Accepted Manuscript

Formation of a bile salt-drug hydrogel to predict human intestinal absorption

Dina S. Shokry, Laura J. Waters, Gareth M.B. Parkes, John C. Mitchell, Martin J. Snowden

PII: S0022-3549(18)30602-6

DOI: 10.1016/j.xphs.2018.10.005

Reference: XPHS 1312

To appear in: *Journal of Pharmaceutical Sciences*

Received Date: 23 July 2018

Revised Date: 2 October 2018

Accepted Date: 3 October 2018

Please cite this article as: Shokry DS, Waters LJ, Parkes GMB, Mitchell JC, Snowden MJ, Formation of a bile salt-drug hydrogel to predict human intestinal absorption, *Journal of Pharmaceutical Sciences* (2018), doi: https://doi.org/10.1016/j.xphs.2018.10.005.

Formation of a bile salt-drug hydrogel to predict human intestinal absorption

Dina S. Shokry[b], Laura J. Waters*[a], Gareth M. B. Parkes[a], John C. Mitchell[b], and Martin J Snowden[b]

[a] School of Applied Sciences, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, UK,

[b] Faculty of Engineering and Science, Medway Centre for Formulation Science, University of Greenwich, Chatham, Kent ME4 4TB, UK.

*Corresponding author: l.waters@hud.ac.uk

ABSTRACT

The unique character of bile salts to self-assemble into hydrogels in the presence of halide salts was exploited in this work to facilitate the prediction of human intestinal absorption (%HIA) for a set of 25 compounds. This was achieved by firstly incorporating each compound separately within the process of gel formation to create a series of gel-drug membranes. Scanning Electron Microscopy (SEM) analysis of the freeze-dried samples of the blank bile salt hydrogels and drug loaded bile salt hydrogels indicated a unique microstructure made of a network of intertwined fibrils. Drug-loaded sodium deoxycholate (NaDC) hydrogels were then utilised as the donor phase to study permeability using flow-through and static diffusion cells. The resulting values of the release-permeability coefficient (K_p) were then analysed, along with other molecular descriptors, for the prediction of human intestinal absorption (%HIA) using multiple linear regression (MLR). Overall, when comparing predicted values (using the systems presented in this study) with known literature values, it can be seen that both methods (i.e. using static and flow through cells) had good predictability with $R^2_{PRED.}$ values of 79.8 % and 79.7 % respectively. This study therefore proposes a novel, accurate and precise way to predict human intestinal absorption for compounds of pharmaceutical interest using a simple *in vitro* permeation system. It is important to develop alternatives to the current methods used in prediction of HIA which are expensive and time consuming or include the use of animals. Therefore, the proposed method in this study being economic and time saving provides superiority over these current methods and suggests the possibility of its use as an alternate to such methods for prediction of HIA.

Statistical Summary: 7648 words, 3 tables, 9 figures, 24 pages

Declaration of Interest: None.

Keywords: sodium deoxycholate; NaDC; human intestinal absorption; %HIA; hydrogels; flow-through cells; static diffusion cells; absorption; permeation

1. Introduction

Due to convenience of the oral route of administration, most of the pharmaceutical compounds are formulated as orally administered drugs. However, the properties of some compounds can be incompatible with oral administration. In fact, the pharmaceutical industry suffers from major financial losses because of the poor bioavailability of some new drugs after their oral administration, only discovered once in the clinical development stage[1-4].Therefore, poor drug candidates with poor biopharmaceutical properties, such as poor oral bioavailability, and aqueous solubility should be identified as soon as possible before entering the clinical development stage in which the cost of research performed for a compound is significantly high. As a result, there has been a growing interest in the early prediction of biopharmaceutical properties by means of experimental and theoretical models. This study provides a rapid and cheap method for the prediction of one of the most important pharmacokinetic properties of pharmaceutical compounds which is intestinal absorption through the use of bile salts. The number of recent publications on the physiological importance of bile salts reflects a growing interest in this area. It has been found that certain bile salts, such as sodium deoxycholate (NaDC), are capable of self-assembling into gels through a process driven by the balance of hydrophobic interactions, hydrogen-bonding, van der Waals forces and steric effects[5-9]. The hydrogels made from bile salts are very different from polymeric gels (which are made from chemical cross-linking) and have specific and vital roles within the body. Bile salts are a group of cholic acid derivatives, known as biosurfactants, that have an amphiphilic structure with a steroidal backbone; a distinctive structure that differentiates this class of surfactants from conventional synthetic surfactants[9, 10]. Some important biological functions, such as cholesterol solubilisation, dietary fat and fat soluble vitamin absorption as well as removal of fatty acids from pancreatic hydrolysis, are carried out by the aggregates resulting from the self-assembly of bile salts occurring as a result of their unusual structure[9, 11-13].

Biocompatible fragment containing hydrogels, such as amino acid derivatives[14], peptides[15, 16], cholic acid[17] derivatives and carbohydrate systems[18, 19], have been given notable attention because of one key benefit, namely their safe use in biomedical applications. The presence of halide salts has been shown to improve the production of bile salt formed hydrogels[20, 21]. The formation of these hydrogels is through a network of intertwined fibrils formed by massive cycles of bile salt molecules attached together by noncovalent interactions, especially hydrogen-bonds. It was found that NaCl had a prominent effect on encouraging the gelation of NaDC solutions to form supramolecular hydrogels with higher gelation capability and mechanical force because of the small radius of hydration of the NaCl ions[20]. An important role is played by both sodium and the chloride ions in the formation of the hydrogels by reducing the electrostatic repulsion between NaDC polar heads therefore contributing to the compression of the thickness of the electric double layer[20]. Furthermore, weak coordination bonds form between sodium ions and carboxylate groups, stimulating connection of the polar head of carboxyl groups via hydrogen-bonding which leads to the formation of a more regular crystalline interface thus shifting the growth of aggregates along one direction towards fibrous aggregate formation[22, 23]. In addition, the chloride ions play a role in hydrogel formation, as well as the weak electrostatic interaction which is thought to exist between the sodium salt anion (chloride ions) and the α-methylene attached to the carboxylate group of the bile salt[24]. NaDC solutions have been reported in literature to give highly viscous gels by the formation of polymer-like aggregates at pH values less than 7.8 (Figure 1)[25, 26]. However, such systems have never been formulated in the presence of pharmaceutical compounds, such as those considered in this work.

