# The "High Solubility" Definition of the Current FDA Guidance on Biopharmaceutical Classification System May Be Too Strict for Acidic Drugs

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***Purpose.*** The purpose of this study was to assess if the definition of high solubility as proposed in the FDA Guidance on Biopharmaceutical Classification System (BCS) is too strict for highly permeable acidic drugs.

***Methods.*** The solubility and permeability values of 20 (18 acidic and 2 non-acidic) nonsteroidal anti-inflammatory drugs (NSAID) were determined. The NSAIDs were grouped into three different sets having acetic acid, propionic acid, or other acidic moieties such as fenamate, oxicam, and salicylate. Two nonacidic NSAIDs (celecoxib and rofecoxib) were also included for comparison purposes. Equilibrium solubility values were determined at pH 1.2, 5.0, 7.4, and in bio-relevant media simulating fed intestinal fluid at pH 5.0. For a select number of acids, we also measured solubility values in media simulating gastric and fasted intestinal fluids. Permeability classification was established relative to that of reference drugs in the Caco-2 cell permeability model. Permeability coefficients for all drugs were measured at concentrations corresponding to the lowest and highest marketed dose strengths dissolved in 250 ml volume, and their potential interaction with cellular efflux pumps was investigated.

***Results.*** All NSAIDs with different acidic functional groups were classified as highly permeable based on their Caco-2 cell permeability. Only ketorolac appeared to have a potential for interaction with cellular efflux pumps. Solubility classification was based on comparison of equilibrium solubility at pH 1.2, 5.0, and 7.4 relative to marketed dose strengths in 250 ml. The p$K_a$ values for the acidic NSAIDs studied were between 3.5 and 5.1, and, as expected, their solubility increased dramatically at pH 7.4 compared to pH 1.2. Only three NSAIDs, ketorolac, ketoprofen, and acetyl salicylic acid, meet the current criteria for high solubility over the entire pH range. However, with the exception of ibuprofen, oxaprozin, and mefenamic acid, the remaining compounds can be classified as Class I drugs (high solubility–high permeability) relative to solubility at pH 7.4. The use of bio-relevant media simulating gastric and intestinal milieu for solubility measurements or increasing the dose volume to 500 ml did not provide for a better boundary for solubility classification.

***Conclusions.*** Based on the current definition of solubility, 15 of the 18 acidic NSAIDs in this study will be classified as Class II compounds as the solubility criteria applies to the entire pH range of 1.2 to 7.4, although the low solubility criteria does not hold true over the entire pH range. Whence, of the 18 acidic drugs, 15 can be classified as Class I based on the pH 7.4 solubility alone. This finding is intriguing because these drugs exhibit Class I behavior as their absorption does not seem to be dissolution or solubility limited. It could then be argued that for acidic drugs, the boundaries for solubility are too restrictive. Solubility at pH > 5 (pH in duodenum) may be more appropriate because most compounds are mainly absorbed in the intestinal region. Consideration for an intermediate solubility classification for highly permeable ionizable compounds that reflects physiological conditions seems warranted.

**KEY WORDS:** Biopharmaceutical Classification System (BCS); Caco-2; permeability; solubility.

## INTRODUCTION

The Biopharmaceutical Classification System (BCS) is a scientific approach for classifying compounds based on solubility as related to dose and permeability of drug substance combined with the dissolution properties of the drug product (1). Several regulatory guidances for industry have been issued by the FDA starting with SUPAC-IR guidance in 1995 (2) and more recently a guidance on biowaiver of *in vivo* bioavailability and bioequivalence studies which allows petitioners to request biowaivers for immediate release oral dosage forms based on the Biopharmaceutical Classification System (1). Specifically, biowaivers may be requested for high solubility–high permeability compounds (Class I) formulated in immediate release (IR) solid oral-dosage forms that exhibit rapid *in vitro* dissolution, provided the following conditions are met: 1. the drug must be stable in the gastrointestinal tract; 2. excipients used in the IR solid oral-dosage forms have no significant effect on the rate and extent of oral drug absorption; 3. the drug must not have a narrow therapeutic index; and 4. the product is designed not to be absorbed in the oral cavity.

Currently, the FDA guidance on biowaivers defines drugs having more than 90% of the orally administered dose absorbed as highly permeable. Drugs are defined as highly soluble "when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1.0–7.5" (1,3). It has recently been suggested that this FDA guidance is conservative with respect to class boundaries of solubility and permeability (4). In particular, the solubility boundary definition may be too strict for highly permeable acidic drugs and does not allow any flexibility based on the dose strength and bioavailability. With respect to solubility, some of the proposed changes and/or areas that would require more research included 1. narrowing the required pH solubility range from 1.0–7.5 to 1.0–6.8, 2. increasing the dose volume for solubility classification from 250 to 500 ml, and 3. including bile salts in the solubility measurements. The potential for defining an intermediate solubility class for Class II (low solubility–high permeability) was also raised in the same article (4).

