

Biorelevant Dissolution of Dipyridamole and Piroxicam Using an Automated UV/Vis Spectrophotometric and Potentiometric Dissolution Testing Platform

Ragna Berthelsen¹, Susan Weng Larsen¹, Anette Müllertz^{1,2}, Karl Box³, and Jesper Østergaard^{1*}

¹Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark.

²Bioneer: FARMA, Copenhagen, Denmark.

³pION Inc, Forest Row, East Sussex, UK.

e-mail: jesper.ostergaard@sund.ku.dk

ABSTRACT

The objective of this study was to investigate the implications of changing dissolution parameters, including pH ramp time, absence and presence of simulated intestinal fluid (SIF), and the addition of a partitioning phase, using an automated dissolution-testing platform. The molar absorption coefficients, pK_a, and dissolution characteristics of dipyridamole and piroxicam were investigated in the UV/Vis spectrophotometric and potentiometric platform, inForm (Pion Inc). Dissolution of dipyridamole and piroxicam from 10-mg compacts (tablets) was studied at pH 2.0 and 6.5 in fasted-state SIF version 2 (v2) and in biphasic media using decanol as the partitioning phase. Transfer through the gastrointestinal tract was simulated by shifting pH from 2.0 to 6.5 during dissolution testing. Dipyridamole (pK_a 6.0) dissolved rapidly at pH 2.0; however, changing the pH to 6.5 brought dipyridamole into a supersaturated state, from which it precipitated. Precipitation was slower in the presence of SIF, and higher dipyridamole concentrations were maintained in solution compared with simple buffer systems. In the biphasic dissolution assay, rapid distribution of dipyridamole into the lipophilic partitioning phase minimized drug precipitation. For piroxicam (pK_a1 1.9; pK_a2 5.3), the dissolution rate increased with increasing pH. The inclusion of SIF and introduction of a partition phase had limited influence on piroxicam dissolution. The automated platform facilitated efficient exploration of dissolution conditions. Tailoring of dissolution assays including pH gradients, SIF, and biphasic partitioning enabled detailed drug characterization, increased biorelevance, and possibly in vivo predictability. The use of a biphasic dissolution assay had a large impact on the in vitro dissolution of dipyridamole. The incorporation of an absorptive sink might be key for unraveling the supersaturation and dissolution behavior of weakly basic drug compounds.

KEYWORDS: Biorelevant medium, biphasic dissolution, supersaturation, dissolution, pH shift, InForm

INTRODUCTION

To reach systemic circulation after oral administration, an active pharmaceutical ingredient (API) must dissolve in the gastrointestinal (GI) fluids and subsequently be absorbed across the intestinal membrane. The physicochemical characteristics of the API, e.g., solubility, pK_a, solid form, and lipophilicity, together with the properties of the drug delivery system and the physiological environment of the GI tract (e.g. pH, content of bile salts, and gastric emptying rate) determine the rate and extent of drug dissolution and absorption (1-4). Many oral drugs are absorbed in the upper part of the small intestine, and thus have to be in solution in

a slightly acidic to neutral environment (pH 5.4-6.5) (5). Biopharmaceutics Classification System (BCS) class II compounds are poorly soluble and may dissolve slowly in the small intestine, but have a high permeability, and consequently exhibit solubility and/or dissolution-limited absorption (6). For such APIs, it may be important to cover the pH range experienced during transit from the acidic stomach to the near neutral small intestine during in vitro dissolution testing. In particular, the pH change during transit from the stomach to the small intestines may lead to a significant decrease in solubility of basic APIs. Weak bases, fully ionized and highly soluble in the acidic environment, may upon transit into the small intestine

*Corresponding author

precipitate upon transient supersaturation (3, 7–9). As the extent and duration of in vivo supersaturation have great influence on the bioavailability, it is crucial to capture these phenomena in an in vivo relevant manner during in vitro dissolution studies (3, 7, 10–14). Biphasic dissolution assays have been proposed to mimic the dissolution and absorption events taking place in vivo (12, 15, 16).

In the present study, the dissolution behavior of dipyrindamole (PubChem CID: 3108) and piroxicam (PubChem CID: 54676228) was investigated using an automated UV/Vis spectrophotometric and potentiometric dissolution testing platform. The pH and composition of the dissolution medium were adjusted to simulate the conditions of the human GI tract. Transport of API into a lipophilic phase during dissolution was used to simulate drug absorption. The objective of the study was to investigate the implications of changing defined dissolution testing parameters; pH, pH ramp time, absence and presence of simulated intestinal fluid (SIF), and absence and presence of a partitioning phase, on the dissolution behavior of dipyrindamole and piroxicam.

