction. Our studies indicated that reversible and pH-dependent drug-excipient binding that was weaker than a drug-drug self-association did not affect oral bioavailability of high dose drugs. In addition, we developed a direct and objective measure of strength of drug-excipient interaction in the solution state, since drug gets absorbed from the solution state. We propose the use of ITC to assess the relative strength of drug-excipient and drug's self-association binding interactions.

The interaction of a model hydrophobic (log P = 2.5), weakly basic ($pK_a = 6.9$), amine drug, brivanib alaninate (BA), with the salt of a weakly acidic ($pK_a = 4.8$) polymeric excipient, CCS, was investigated by dissolution studies.[16] Strength of BA-CCS interaction was assessed by Langmuir adsorption modelling and ITC. Non-biorelevance of BA-CCS interaction was predicted using oral drug absorption modelling and confirmed by an oral drug pharmacokinetic study in monkeys.

Materials and Methods

Materials

Microcrystalline cellulose and CCS were procured from FMC Biopolymer (Philadelphia, PA, USA); hydroxypropyl cellulose from Aqualon (Wilmington, DE, USA); crospovidone from BASF Corporation (Florham Park, NJ, USA); colloidal silicon dioxide from Cabot Corporation – Becca Golden (Alpharetta, GA, USA); and magnesium stearate from Mallinckrodt, Inc. (St Louis, MO, USA). A model amine drug, brivanib alaninate (BA), was obtained from Bristol-Myers Squibb, Co. (New Brunswick, NJ, USA). Sodium acetate, potassium phosphate, Triton X-100, sodium taurocholate, and all other reagents were procured from Sigma-Aldrich, Inc. (St Louis, MO, USA).

Tablet manufacturing

An immediate release oral tablet formulation containing BA was manufactured by a wet granulation process using conventional excipients. Briefly, BA was mixed with intra-granular disintegrant (CCS or crospovidone), binder, and filler in a Diosna high shear mixer (Diosna Bierks & Sohne GmbH, Osnabruck, Germany), followed by granulation with water. The granules were sized using Comil (Quadro Engineering Corp., Waterloo, ON, Canada) and blended with extra-granular excipients in a bin blender (A&M Process Equipment Ltd, Toronto, ON, Canada). Tablets were compressed on a Korsch press (PH106, 6-stations, Korsch

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Maschinenfabrik, Berlin, Germany) at target 800 mg press weight and 28 Strong-Cobb unit hardness. Tablets were coated with a 15% w/w hydroxypropyl methylcellulose-based coating suspension (Opadry, Colorcon, Inc., Harleysville, PA, USA) in a Vector LDCS Hi-Coater (Vector Corporation, Marion, IA, USA) using standard operating parameters.

Drug release studies

In-vitro drug release of 400 mg strength BA tablets was studied at pH 1.2, 4.5, and 6.8, using 1000 ml of an aqueous medium in compendial (United States Pharmacopeia) dissolution Apparatus 2 (paddle) at 75 rev/min and 37°C. Dissolution was carried out in 0.1 M HCl (pH 1.2), 22 mM sodium acetate buffer (pH 4.5), and 50 mM potassium phosphate buffer (pH 6.8) containing 1% w/v Triton X-100. In addition, to assess the strength of binding, dissolution in pH 4.5 acetate buffer was carried out at higher buffer concentrations (66, 170, and 250 mM). Samples (10 ml) were collected at 5, 10, 15, 20, 30, 45, and 60 min and replaced with fresh medium equilibrated to 37°C. At 60 min, the paddle speed was increased to 200 rev/min. Dissolution was continued until 90 min, at which point a final 10-ml sample was collected. All the samples were diluted appropriately and analysed for drug content using a validated high performance liquid chromatography (HPLC) method.

Binding study

Binding of BA to CCS was studied in 105 mM acetate buffer with 45 mM sodium chloride at different drug/excipient molar ratios. Briefly, CCS was dispersed in the buffer in a beaker using a magnetic stirrer at different concentrations, followed by the addition of BA at 4.5 mM, and equilibration at room temperature for 4 h. The solution was filtered through a 0.45-μm pore diameter polytetrafluoro ethylene (Teflon) syringe filter, followed by HPLC analysis for BA content. In some studies, sodium taurocholate was dissolved in the buffer solution at different concentrations before the addition of BA and CCS.

Modelling and simulation

CCS is a hydrophilic polymeric excipient that forms an insoluble colloidal dispersion in the aqueous medium. Isothermal adsorption of a solute (BA) in the solution phase on the solid substrate (CCS) can be represented as:

$$BA + CCS \rightleftarrows BA\text{-}CCS$$

The association (binding) equilibrium constant, b, is given by:

$$b = \frac{[BA-CCS]}{[BA][CCS]} \quad (1)$$

The dissociation equilibrium constant, k, is given by the inverse:

$$k = \frac{[BA][CCS]}{[BA-CCS]} \quad (2)$$

This binding was modelled by Langmuir isotherm:

$$y = y_m\left(\frac{bc}{1+bc}\right) \quad (3)$$

where y is the amount of BA (mmol) adsorbed on 1 mmol CCS, y_m is the maximum adsorption capacity of BA on CCS (mmol BA/mmol CCS), c is the concentration of free BA in solution (mM), and b represents the association (binding) equilibrium constant (1/mM).

Rearranging,

$$\frac{c}{y} = \frac{1}{y_m b} + \frac{c}{y_m} \quad (4)$$

Thus, a plot of c/y against c gives a straight line, from which y_m and b can be estimated.

The effect of drug-excipient binding on oral drug absorption was estimated by simultaneously solving the equations for free drug concentration in the gut and the drug absorption, as described below. The resulting plasma drug concentrations were estimated using the drug's pharmacokinetic parameters in a two-compartmental model. Thus, amount of drug absorbed into the plasma (central compartment, or compartment 1) (Figure 1) is given by:

$$\frac{dXA}{dt} = k_a \times XC \quad (5)$$

where XA is the amount of drug absorbed, dXA/dt is the rate of drug absorption per unit time t, k_a is the absorption rate constant, and XC is the amount of free drug available in the gut.

Amount of free drug in the gut (XC) is given by:

$$XC = X - XA - XY \quad (6)$$

where X is the administered dose, XA is the amount absorbed, and XY is the amount of drug bound to CCS – which is estimated using the Langmuir isotherm.