We evaluated the potential impact of such changes in the solubility definition on the classification of compounds by studying a number of small molecular weight nonsteroidal anti-inflammatory drugs (NSAIDs). The NSAIDs were selected as they are well-characterized and studied in the literature and have generally very high oral absorption in humans (>90%) (5–10). It has also been suggested that NSAIDs exhibit behavior similar to Class I (high solubility–high permeability) despite their low solubility at low pH values though most would be classified as Class II (low solubility–high permeability) based on the current FDA definition of solubility (4).

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The question is then if the solubility boundaries were to be changed, would the classification of these compounds also change and would that provide any additional insight into their biopharmaceutical behavior that could aid in redefining the solubility criteria?

To address this question, we determined solubility and permeability values of 20 (18 acidic and 2 non-ionizable) NSAIDs in our laboratories to provide for a standardized database. Solubility was determined at pH 1.2 and pH 7.4 as these values bracket the pH range. Solubility was also determined at pH 5.0 buffer and fed-state simulated intestinal fluid at pH 5.0 to approximate the pH in the duodenum where intestinal absorption takes place. Permeability classification was determined using the Caco-2 cell *in vitro* model.

The NSAIDs were grouped into three different sets having acetic acid, propionic acid, and other acidic NSAIDs containing fenamate, oxicam, and salicylic acid moieties. For comparison purposes, two NSAIDs (celecoxib and rofecoxib) non-ionizable in the pH 1.0–7.5 range were also included. The compounds were then classified based on the currently approved solubility criteria (250 ml over the pH range 1.0–7.5) and using alternate boundaries as suggested in Ref. 4 and summarized above.

In addition, and for clarity of the following discussions, the term *equilibrium solubility* will be used to refer to solubility measured in buffer and media whereas the term *dose-relative solubility* will be used for solubility as defined in the guidance; that is, concentrations corresponding to the (highest) dose strength in 250 ml.

## MATERIALS AND METHODS

### Materials

Fenoprofen, flurbiprofen, ibuprofen, ketoprofen, naproxen, diclofenac, etodolac, indomethacin, ketorolac, sulindac, tolmetin, mefenamic acid, acetylsalicylic acid, diflunisal, salicylic acid, sodium taurocholate, and Triton X-100 were purchased from Sigma Aldrich (Milwaukee, WI, USA). Potassium phosphate monobasic, potassium chloride, and sodium chloride were purchased from EM Science (Gibbstown, NJ, USA). Sodium hydroxide was purchased from J.T. Baker (Philipsburg, NJ, USA), and acetic acid was purchased from Fisher Scientific (Pittsburg, PA, USA). Oxaprozin was supplied by Wyckoff, Inc (South Haven, MI, USA). Celecoxib was supplied by Desynth S.A (Buenos Aires, Argentina). Rofecoxib was isolated from Vioxx Tablets (Merck & Co., Whitehouse Station, NJ, USA). Meloxicam was from Boehringer Ingelheim Pharmaceuticals Inc. (Ridgefield, CT, USA). Caco-2 cells were obtained from American Tissue Culture Collection (Rockville, MD, USA). Culture media and reagents were purchased from Gibco BRL Products (Gaithersburg, MD, USA). Lecithin, L-α-phosphatidycholine (egg) 99% purity was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA).

### Methods

#### *Equilibrium Solubility Determination*

Equilibrium solubility values were determined at pH 1.2 (0.1 N HCl), pH 5.0 (0.02 M citric acid), and pH 7.4 (0.02 M $Na_2HPO_4$, 0.02 M $NaH_2PO_4$) for all of the NSAIDs. In addition, solubility values for two drugs in the propionic acid group (ibuprofen and naproxen) and two drugs in the acetic acid group (indomethacin and sulindac) were determined in "bio-relevant" media simulating the gastric fluid in the stomach and the fed and fasted state conditions in the small intestine. Furthermore, the solubility of these four drugs at pH 6.5 (0.02 M $NaH_2PO_4$) was also determined.

A known amount of drug, generally 10 to 20 mg, was added to 10 ml of buffer, and the resulting suspension was stirred for 24 h and filtered through a 0.45-μm filter. The filtered suspension was then assayed by HPLC to determine the drug concentration. In cases where the drug had completely dissolved in the buffer, the value for equilibrium solubility was assumed to be higher than the value determined by HPLC and was reported as such. All solubility experiments were performed at ambient temperature. All determinations were made in triplicate.

