# The use of bile salt micelles for the prediction of human intestinal absorption

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**Abstract**

Human intestinal absorption (HIA) will dictate biopharmaceutical performance through its influence on ADME (Absorption, Distribution, Metabolism and Elimination) and can vary significantly depending upon the nature of the compound under consideration. In this study, an *in vitro* assay method is proposed for the prediction of HIA through the measurement of drug solubility in an aqueous phase containing micellar bile salt, namely sodium deoxycholate (NaDC). A series of twenty compounds, displaying a range of physicochemical properties and known HIA values, were analysed using UV spectroscopy to determine a solubilisation ratio (SR) for each compound. A micelle/water partition coefficient ($K_{xm/a}$) was calculated and then used to develop an equation through simple linear regression; logit HIA = -0.919 + 0.4618 log$K_{xm/a}$ ($R^2$ = 0.85). From this equation a value for % HIA was determined which compared well with literature. Furthermore, four additional drugs were then analysed using the developed equation and found to match well with literature, confirming the suitability of the method. Using a simple, economic and robust UV bile salt assay allows prediction of human intestinal absorption and avoids many of the disadvantages of other techniques, such as animal based methods.

**Keywords**

Human Intestinal absorption; HIA; solubilisation; UV; bile salts

## Introduction

Human intestinal absorption (HIA) is the mechanism through which drugs traverse from the intestine into the bloodstream. The vast majority of active pharmaceutical ingredients are administered orally thus it is essential that they are absorbed within the intestine to reach the intended site of action. Although it is possible to measure the percent HIA (% HIA) during clinical studies, it is far more useful to be able to predict the value much earlier on during drug development. It is for this reason that a significant amount of research has been undertaken in an attempt to develop a reliable, robust and accurate method to predict % HIA.

Several different predictive approaches have been undertaken, including computational (*in silico*) methods[1], such as quantitative structure-activity relationships (QSARs)[2] and physiologically-based pharmacokinetic (PBPK) modelling[3]. These techniques have a clear advantage in that they remove the need for costly laboratory based experimental measurement yet their predictive ability can be limited.

*In vitro* models for the prediction of absorption include the application of dissolution analysis[4], chromatographic analysis[5] and dynamic gastric models[6]. Many of these *in vitro* models have included the presence of physiologically relevant solvent compositions, mainly because it is known that solvent composition dictates intestinal drug solubility which, in turn, is an important factor in determining the rate, and extent, of absorption[7]. The specific components within human intestinal fluids that dramatically alter drug solubility are bile salts. The main biological function of bile salts is to solubilise lipids and vitamins in the intestine with a similar effect encountered for orally administered drugs. For a full review of the absorption-enhancing effects of bile salts see Ref 8[8].

In humans, the composition of bile salts is rather complex and for the purposes of this study was simplified to consider one bile salt in particular, namely sodium deoxycholate (NaDC). NaDC is a well-characterised amphiphilic molecule which can undergo micellar aggregation[9], stabilised by polar interactions[10], with comparatively small aggregation numbers as a result of the rigid molecular structure[11]. Previous research within our group has shown that NaDC, when in the presence of drugs, will exhibit modified physicochemical properties, for example a variable (drug-specific) reduction in critical micellar concentration (CMC)[12].

When quantifying (or comparing) enhancement in solubility for a specific drug, or series of drugs, it is possible to evaluate the solubilisation ratio (SR), where SR is equal to the moles of drug solubilised per mole of bile salt. One study in particular calculated SR for a series of steroids and then used this data to calculate micelle/water partition coefficients ($K_{m/w}$) which were then correlated with octanol/water partition coefficients ($P_{o/w}$)[13]. Using this same theory as a basis for drug-NaDC measurement, this paper describes the evolution of measuring SR and then using these values as the basis to form an equation to permit prediction of % HIA, thereby presenting an *in vitro* method to predict *in vivo* behaviour.