In addition, if a drug's pharmacokinetic profile is defined by a two-compartmental model, drug concentration in the plasma and the tissue compartment can be obtained by:

$$\frac{dXP}{dt} = k_a \times XC - k_{12} \times XP - k_e \times XP + k_{21} \times XT \quad (7)$$

$$\frac{dXT}{dt} = k_{12} \times XP - k_{21} \times XT \quad (8)$$

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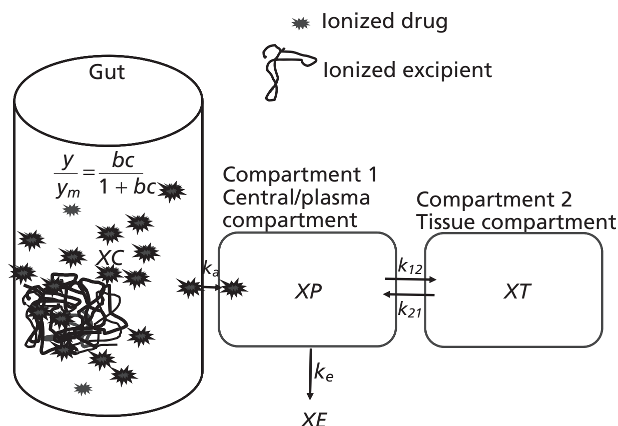

Figure 1 Schematic of a model for assessing the effect of drug-excipient binding interaction on oral absorption and plasma pharmacokinetics. This figure shows the presence of both the drug and the polymeric excipient in the gut compartment with the Langmuir binding isotherm equation (see text for details) and the transport of drug between the gut, the central/plasma compartment (compartment 1), the tissue compartment (compartment 2), and the elimination pathways. See text for explanation of abbreviations.

$$\frac{dXE}{dt} = k_e \times XP \quad (9)$$

where XP is the amount of drug in plasma, XE is the amount of drug eliminated from the plasma compartment, k_{12} is the rate of drug transport from compartment 1 (central or plasma) to compartment 2 (tissue), k_{21} is the rate of drug transport from compartment 2 to compartment 1, k_e is the elimination rate constant from the central compartment, and XT is the amount of drug in the tissue compartment.

Solving these equations simultaneously estimates the amount of free drug in the gut, amount of drug absorbed, and the plasma drug concentration as a function of time (Figure 1). This model allows parameter sensitivity analysis using a range of biopharmaceutical and pharmacokinetic parameters to identify cases where drug-excipient interaction may or may not pose significant bioavailability risk. For example, the effect of drug-excipient binding interaction on the plasma concentration-time profile can vary significantly depending on drug's dose and absorption rate constant. Numerical solution of the equations shown above was carried out using Scientist 3.0 software (Micromath Research, St Louis, MO, USA).

Isothermal titration calorimetry

Thermal changes associated with the BA-CCS binding interaction were studied by isothermal titration calorimetry (ITC) using iTC-200 (Microcal; GE Healthcare, Piscataway, NJ, USA). Briefly, 200 μl CCS solution at 0, 1, 3, 9, 20, 30, 40, 50, 60, 70, 85, or 100 mM concentration in 50 mM pH 4.5 acetate buffer was loaded in the cell and 35 μl of 10 mM BA solution in the same buffer was loaded in the syringe. Titration was carried out at 25°C by making twenty 2.5-μl injections every 180 s at a rate of 30 μl/min, using the in-built paddle at 1000 rev/min to mix the contents in the cell. Enthalpy change associated with each injection was recorded. ITC data were analysed using Microcal-Origin 7.0383 software (OriginLab Corporation, Northampton, MA, USA).

Animal pharmacokinetic study

A two-way crossover pharmacokinetic study was carried out between tablets manufactured with or without CCS in healthy male cynomolgus monkeys after a single oral dose. Eight monkeys (3.18–4.94 kg) were dosed with 400-mg tablets. A seven-day washout period was observed between crossover dosing. Blood samples were collected for up to 72 h postdose. BA concentration in plasma was determined using a validated liquid chromatography followed by tandem mass spectroscopy (LC-MS/MS) method.

Ethical committee approval for the animal study

The animal study was carried out at WuXi AppTec, Suzhou, Jiangsu Province 215004, China with approval of the institutional animal care and use committee under the study #BMS-20090417 obtained in April, 2009.

Statistical methods

Statistical analysis of the significance of differences in drug release rates in different dissolution media (Figure 2a) was carried out by analysis of variance and for the effect of formulation on drug release from two formulations (Figure 2b) by unpaired two-tailed *t*-test, after confirming the assumption of equal variances by the *F*-test, at $\alpha = 0.05$ for all time points using JMP 8.0 software (SAS Institute, Inc., Cary, NC, USA). The dissolution profiles did not meet the requirements for comparison by the similarity factor.[17] Pharmacokinetic parameters from the animal study were determined by non-compartmental analysis using WinNonlin v5.2 (Pharsight Corporation, Mountain View, CA, USA). A bioequivalence analysis between the two profiles was conducted by calculating the 90% confidence interval for the ratio of log-transformed pharmacokinetic parameters of maximum concentration reached in plasma (C_{max}), the time of maximum plasma concentration (T_{max}), and area under the plasma concentration-time curve from dosing till time of last sampling (72 h) (AUC_{0-t}). The plasma concentrations between the two groups were compared at each time point by repeated measures analysis of variance at $\alpha = 0.05$.

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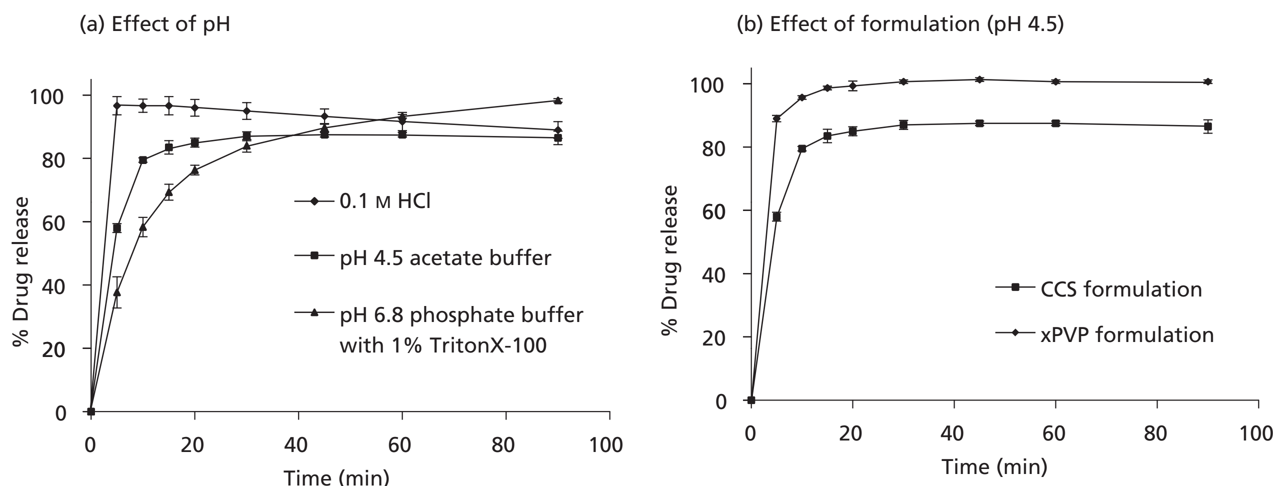

Figure 2 Comparison of drug release from a brivanib alaninate formulation containing croscarmellose sodium in different pH media (a) and with a formulation not containing croscarmellose sodium in pH 4.5 acetate buffer (b). The data plotted is the average ± (SD) of $n = 3$ tablets. In comparison of the effect of different pH media (a), data in the three pH media at all time points (except 60 min) were statistically significantly different. In comparison of the effect of formulation (b), data from the two formulations was statistically significantly different at all time points. CCS, croscarmellose sodium; xPVP, crospovidone.