Determining human intestinal absorption is a complex, expensive and lengthy process yet is necessary to understand the behaviour of pharmaceutical compounds *in vivo*. Developed systems to predict absorption are highly sought-after and seek to provide a simpler, less expensive and more ethically acceptable method to predict intestinal absorption. Several techniques have previously been employed to predict intestinal absorption including cell culture based models[27], such as Caco-2 cells[28-35], membrane based models, such as PAMPA[36-39], *ex-vivo* models, such as Ussing chambers[40-42], *in situ* intestinal perfusion models[43-46] and everted intestinal ring/sac[47]. Models using spectroscopic[48] and chromatographic[49] properties have previously been developed by the authors of this study. This work however, presents the creation of a novel drug-gel bile salt based product as the

basis of the analytical donor phase to determine permeation which can ultimately lead to prediction of intestinal absorption. The synthesised hydrogel was used as a donor phase in both static diffusion and flow-through cells, with a permeable dialysis membrane for support, to determine the release-permeability coefficient (K_p) of the studied drugs. This was then statistically analysed for developing models for prediction of human intestinal absorption.

2. Experimental

2.1 Chemicals and materials

Sodium deoxycholate (NaDC, 97 %), sodium chloride (NaCl, analytical grade), sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$, 99 %), disodium hydrogen orthophosphate anhydrous (Na_2HPO_4, 99 %) were purchased from Fisher Scientific, Loughborough, UK. The compounds of pharmaceutical interest considered in this work were: caffeine 97 % (Sigma Aldrich, Dorset, UK), fenoprofen 97 % (Fluka, Dorset, UK), quinine 96 % (Fluka, Dorset, UK), acetaminophen 99 % (Sigma Aldrich, Dorset, UK), leflunomide 98 % (Sigma Aldrich, Dorset, UK), linezolid >98 % (Sigma Aldrich, Dorset, UK), ketoprofen 98 % (Sigma Aldrich, Dorset, UK), lidocaine 98 % (Sigma Aldrich, Dorset, UK), indomethacin 99 % (Sigma Aldrich, Dorset, UK), phenylbutazone 99 % (Sigma Aldrich, Dorset, UK), fluconazole 98 % (Sigma Aldrich, Dorset, UK), carbamazepine 99 % (Sigma Aldrich, Dorset, UK), cimetidine (Sigma Aldrich, Dorset, UK), moexipril >98 % (Sigma Aldrich, Dorset, UK), naproxen 98 % (Sigma Aldrich, Dorset, UK), piroxicam 98 % (Sigma Aldrich, Dorset, UK), zolmitriptan >98 % (Sigma Aldrich, Dorset, UK), haloperidol 99 % (Sigma Aldrich, Dorset, UK), fosinopril >98 % (Sigma Aldrich, Dorset, UK), ibuprofen 98 % (BASF, Cheshire, UK), diclofenac 98 % (TCI Europe, Zwijndrecht, Belgium), flurbiprofen 98 % (TCI Europe), gemfibrozil 98 % (TCI Europe), theophylline 98 %, (TCI, Oxford, UK) and meloxicam 98 % (TCI Europe). Dialysis membrane was a high retention, seamless, cellulose tubing with an average flat width of 23 mm and molecular weight cut off (MWCO) of 12400.

2.2 Saturated solubility analysis

Wavelength of maximum absorption (λ_{max}) of each compound was determined by scanning its solution within (200-400 nm) using UV spectrophotometer. An excess amount of each of the drugs was added to 5 mL of phosphate buffer (0.2 M disodium orthophosphate, sodium dihydrogen orthophosphate and sodium chloride at a pH of 7.4) in 7 mL vials, then sealed and stored at 37 ºC. The solutions were then filtered through 0. 45 µm Nylon filters to

remove excess solid, diluted by known amounts using buffer, then assayed using UV spectrophotometry at the pre-determined λ_{max} and compared with an already established calibration plot. A calibration plot was established for each compound by preparing a standard stock solution of each compound, in the previously mentioned buffer/salt mixture at pH 7.4, from which different volumes were aliquoted out and diluted accordingly for preparation of different dilutions of the compound. The dilutions were then scanned for measuring the absorbance and a plot of the compound concentrations against its corresponding absorbances was then constructed.

2.3 Preparation of bile salt hydrogel with infinite dose of drug

An NaDC-based hydrogel (70 mM) was prepared by gradually adding a specified volume of phosphate buffer (composition described above). Based on the saturation solubility results, the infinite dose for each of the drugs under study was calculated where anaccurately weighed amount of each of the drugs under study was added to an accurately weighed amount of NaDC (580.4 mg) in a 50 mL beaker for the formation of a 20 mL drug saturated hydrogel. The mixture of NaDC and drug in buffer was then sonicated in an ultrasonic water bath for 2 minutes at room temperature until the consistency of the solution thickened and the gel began to form so the gelation process was visually judged. The gel was left stirring after sonication and then allowed to stand for 24 hours to ensure homogenous distribution of the drug throughout the gel.

2.4 Analytical Instrumentation

2.4.1 Diffusion cells

Diffusion cells have been classically considered to be one of the more popular methods used in prediction of permeation of drugs and chemicals across the skin[50, 51]. Both static (Franz-type) cells and continuous flow (flow-through) cells were used in this study whereby the setup of both types of diffusion cells have donor and receptor compartments with a membrane mounted between and a water jacket to achieve a consistent temperature of 37 ^{o}C. Sink conditions were maintained in both types of cells.

2.4.1.1 Static diffusion cells

A set up of six 30 mL vertical Franz cells, bespoke, were used in the study of the permeation of drugs from the drug-saturated hydrogels in the donor chamber to buffer in the receptor chamber per experiment. Each cell was formed of two chambers; donor and receptor chambers held together by clamps with a dialysis membrane fitted to cover the diffusion area

(3.14 cm^2) mounted between the two chambers as a support for the hydrogel. A 5 mL sample of the drug-saturated hydrogel was placed in the donor chamber while the clean, dried receptor chamber was filled with deaerated buffer and allowed to equilibrate at 37 ºC. All openings, including donor top and receptor arm, were occluded to prevent evaporation. The receptor compartment was stirred at 450 rpm using a magnetic stirrer. Samples (1 mL) were extracted at 45 minute intervals over a 6 hour period and analysed using UV spectrophotometry at the λ_{max} of each drug. After each extraction, 1mL of fresh, deaerated buffer was introduced into the receptor.