#### *Composition of "Bio-Relevant" Media*

Media simulating conditions in the proximal small intestine in the fasted state and the fed state were prepared as previously reported (5). Briefly, fasted state simulated intestinal fluid (FaSSIF) contained 3 mM sodium taurocholate and 0.75 mM lecithin and had a pH of 6.5. Fed state simulated intestinal fluid (FeSSIF) contained 15 mM sodium taurocholate and 3.75 mM lecithin and had a pH of 5.0. Simulated gastric fluid (SGF), fluid simulating fasted state condition in the stomach, was prepared as the simulated gastric fluid without pepsin with an additional 0.1% w/v Triton X 100 and had a pH of 1.2 (6,7).

#### *Caco-2 Cell Culture*

Caco-2 cell permeability studies were conducted as previously reported (8). Caco-2 cells obtained from American Type Culture Collection were grown at 37°C in an atmosphere of 5% $CO_2$ in Dulbecco's Modified Eagle Medium (DMEM) growth medium supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) nonessential amino acids, penicillin (100 U/ml), and streptomycin (100 μg/ml). Confluent cell monolayers were subcultured every 7 days by treatment with 0.25% trypsin containing 1 mM EDTA. Caco-2 cells were seeded at a density of 80,000 cells/$cm^2$ in 6-well plates on Transwell polycarbonate filters (Costar Corning, NY, USA; diameter 24.5 mm, pore size 0.4 μm). Cells were grown until fully differentiated after 21 days, and all experiments were conducted between 21 and 25 days. Cells of passage numbers 30 to 50 were used throughout.

#### *Permeability Studies*

Caco-2 cell monolayers were preconditioned by incubating with Hank's Balanced Salt Solution (HBSS) (pH 7.4) consisting of 1.3 mM $CaCl_2$, 5.4 mM KCl, 0.44 mM $KH_2PO_4$, 0.49 mM $MgCl_2$, 0.41 mM $MgSO_4$, 137 mM NaCl, 0.34 mM $Na_2HPO_4$, 5.5 mM D-glucose, and 4.2 mM $NaHCO_3$ at 37°C for 30 min. NSAIDs at concentrations corresponding to the highest and lowest available strengths in 250 ml of buffer were used in permeability studies (9). For apical to basolateral (A to B) experiments, the solution was placed on the apical side of the cells, and samples were taken from the basolateral side. In contrast, for basolateral to apical (B to A) experiments, the solution was placed on the basolateral side and samples were

taken from apical side of the cells. Samples were analyzed by HPLC (Hewlett Packard, HP1100). The pH of the transport medium after the addition of tested compounds did not change by more than 0.2 pH units over the duration of the entire experiment. All permeability studies were performed at 37°C. Permeability coefficient ($P_{Caco\text{-}2}$) was determined according to Eq. (1):

$$P_{Caco\text{-}2} = \frac{J}{A * C_i} \quad (1)$$

where $J$ is the transport rate determined by plotting cumulative amounts of drug permeated to the receiver chamber as a function of time, $A$ is the surface area of the filter, and $C_i$ is the initial concentration of the solution in the donor chamber.

To determine whether the drugs were substrate for the apically polarized efflux systems in Caco-2 cell monolayers, $P_{Caco\text{-}2}$ of each drug from both A to B ($P_{A\ to\ B}$) and B to A ($P_{B\ to\ A}$) at each concentration was measured. For cellular efflux pump substrates, $P_{Caco\text{-}2}$ values are expected to be higher from B to A than from A to B (10). All experiments were performed in at least triplicate, and data are expressed as mean ± standard deviation.

## RESULTS AND DISCUSSION

### Caco-2 Cell System Suitability

The Caco-2 cell permeability model was used to determine the permeability class of the drugs used in this study. We had previously validated our model using a large set of small-molecular-weight marketed drugs (n = 37) (8). Our data demonstrated that our Caco-2 cell model was discriminating and that those compounds with permeability coefficients higher than ~1 × $10^{-6}$ cm/s had 90% or more of the administered dose absorbed (8). The suitability of the Caco-2 cell method over the course of this study was established by measuring the permeability of nine reference drugs selected from the list provided in the FDA Guidance (3). Thus, four highly permeable drugs (caffeine, metoprolol, propanolol, and verapamil) and five low permeability drugs (atenolol, ranitidine, hydrochlorothiazide, mannitol, and furosemide) were used as markers at 100 mM (Table I). Permeability values were determined from both apical to basolateral ($P_{A\ to\ B}$) and basolateral to apical ($P_{B\ to\ A}$) directions. The permeability directional ratio (PDR) defined as the ratio of $P_{B\ to\ A}$ to $P_{A\ to\ B}$ values was used to assess the potential interaction of drugs with cellular efflux pumps (10). For instance, compounds that are transported only by passive diffusion should have similar permeability values in either direction, and their PDR values should be about 1. However, for drugs that have an affinity for cellular efflux pumps such as *p*-glycoproteins and are actively transported out of the cells such that $P_{B\ to\ A}$ is greater than $P_{A\ to\ B}$, the PDR values should be greater than unity. For the purpose of this work, compounds with PDR values greater than 2 were considered to be interacting with cellular efflux pumps.