Results

Drug-excipient binding in tablets

Dissolution studies on a tablet formulation containing 400 mg BA and 24 mg CCS (BA/CCS *m/m* ratio = 9.8) showed incomplete (~80%) drug release in pH 4.5 acetate buffer, although nearly complete release was obtained in 0.1 M HCl and pH 6.8 phosphate buffer with 1% w/w Triton X-100 (Figure 2a). Complete drug release in 0.1 M HCl was followed by decrease in drug concentration due to degradation at highly acidic pH (data not shown). On the other hand, drug release studies at pH 6.8 required the addition of a surfactant to generate sink conditions in the dissolution vessel, since drug solubility at this pH is extremely low (< 10 μg/ml). The intermediate pH of 4.5 offered both adequate solubility and stability of BA. Thus, sink condition was present in the dissolution vessel at pH 4.5 and incomplete drug release at this pH was not a result of the dissolution method. Also, pH 4.5 fell within the range where both BA and CCS were substantially ionized (discussed later).

When drug release from a formulation with CCS was compared with another formulation with crospovidone as a non-ionizable intragranular disintegrant at pH 4.5, complete drug release was obtained from the crospovidone-based formulation but not from the CCS-based formulation (Figure 2b). These results indicated that incomplete drug release at pH 4.5 was due to BA-CCS interaction.

To further confirm that BA-CCS interaction was the underlying cause of incomplete drug release in a pH 4.5 dissolution medium and investigate its reversibility, residues in dissolution vessels ($n = 3$), after conducting drug release study in pH 4.5 acetate buffer for 60 min (~80% drug released), were collected and transferred to 1000 ml of fresh pH 4.5 acetate buffer, pH 4.5 acetate buffer with a small amount of surfactant (0.1% w/v Triton X-100) to assess whether surfactant disrupted the interaction, or pH 6.8 phosphate buffer with 1% w/v Triton X-100 at 37°C in three separate dissolution vessels ($n = 1$ each). Drug release in these media was measured after rotating the paddle at 200 rev/min for 20 min. Only approximately 7% more drug was released (total ~87% released) in pH 4.5 acetate buffer, whereas complete release of the remaining amount was observed in both pH 4.5 acetate buffer with 0.1% Triton X-100 and pH 6.8 phosphate buffer with 1% Triton X-100. These results confirmed that BA-CCS interaction was the underlying cause of incomplete drug release in a pH 4.5 dissolution medium and indicated that this binding interaction was reversible.

Strength of BA-CCS binding in tablets was investigated by using different concentrations of the acetate buffer at pH 4.5 as a dissolution medium for $n = 3$ tablets each. Drug release from CCS-containing tablets did not change at different (66, 170, and 250 mM) buffer concentrations (data not shown). The drug release values at all time points were almost overlapping and statistically not significantly different.

Drug-excipient binding in solution

Addition of BA solution to different concentrations of CCS dispersion in pH 4.5 acetate buffer under continuous stirring, followed by equilibration at room temperature for 4 h, and

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analysis of drug content in the filtered solution by HPLC showed incomplete recovery of the drug. The proportion of BA bound to the CCS decreased with increasing BA to CCS ratio. Thus, 85.7% w/w of BA was bound to CCS at 1 : 1 BA/CCS *m/m* ratio, which decreased to 22.2% w/w at 4 : 1 BA/CCS *m/m* ratio, and to 5.8% w/w at 12 : 1 BA/CCS *m/m* ratio.

The proportion of drug bound was in agreement with the theoretical range predicted for a 1 : 1 ionic interaction of drug with repeating units of CCS, after correcting for the range of degree of substitution of CCS (0.60–0.85), as per the United States National Formulary monograph.[18] The predicted range was 60–85% bound drug for the interaction at 1 : 1 BA/CCS *m/m* ratio, 15–21.25% bound drug for the interaction at 4 : 1 BA/CCS *m/m* ratio, and 5–7.08% bound drug for the interaction at 12 : 1 BA/CCS *m/m* ratio. This indicated concentration dependence and the involvement of ionic interaction in the extent of binding.

These data were used to calculate the Langmuir adsorption parameters of BA/CCS binding, as outlined in the Methods section. The Langmuir parameters obtained were $b = 8.34\ \text{mM}^{-1}$ and $y_m = 0.85$ mmol BA/mmol CCS.

Reversibility and in-vivo relevance of this interaction was investigated by dilution of bound BA-CCS at 4 : 1 BA/CCS *m/m* ratio in fresh pH 4.5 acetate buffer with or without the addition of sodium taurocholate in a concentration range representative of fasted (3 mM) or fed (10 mM) state human intestinal fluids.[19] While 50-fold dilution of a solution containing BA and CCS with pH 4.5 acetate buffer did not result in any substantial change in the amount of bound drug (27.5% bound before dilution and 27.2% bound after dilution), dilution with a buffer containing 3.0, 7.5, or 15.0 mM sodium taurocholate resulted in complete drug release (0% bound after dilution). These results were indicative of free BA in solution partitioning into soluble sodium taurocholate micelles, thereby shifting the equilibrium of BA bound to the insoluble CCS in BA-CCS complexes towards releasing the free BA. Thus, partitioning of BA into sodium taurocholate micelles at high concentration led to higher drug recovery. These results indicated that the binding was reversible upon dilution with a surfactant, which is expected in-vivo.

In contrast, in the undiluted state, addition of sodium taurocholate resulted in a complex behaviour that showed increase in the proportion of bound drug from 27.5% to 96.3% at 3 mM sodium taurocholate, and 48.8% at 7.5 mM sodium taurocholate, but decrease to 5.1% at 15 mM sodium taurocholate concentration, respectively. Increase in fraction of BA bound to CCS at lower sodium taurocholate concentrations while decrease at high concentration suggested the presence of insoluble BA-CCS-sodium taurocholate species, in addition to the insoluble BA-CCS and the soluble BA-sodium taurocholate complexes, in solution. Thus, at low sodium taurocholate concentrations, the formation of BA-CCS-sodium taurocholate species led to increased fraction of bound drug, while the formation of sodium taurocholate micelles and BA-sodium taurocholate complexes at high sodium taurocholate concentration led to increased recovery of the unbound drug. These hypotheses about the nature and type of species in solution, however, are unverified. Nevertheless, the results indicated some involvement of hydrophobic interactions in BA-CCS binding, which was expected since CCS is polymeric and BA is an amphiphilic ionizable hydrophobic molecule that may act as a surfactant.