2.4.1.2. Flow-through diffusion cells

A set up of six flow through cells were used per experiment (purchased from PermeGear Inc., Hellertown, PA 18055 USA). Each cell consisted of two compartments; the donor and the receptor compartments fixed together by clamps and screws with a dialysis membrane cut down to cover the diffusion area (0.554 cm^2) as a support for the hydrogel. 0.8 mL of the drug saturated hydrogel was placed in the donor chamber while buffer was pumped continuously through the six receptor compartments at a flow rate of 0.52 mL/min using a peristaltic pump. All the cells were kept at a temperature of 37 ºC using a heat conducting cell holder. The donor compartments were covered with moisture-resistant film (Parafilm M, Alcan packaging) to avoid drying of the hydrogel. Samples from the six cells were collected in 7 mL vials every 45 minutes over a 6-hour period and analysed using UV spectrophotometry at the λ_{max} of each drug.

All spectrophotometric analysis was undertaken using an Agilent Model Cary 60 UV-Vis fitted with a Cary single cell Peltier accessory to keep the samples in the sample compartment at the specified temperature. A quartz cuvette of 10 mm internal thickness was used in all measurements.

2.4.2 Scanning Electron Microscopy (SEM)

Electron micrographs of hydrogel with no drug as well as hydrogels saturated with each of the following drugs (carbamazepine and meloxicam) were obtained using a scanning electron microscope (Leica Cambridge S360, UK) operating at 15 kV. The hydrogel samples were freeze dried using (CHRIST ALPHA 2 - 4 LD plus) and mounted on a metal stub with double-sided adhesive tape and coated under vacuum with gold in an argon atmosphere prior to observation. Micrographs with different magnifications were taken to facilitate the study of the morphology of the hydrogels.

2.4.3 Fourier transform infrared (FT-IR)

The FT-IR spectra (650-4000 cm−1) of hydrogels saturated with (caffeine, carbamazepine, fluconazole, meloxicam and piroxicam) each of the previously mentioned drugs under study were air dried then recorded using ATR with a FT-IR spectrophotometer (PerkinElmer, UK). Spectra with sharp peaks of reasonable intensity were obtained to consider the stability of the hydrogel after the addition of the drugs.

2.5 Calculations:

2.5.1 Calculation of permeability coefficient (K_p):

Since the drug was added to the hydrogel in an infinite dose, the permeability coefficient (K_p) can be calculated from the following relationship [52] :

$$K_p = Q / [A \,.t.\, (C_o - C_i)]$$

Where:

Q: the quantity of drug transported through the hydrogel in time t (min).

C_o: the concentration of the drug in the donor chamber (saturated solubility concentration in the buffer used).

C_i: is the concentration of the drug in the receptor chamber.

A: the area of the exposed hydrogel in cm^2.

Since the drug was applied to the hydrogel in an infinite dose, C_i can be simplified to zero. K_p, defined as the permeant penetration rate per unit concentration, is given in cm/min and assumed to be a first order process.

2.5.2 Statistical analysis:

Data analysis was conducted using Minitab 17. Multiple linear regression analysis was conducted where all the molecular descriptors were included and regressed against the dependant variable %HIA and backward elimination modelling strategy. Variables with high variance inflation factors (VIF) were removed to take (VIF) to acceptable limits. At the end an optimum model was obtained that provided a good summary of data. The variables remaining in the optimal model were assessed for significance and relative importance by standardised coefficients and the associated p-values.

The predictive ability of the preferred model was assessed using adjusted-R^2 and R^2 for prediction (R^2_{PRED}) which can indicate the predictive ability of the model itself and consequently reflects the far wider ability to apply the model.

3. Results and discussion

3.1 Hydrogel formation and characterisation

The microstructure of the hydrogels formed by NaDC at pH 7.4 was investigated for 'blank' NaDC hydrogel, as well as drug-loaded NaDC hydrogel, using scanning electron microscopy (SEM). The obtained SEM observations for the blank NaDC hydrogel freeze dried samples showed a network structure of intertwined fibrils with medium size pockets between (Figure 3).

Furthermore, the microstructure of the freeze dried samples of two selected drug loaded hydrogels (carbamazepine and meloxicam) showed the same network structure as the freeze dried sample of 'blank' NaDC hydrogel (Figure 4).

The difference in the structure of carbamazepine and meloxicam hydrogels could be attributed to carbamazepine being more hydrophobic than meloxicam where the log D of carbamazepine at pH 7.4 is 2.28 [53] while that of meloxicam at the same pH is 1.04. As a result, carbamazepine may have become more involved in the construction of the hydrogel network thus partially interrupting the crystalline-like arrangement of NaDC molecules together in the gel.

In contrast, the anionic drug meloxicam is less hydrophobic (log D at pH 7.4=1.04) [53] and has a comparatively high molecular weight of 351.40 g/mol [53] so is less involved in the main structure of hydrogel formation. Therefore, in this product the network was more compact with the presence of the drug entrapped inside the network structure. This was later confirmed by the higher K_p value obtained for carbamazepine than that obtained for meloxicam.

FT-IR spectra were obtained upon drying of the sample to confirm the stability of the hydrogel with the addition of the drugs. These were compared with spectra obtained from the blank hydrogel samples to identify changes in the characteristic peaks (spectra presented in Supplementary Information).

It was observed that upon the inclusion of drugs to the NaDC hydrogel there was a decrease in the wave number of the O-H broad peak appearing at 3334 cm^{-1} for the blank NaDC hydrogel sample. This decrease shows destruction of H-bonding between the NaDC molecules and the formation of new H-bonding between the NaDC and each drug molecule [20]. The decrease was the highest in the case of carbamazepine (3233 cm^{-1}) indicating carbamazepine was more involved in the hydrogel structure confirming the previous SEM

results for this drug. In contrast, the decrease was the least in the case of meloxicam (3327 cm^{-1}) showing less inclusion of this drug in the NaDC hydrogel structure thus confirming the previous SEM results for meloxicam (Figure 4). No appearance of new peaks or disappearance of existing peaks was observed suggesting no chemical interaction between the added drugs and NaDC gel.

3.2 Bile salt concentration effects

In this work, a 70 mM NaDC concentration was picked for the preparation of all the drug loaded hydrogels used in all the permeation studies carried out for the prediction of human intestinal absorption (HIA). The selection of such value was attributed to the rate of drug permeation being the highest at this concentration. Such finding was based on testing the effect of the change in the hydrogel concentration from 50 to 100 mM on the release of different compounds (neutral, anionic and cationic), as shown in Figure 5.