The $P_{A\ to\ B}$ values for all highly permeable reference drugs were higher than 15.8 × $10^{-6}$ cm/s and none appeared to have an affinity for cellular efflux pumps (PDR values in the range of 0.7 to 1.3) (Table I). On the other hand, for the low permeable reference drugs, the $P_{A\ to\ B}$ values were less than 1.57 × $10^{-6}$ cm/s. Of the five low permeability reference compounds, ranitidine, hydrochlorothiazide, and furosemide appeared to have an affinity for cellular efflux pumps (PDR > 2). Furosemide, in particular, had an exceptionally high PDR value of 687 that may explain its highly variable oral absorption in humans (11). In this study, we classified the drugs relative to atenolol (50% absorption in human), and compounds were considered as having high permeability when their $P_{A\ to\ B}$ values were higher than that of atenolol.

### Permeability Class Evaluation

#### *NSAIDs with Acetic Acid Moieties*

Six NSAIDs (diclofenac, etodolac, indomethacin, ketorolac, sulindac, and tolmetin) containing a single acetic acid moiety and no other ionizable functional groups were studied. Structures, molecular weight, p$K_a$, marketed dose strengths, and Caco-2 cell permeability values are presented in Table II. These drugs are rapidly and completely absorbed with bioavailability exceeding 90% with the exception of diclofenac, which undergoes significant first-pass effect and has a bioavailability of ~54% (12).

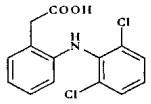
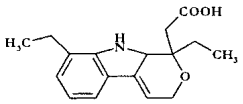
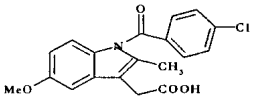
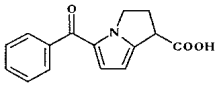
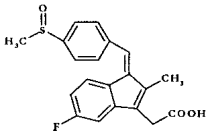
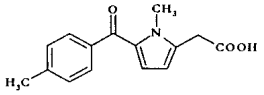
As expected from the *in vivo* human data, the Caco-2 cell permeability values for these NSAIDs were above that of atenolol and were therefore classified as highly permeable. Ketorolac was the only drug that showed a potential for interacting with cellular efflux pumps and had a PDR value of approximately 4.

**Table I.** The Caco-2 Cell Permeability of Reference Markers from the FDA Guidance*

| Drugs | Molecular weight | Permeability A to B × $10^6$ (cm/s) | Permeability B to A × $10^6$ (cm/s) | PDR | % Absorption in humans |
|---|---|---|---|---|---|
| High permeability drugs | | | | | |
| Caffeine | 194 | 47.8 ± 0.1 | 37.6 ± 1.2 | 0.8 | 100 (16) |
| Metoprolol | 267 | 43.4 ± 0.7 | 34.1 ± 0.6 | 0.8 | >95 (16) |
| Propranolol | 259 | 33.9 ± 1.8 | 25.0 ± 0.8 | 0.7 | 100 (16) |
| Verapamil | 455 | 15.8 ± 1.2 | 21.1 ± 2.0 | 1.3 | 100 (16) |
| Low permeability drugs | | | | | |
| Atenolol | 266 | 1.57 ± 0.25 | 1.90 ± 0.30 | 1.2 | 50 (16) |
| Ranitidine | 314 | 1.24 ± 0.25 | 5.98 ± 0.65 | 4.8 | 50 (17) |
| Hydrochlorothiazide | 298 | 0.86 ± 0.12 | 3.46 ± 0.32 | 4.0 | 90 (16) |
| Mannitol | 182 | 0.18 ± 0.01 | 0.16 ± 0.01 | 0.9 | 16 (18) |
| Furosemide | 331 | 0.03 ± 0.00 | 19.5 ± 0.1 | 687 | Variable (11) |

PDR = permeability directional ratio.
* Ref. 3.