Isothermal titration calorimetry

To investigate the strength of BA-CCS interaction, enthalpy changes associated with the addition of BA to CCS at different BA/CCS ratios were measured by ITC. A 10 mM BA solution in 50 mM pH 4.5 acetate buffer was loaded in the syringe of the iTC-200 apparatus and added into buffer (control sample) or CCS in buffer at different CCS concentrations (test samples) in the cell. Titration of BA solution into buffer resulted in a strong endothermic signal (Figure 3, 0 mM CCS plot). This signal reflected the heat required for the dissociation of a self-associated form of the drug.[20] Dissociation of a self-associated state of BA at 10 mM in the syringe upon dilution to ~0.1 mM in the cell was consistent with concentration-dependent changes in ^{1}H nuclear magnetic resonance (NMR) spectroscopy shifts (data not shown).

Titration of BA into CCS in the cell clearly showed an exothermic heat release due to the binding of BA to CCS. Also, the exothermic enthalpy of BA-CCS interaction was lower than the endothermic enthalpy of self-association of the drug. As the CCS concentration in the cell was increased in separate titrations (Figures 3 and 4), the increasing heat released due to BA-CCS binding progressively blunted the endothermic response of BA dilution. These results indicated that the affinity of the BA-CCS interaction was in the range of the concentrations of CCS being studied (i.e., mM range).

Animal pharmacokinetic study

Following a single oral dose of 400 mg (~0.900 mmol) tablets (manufactured with or without CCS as the intragranular disintegrant) to eight male cynomolgus monkeys, the value for C_{max} was 27.4 ± 17.3 and 25.4 ± 26.5 μM at (T_{max}) 5.25 ± 2.1 and 4.75 ± 4.75 h postdose, respectively (mean ±SD). The drug's plasma half-life ($t^1/_2$) was 6.97 ± 1.4 and 7.15 ± 1.7 h, and the values for AUC_{0-t} were 278.1 ± 165.2 and 374.6 ± 379.9 μM h, for the formulation with or without CCS, respectively. The 90% confidence intervals for the ratio of log transformed pharmacokinetic parameters C_{max}, T_{max}, and AUC_0 were outside the 80–125% range, which is generally accepted as a criterion for human bioequivalence. This was because of the high variability

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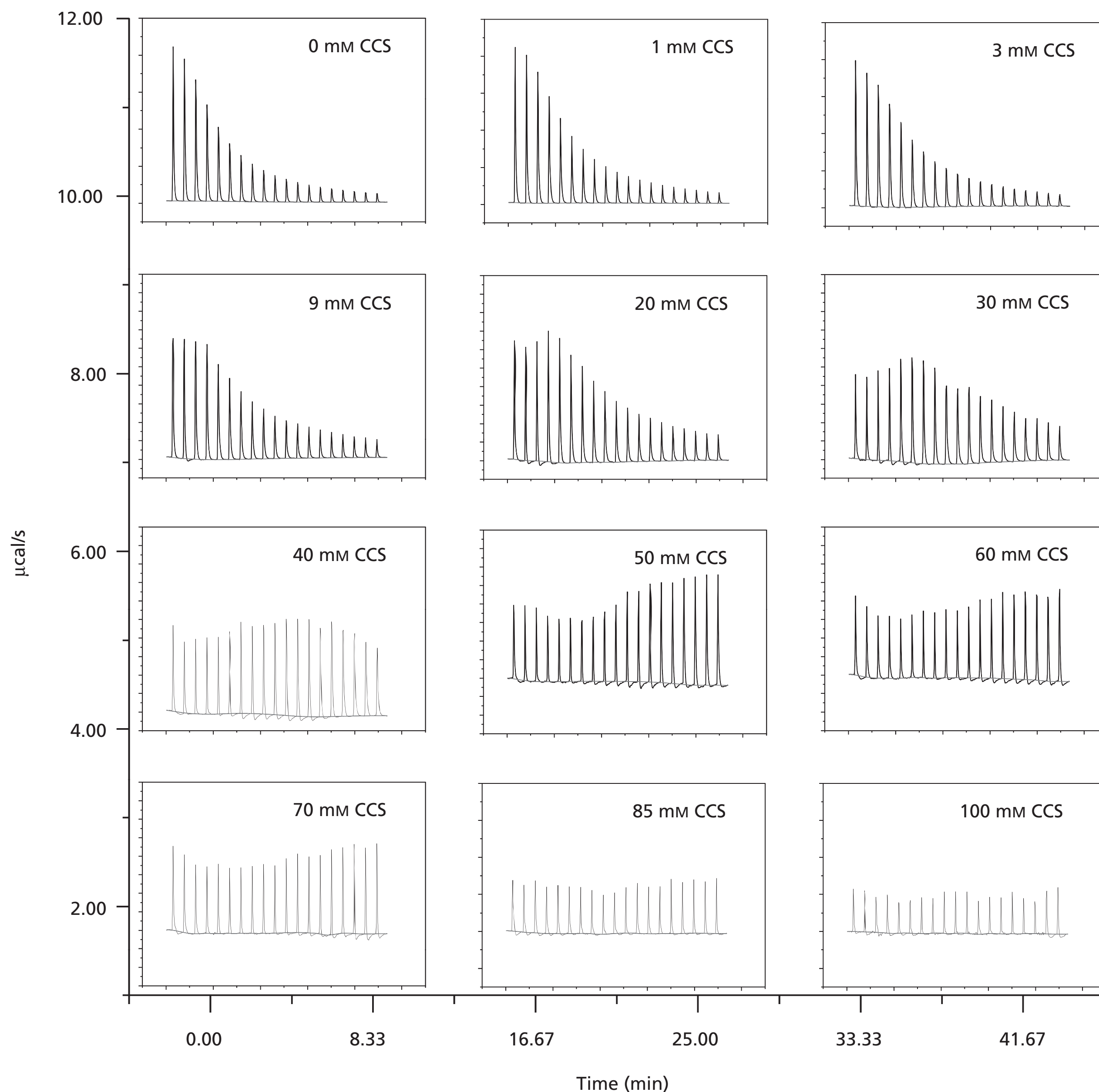

Figure 3 Isothermal titration calorimetry. Heat change (endotherm on the positive y-axis in μcal/s) at each injection of the drug solution in the syringe at 10 mM concentration into the croscarmellose sodium (CCS) suspension in the cell at concentrations noted in each sub-figure plotted on the y-axis against time on the x-axis. Each injection is associated with a distinct endothermic peak.

leading to low power of the study at the selected sample size. Although this study was not powered as a bioequivalence study, the mean BA plasma concentrations at all time points were similar (no statistically significant difference) between the formulations with or without CCS (Figure 5). The pharmacokinetic study indicated that the oral bioavailability of BA from the two formulations was similar.