According to Figure 5, it can be observed that the permeation rate of anionic drugs (flurbiprofen, gemfibrozil, ibuprofen and piroxicam) is less affected by the change in the concentration of NaDC hydrogel than neutral (acetaminophen, carbamazepine and fluconazole) and cationic (lidocaine) drugs. However, all the selected drugs showed the highest permeation rate at 70 mM concentration of NaDC.

3.3 Determination of release-permeability

Based on the previous findings, an NaDC concentration of 70 mM was selected to be used in a permeation study of 25 compounds where the permeation experiment for each compound was carried out in triplicate. Calculated K_p values were then used in the statistical modelling of human intestinal absorption. The permeability coefficient (K_p) for twenty-five compounds was calculated from the slopes of the plots of cumulative amount of drug permeated through the hydrogel ($\mu g/cm^2$) against time (min) obtained using both the flow through and static cell techniques as shown in Figures 6 and 7. The dialysis membrane works only as a support for the hydrogel to prevent it from falling into the receiver chamber. Since the MWCO of the used dialysis support is 12400, it will retain compounds of a molecular weight of 12400 or greater so the studied compounds in solution or in hydrogel are expected to move freely with the buffer movement from the donor to the receiver chamber without being retained. Although the hydrogels were prepared in a manner such that the compounds under study saturated it, these compounds are completely soluble in the hydrogel matrix therefore allowing the drug molecules to move with the buffer from the gel donor chamber to the receiver chamber without any hindrance from the dialysis support. For comparison, the

permeability coefficient was determined for eight selected drugs using only buffer pH 7.4 with no NaDC hydrogel and it was found to be always higher in buffer than in hydrogel except for acetaminophen which could be attributed to that acetaminophen was the only drug with significant aqueous solubility, a low molecular weight (151.2 g/mol) [53] and was the least lipophilic (log $P_{o/w}$ = 0.46) of all the studied drugs [54].

Although the sample volume in the donor chamber when using static cells (5 mL) was higher than when using flow through cells (0.8 mL), less permeation of the studied compounds was observed with the static cells than the flow through cells. This could be attributed to the bigger receptor chamber volume of the static cells used (30 mL) than the flow through cells (1.5 mL of the receptor phase was collected every 45 min).

3.4 Statistical modelling of human intestinal absorption (HIA)

For the prediction of human intestinal absorption (HIA), the obtained permeability coefficients (K_p) from both flow through and static cells were statistically analysed alongside the reported values of some molecular descriptors such as molecular weight (Mwt), polar surface area (PSA), freely rotating bonds (FRB), molar volume (VM), dissociation constant (pK_a), aqueous solubility (S_w), number of hydrogen bond donors (nHD) and number of hydrogen bond acceptors (nHA) against literature values of human intestinal absorption (HIA) using multiple linear regression. Twenty-five drugs were selected to be used in the statistical modelling of human intestinal absorption. These drugs are of a wide variety of physicochemical properties (Mwt, PSA, S_w, RB, nHD, nHA and V_M) and having a wide range of %HIA reported values within (23-100 %) (Tables 1-3).

A linear relationship was found between reported (%HIA) and experimental *Kp* values but logit(HIA) was used to improve this relationship as seen in studies of a similar type [55-57]. The human intestinal absorption values were transformed to logit by substitution in the following equation, where %HIA = %Human Intestinal Absorption.

$$\text{logit}\ (\%HIA) = \log\ (\%HIA / (100-\%HIA))$$

Therefore, all drugs with % HIA values of 100 or 0 % from the training set were removed for simplification.

Eighteen drugs were used in the development of the final models. $K_{p,}$ alongside the molecular descriptors (nHD and V_M) were included in the final models of both techniques.

Modelling of Human intestinal absorption using flow-through cells:

The following model was obtained for the prediction of HIA using flow-through cells:

logit HIA = -0.59 - 0.5522 nHD – 0.006085 V_M - 0.765 log K_p

The model's R^2 = 87.58 %, $R^2_{adjust.}$= 84.92 % , R^2_{PRED} = 79.80 %, S= 0.267

A 95 % confidence interval for log K_p is given by (-1.19, -0.34). t-statistic and standardised coefficient of log K_p are -3.86 ($p<0.05$) and -0.397 respectively suggesting the statistical significance of log K_p as a predictor. Also the F-ratio of the overall model is statistically significant, F=32.90 and P value 0.000 ($p<0.05$). Absence of autocorrelation in the current regression model was proved by a Durbin- Watson statistic value of 2.532. There was no marked relationship between residuals and predicted values. Seven compounds (carbamazepine, fenoprofen, linezolid, naproxen, piroxicam, quinine and zolmitriptan) were used for testing the obtained model as shown in Table 2. The model was able to successfully predict the %HIA for six compounds in the test set within a minimum of 0.29 % and a maximum of 10.97 % difference between the predicted %HIA and the published %HIA. The model underestimated the %HIA for piroxicam where its predicted value for human intestinal absorption was found to be 82.65 % against a literature value of 99 % experimentally obtained in humans. However, the obtained predicted value was found to be closer to a literature value of 89 % for piroxicam's intestinal absorption in dogs [71]. An overall close agreement was shown between literature and predicted values of %HIA in Figure 8.

Prediction of HIA using static cells:

logit HIA = 0.515 - 0.4294 nHD – 0.006005 V_M - 0.453 log K_p

The model's R^2 = 86.61 %, $R^2_{adjust.}$= 83.74 % , R^2_{PRED} = 79.67 %, S= 0.253

A 95 % confidence interval for log *Kp* is given by (-0.874, -0.031). t-statistic and standardised coefficient of log *Kp* are -2.3 ($p<0.05$) and -0.261 respectively suggesting the statistical significance of log *Kp* as a predictor. Also the F-ratio of the overall model is statistically significant, F= 30.19 and P value 0.000 ($p<0.05$). A Durbin- Watson statistic value of 2.105 proved the absence of autocorrelation in the current regression model. There was no marked relationship between residuals and predicted values. Seven compounds (carbamazepine, fenoprofen, indomethacin, linezolid, piroxicam, quinine and zolmitriptan) were used for testing the obtained model. As shown in Table 3, the model was able to successfully predict the %HIA for six compounds in the test set within a minimum of 0.6 % and a maximum of 12.60 % difference between the predicted %HIA and the published

%HIA. The model underestimated the %HIA for piroxicam where its predicted value for %HIA was found to be 80.73 % against a literature value of 99 % experimentally obtained in humans (see reference in Table 2). However, the obtained predicted value was found to be closer to a literature value of 89 % for piroxicam's intestinal absorption in dogs [71]. The model's good predictive power is shown in Figure 9.