**Table II.** Structure, Properties, Dose, and Caco-2 Permeability of NSAIDs with Acetic Acid Moieties

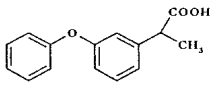
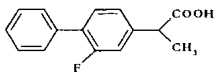
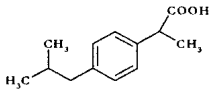
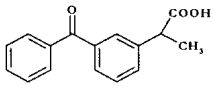
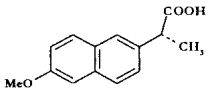
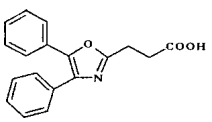
| Compound | Structure | Molecular weight | p$K_a$ | Human BA (%) | High & low doses (mg) | $P_{Caco\text{-}2} \times 10^6$ cm/s | | PDR |
|---|---|---|---|---|---|---|---|---|
| | | | | | | A to B | B to A | |
| Diclofenac |  | 295.1 | 4.2 (16) | 54 ± 2 (12) | 50 | 20.2 ± 1.7 | 21.3 ± 1.3 | 1.1 |
| Etodolac |  | 287.4 | 4.7 (19) | >80 (12) | 200<br>400 | 23.4 ± 2.1<br>21.9 ± 2.3 | 26.8 ± 2.0<br>23.9 ± 2.6 | 1.1<br>1.1 |
| Indomethacin |  | 357.8 | 4.5 (16) | 98 ± 21 (12) | 25<br>50 | 23.8 ± 1.4<br>10.4 ± 1.0 | 33.0 ± 0.6<br>24.5 ± 2.6 | 1.4<br>2.4 |
| Ketorolac |  | 255.3 | 3.5 (16) | 100 ± 20 (12) | 10<br>20 | 4.3 ± 0.2<br>4.3 ± 0.1 | 17.9 ± 1.3<br>18.6 ± 0.7 | 4.2<br>4.3 |
| Sulindac |  | 356.4 | 4.5 (16) | 88 (20) | 150<br>200 | 4.9 ± 0.4<br>6.3 ± 1.0 | 9.6 ± 0.7<br>12.2 ± 1.4 | 2.0<br>1.9 |
| Tolmetin |  | 257.3 | 3.5 (16) | >90 (12) | 200<br>600 | 8.4 ± 1.0<br>7.7 ± 0.2 | 9.7 ± 0.3<br>8.7 ± 0.3 | 1.2<br>1.1 |

PDR, permeability directional ratio.

*NSAIDs with Propionic Acid Moieties*

Six NSAIDs (fenoprofen, flurbiprofen, ibuprofen, ketoprofen, naproxen, and oxaprozin) containing a single propionic acid moiety and no other ionizable functional groups were studied. Structures, molecular weight, p$K_a$, marketed dose strength, and Caco-2 cell permeability values are presented in Table III. These NSAIDs are rapidly and completely absorbed with bioavailability values above 80%. The reported p$K_a$ values for these drugs were in the range of 4.2 to 4.6.

**Table III.** Structure, Properties, Dose, and Caco-2 Cell Permeability of NSAIDs with Propionic Acid Moieties

| Compound | Structure | Molecular weight | p$K_a$ | Human BA (%) | High & low doses (mg) | $P_{Caco\text{-}2} \times 10^6$ cm/s | | PDR |
|---|---|---|---|---|---|---|---|---|
| | | | | | | A to B | B to A | |
| Fenoprofen |  | 242.3 | 4.5 (16) | 85% Absorbed (17) | 200<br>600 | 11.3 ± 0.7<br>7.4 ± 0.2 | 13.7 ± 0.4<br>7.7 ± 0.2 | 1.2<br>1.0 |
| Flurbiprofen |  | 244.3 | 4.3 (16) | ~92 (12) | 50<br>100 | 12.3 ± 1.4<br>20.1 ± 2.7 | 29.5 ± 3.9<br>18.8 ± 0.3 | 2.4<br>0.9 |
| Ibuprofen |  | 206.3 | 4.4 (16) | >80 (12) | 200<br>800 | 10.1 ± 0.2<br>9.6 ± 0.4 | 19.8 ± 0.3<br>19.2 ± 1.8 | 2.0<br>2.0 |
| Ketoprofen |  | 254.3 | 4.6 (16) | ~100 (12) | 12.5<br>75 | 12.8 ± 2.1<br>20.1 ± 1.0 | 22.9 ± 0.5<br>25.6 ± 2.5 | 1.8<br>1.3 |
| Naproxen |  | 230.3 | 4.2 (16) | 99 Estimated (12) | 200<br>500 | 11.6 ± 0.2<br>12.3 ± 0.4 | 20.0 ± 0.5<br>20.0 ± 0.6 | 1.7<br>1.6 |
| Oxaprozin |  | 293.3 | 4.3 (17) | 95–100 (12) | 600 | 33.4 ± 2.6 | 39.4 ± 3.4 | 1.2 |

PDR, permeability directional ratio.

As expected from the *in vivo* human data, the Caco-2 cell permeability values for these NSAIDs were above that of atenolol, and compounds were classified as highly permeable. None showed a potential for interacting with cellular efflux pumps at the concentration ranges studied.