Discussion

The effect of drug-excipient binding in the dosage form on the oral bioavailability of high dose drugs was mathematically modelled and experimentally studied using BA as a model amine drug and CCS as an anionic excipient. BA is a hydrophobic (log P = 2.5), weakly basic ($pK_a = 6.9$), amine

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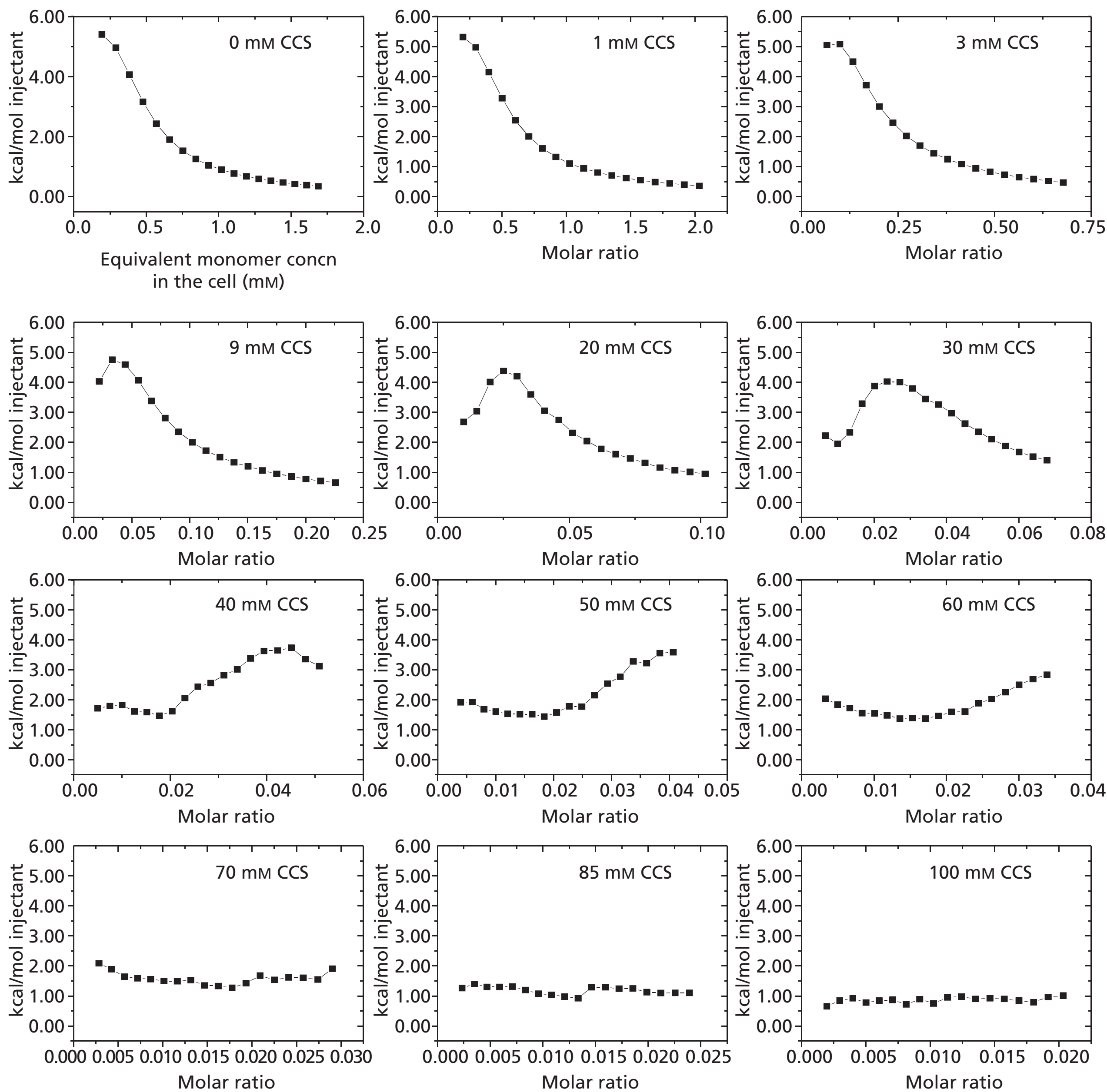

Figure 4 Isothermal titration calorimetry. Heat change per mol injectant (endotherm in kcal/mol injectant) at each injection of the drug solution in the syringe into the croscarmellose sodium (CCS) suspension in the cell plotted on the y-axis against the molar ratio of BA/CCS in the cell at each injection on the x-axis.

compound with self-association behaviour in acidic aqueous solutions. It shows pH dependent solubility – with very low solubility (< 10 µg/ml) at high pH and high solubility (> 4 mg/ml) under acidic pH conditions. Although the drug is stable over a wide pH range, it degrades in a highly acidic pH environment (e.g., 0.1 M HCl).

An oral tablet formulation of BA manufactured using CCS as a disintegrant showed incomplete drug release in pH 4.5 acetate buffer but not in 0.1 M HCl at pH 1.2 and in pH 6.8 phosphate buffer with 1% Triton X-100 as a nonionic surfactant (Figure 2a). High BA solubility at pH 4.5 assured sink conditions in the dissolution medium. However, at pH 4.5, both BA and CCS were substantially ionized. Thus, using the Henderson-Hasselbalch equation, 99.6% BA and 33.4% CCS were expected to be ionized at pH 4.5; while ionization of BA and CCS at pH 1.2 would be 100.0% and 0.0%, respectively.

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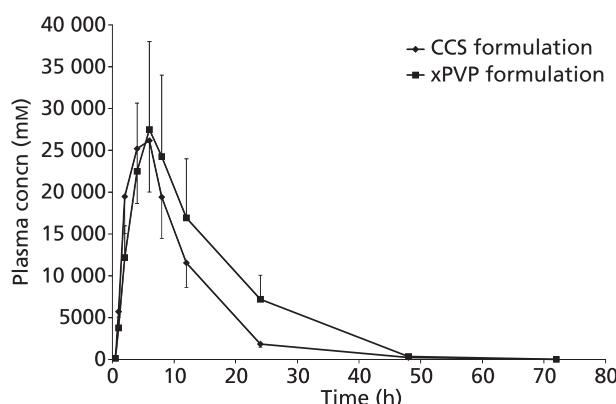

Figure 5 Mean plasma concentration-time profiles of brivanib alaninate tablet formulations with or without croscarmellose sodium after a single oral dose in male cynomolgus monkeys. The data represents mean ± standard error (SE) of $n = 7$ animals. Error bars are bidirectional but plotted unidirectionally only to facilitate data review. The study was carried out on eight animals, but one animal rejected tablet dosing. Therefore, one animal's data was excluded from the calculations. The plasma concentrations were statistically not significantly different at any time points. CCS, croscarmellose sodium; xPVP, crospovidone.