The final model equation included permeability coefficient, number of hydrogen bond donors and molar volume which all correlate well with the absorption values in the human intestine. Since the permeability coefficient is independent of the donor concentration [82] and it describes an intrinsic property of the solute to permeate across a specific medium (e.g. intestine) which is independent of the dose but influenced by the applied vehicle [83]. Therefore, this intrinsic property could be related to one or more of the previously reported physicochemical properties of the permeating solutes in this study. According to literature, molar volume (V_M) encodes for hydrophobic and dispersion forces while hydrogen bond acceptor basicity (nHA) accounts for orientation and induction forces[84]. Both of these parameters are considered as factors of solute lipophilicity as it can be factorised in to two sets which are hydrophobicity which accounts for hydrophobic and dispersion forces and also polar terms which account for hydrogen bonds, orientation and induction forces[84]. The mechanisms of drug-biomembrane interactions involve partitioning in to the hydrophobic core of the membrane which consequently involves the lipophilicity term. Therefore, the descriptors involved in the final model equation show an important impact in the permeation process and also confirm the success of such a technique in the simulation of intestinal membrane for prediction of HIA. However, it should be noted that the majority of the compounds analysed in this work displayed a comparatively high percentage absorption (as is often the case for pharmaceutical compounds) and therefore the predictive ability for compounds with a low absorption should be carefully considered.

4. Conclusion

Drug-loaded sodium deoxycholate (NaDC) hydrogels were utilised as the donor phase to study release-permeability using flow-through and static diffusion cells. Furthermore, determination of K_p from the permeation of a number of compounds from the prepared NaDC hydrogels using flow-through cells and static cells was successful in the development of models of high predictive capabilities for human intestinal absorption by using the experimentally obtained K_p. Overall, the two permeation methods yielded highly predictive models of %HIA. Although static cells presented a cheaper option, flow through cells could

be considered as a better method as it requires smaller volumes of buffer solution and tested samples in addition to the easier sample collection. NaDC, being a natural physiological surfactant and having gelation properties in the presence of certain factors, has facilitated the creation of a simulation of a biological membrane to mimic the absorption process inside the human intestine which makes it a good alternative for the use of animals in the prediction of human intestinal absorption of pharmaceutical compounds. At present, the proposed system may require more drug than ideally desired within early stage development in industry to complete the analysis yet it does present clear advantages as a novel analytical system. For example, this technique has the advantage of being a much cheaper alternative to the classical *in vitro* cell culture based models (e.g. Caco-2 cells) and membrane based models (e.g. PAMPA) also it is less time consuming and laboratory intensive.

References:

1. Prentis, R., Y. Lis, and S. Walker, *Pharmaceutical innovation by the seven UK-owned pharmaceutical companies (1964-1985).* British journal of clinical pharmacology, 1988. **25**(3): p. 387-396.
2. Kennedy, T., *Managing the drug discovery/development interface.* Drug discovery today, 1997. **2**(10): p. 436-444.
3. Venkatesh, S. and R.A. Lipper, *Role of the development scientist in compound lead selection and optimization.* Journal of pharmaceutical sciences, 2000. **89**(2): p. 145-154.
4. Arlington, S., *Pharma 2005-An industrial revolution in R&D.* Pharmaceutical Executive, 2000. **20**(1): p. 74-85.
5. Qiao, Y., et al., *Unique temperature-dependent supramolecular self-assembly: from hierarchical 1D nanostructures to super hydrogel.* The Journal of Physical Chemistry B, 2010. **114**(36): p. 11725-11730.
6. Dastidar, P., *Supramolecular gelling agents: can they be designed?* Chemical Society Reviews, 2008. **37**(12): p. 2699-2715.
7. van Esch, J.H., *We can design molecular gelators, but do we understand them?* Langmuir, 2009. **25**(15): p. 8392-8394.
8. Song, F., et al., *Viscoelastic and fractal characteristics of a supramolecular hydrogel hybridized with clay nanoparticles.* Colloids and Surfaces B: Biointerfaces, 2010. **81**(2): p. 486-491.
9. Löfman, M., et al., *Bile acid alkylamide derivatives as low molecular weight organogelators: Systematic gelation studies and qualitative structural analysis of the systems.* Journal of colloid and interface science, 2011. **360**(2): p. 633-644.
10. Rub, M.A. and A.Z. Naqvi, *Aqueous amphiphilic drug (amitriptyline hydrochloride)–bile salt mixtures at different temperatures.* Colloids and Surfaces B: Biointerfaces, 2011. **84**(2): p. 285-291.
11. Song, S., et al., *Temperature regulated supramolecular structures via modifying the balance of multiple non-covalent interactions.* Soft Matter, 2013. **9**(16): p. 4209-4218.
12. Sievänen, E., *Exploitation of bile acid transport systems in prodrug design.* Molecules, 2007. **12**(8): p. 1859-1889.
13. Mukhopadhyay, S. and U. Maitra, *Chemistry and biology of bile acids.* Current Science, 2004. **87**(12): p. 1666-1683.