METHODS

Materials

Dipyrindamole, methanol, N-methyl-2-pyrrolidinone (NMP), and piroxicam (anhydrate) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride, 0.50 M HCl, and 0.50 M NaOH were from Fisher Scientific (Leics, UK); n-Decanol was obtained from Alfa Aesar (Heysham, UK); and SIF-v2 powder was purchased from biorelevant.com (Croydon, UK). Purified water was obtained from a Purite Select deionization unit (Ondeo Industrial Solutions, Grangemouth, UK)

Instrumentation

Determination of molar absorption coefficients and pKa values, as well as the dissolution studies on dipyrindamole and piroxicam were performed using the inForm instrument from Pion Inc. (Forest Row, UK), which is an automated platform based on potentiometric titration as well as UV-metric measurements. Figure 1 shows a schematic presentation of the 100-mL measurement vessel (46-mm inner diameter, 75-mm height) containing two fiber-optic UV probes, a pH electrode, temperature probe, flat blade type overhead stirrer, compact holder, and capillaries for dispensing acid, base, and media. Temperature was maintained using a Peltier element. The autosampler, having two robotic arms, allows for automated washing, sample handling, and measurement procedures. The instrument was controlled using inForm software version 1.1.3.6 (Pion Inc.).

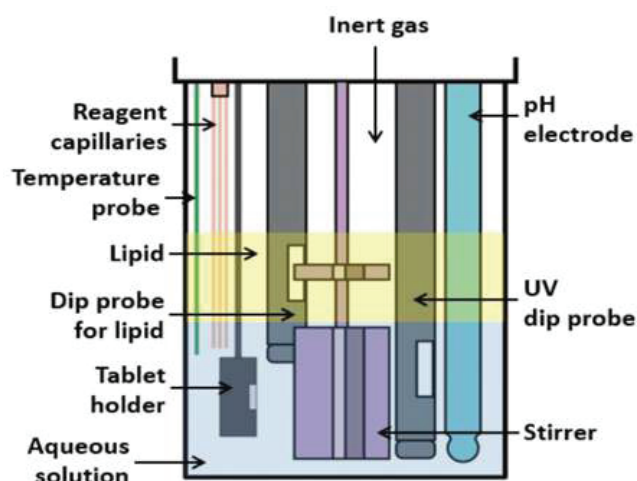


Figure 1. Schematic representation of potentiometric and UV-metric measurement vessel used for determination of molar absorption coefficients, pK_a, and dissolution experiments.

Molar Absorption Coefficient and pKa Determination

Molar absorption coefficients, as a function of wavelength (185–750 nm) and pH ranging from 2.0–12.0, were determined at 37 °C for dipyrindamole and piroxicam. An API solution in NMP at a concentration of 20 mM was added to 36 mL of an ionic strength-adjusted buffer solution ($I = 0.172$ M) containing acetate, phosphate, and sodium chloride. For both dipyrindamole and piroxicam, experiments were performed in neat aqueous medium (three titrations in each experiment, $n = 3$) as well as in methanol-buffer solutions (45, 30, and 22% methanol). Drug concentrations in the solutions ranged from 12–350 μ M and 4–27 μ M for dipyrindamole and piroxicam, respectively. During the measurements, the sample solution was stirred at a rate of 300 rpm. Potentiometric titrations from high to low pH and low to high pH were performed by the addition of 0.50 M HCl and 0.50 M NaOH, respectively. UV/Vis spectra were recorded during the titrations using the fiber optic probe with a light path of 10 or 20 mm connected to the inForm diode array spectrometer.

Molar absorption coefficients of the APIs in decanol were determined using the fiber optic probe by addition of aliquots of API stock solution in NMP to 40 mL of decanol while stirring at 300 rpm, providing dipyrindamole and piroxicam concentrations ranging from 50–250 μ M and 14–71 μ M, respectively.

Preparation of Compacts

Compacts with a surface diameter of 3 mm, comprising 7.5–10.8 mg of drug substance, were prepared in stainless steel dies using a manual screw press (Pion Inc.). The dimensions of the compact holding dies were 3, 12,

and 6 mm for the inner diameter, outer diameter, and thickness, respectively; the back side was sealed with a silicon rubber stopper. Compacts were prepared under a weight of 120 kg applied for 6 min. The compacts were visually inspected and ensured to have a smooth surface free of visible defects.