Thus, ionic interaction of BA and CCS was favoured at pH 4.5. Ionic interaction as the reason for incomplete drug release at pH 4.5 was supported by the observation that removing CCS from the formulation led to complete drug release (Figure 2b).

The absence of BA-CCS binding at pH 1.2 correlated with the absence of CCS ionization. At pH 6.8, although significant proportion of both BA and CCS were expected to be ionized (55.7% BA and 99.0% CCS), drug solubility at this pH was very low (< 10 μg/ml). Hence, BA-CCS binding in the buffer alone was not observable. For dissolution studies, a surfactant (1% w/v Triton X-100, or ~15.5 mM) was used to increase drug solubility and create sink conditions. The presence of surfactant significantly reduced BA-CCS interaction, which could account for complete drug release in pH 6.8 medium.

When the undissolved solid residue in the pH 4.5 acetate buffer dissolution vessel of the CCS-containing formulation was transferred to fresh media, 7% additional drug was released. When the material was transferred to fresh media at pH 4.5 or pH 6.8 containing a surfactant, all the remaining drug in the residue was released. These results indicated that the drug-excipient binding interaction in the tablet was reversible.

The impact of a drug's reversible binding interaction in the dosage form on its oral bioavailability is expected to depend on several biopharmaceutical factors, such as its solubility and ionization status; affinity and capacity for binding with the excipient; and the absorption rate constant. Ionization of drug and excipient as a function of pH was estimated at different pH values, using the pK_a values of the molecules and the Henderson-Hasselbalch equation. These results identified an 'interaction region' where both the drug and the excipient were substantially soluble and ionized.[3,4] The pH-dependence of drug-excipient interaction was expected to reduce its impact on the drug's oral absorption given the changing pH of the gastrointestinal tract. A passively-absorbed weakly basic amine drug would be positively charged in the acidic pH in the stomach. However, certain treatments, such as antacids, can increase gastric pH.[21,22] Therefore, drug-excipient interaction was studied at pH 4.5, since it was close to the pH of the upper small intestine and the antacid-treated gastric pH.[23] For a drug without a narrow absorption window, pH 4.5–6.8 represents the pH of gastrointestinal regions of maximum drug absorption. Also, secretion of bile in the duodenum leads to the presence of surfactants in the intestinal fluids ~pH 6.8, which may disrupt reversible drug-excipient ionic binding. Therefore, pH 4.5 was selected for in-vitro investigation of BA-CCS binding and modelling its effect on oral absorption.

Drug-CCS binding can be described by an adsorption isotherm since polymeric CCS forms an insoluble colloidal dispersion and binds a soluble drug (solute) from an aqueous medium. Chien *et al.*[3] described the binding of oxymorphone derivatives to CCS and sodium starch glycolate using the Freundlich adsorption isotherm:

$$\frac{x}{m} = kc^{1/n} \tag{10}$$

where x is the mass of solute in the medium or adsorbate, m is the mass of adsorbing material, c is the equilibrium concentration of the solute in solution, and k and n are constants for specific adsorbate and adsorbent, respectively, at a given temperature.

The Freundlich adsorption isotherm is an empirically derived adsorption model, which assumes that solute adsorption on a substrate can proceed via substrate-solute interactions and solute-solute interactions. The Freundlich model does not allow for the saturation of adsorptive surface, thus modelling multilayer adsorption. On the other hand, the Langmuir adsorption model assumes that the saturation of adsorptive surface is possible, with y eventually reaching y_{max}.[24,25]

Conventionally, both the Freundlich and the Langmuir adsorption isotherms are used as a convenient empirical representation of a single-component experimental data, without implying the inherent validity of any particular physical model that may underlie the derivation of either isotherm.[26] Nevertheless, the Langmuir monolayer adsorption model is mechanistically more appropriate for analysing specific drug-excipient binding interactions. Therefore, this model (eqn 3) was used in our work. In the absence of sodium

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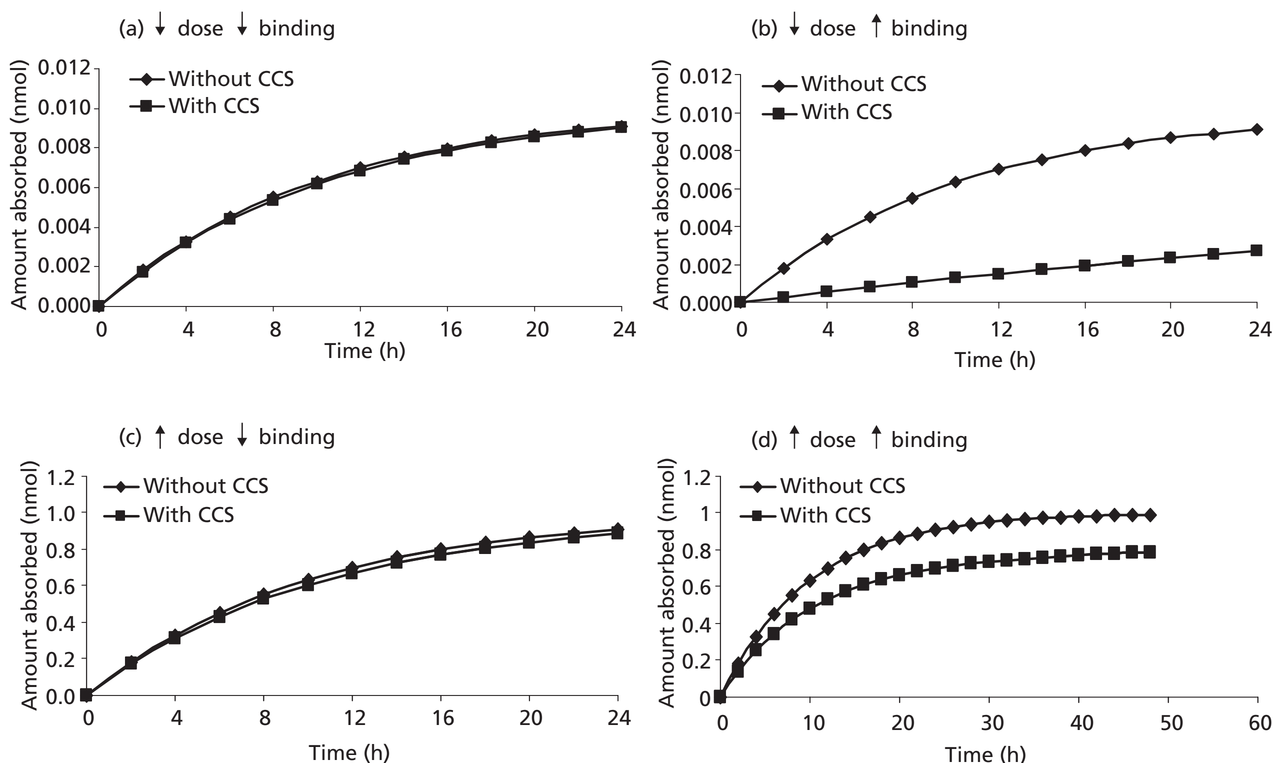