14. Kiyonaka, S., et al., *Semi-wet peptide/protein array using supramolecular hydrogel.* Nature materials, 2004. **3**(1): p. 58.
15. Chen, L., et al., *Effect of molecular structure on the properties of naphthalene− dipeptide hydrogelators.* Langmuir, 2010. **26**(16): p. 13466-13471.
16. Lin, Y., et al., *Self-assembled laminated nanoribbon-directed synthesis of noble metallic nanoparticle-decorated silica nanotubes and their catalytic applications.* Journal of Materials Chemistry, 2012. **22**(35): p. 18314-18320.
17. Travaglini, L., et al., *Twisted nanoribbons from a RGD-bearing cholic acid derivative.* Colloids and Surfaces B: Biointerfaces, 2017. **159**: p. 183-190.
18. Tsirigotis-Maniecka, M., R. Gancarz, and K.A. Wilk, *Polysaccharide hydrogel particles for enhanced delivery of hesperidin: Fabrication, characterization and in vitro evaluation.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2017. **532**: p. 48-56.
19. Cheng, X., et al., *An injectable, dual pH and oxidation-responsive supramolecular hydrogel for controlled dual drug delivery.* Colloids and Surfaces B: Biointerfaces, 2016. **141**: p. 44-52.
20. Sun, X., et al., *Manipulation of the gel behavior of biological surfactant sodium deoxycholate by amino acids.* The Journal of Physical Chemistry B, 2014. **118**(3): p. 824-832.
21. Calabresi, M., P. Andreozzi, and C. La Mesa, *Supra-molecular association and polymorphic behaviour in systems containing bile acid salts.* Molecules, 2007. **12**(8): p. 1731-1754.
22. Wang, D. and J. Hao, *Self-assembly fibrillar network gels of simple surfactants in organic solvents.* Langmuir, 2011. **27**(5): p. 1713-1717.
23. Yuan, Z., et al., *Gel phase originating from molecular quasi-crystallization and nanofiber growth of sodium laurate–water system.* Soft Matter, 2008. **4**(8): p. 1639-1644.
24. CAO, X.-L., et al., *Interactions between anionic surfactants and cations.* Acta Physico-Chimica Sinica, 2010. **26**(7): p. 1959-1964.
25. Jover, A., et al., *Dynamic rheology of sodium deoxycholate gels.* Langmuir, 2002. **18**(4): p. 987-991.
26. Murata, Y., et al., *Study of the micelle formation of sodium deoxycholate. Concentration dependence of carbon-13 nuclear magnetic resonance chemical shift.* The Journal of Physical Chemistry, 1982. **86**(24): p. 4690-4694.
27. Rothen-Rutishauser, B., et al., *A newly developed in vitro model of the human epithelial airway barrier to study the toxic potential of nanoparticles.* Altex, 2008. **25**(3): p. 191-196.
28. Paixão, P., L.F. Gouveia, and J.A. Morais, *Prediction of the in vitro permeability determined in Caco-2 cells by using artificial neural networks.* European Journal of Pharmaceutical Sciences, 2010. **41**(1): p. 107-117.
29. Thomas, S., et al., *Simulation modelling of human intestinal absorption using Caco-2 permeability and kinetic solubility data for early drug discovery.* Journal of pharmaceutical sciences, 2008. **97**(10): p. 4557-4574.
30. Camenisch, G., G. Folkers, and H. van de Waterbeemd, *Comparison of passive drug transport through Caco-2 cells and artificial membranes.* International journal of pharmaceutics, 1997. **147**(1): p. 61-70.
31. Subramanian, G. and D.B. Kitchen, *Computational approaches for modeling human intestinal absorption and permeability.* Journal of molecular modeling, 2006. **12**(5): p. 577-589.
32. Hou, T., et al., *ADME evaluation in drug discovery. 5. Correlation of Caco-2 permeation with simple molecular properties.* Journal of chemical information and computer sciences, 2004. **44**(5): p. 1585-1600.
33. Reynolds, D.P., et al., *Ionization-specific analysis of human intestinal absorption.* Journal of pharmaceutical sciences, 2009. **98**(11): p. 4039-4054.
34. Bock, U., T. Flötotto, and E. Haltner, *Validation of cell culture models for the intestine and the blood brain barrier and comparison of drug permeation.* Altex, 2004. **21**(Suppl 3): p. 57-64.
35. Augustijns, P. and R. Mols, *HPLC with programmed wavelength fluorescence detection for the simultaneous determination of marker compounds of integrity and P-gp functionality in*

the Caco-2 intestinal absorption model. Journal of pharmaceutical and biomedical analysis, 2004. **34**(5): p. 971-978.
36. Bujard, A., et al., *Predicting both passive intestinal absorption and the dissociation constant toward albumin using the PAMPA technique.* European Journal of Pharmaceutical Sciences, 2014. **63**: p. 36-44.
37. Zhu, C., et al., *A comparative study of artificial membrane permeability assay for high throughput profiling of drug absorption potential.* European journal of medicinal chemistry, 2002. **37**(5): p. 399-407.
38. Akamatsu, M., et al., *In silico prediction of human oral absorption based on QSAR analyses of PAMPA permeability.* Chemistry & biodiversity, 2009. **6**(11): p. 1845-1866.
39. Nielsen, P.E. and A. Avdeef, *PAMPA—a drug absorption in vitro model: 8. Apparent filter porosity and the unstirred water layer.* European journal of pharmaceutical sciences, 2004. **22**(1): p. 33-41.
40. Rogers, S.M., D. Back, and M. Orme, *Intestinal metabolism of ethinyloestradiol and paracetamol in vitro: studies using Ussing chambers.* British journal of clinical pharmacology, 1987. **23**(6): p. 727-734.
41. Smith, P., et al., *Intestinal 5-fluorouracil absorption: Use of Ussing chambers to assess transport and metabolism.* Pharmaceutical research, 1988. **5**(9): p. 598-603.
42. Annaert, P., et al., *In vitro, ex vivo, and in situ intestinal absorption characteristics of the antiviral ester prodrug adefovir dipivoxil.* Journal of pharmaceutical sciences, 2000. **89**(8): p. 1054-1062.
43. Kim, J.-S., et al., *The suitability of an in situ perfusion model for permeability determinations: utility for BCS class I biowaiver requests.* Molecular pharmaceutics, 2006. **3**(6): p. 686-694.
44. Zakeri-Milani, P., et al., *Predicting human intestinal permeability using single-pass intestinal perfusion in rat.* J Pharm Pharm Sci, 2007. **10**(3): p. 368-379.
45. Sun, L., et al., *Structure-based prediction of human intestinal membrane permeability for rapid in silico BCS classification.* Biopharmaceutics & drug disposition, 2013. **34**(6): p. 321-335.
46. Doluisio, J.T., et al., *Drug absorption I: An in situ rat gut technique yielding realistic absorption rates.* Journal of pharmaceutical sciences, 1969. **58**(10): p. 1196-1200.
47. Neirinckx, E., *Towards a veterinary biopharmaceutics classification system: oral bioavailability and ex vivo intestinal permeability studies of paracetamol and ketoprofen in different animal species*. 2010, Ghent University.
48. Waters, L.J., et al., *The use of bile salt micelles for the prediction of human intestinal absorption.* Journal of pharmaceutical sciences, 2016. **105**(12): p. 3611-3614.
49. Waters, L.J., D.S. Shokry, and G. Parkes, *Predicting human intestinal absorption in the presence of bile salt with micellar liquid chromatography.* Biomedical Chromatography, 2016. **30**(10): p. 1618-1624.
50. Franz, T.J., *Percutaneous absorption. On the relevance of in vitro data.* Journal of Investigative Dermatology, 1975. **64**(3): p. 190-195.
51. Bartosova, L. and J. Bajgar, *Transdermal drug delivery in vitro using diffusion cells.* Current medicinal chemistry, 2012. **19**(27): p. 4671-4677.
52. PermeGear, *Diffusion Testing Fundamentals*. 2015.
53. http://www.chemspider.com/, December, 2014.
54. http://www.drugbank.ca/, December, 2014.
55. Norinder, U., T. Österberg, and P. Artursson, *Theoretical calculation and prediction of intestinal absorption of drugs in humans using MolSurf parametrization and PLS statistics.* European journal of pharmaceutical sciences, 1999. **8**(1): p. 49-56.
56. Raevsky, O.A., et al., *Quantitative Estimation of Drug Absorption in Humans for Passively Transported Compounds on the Basis of Their Physico-chemical Parameters.* Quantitative Structure-Activity Relationships, 2000. **19**(4): p. 366-374.