Dissolution Studies

The dissolution behavior of dipyrindamole and piroxicam from 3-mm diameter compacts was investigated at 37 °C. Experiments were initiated by lowering the compact into 40 mL pre-heated dissolution medium at 37 °C while stirring the medium at 100 rpm. UV/Vis spectra and solution pH levels were recorded every 30 s. Drug dissolution was investigated in buffered solutions at pH 2.0 and pH 6.5 ($I = 0.15\text{--}18\text{ M}$), in FaSSIF v2 (prepared from SIF-v2 powder and added as a 10x concentrate), and in biphasic medium with decanol as the organic layer. The aqueous dissolution medium comprised of acetate-phosphate buffer (0.10 M sodium acetate, 0.10 M sodium dihydrogen phosphate) for maintaining pH with NaCl (0.15 M) added for ionic strength adjustment. The dissolution was investigated utilizing a range of different experimental conditions as summarized in Table 1. In general, drug dissolution was followed for 30 min after which the experiment was terminated or the dissolution conditions altered in terms of pH, media change and/or addition of an absorptive/partitioning phase. Transfer through the GI tract was simulated by shifting pH from 2.0 to 6.5 during dissolution. The pH was either ramped from acidic to neutral pH over 60 s or changed gradually (linear relationship of pH against time) over a period of 30 min. In selected experiments, a partitioning phase consisting of decanol (30 mL) was added to simulate an absorptive step. The nominal interfacial area between the aqueous and decanol phases was 16.6 cm². The decanol phase was also subjected to agitation (100 rpm) and the amount of drug substance partitioning into the decanol phase was

measured using a second fiber optic probe (light path 10 mm).

Data Processing

Analysis of the dissolution experiments was based on the Noyes-Whitney equation:

$$\frac{dC}{dt} = k(S - C) \quad (1)$$

where S represents the solubility of the drug substance, C is the drug substance concentration in solution at time t , and k is a constant. Integration of Eq. (1) provides:

$$C = S(1 - e^{-kt}) \quad (2)$$

To account for a temporal offset, the exponential Eq. (2) was modified as follows:

$$C = S(1 - e^{-k(t-t_0)}) \quad (3)$$

where t_0 allows for the temporal offset (17, 18). The intrinsic dissolution rate, J , was calculated according to (18):

$$J = \frac{dC}{dt} \frac{V}{A} = \frac{V}{A} kS \quad (4)$$

where V is the volume of the dissolution medium and A is the surface area of the compressed drug disk.

The precipitation rate, $\frac{dM}{dt}$, was determined by fitting a first order expression to the relevant part of the concentration – time profile:

$$C = C_{\text{onset}} e^{-k'(t-t_{\text{onset}})} \quad (5)$$

followed by substitution of the empirical precipitation rate constant k' :

$$\frac{dM}{dt} = \frac{dC}{dt} V = -k'VC_{\text{onset}} \quad (6)$$

where C_{onset} is the drug substance concentration in solution at the time where precipitation starts (is detected), t_{onset} .

Table 1. Outline of Dissolution and Partitioning Studies Performed^a

Experiment no.	Sector 1	Sector 2	Sector 3
I	Buffer pH 6.5	-	-
II	Buffer pH 2.0	Buffer pH 6.5	-
III	Buffer pH 2.0	Linear pH gradient	Buffer pH 6.5
IV	Buffer pH 2.0	Linear pH gradient + addition of FaSSIF v2	FaSSIF v2 pH 6.5
V	Buffer pH 2.0	Buffer pH 6.5 + decanol partition phase	-
VI	Buffer pH 2.0	Linear pH gradient + decanol partition phase	Buffer pH 6.5 + decanol partition phase
VII	Buffer pH 2.0	Linear pH gradient + addition of FaSSIF v2 + decanol partition phase	FaSSIF v2 + decanol partition phase

^aDissolution experiments were conducted at 37 °C and 100 rpm. The duration of each sector was 30 min. FaSSIF v2: fasted-state simulated intestinal fluid, version 2.

Data processing was conducted using Sirius Refine Software version 1.1.3.6 (Pion Inc.). Absorbance spectra were converted to drug concentration or the absolute sample amount of drug substance dissolved using the molar absorption coefficients determined using inForm. Subsequently, k , S , and J (k' , C_{onset} , and $\frac{dM}{dt}$) were calculated utilizing a refinement process in which, k , S , and t_0 (k' , C_{onset} , and t_{onset}) were varied to minimize the root mean square deviation between the modelled and measured drug substance concentrations.