*NSAIDs with Other Acidic Moieties*

Seven additional NSAIDs containing fenamate, oxicam, and salicylic acid moieties and two non-ionizable compound NSAIDs (celecoxib and rofecoxib) were also studied. Structures, molecular weights, p$K_a$, marketed dose strengths, and Caco-2 cell permeability values are presented in Table IV.

Bioavailability and absorption data were not available for all compounds, however, based on the permeability coefficients in the Caco-2 cell model, all seven drugs exhibit high permeability and no apparent interactions with cellular efflux pumps.

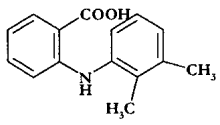
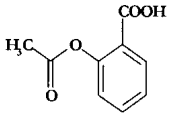
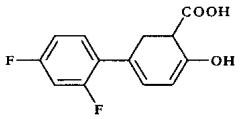
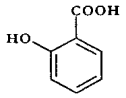
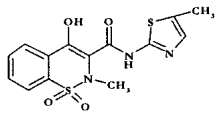
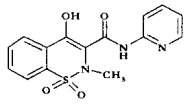
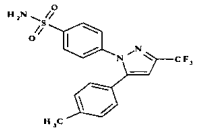
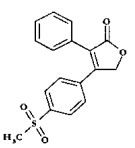
**Solubility Class Evaluation**

The equilibrium solubility, dose-relative solubility (concentrations corresponding to the highest strengths in 250 ml), and solubility class evaluation for all NSAIDs are presented in Table V.

As expected of acidic compounds (p$K_a \approx 3.5$ to 5.1), solubility values increased dramatically at the highest pH value studied. Whence, looking at equilibrium solubility values at pH 7.4 alone, where the pH is more than 2 U above the p$K_a$ and the drugs are expected to be fully ionized, all of the acidic NSAIDs, with the exception of mefenamic acid, ibuprofen, and oxaprozin, met the high solubility criteria as these values were equal to or greater than the dose-relative solubility values. At pH 5.0, the solubility values are considerably lower, and only 8 out of 18 acidic NSAIDs met the high solubility criteria, and the addition of sodium taurocholate and lecithin (i.e., in FeSSIF at pH 5.0) did not significantly increase the solubility values or solubility classification. Interestingly, the classification for diclofenac changed from "high" at pH 5.0 to "low" in FeSSIF whereas that of naproxen was reversed.

At pH 1.2, where the pH is more than 2 U below the p$K_a$ and the drugs are expected to be completely non-ionized, 15 of 18 NSAIDs can be classified as having low solubility (Class

**Table IV.** Structure, Properties, Dose, and Caco-2 Cell Permeability of NSAIDs with Anthranilic and Salicylic Acids and Oxicam Moieties and Cox-2 Inhibitors

| Compound | Structure | Molecular weight | p$K_a$ | Human BA (%) | High & low doses (mg) | $P_{Caco\text{-}2} \times 10^6$ cm/s | | PDR |
|---|---|---|---|---|---|---|---|---|
| | | | | | | A to B | B to A | |
| Mefenamic Acid |  | 241.3 | 4.2 (19) | Rapidly Absorbed (17) | 250 | 17.9 ± 0.4 | 22.2 ± 2.1 | 1.2 |
| Acetyl-salicylic acid |  | 180.2 | 3.5 (19) | 68 ± 3 for unchanged drug (12) | 325<br>975 | 20.1 ± 6.3<br>25.5 ± 3.5 | 21.4 ± 0.8<br>19.1 ± 2.9 | 1.1<br>0.7 |
| Diflunisal |  | 250.2 | 3.0 (19) | ~90 (12) | 250<br>500 | 18.4 ± 1.8<br>12.5 ± 1.2 | 22.1 ± 2.1<br>17.0 ± 0.2 | 1.2<br>1.4 |
| Salicylic acid |  | 138.1 | 3.0 (19) | 100 (12) | 500<br>750 | 25.4 ± 2.1<br>17.6 ± 0.8 | 24.9 ± 1.7<br>20.5 ± 0.7 | 1.0<br>1.2 |
| Meloxicam |  | 351.4 | 1.1, 4.2 (17) | 89 (12) | 7.5<br>15 | 17.6 ± 1.3<br>13.8 ± 1.3 | 15.1 ± 0.6<br>15.3 ± 0.9 | 0.9<br>1.1 |
| Piroxicam |  | 331.4 | 1.8, 5.1 (17) | Rapidly Absorbed (17) | 10<br>20 | 24.1 ± 1.3<br>23.5 ± 1.2 | 19.7 ± 1.3<br>21.0 ± 1.0 | 0.8<br>0.9 |
| Celecoxib |  | 381.4 | — | — | 100<br>200 | 17.6 ± 1.3<br>13.8 ± 1.3 | 15.1 ± 0.6<br>15.3 ± 0.9 | 1.2<br>1.1 |
| Rofecoxib |  | 314.4 | — | 93 (21) | 12.5<br>25 | 24.1 ± 1.3<br>23.5 ± 1.2 | 19.7 ± 1.3<br>21.0 ± 1.0 | 1.4<br>1.2 |

PDR, permeability directional ratio.