Figure 6 Parameter sensitivity analysis for modelling the effect of drug-croscarmellose sodium binding on oral drug absorption. Simulated amount of drug absorbed as a function of time for: (a) a low dose drug with low croscarmellose sodium (CCS) binding affinity and capacity (using parameters drug = 5 mg per tablet, CCS = 5 mg per tablet, y_m = 0.5 mmol drug/mmol CCS, b = 1 /mM, and k_a = 0.1 h-1); (b) low dose drug with high CCS binding affinity and capacity (using parameters drug = 5 mg per tablet, CCS = 5 mg per tablet, y_m = 2.0 mmol drug/mmol CCS, b = 50 1/mM, and k_a = 0.1 h-1); (c) high dose drug with low CCS binding affinity and capacity (using parameters drug = 500 mg per tablet, CCS = 30 mg per tablet, y_m = 0.5 mmol drug/mmol CCS, b = 1 1/mM, and k_a = 0.1 h-1); and (d) high dose drug with high CCS binding affinity and capacity (using parameters drug = 500 mg per tablet, CCS = 30 mg per tablet, y_m = 2.0 mmol drug/mmol CCS, b = 50 1/mM, and k_a = 0.1 h-1).

taurocholate, the BA-CCS binding profile in acidic aqueous solution fitted the Langmuir isotherm. The Langmuir parameters were $b = 8.34$ mM^{-1} and $y_m = 0.85$ mmol BA/mmol CCS.

The drug-excipient binding interaction parameters can be used to solve equations 3–9 to simulate the effect of various variables on the drug's plasma concentration following oral administration (Figure 1). Thus, the effect of changing parameters within realistic ranges on the drug's oral pharmacokinetics can be estimated using this model.

Oral drug absorption was simulated for a hypothetical low dose drug of molecular weight 500 g/mol at a dose of 5 mg per tablet with the assumption of 100 mg tablet weight and 5 mg per tablet CCS amount. A high dose formulation was simulated at a drug dose of 500 mg per tablet with the assumption of 600 mg tablet weight and 30 mg per tablet CCS amount. Similarly, low CCS binding was assumed to have $y_m = 0.5$ mmol drug/mmol CCS and $b = 1$ mM^{-1}, while high CCS binding was assumed to have $y_m = 2.0$ mmol drug/mmol CCS and $b = 50$ mM^{-1}. The pharmacokinetic parameters were assumed constant for all simulations. Thus, the absorption rate constant, k_a, was assumed 0.1 h-1; elimination rate constant, k_e, was assumed 1 h-1; inter-compartment transfer rate constants, k_{12} and k_{21} were assumed 0.1 h-1 and 10 h-1, respectively; and the volume of distribution, V_d, was assumed 1 l. These simulations (Figure 6) indicated that a drug's dose and extent of drug-excipient binding were the key determinants of its oral absorption. When the binding constant and capacity were low, the impact of drug-excipient binding on a drug's oral bioavailability was low – even for low dose drugs. At the same time, at high binding constant and capacity, the drug-excipient binding could affect a drug's oral bioavailability – and this effect was more pronounced for low dose drugs.

Using Langmuir adsorption parameters and pharmacokinetic parameters of BA from previous clinical studies (data not shown), the plasma concentration profile of BA in a tablet formulation with or without CCS was simulated (Figure 7). In line with the expectation for high dose drugs, these results predicted minor effect of BA-CCS binding on the drug's pharmacokinetic profile. These results were consistent with

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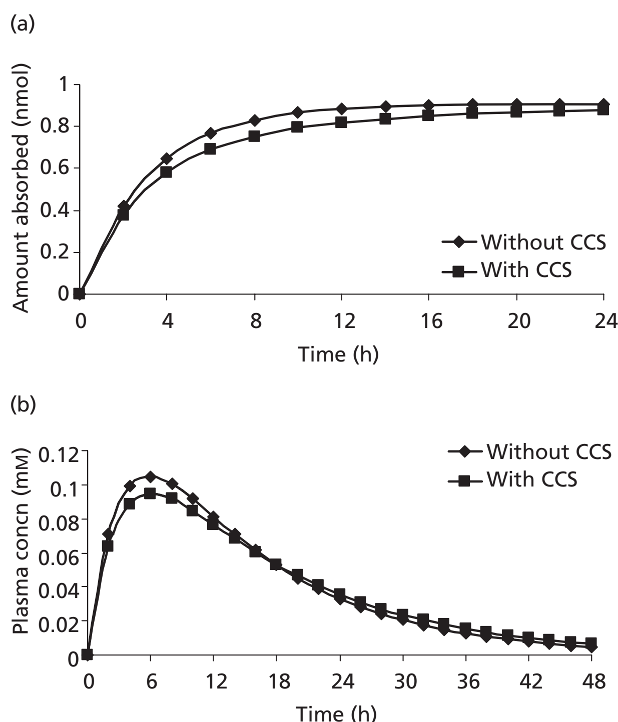


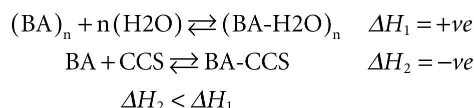
Figure 7 Simulation of the effect of brivanib alaninate (BA) cros carmellose sodium (CCS) binding on plasma concentration profiles of BA after a single oral dose of 400-mg tablet. The pharmacokinetic parameters of BA used were: volume of distribution, $V_d = 5.4$ l, $k_a = 0.31$ h-1, $k_{12} = -0.11$, $k_{21} = 112$, and $k_e = 0.08$). Binding parameters observed using Langmuir isotherm were: $y_m = 0.85$ mmol BA/mmol CCS and $b = 8.34$ mM^{-1}. (a) The amount of drug absorbed as a function of time. (b) The plasma concentration–time profile.

the expectation of BA-CCS binding being disrupted in-vivo due to other components of the gastrointestinal fluid, such as sodium taurocholate.