Statistical comparison of intrinsic dissolution rates as well as maximum concentrations (estimated solubilities) was conducted by use of a two-way ANOVA test followed by multiple comparison using the Tukey method ($\alpha = 0.05$). The statistical tests were performed using GraphPad Prism, 9.2.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Both model compounds, dipyridamole and piroxicam, are categorized as BCS class II drugs and are relatively well-characterized in the literature with respect to their in vitro and in vivo behavior (3, 12, 19–26).

Absorbance Spectra, Molar Absorption Coefficients and pK_a

Figure 1 shows a schematic presentation of the inForm potentiometric and UV-metric measurement vessel, which together with the autosampler allow unattended absorbance measurements and subsequent determination of molar absorption coefficients, pK_a , and dissolution rates of up to 20 samples. The application of UV/Vis fiber optic probes and multivariate chemometric approaches to pK_a determination and dissolution testing is well-established (27–33). Thus, performance verification was limited to the assessment of the pK_a values for dipyridamole and piroxicam.

For dipyridamole, a mean $\pm$ SD ($n = 3$) pK_a of 6.04 ± 0.14 (fully aqueous medium) and 6.24 ± 0.02 (mixed solvent using Yasuda-Shedlovsky [Y-S] extrapolation) was determined at 37 °C and 25 °C, respectively ($I = 0.17$ M). Dipyridamole is a weak base having two basic functional groups (pK_a 6.2 and ~ 0.8 at 25 °C and $I = 0.15$ M) (18). Only the least acidic pK_a was within the pH range of the conducted UV titration. The pK_a determined was consistent with most published values, e.g., 6.23 (25 °C; $I = 0.15$ M) and 6.22 (25 °C; $I = 0.15$ M), with exception of 4.93 (37 °C; $I = 0.15$ M) (34–36). For piroxicam, the mean $\pm$ SD pK_a values were determined as 1.94 ± 0.03 and 5.28 ± 0.02 ($n = 2$, Y-S extrapolation) and 1.84 ± 0.03 and 5.29 ± 0.02 ($n = 4$, Y-S extrapolation) at 37 °C and 25 °C, respectively ($I = 0.17$ M). These values correlated well

with the published pK_a values, e.g., 1.88 and 5.23 (25 °C; $I = 0.15$ M), 1.88 and 5.29 (25 °C; $I = 0.15$ M), 1.89 and 5.34 (25 °C; $I = 0.15$ M), and 5.34 ± 0.02 (37 °C and $I = 0.15$ M) (17, 18, 34, 37). Overall, the results indicated that the UV-metric measuring technique was reliable and robust.

Dipyridamole Dissolution

Figures 2 and 3 display the dissolution profiles for dipyridamole obtained applying the experimental conditions listed in Table 1, with the measured drug concentration as a function of time. When dipyridamole dissolved at pH 6.5 (Fig. 2), the intrinsic dissolution rate was low ($3.7 \times 10^{-3} \pm 0.4 \times 10^{-3}$ mg/min cm^{-2}) and the maximal concentration reached within 30 min was low (0.22 ± 0.02 $\mu\text{g/mL}$). This low concentration corresponds well to the fact that dipyridamole is a weak base with a pK_a of 6.0, and therefore is predominantly neutral at pH 6.5, displaying poor aqueous solubility. In addition, the low concentration measured in the aqueous buffer at pH 6.5 is consistent with reported solubilities of 8.1 ± 0.4 $\mu\text{g/mL}$ (50-mM phosphate buffer, pH 6.5) and 6.9 ± 0.2 $\mu\text{g/mL}$ (phosphate buffered saline [PBS], pH 6.8) (38, 39). At pH 2.0 (Fig. 3), the intrinsic dissolution rate of dipyridamole was much higher (overall mean 6 ± 2 mg/min cm^{-2}), leading to an average maximum concentration of dissolved drug of 163 ± 24 $\mu\text{g/mL}$, corresponding to $75\% \pm 8\%$ w/w of the dose dissolved after 30 min of dissolution. Based on the dissolution profiles obtained at pH 2.0, the dipyridamole solubility was estimated to 245 ± 42 $\mu\text{g/mL}$ in this medium at 37 °C using Eq. (3). There was no statistical difference between the intrinsic dissolution rates, the estimated solubility, or the maximum concentration measured in aqueous buffer at pH 2.0 when determined after applying the different experimental settings (Table 1). As the first sector settings were analogous for the studies II-VII (dissolution at pH 2), the results were expected to be similar, and the lack of a statistical difference simply indicates that the model design and data analysis were robust. The estimated solubility of dipyridamole in aqueous buffer pH 2.0 also correlates well with published values (e.g., 234 ± 27 $\mu\text{g/mL}$ in diluted simulated gastric fluid at pH 2.0 and 37 °C) (38).