## Materials and Methods

### Materials

Aqueous solutions of sodium deoxycholate (NaDC), used as purchased from Sigma Aldrich, Dorset, UK (97 %), were prepared by dilution from a 20 mM stock solution with distilled water as necessary to achieve concentrations of 7, 9, 11, 13, 17 and 20 mM (i.e. always at concentrations greater than the stable micelle CMC concentration of NaDC[9b]). The

24 compounds considered in this work were: acetaminophen (99 %, Sigma Aldrich, Dorset, UK), acetyl salicylic acid (99 %, Acros organics, Geel, Belgium), alprenolol (98 %, Sigma Aldrich, Dorset, UK), amitriptyline (98 %, Sigma Aldrich, Dorset, UK), carbamazepine (99 %, Sigma Aldrich, Dorset, UK), cimetidine (Sigma Aldrich, Dorset, UK), diclofenac (98 %, TCI, Europe), diphenhydramine (98 %, TCI, Europe), fenoprofen (97 %, Fluka, Dorset, UK), fluconazole (98 %, Sigma Aldrich, Dorset, UK), flurbiprofen (98 %, TCI, Europe), gemfibrozil (98 %, TCI, Europe), ibuprofen (98 %, BASF, Cheshire, UK), indomethacin (99 %, Sigma Aldrich, Dorset, UK), ketoprofen (98 %, Sigma Aldrich, Dorset, UK), lidocaine (98 %, Sigma Aldrich, Dorset, UK), mannitol (98 %, Sigma Aldrich, Dorset, UK), meloxicam (98 %, TCI, Europe), naproxen (98 %, Sigma Aldrich, Dorset, UK), phenylbutazone (99 %, Sigma Aldrich, Dorset, UK), piroxicam (98 %, Sigma Aldrich, Dorset, UK), propranolol (99 %, Sigma Aldrich, Dorset, UK), quinine (96 %, Fluka, Dorset, UK), terbutaline (96 % Sigma Aldrich, Dorset, UK), used as purchased**.** All experimental work was conducted without altering the pH or ionic strength to avoid the formation of a surfactant-gel hydropolymer.

**Method**

A calibration plot was established at each of the 6 bile salt concentrations using the Agilent Cary 60 UV-Vis spectrophotometer set at wavelength of maximum absorbance for each drug as follows (acetaminophen $\lambda_{max}$. 243 nm, acetyl salicylic acid $\lambda_{max}$. 295 nm, alprenolol $\lambda_{max}$. 270 nm, amitrityline $\lambda_{max}$. 240 nm, carbamazepine $\lambda_{max}$. 284 nm, cimetidine $\lambda_{max}$. 218 nm, diclofenac $\lambda_{max}$. 276 nm, diphenhydramine $\lambda_{max}$. 221 nm, fenoprofen $\lambda_{max}$. 271 nm, fluconazole $\lambda_{max}$. 260 nm, flurbiprofen $\lambda_{max}$. 247 nm, gemfibrozil $\lambda_{max}$. 274 nm, ibuprofen $\lambda_{max}$. 272 nm, indomethacin $\lambda_{max}$. 320 nm, ketoprofen $\lambda_{max}$. 261 nm, lidocaine $\lambda_{max}$. 262 nm, mannitol $\lambda_{max}$. 295 nm, meloxicam $\lambda_{max}$. 362 nm, naproxen $\lambda_{max}$. 230 nm, phenylbutazone

$\lambda_{max}$. 264 nm, piroxicam $\lambda_{max}$. 355 nm, propranolol $\lambda_{max}$. 292 nm, quinine $\lambda_{max}$. 332 nm, terbutaline $\lambda_{max}$. 280 nm), also the sample cell was thermostated at 37 $^{0}$C. Separately, an excess of drug was added to 1 mL of each bile salt concentration in a microcentrifuge tube and placed in a shaking water bath for 48 hours at 37 ºC, then centrifuged at 13,000 rpm, filtered and diluted using the corresponding bile salt concentration. Using the regression equation obtained from the established calibration plot of each drug at each bile salt concentration, the concentration of solubilised drug was determined. A plot of the amount solubilised with bile salt concentration facilitated calculation of the solubilisation ratio (SR) whereby the mole fraction solubilised ($X_m$) is equal to SR/(1 + SR) and can be combined with the literature-based calculated mole fraction aqueous solubility ($X_a$) to determine the micelle/water partition coefficient ($K_{xm/a}$) as follows[14]:

$$K_{xm/a} = X_m / X_a$$

Results from the UV analysis permitted the development of a dataset that contained $\log K_{xm/a}$ values for 20 compounds along with their physicochemical parameters (e.g. molecular weight, rotatable bonds, molar volume, number of hydrogen bond acceptors) published human intestinal absorption (HIA) values facilitating development of an equation to relate $\log K_{xm/a}$ with HIA using simple linear regression in combination with the established equation:

$$\text{Logit HIA} = \log [\% \text{ HIA} / (100 - \% \text{ HIA})] \quad [15]$$

A further four compounds were then similarly analysed by measuring $\log K_{xm/a}$, to predict % HIA. A comparison was then made between the predicted values with those published in literature. Simple linear regression analysis was carried out using Minitab 17® (Minitab Inc., State college, PA, USA; licensed to the University of Huddersfield) where the previously mentioned dataset was imported into it. The final model was obtained by excluding molecular descriptors which were not statistically significant (P-value > 0.05) and those with

unacceptably high levels of variance inflation factor (VIF), which is considered as a multicollinearity indicator, were not included in the final model. Cook's distance and residuals were used to detect whether any of the model variables had high leverage. The optimal final model was then obtained including only $\log K_{xm/a}$ as a predictor for logit HIA, the model was then validated using four compounds.

## Results and Discussion

In total, twenty four drugs were analysed to determine the concentration of drug in solution as a function of NaDC concentration, these were selected to cover a range of physicochemical properties, such as reported HIA, log $P_{o/w}$ and other properties. All data were then plotted to determine a SR value for each drug (i.e. the slope): a selection of which can be seen in Figure 1.

Figure 1 clearly shows a linear relationship between the concentration of drug and the concentration of NaDC. Only linear sections of the plots were incorporated to calculate SR, some were deemed to be non-linear, such as the lower concentrations of quinine and the

147 higher concentrations of acetaminophen (data not shown). These non-liner relationships may
148 be due to preferential drug-drug interactions rather than drug-NaDC interactions as such
149 drugs are known to self-associate [16]. The majority of the compounds did exhibit a linear
150 relationship over the concentration range studied (7 – 20 mM) ensuring confidence in the
151 experimental system. Using the calculated SR value, along with the mole fraction of aqueous
152 solubility for the drug, facilitated calculation of a micelle/aqueous partition coefficient for
153 each drug. By analysing these values alongside reported % HIA literature data (Table 1)
154 enabled the application of simple linear regression to construct an equation to permit
155 calculation of % HIA for any compound through measurement of its solubility in NaDC.

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165 Using data presented in Table 1, simple linear regression analysis was utilised to create an
166 optimised equation to predict HIA:

167 $$\text{Logit HIA} = -0.919 + 0.4618 \log K_{xm/a}$$

S= 0.236264 $R^2$ = 0.8492, $R^2_{adj.}$= 0.8409, $R^2_{Pred.}$= 0.8232, F=101.388. The P-values obtained for this model indicate that the relationship between % HIA and $logK_{xm/a}$ values was statistically significant at the 95 % confidence level where they were < 0.05, also the model's F-ratio was found to be statistically significant. $logK_{xm/a}$ was found to have a 95 % confidence interval of (0.365, 0.558) and a t-value of (10.069). According to Cook's distance and residuals, no drug among the model's dataset was found to be influential or having high leverage. The unadjusted $R^2$ of 0.8492 derived from the current data indicates that the fit of the sampled drugs to the model is good. The $R^2_{Pred.}$ value of 0.8232 indicates that the fit of the drugs to the model is valid and confirms the potential suitability of UV measurement of solubility using NaDC to predict oral drug absorption in the human GI tract. The close values of $R^2_{adj}$ and $R^2_{Pred.}$ show no evidence of the current model over-fitting the data. A Durbin-Watson statistic value of 2.309 proves the absence of autocorrelation in the current regression model. A summary of predicted % HIA values using the established equation alongside published literature values for % HIA can be seen in Table 2 including validation compounds.

A plot of calculated % HIA with corresponding literature values can be seen in Figure 2.

## Conclusions

Overall, the development of an equation to predict % HIA using a simple UV based technique via calculation of the micelle/water partition coefficient, has been shown to be statistically appropriate and reliable as a method to determine intestinal absorption. Using a simple, economic and robust UV bile salt assay allows prediction of human intestinal absorption and avoids many of the disadvantages of other techniques, such as animal based methods.

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