57. Zhao, Y.H., et al., *Rate-limited steps of human oral absorption and QSAR studies.* Pharmaceutical research, 2002. **19**(10): p. 1446-1457.
58. Rhodes, H., et al., *Differentiating nonaqueous titration of aspirin, acetaminophen, and salicylamide mixtures.* Journal of pharmaceutical sciences, 1975. **64**(8): p. 1386-1388.
59. Martin, A., *Physical pharmacy: Physical chemical principles in the pharmaceutical sciences*. 2nd ed. ed. 1969, Philadelphia: Lea & Febiger.
60. Shayanfar, A., S. Velaga, and A. Jouyban, *Solubility of carbamazepine, nicotinamide and carbamazepine–nicotinamide cocrystal in ethanol–water mixtures.* Fluid Phase Equilibria, 2014. **363**: p. 97-105.
61. http://www.pharminfotech.co.nz/manual/Formulation/mixtures/fluconazole.html, April, 2015.
62. https://pubchem.ncbi.nlm.nih.gov/, December, 2014.
63. http://www.chemicalbook.com/ProductMSDSDetailCB9154247_EN.htm, April, 2015.
64. Janssen, P.A., et al., *Chemistry and pharmacology of CNS depressants related to 4-(4-hydroxy-4-phenylpiperidino) butyrophenone. Part I: Synthesis and screening data in mice.* J. med. pharm. Chem, 1959. **1**: p. 281-297.
65. information, U.p., 1975.
66. NARAHASHI, T., D.T. FRAZIER, and M. YAMADA, *The site of action and active form of local anesthetics. I. Theory and pH experiments with tertiary compounds.* Journal of Pharmacology and Experimental Therapeutics, 1970. **171**(1): p. 32-44.
67. https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID1045166#toxval, April, 2015.
68. Stella, V. and J. Pipkin, *Phenylbutazone ionization kinetics.* Journal of pharmaceutical sciences, 1976. **65**(8): p. 1161-1165.
69. Ballard, B.E. and E. Nelson, *Physicochemical properties of drugs that control absorption rate after subcutaneous implantation.* Journal of Pharmacology and Experimental Therapeutics, 1962. **135**(1): p. 120-127.
70. Patel, S.R., et al., *Controlled non-invasive transdermal iontophoretic delivery of zolmitriptan hydrochloride in vitro and in vivo.* European Journal of Pharmaceutics and Biopharmaceutics, 2009. **72**(2): p. 304-309.
71. Musther, H., et al., *Animal versus human oral drug bioavailability: Do they correlate?* European Journal of Pharmaceutical Sciences, 2014. **57**: p. 280-291.
72. Castillo-Garit, J., et al., *Prediction of ADME properties, Part 1: Classification models to predict Caco-2 cell permeability using atom-based bilinear indices.* Afinidad, 2014. **71**(566).
73. Yan, A., Z. Wang, and Z. Cai, *Prediction of human intestinal absorption by GA feature selection and support vector machine regression.* International journal of molecular sciences, 2008. **9**(10): p. 1961-1976.
74. Varma, M.V., K. Sateesh, and R. Panchagnula, *Functional role of P-glycoprotein in limiting intestinal absorption of drugs: contribution of passive permeability to P-glycoprotein mediated efflux transport.* Molecular pharmaceutics, 2005. **2**(1): p. 12-21.
75. Paixão, P., L.F. Gouveia, and J.A. Morais, *Prediction of the human oral bioavailability by using in vitro and in silico drug related parameters in a physiologically based absorption model.* International journal of pharmaceutics, 2012. **429**(1): p. 84-98.
76. Hou, T., et al., *ADME evaluation in drug discovery. 7. Prediction of oral absorption by correlation and classification.* Journal of chemical information and modeling, 2007. **47**(1): p. 208-218.
77. Molero-Monfort, M., et al., *Biopartitioning micellar chromatography: an in vitro technique for predicting human drug absorption.* Journal of Chromatography B: Biomedical Sciences and Applications, 2001. **753**(2): p. 225-236.

78. Newby, D., A.A. Freitas, and T. Ghafourian, *Decision trees to characterise the roles of permeability and solubility on the prediction of oral absorption.* European journal of medicinal chemistry, 2015. **90**: p. 751-765.
79. Veber, D.F., et al., *Molecular properties that influence the oral bioavailability of drug candidates.* Journal of medicinal chemistry, 2002. **45**(12): p. 2615-2623.
80. Chu, K.A., *Predicting Passive Intestinal Drug Absorption: An Interesting Relationship between Fraction Absorbed and Melting Point.* 2009.
81. Kansy, M., F. Senner, and K. Gubernator, *Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes.* Journal of medicinal chemistry, 1998. **41**(7): p. 1007-1010.
82. Koláčková, L., *Comparative in vitro study of permeation of selected drugs from lipophilic solutions through human skin.* 2007: p. 10.
83. Dragicevic, N. and H.I. Maibach, *Percutaneous Penetration Enhancers Drug Penetration Into/through the Skin: Methodology and General Considerations*. 2017: Springer.
84. Liu, X., B. Testa, and A. Fahr, *Lipophilicity and its relationship with passive drug permeation.* Pharmaceutical research, 2011. **28**(5): p. 962-977.
85. Deconinck, E., et al., *Evaluation of chromatographic descriptors for the prediction of gastro-intestinal absorption of drugs.* Journal of Chromatography A, 2007. **1138**(1): p. 190-202.