In addition, ITC was used to assess the strength of binding.[27] Binding of two species is thermodynamically driven by a decrease in the free energy (ΔG) of the complex over that of the reactants. Thus, binding can be driven by either negative enthalpy (ΔH) or positive entropy (ΔS), or a combination of the two. In addition, positive enthalpy could be an energy barrier to the progress of a binding interaction.[28] ITC involves sequential injection of a small volume (e.g., 2.5 µl) of a binding reactant in the syringe at high concentration (e.g., 10 mM BA) into a larger volume (e.g., 200 µl) of the other binding reactant in the microcalorimeter cell (e.g., CCS). A binding interaction is associated with a release of heat from the system (exotherm), which is registered as a negative *y*-axis peak since the heat is absorbed from the cell by the ITC instrument to maintain the temperature constant (isothermal). Similarly, phenomena that are associated with gain of heat by the system (endotherm), such as dissociation of a self-associated species in the syringe that gets diluted upon injection into a buffer in the cell, are registered as a positive *y*-axis peak since the heat is provided to the cell by the ITC instrument to maintain isotherm.

When BA was titrated into buffer (Figures 3 and 4, top left, 0 mM CCS plot), endothermic heats of dilution were observed. This reflected the dissociation of self-associated state of BA upon dilution (approximately 100-fold) into the cell. Dilution thermograms of dimers show a typical hyperbolic shape, whereas higher order association structures show sigmoidal dilution endotherms with an inflection.[29] The shape of the ITC data did not perfectly fit to a simple dimer-monomer model (Microcal-Origin 7 software, data not shown). This suggested that self-association of BA could involve higher order oligomeric forms in the syringe at 10 mM concentration. Self-association of BA was evident in ^{1}H NMR studies that showed BA self-association progressively increasing from 0.1 to 5 mM and the presence of a self-associated state at 10 mM (data not shown). Nevertheless, approximating the equilibrium with a dimer-monomer model yielded an enthalpy, ΔH, of 25.5 ± 3.0 kcal/mol. By comparison, this indicated strong self-association of BA relative to the dissociation of insulin dimers, which had $\Delta H = 9.8$ kcal/mol.[30] This strong self-association of BA reflected cooperative binding interactions, which may have included hydrophobic domains, aromatic resonance structure with planar π electron clouds, and/or electronegative atoms that could hydrogen bond.

When BA was titrated into CCS (Figures 3 and 4), dilution of BA and its binding with CCS occurred simultaneously, and the contents of the cell re-equilibrated before the next ITC injection (in 3 min). Thus, the heat changes in the ITC experiment were attributed to the equilibria of: dilution of *n* molecules of self-associated BA leading to the breakage of BA-BA bonds and the formation of BA-H_2O bonds with a positive enthalpy (+ ve ΔH_1, takes heat, endotherm); and formation of BA-CCS complex with a negative enthalpy (-ve ΔH_2, releases heat, exotherm), as summarized below.



$$(\mathrm{BA})_n + n(\mathrm{H2O}) \rightleftarrows (\mathrm{BA\text{-}H2O})_n \quad \Delta H_1 = +ve$$
$$\mathrm{BA} + \mathrm{CCS} \rightleftarrows \mathrm{BA\text{-}CCS} \quad \Delta H_2 = -ve$$
$$\Delta H_2 < \Delta H_1$$

Titration of BA into increasing concentrations of CCS in the cell (Figures 3 and 4) generated ITC curves that were characterized by: progressive reduction in the initial endotherm of BA dilution; shifting of the BA dilution endotherm to the right with increasing BA/CCS ratios; and reduction of the initial declining slope of the drug dilution endotherm. For example, only blunting of the initial endotherm was observed at 3 mM CCS concentration, while shifting of the maximum heat released along with lowering of the kcal/mol injectant was observed between 9 and 30 mM CCS concentrations

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(Figure 4). These observations were consistent with $\Delta H_2 < \Delta H_1$ and the BA-CCS binding exotherm partially compensating for the heat required for the drug dilution endotherm. Thus, the initial reduction in the endothermic signal of BA dilution was due to exothermic BA-CCS binding, which, at lower CCS concentrations, was followed by the endothermic heat of dissociation of BA at later titration points. At very high CCS concentrations, the endothermic heat of dissociation became progressively less pronounced as the extent of BA-CCS binding increased and dominated the profile.

Overall, the ITC data indicated a weak exothermic interaction between BA and CCS under these conditions. The affinity of the BA-CCS interaction was in the range of the CCS concentration used in these experiments. Therefore, the dissociation constant for BA-CCS interaction was expected to be in the mM range.[31] This was in-line with the Langmuir isotherm analysis, which showed an association constant of 8.34 mM^{-1} (which corresponded to a dissociation constant of 0.12 mM). This interaction is much weaker than typical drug-protein interactions, which are in the μM to pM range.[32] Binding partners with weak binding affinity constants are postulated to readily dissociate in-vivo.[33] Thus, the ITC data indicated that BA-CCS interaction was weak and could be displaced in-vivo by other components in the solution. In line with this expectation, the presence of sodium taurocholate in the aqueous medium disrupted BA-CCS interaction in a concentration-dependent manner, as discussed earlier.

Thus, both ITC and Langmuir adsorption-oral absorption modelling predicted non-biorelevance of BA-CCS interaction on oral BA absorption. The in-vivo pharmacokinetic study of BA comparing a tablet formulation with or without CCS as a disintegrant indicated similar plasma concentration–time profiles of the two formulations (Figure 5). These results confirmed the predictions based on modelling and simulation, and weak binding, as indicated by calorimetric titrations.

We propose the use of ITC and Langmuir binding analysis to assess the strength and biorelevance of drug-excipient binding interactions. The results of our in-vitro and in-vivo studies with BA-CCS interaction support this proposition. Although the Langmuir adsorption-oral pharmacokinetic model proposed in this work incorporated the effect of drug dose directly and the effect of physicochemical properties of the drug indirectly (by their effect on the drug's pharmacokinetic parameters), it did not take into consideration the effect of surfactant, salt concentration, and other components of the gastrointestinal tract such as food. This model is also limited in not accounting for the effect of active or site-specific drug absorption. Further studies with different drugs, excipients, and types of interactions would help confirm the value of ITC and Langmuir binding as in-vitro tools to assess the strength and biorelevance of a binding interaction.

Conclusions

Biorelevance of drug-excipient binding interaction is conventionally assessed by the stability of interaction in the presence of physiologically relevant concentration of salt/buffer solution. Using a model drug (BA), we observed that the drug-excipient (CCS) interaction was not disrupted by higher concentrations of buffer solution. In contrast, microcalorimetry, Langmuir adsorption modelling, and pharmacokinetic simulation indicated that the binding interaction was weak and should not be biorelevant. In-vivo studies confirmed the lack of biorelevance of BA-CCS binding interaction. These results indicated that ITC, Langmuir adsorption modelling, and pharmacokinetic simulation could be better tools to assess the biorelevance of drug-excipient binding interactions than ion displacement studies. In addition, the simulation and experimental studies indicated that most reversible and pH-dependent weak drug-excipient binding interactions are unlikely to affect a drug's oral bioavailability.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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