**Table V.** Equilibrium Solubility and Solubility Classification for NSAIDs

| Compound | Highest dose (mg) | Dose-relative solubility in: | | Equilibrium solubility (mg/ml) | | | | Solubility classification at 250 ml | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 250 ml | 500 ml | pH 1.2 | pH 5.0 | FeSSIF (pH 5) | pH 7.4 | pH 1.2 | pH 5.0 | FeSSIF (pH 5) | pH 7.4 |
| Diclofenac | 50 | 0.2 | 0.1 | 0.001 | 2.8 | 0.14 | 15.9 | Low | High | Low | High |
| Etodolac | 400 | 1.6 | 0.8 | 0.04 | 0.14 | 0.74 | 4.5 | Low | Low | Low | High |
| Indomethacin | 50 | 0.2 | 0.1 | 0.001 | 0.01 | 0.07 | 1.3 | Low | Low | Low | High |
| Ketorolac | 20 | 0.08 | 0.04 | 0.11 | 5.74 | 15.6 | >1.3 | High | High | High | High |
| Sulindac | 200 | 0.8 | 0.4 | 0.007 | 0.35 | 0.40 | >1.3 | Low | Low | Low | High |
| Tolmetin | 600 | 2.4 | 1.2 | 0.02 | 5.65 | 1.26 | >10 | Low | High | Low | High |
| Fenoprofen | 600 | 2.4 | 1.2 | 0.1 | 3.23 | 4.8 | >3.1 | Low | High | High | High |
| Flurbiprofen | 100 | 0.4 | 0.2 | 0.007 | 0.06 | 0.43 | 2.6 | Low | Low | High | High |
| Ibuprofen | 800 | 3.2 | 1.6 | 0.06 | 0.14 | 0.65 | 2.3 | Low | Low | Low | Low |
| Ketoprofen | 75 | 0.3 | 0.2 | 0.13 | 0.38 | 0.84 | >1.4 | Low | High | High | High |
| Naproxen | 500 | 2.0 | 1.0 | 0.005 | 0.09 | 0.20 | >2.5 | Low | Low | High | High |
| Oxaprozin | 600 | 2.4 | 1.2 | 0.004 | 0.007 | 0.08 | 1.7 | Low | Low | Low | Low |
| Mefenamic acid | 250 | 1.0 | 0.5 | 0.0002 | 0.0005 | 0.018 | 0.1 | Low | Low | Low | Low |
| Acetyle-salicylic acid[a] | 975 | 3.9 | 2.0 | 6.2 | 5.5 | >8 | 6.4 | High | High | High | High |
| Diflunisal | 500 | 2.0 | 1.0 | 0.003 | 0.18 | 0.65 | 2.4 | Low | Low | Low | High |
| Salicylic acid | 750 | 3.0 | 1.5 | 1.8 | 3.1 | >8 | >8 | Low | High | High | High |
| Meloxicam | 15 | 0.06 | 0.03 | 0.0013 | 0.002 | 0.007 | 0.46 | Low | Low | Low | High |
| Piroxicam | 20 | 0.08 | 0.04 | 0.09 | 0.012 | 0.026 | 0.26 | Low | Low | Low | High |
| Celecoxib | 200 | 0.8 | 0.4 | 0.003 | — | — | 0.005 | Low | — | — | Low |
| Rofecoxib | 25 | 0.10 | 0.05 | 0.0008 | — | — | 0.0009 | Low | — | — | Low |

FeSSIF, fed state simulated intestinal fluid.
[a] Degradation peaks in solubility samples.

II) drugs. Ketorolac, acetylsalicylic acid, and piroxicam were the only NSAIDs that met the high solubility criteria at all conditions and therefore would be classified as Class I.

Ketoprofen is a good example of dose effect on classification as it would be classified as low solubility (Class II) at the high dose (75 mg) and high solubility (Class I) at the lower dose (12.5 mg). Similarly, and based on the pH 7.4 solubility value alone, ibuprofen would meet the criteria of high solubility at the low dose (200 mg) and that of low solubility at the high dose (800 mg).

We also measured solubility and permeability values of two nonacidic NSAIDs: Cox-2 inhibitors celecoxib and rofecoxib. As expected, there were no differences in solubility values of either drug at pH 1.2 and pH 7.4, and both compounds can then be readily classified as Class II due to their low solubility relative to dose strength.