LEGENDS

Table 1. A summary of molecular descriptors for the selected twenty-five drugs.

Table 2: Permeability coefficient (log K_p) experimentally obtained from flow-through cells, predicted %HIA (Pred. %HIA) and experimentally determined literature %HIA (Expt. % HIA) values for the compounds analysed including seven validation compounds (*).

Table 3: Permeability coefficient (log K_p) experimentally obtained from static cells, predicted %HIA (Pred. %HIA) and experimentally determined literature %HIA (Expt. %HIA) values for the compounds analysed including seven validation compounds (*).

Fig. 1. Schematic representation of the formed salt-induced NaDC gels[20].

Fig. 2. A diagrammatic representation of a static cell (left) and flow through cell (right) (reference [52]).

Fig. 3. SEM images of gel formed by 70 mM NaDC of magnification power x1000 (left) and x1300 (right).

Fig. 4. SEM images for a) carbamazepine 70 mM hydrogel and b) meloxicam 70 mM hydrogel

Fig. 5. Permeability coefficients (K_p) of eight selected drugs at three different concentrations of NaDC hydrogels.

637 **Fig. 6.** Plot of cumulative permeated amount of eight selected drugs against time obtained
638 using flow-through cells.
639
640 **Fig. 7.** Plot of cumulative permeated amount of eight selected drugs against time obtained
641 using static cells.
642
643 **Fig. 8.** Regression plot of predicted %HIA values against literature %HIA.
644
645 **Fig. 9.** Regression plot of predicted %HIA values against literature %HIA.
646
647
648
649
650
651

Table 1

Drug	**Mwt** [53]	**pK_a** [54]	**S_w** [54]	**HD** [53]	**HA** [53]	**RB** [53]	**V_M** [53]
Acetaminophen	151.20	9.9 [58]	14	2	3	1	131.1
Caffeine	194.20	14 [59]	21.6	0	6	0	133.4
Carbamazepine	236.36	13.9	0.21 [54, 54, 60]	2, 1 [54]	3	0	186.6
Cimetidine	252.34	6.8	9.38	3	6	8	198.2
Diclofenac	296.20	4.15	0.00237	2	3	4	206.8
Fenoprofen	242.27	4.5	0.033 [53]	1	3	4	204.7
Fluconazole	306.27	12.71	9 [61]	1	7	5	205.3
Flurbiprofen	244.26	4.42	0.008	1	2	3	203.6
Fosinopril	563.66	-4.4	0.00101	1	8	15	480.4
Gemfibrozil	250.33	4.5 [62]	0.13 [63]	1	3	6	239.7
Haloperidol	375.86	8.3 [64]	0.014	1	3	6	303.3
Ibuprofen	206.30	5.2 [65]	0.0684	1	2	4	200.3
Indomethacin	357.79	4.5	0.000937	1	5	4	269.6
Ketoprofen	254.30	3.88	0.051	1	3	4	212.2
Leflunomide	270.21	-0.45	0.021	1	4	3	194.1
Lidocaine	234.40	7.9 [66]	0.2337 [67]	1	3	5	238.8
Linezolid	337.35	-0.66	1.44	1	7	4	259.0
Meloxicam	351.40	4.08	0.00715	2	7	2	220.3
Moexipril	498.57	5.2	0.00585	2	9	12	408.1
Naproxen	230.26	4.15	0.0159	1	3	3	192.3
Phenylbutazone	308.37	4.4 [68]	0.7 [62]	0	4	5	262.8
Piroxicam	331.35	6.3	0.023	2	7	2	222.8
Quinine	324.42	4.2	0.5	1	4	4	266.4
Theophylline	180.16	8.8 [69]	22.9	1	6	0	122.9
Zolmitriptan	287.36	9.52 [70]	0.19	2	5	5	236.1

Table 2

Drug	Expt. %HIA	Pred. %HIA	Expt. %HIA- Pred. %HIA
Acetaminophen	80[72]	84.32	-4.32
Caffeine	99[73]	98.87	0.13
Carbamazepine*	70[74]	69.71	0.29
Cimetidine	60[72]	57.13	2.87
Diclofenac	81[75, 76]	88.02	-7.02
Fenoprofen*	85[76]	95.97	-10.97
Fluconazole	94[73]	92.76	1.24
Flurbiprofen	95[77]	93.69	1.31
Fosinopril	35[78]	35.68	-0.68
Gemfibrozil	95[75]	92.66	2.34
Haloperidol	60[79]	49.70	10.3
Ibuprofen	85[75]	94.07	-9.07
Indomethacin	98[79]	95.38	2.62
Ketoprofen	96[72]	93.67	2.33
Leflunomide	80[78]	89.14	-9.14
Lidocaine	90[77]	88.14	1.86
Linezolid*	100[78]	91.16	8.84
Meloxicam	90[72]	76.70	13.3
Moexipril	23[78]	37.83	-14.83
Naproxen*	94[72]	95.06	-1.06
Phenylbutazone	96[73, 79, 80]	97.45	-1.45
Piroxicam*	99[80]	82.65	16.35
Quinine*	95[77]	96.72	-1.72
Theophylline	98[81]	98.41	-0.41
Zolmitriptan*	70[71, 78]	68.01	1.99

Table 3

Drug	Expt. %HIA	Pred. %HIA
Acetaminophen	80[72]	88.97
Caffeine	99[73]	98.24
Carbamazepine*	70[74]	70.60
Cimetidine	60[72]	57.16
Diclofenac	81[75, 76]	85.47
Fenoprofen*	85[76]	92.37
Fluconazole	94[73]	93.77
Flurbiprofen	95[77]	91.79
Fosinopril	35[78]	34.68
Gemfibrozil	95[75]	90.64
Haloperidol	60[79]	62.80
Ibuprofen	90[75, 85]	92.01
Indomethacin*	98[79]	86.36
Ketoprofen	90[77]	89.69
Leflunomide	80[78]	86.12
Lidocaine	81[73, 74, 77, 80]	86.71
Linezolid*	100[78]	87.40
Meloxicam	90[72]	77.64
Moexipril	23[78]	26.55
Naproxen	97[72, 77]	91.95
Phenylbutazone	90[79]	94.44
Piroxicam*	99[80]	80.73
Quinine*	95[77]	93.94
Theophylline	96[85]	97.95
Zolmitriptan*	70[71, 78]	70.94