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 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. A series of twenty compounds, displaying a range of physico- chemical properties and known HIA values, were analyzed using UV spectroscopy to determine a solubilization ratio 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, 4 additional drugs were then analyzed 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 HIA and avoids many of the disadvantages of other techniques, such as animal-based methods.

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,[1,2] such as quantitative structure-activity relationships[3,4] and physiologically based pharmacokinetic modelling.[5] 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,[6] chromatographic analysis,[7] and dynamic gastric models.[8] 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.[9] The specific components within human intestinal fluids that dramatically alter drug solubility are bile salts. The main biological function of bile salts is to solubilize 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.[10]

In humans, the composition of bile salts is rather complex and for the purposes of this study was simplified to consider 1 bile salt in particular, namely sodium deoxycholate (NaDC). NaDC is a well-characterized amphiphilic molecule which can undergo micellar aggregation,[11,12] stabilized by polar interactions,[13] with comparatively small aggregation numbers as a result of the rigid molecular structure.[14] 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.[15]

When quantifying (or comparing) enhancement in solubility for a specific drug, or series of drugs, it is possible to evaluate the solubilization ratio (SR), where SR is equal to the moles of drug solubilized per mole of bile salt. One study in particular calculated SR for a series of steroids and then used these data to calculate micelle/water partition coefficients ($K_{m/w}$) which were then correlated with octanol/water partition coefficients ($P_{o/w}$).[16] Using this same theory as a basis for drug-NaDC measurement, this article 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* behavior.

Materials and Methods

Materials

Aqueous solutions of NaDC (97%), used as purchased from Sigma-Aldrich (Dorset, UK), 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 con- centrations greater than the stable micelle critical micellar con-

centration of NaDC[12]). The following 24 compounds considered in

this work were used as purchased: acetaminophen (99%; Sigma- Aldrich), acetyl salicylic acid (99%; Acros Organics, Geel, Belgium), alprenolol (98%; Sigma-Aldrich), amitriptyline (98%; Sigma- Aldrich), carbamazepine (99%; Sigma-Aldrich), cimetidine (Sigma- Aldrich), diclofenac (98%; TCI Europe, Zwijndrecht, Belgium), diphenhydramine (98%; TCI Europe), fenoprofen (97%; Fluka, Dorset, UK), fluconazole (98%; Sigma-Aldrich), flurbiprofen (98%; TCI Europe), gemfibrozil (98%; TCI Europe), ibuprofen (98%; BASF, Cheshire, UK), indomethacin (99%; Sigma-Aldrich), ketoprofen (98%; Sigma-Aldrich), lidocaine (98%, Sigma-Aldrich), mannitol (98%; Sigma-Aldrich), meloxicam (98%; TCI Europe), naproxen (98%; Sigma-Aldrich), phenylbutazone (99%; Sigma-Aldrich), pir-

oxicam (98%, Sigma-Aldrich), propranolol (99%; Sigma-Aldrich), quinine (96%; Fluka), and terbutaline (96% Sigma-Aldrich). 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 Spectrophotom- eter set at wavelength of maximum absorbance for each drug as follows: acetaminophen λ_{max}. 243 nm, acetyl salicylic acid λ_{max}. 295 nm, alprenolol λ_{max}. 270 nm, amitrityline λ_{max}. 240 nm, carba- mazepine λ_{max}. 284 nm, cimetidine λ_{max}. 218 nm, diclofenac λ_{max}. 276 nm, diphenhydramine λ_{max}. 221 nm, fenoprofen λ_{max}. 271 nm, fluconazole λ_{max}. 260 nm, flurbiprofen λ_{max}. 247 nm, gemfibrozil λ_{max}. 274 nm, ibuprofen λ_{max}. 272 nm, indomethacin λ_{max}. 320 nm, ketoprofen λ_{max}. 261 nm, lidocaine λ_{max}. 262 nm, mannitol λ_{max}. 295 nm, meloxicam λ_{max}. 362 nm, naproxen λ_{max}. 230 nm, phen- ylbutazone λ_{max}. 264 nm, piroxicam λ_{max}. 355 nm, propranolol λ_{max}. 292 nm, quinine λ_{max}. 332 nm, terbutaline λ_{max}. 280 nm; also,