### Solubility in Bio-Relevant Media

Even though dissolution, transit, and absorption of drugs form the gastrointestinal tract is a heterogeneous and complex process (13), the use of simple bio-relevant media to simulate the intraluminal environment of the gastrointestinal tract for *in vitro* measurements has been advocated for better modeling of the *in vivo* dissolution of drugs (5–7). We selected four NSAIDs, two with acetic acid (indomethacin and sulindac) and two with propionic acid (ibuprofen and naproxen) functional groups. The equilibrium solubility of the four drugs was measured in SGF, FeSSIF, and FaSSIF and compared to simple buffers at the same pH values (Table VI). The solubility of all compounds was consistently higher in the bio-relevant solvent relative to that in simple aqueous buffer at the same pH indicating that solubility in simple buffers may underestimate the *in vivo* solubilization. However, the increase in solubility did not impact on the classification of these compounds. This in turn may be due to an inherent limitation in solubility classification based on equilibrium solubility determination, which is static and does not take into account the dynamic nature of absorption.

Increasing the dose volume for solubility classification from 250 to 500 ml does not have an impact on the classifi-

**Table VI.** Solubility of Select Acidic NSAIDs in Bio-Relevant Buffers

| Compound | Solubility (mg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Buffer (pH 1.2) | SGF (pH 1.2) | Relative increase in SGF | Buffer pH 5.0 | FeSSIF (pH 5.0) | Relative increase in FeSSIF | Buffer (pH 6.5) | FaSSIF (pH 6.5) | Relative increase in FaSSIF | Buffer (pH 7.4) |
| Indomethacin | 0.001 | 0.02 | 20 | 0.01 | 0.07 | 7 | 0.14 | 0.23 | 1.6 | 1.30 |
| Sulindac | 0.007 | 0.03 | 4.6 | 0.35 | 0.40 | 1.1 | 0.53 | 0.77 | 1.5 | >1.3 |
| Ibuprofen | 0.06 | 0.20 | 3.3 | 0.14 | 0.65 | 4.6 | 0.93 | 1.46 | 1.6 | 2.30 |
| Naproxen | 0.005 | 0.10 | 20 | 0.09 | 0.20 | 2.2 | 0.77 | 1.21 | 1.6 | >2.5 |

FeSSIF, fed state simulated intestinal fluid; FaSSIF, fasted state simulated intestinal fluid.

cation of the NSAIDs except for the classification of salicylic acid, which then changes from Class II to Class I. Interestingly, considering solubility values at pH 7.4 alone and increasing the dose volume to 500 ml results in classification of all NSAIDs as Class I drugs. This is not surprising as the calculated volume required to solubilize the entire dose at pH 1.2 for most NSAIDs needs to be in the liters ranging from 6 l for fenoprofen to as high as 1250 l for mefenamic acid, far exceeding the factor of 2 introduced by the use of 500 ml.

## CONCLUSIONS

Based on the current definition of solubility, 15 of the 18 acidic NSAIDs in this study will be classified as Class II compounds as the solubility criteria applies to the entire pH range of 1.2 to 7.4 though the low solubility criteria does not hold true over the entire pH range. Of the 18 compounds, 15 compounds would have been classified as Class I based on the pH 7.4 solubility alone. This finding is intriguing as some of these compounds do indeed exhibit Class I behavior, and their absorption does not seem to be dissolution/solubility limited. Increasing the dose volume to 500 ml or determining solubility values in bio-relevant media do not seem to impact on the solubility classification of the NSAIDs. Narrowing the required pH solubility range from 1.0–7.5 to 1.0–6.8 would again not impact on the classification of acidic drugs. It could be argued that for acidic drugs, the boundaries for solubility are too restrictive. Solubility at pH > 5 (pH in duodenum) may be more appropriate because most compounds are mainly absorbed in the intestinal region. This should also hold true for basic and neutral compounds too, and the required pH solubility range should be narrowed to about 5–7.4 for all compounds, if any.

It we were to consider an intermediate solubility classification for highly permeable ionizable compounds, then the criteria should reflect physiological conditions such as the use of bio-relevant media (fed or fasted) in the intestinal pH range of 5 to 7. Whether this classification can also be applied to poorly permeable compounds warrants further research.

In the end, an inherent limitation in the solubility classification is that it relies on equilibrium solubility determination, which is static and does not take into account the dynamic nature of absorption. For example, the use of intrinsic dissolution rates or dissolution-absorption models have recently been suggested (14,15). Additional research ought to focus in part on dynamic solubility measurement techniques, whether in simple buffer or bio-relevant media, to better define the solubility boundary for classification.

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