the sample cell was thermostated at 37^{o}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 h at 37^{o}C, then centrifuged at 13,000 rpm, filtered, and diluted using the corresponding bile salt concentration. Using the regression equa- tion obtained from the established calibration plot of each drug at each bile salt concentration, the concentration of solubilized drug was determined. A plot of the amount solubilized with bile salt concentration facilitated calculation of the SR whereby the mole fraction solubilized (X_m) is equal to SR/(1 SR) and can be com- bined with the literature-based calculated mole fraction aqueous solubility (X_a) to determine the micelle/water partition coefficient

($K_{xm/a}$) as follows[17]: $Kxm/a=Xm/Xa$

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 accep- tors) and published 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:

Logit HIA = log [% *HIA*/(100 — % *HIA*)][18]

A further 4 compounds were then similarly analyzed by measuring log$K_{xm/a}$ to predict % HIA. A comparison was then made between the predicted values and those published in literature. Simple linear regression analysis was carried out using Minitab 17® (Minitab Inc., State College, PA; licensed to the University of Hud- dersfield) where the previously mentioned dataset was imported into it. The final model was obtained by excluding molecular de- scriptors which were not statistically significant (*p* value > 0.05), and those with unacceptably high levels of variance inflation factor, 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, and the model was then vali- dated using 4 compounds.

Results and Discussion

In total, 24 drugs were analyzed 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 con- centration of drug and the concentration of NaDC. Only linear sections of the plots were incorporated to calculate SR, some were deemed to be nonlinear, such as the lower concentrations of qui- nine and the higher concentrations of acetaminophen (data not shown). These nonlinear relationships may be due to preferential drug-drug interactions rather than drug-NaDC interactions as suchdrugs are known to self-associate.[19] The majority of the compounds did exhibit a linear relationship over the concentration range studied (7-20 mM), ensuring confidence in the experimental sys- tem. Using the calculated SR value, along with the mole fraction of aqueous solubility for the drug, facilitated calculation of a micelle/ aqueous partition coefficient for each drug. Analyzing these values alongside reported % HIA literature data (Table 1) enabled the application of simple linear regression to construct an equation to permit calculation of % HIA for any compound through measure- ment of its solubility in NaDC.

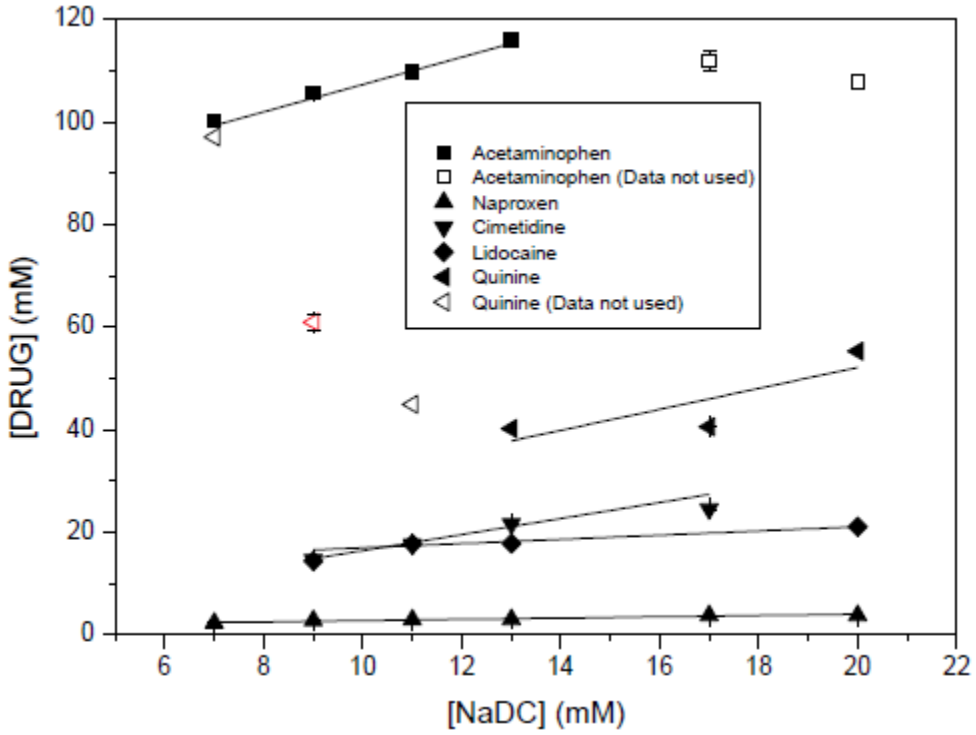

Figure 1. Plots of the concentration of a selection of analyzed compounds in solution with NaDC concentration to determine the SR. Each data point = mean, n = ≥3, ±SD .

Table 1
Calculated SR, X_m, X_a, and $K_{xm/a}$ for the 20 Compounds Studied to Create the Database for Subsequent Simple Linear Regression Analysis

Drug	SR	X_m	X_a	$\log K_{xm/a}$
Acetaminophen	2.5694	0.7198	4.94E−04	3.164
Alprenolol	0.0751	0.0699	1.36E−05	3.711
Amitriptyline	0.2163	0.1777	6.30E−07	5.450
Carbamazepine	0.0550	0.0521	1.35E−06	4.587
Cimetidine	1.2403	0.5536	6.69E−4	2.918
Diclofenac	0.0790	0.0732	1.34E−07	5.737
Diphenhydramine	5.8665	1.2055	2.16E−04	3.747
Fluconazole	0.0400	0.0385	5.88E−08	5.816
Flurbiprofen	0.1028	0.0932	5.89E−07	5.199
Gemfibrozil	0.0561	0.0531	2.00E−06	4.424
Ibuprofen	0.1292	0.1144	5.97E−06	4.283
Ketoprofen	0.1407	0.1233	3.61E−06	4.534
Lidocaine	0.5387	0.3501	3.15E−04	3.046
Mannitol	3.1794	0.7607	2.09E−02	1.561
Meloxicam	0.0311	0.0302	3.66E−07	4.916
Naproxen	0.1151	0.1032	1.24E−06	4.919
Phenylbutazone	0.0903	0.0828	8.40E−06	3.992
Propranolol	0.1166	0.1044	4.28E−06	4.387
Quinine	2.0472	0.6718	2.77E−05	4.384
Terbutaline	5.7796	0.8525	1.67E−02	1.707

Using data presented in Table 1, simple linear regression analysis was used to create an optimized equation to predict HIA:

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

$$S = 0.236264,\ R^2 = 0.8492,\ R^2_{adj.} = 0.8409,\ R^2_{Pred.} = 0.8232,$$

The p values obtained for this model indicate that the relationship between % HIA and logKxm/a values was statistically significant at the 95% confidence level where they were <0.05, and the model's F-ratio was found to be statistically significant. Also, logKxm/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 R2 of 0.8492 derived from the current data indicates that the fit of the sampled drugs to the model is good. The View the MathML source R2 (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 View the MathML source R2 (adj.) and View the MathML source R2 (Pred) show no evidence of the current model overfitting 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.

Table 2
Experimental $logK_{xm/a}$, % HIA_{calc}, and % HIA_{lit} Values for the Compounds Analyzed Including 4 Validation Compounds (*)

Drug	$logK_{xm/a}$	% HIA_{calc}	% HIA_{lit}
Acetaminophen	3.164	77.71	80.00[20]
Alprenolol	3.711	86.18	93.00[20]
Amitriptyline	5.450	97.54	95.00[21]
Aspirin*	2.671	67.37	68.00[22]
Carbamazepine	4.587	94.06	97.00[23]
Cimetidine	2.918	72.86	68.00[24]
Diclofenac	5.737	98.17	99.00[25]
Diphenhydramine	3.747	86.63	72.00[26]
Fenoprofen*	4.398	92.83	85.00[27]
Fluconazole	5.816	98.32	97.50[20]
Flurbiprofen	5.199	96.81	95.00[28]
Gemfibrozil	4.424	93.01	95.00[26]
Ibuprofen	4.283	91.97	85.00[25,29]
Indomethacin*	5.785	98.26	100.00[20]
Ketoprofen	4.534	93.74	95.00[20,28]
Lidocaine	3.046	75.54	75[26,27,30]
Mannitol	1.561	38.81	38.67[31]
Meloxicam	4.916	95.74	97.00[26]
Naproxen	4.919	95.75	97.67[20]
Phenylbutazone	3.992	89.37	90.00[22]
Piroxicam*	3.995	89.40	99.00[20]
Propranolol	4.387	92.75	95.00[20]
Quinine	4.384	92.73	85.00[32]
Terbutaline	1.707	42.55	44.00[29,33]

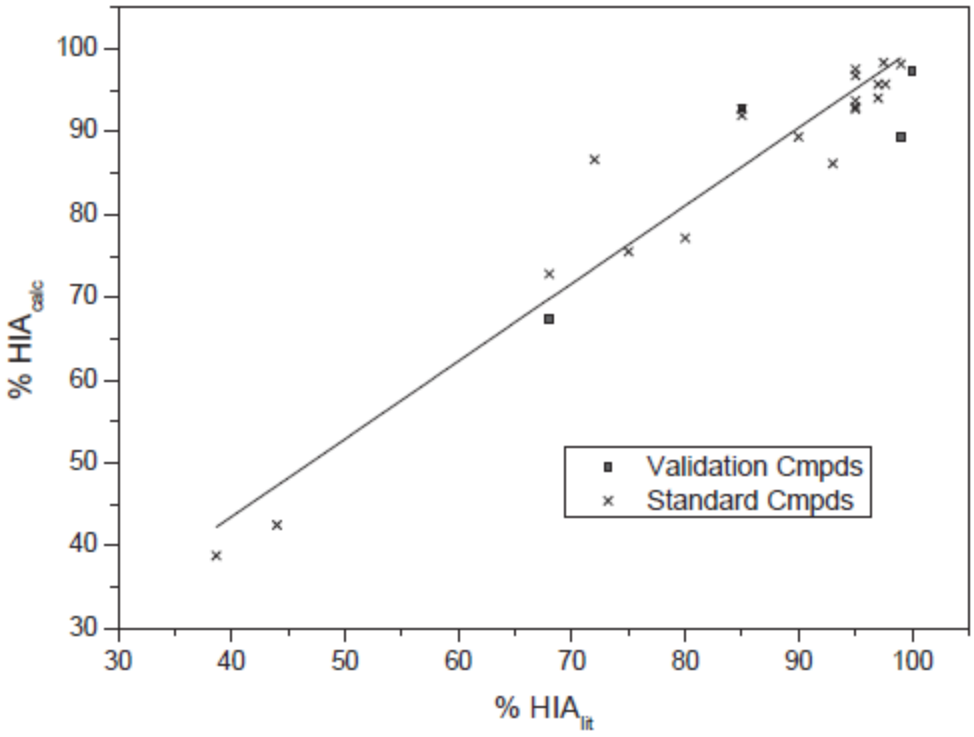

Figure 2. A plot of calculated % HIA (% HIA_{calc}) versus published literature % HIA (% HIA_{lit}), standard compounds used to derive the equation (×) and validation compounds (■).

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 HIA and avoids many of the disadvantages of other techniques, such as animal-based methods.

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