The results from the dissolution experiments conducted using different experimental conditions reveal several interesting points. When the pH ramped from 2.0 to 6.5 within 60 s (Fig. 3a), dipyridamole precipitated instantaneously. However, when the pH was shifted gradually using a linear gradient over 30 min (Fig. 3b), precipitation was not observed before the pH shift was complete, displaying a 30-min lag phase. The observed dipyridamole precipitation rate in aqueous buffer pH

6.5 was found to be independent of how the pH was shifted (setting II and III), with an average value of 1.3 ± 0.4 mg/min for the empirical precipitation rate constant (Fig. 3a and b). In terms of in vivo relevance, the gradual pH shift mimics the fasted-state gastric emptying profile more closely ($t_{1/2} = 13 \pm 1$ min for 240 mL of water) (40). Because the gradual pH shift merely introduced a lag phase for the drug precipitation, which was initiated at pH 6.5 producing a similar precipitation rate as compared to experiments using a fast pH shift, it may be argued that the slow pH shift, at least in this case, complicated the dissolution model without adding supplementary information. The use of a more physiologically relevant dissolution medium (FaSSIF v2) compared to aqueous buffer at pH 6.5 (experiments IV vs III) led to a significant decrease in the drug precipitation rate ($p < 0.05$) (Fig. 3c vs 3b). In FaSSIF v2, the drug precipitation rate at pH 6.5 was calculated to be $7 \times 10^{-2} \pm 2 \times 10^{-2}$ mg/min. As a consequence of the slower precipitation rate, the duration of apparent supersaturation in FaSSIF v2 was increased, with $64\% \pm 2\%$ of the dose still solubilized after 30 min at pH 6.5 (Fig. 3c).

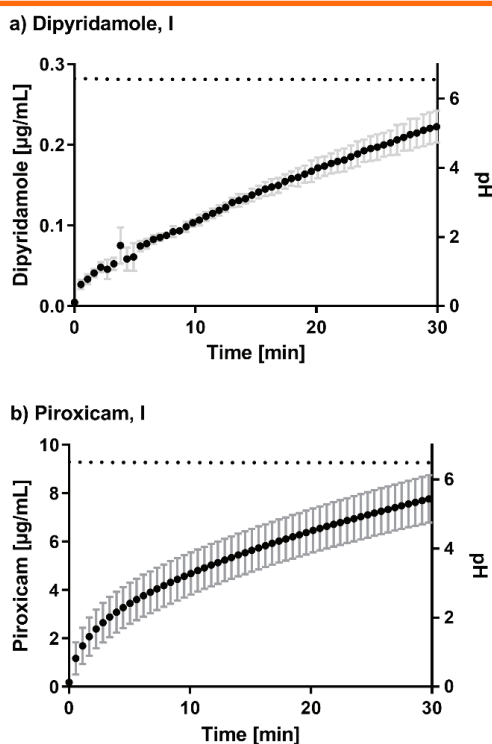


Figure 2. Dipyridamole (a) and piroxicam (b) dissolution profiles obtained at 37°C with monophasic dissolution in aqueous buffer at pH 6.5. The dotted line indicates the pH, black circles represent the amount of dipyridamole dissolved in the aqueous solutions (mean $\pm$ SD, $n = 3$).

Psachoulis et al. studied the in vivo precipitation of dipyridamole in human adults after administration of the drug in solution directly into the antrum of the stomach (24). Drug precipitation was measured after GI

transfer by aspirating fluid samples from the ligament of Treitz. Two doses were evaluated (30 and 90 mg), yet the study showed minimal drug precipitation in the small intestine; i.e., the mean precipitated fraction was below 7% (24). These results indicate that simulating the GI transfer using simple aqueous buffers (experiments II and III) significantly overestimated the extent of drug precipitation in the small intestine; i.e., only $14\% \pm 2\%$ of the dose was solubilized after 30 min of dissolution at pH 6.5 (Fig. 3a and b). Furthermore, the results suggest that the use of a biorelevant dissolution media containing bile salts and phospholipids may improve the predictive performance of the in vitro setup. This finding correlates well with studies supporting the use of biorelevant dissolution media for predicting in vivo behavior of orally administered drugs (5, 19, 41–43).

In 2004, Kostewicz et al. investigated the influence of hydrodynamics, transfer rates, and composition of the SIF (fed state vs fasted state) on the precipitation behavior of dipyridamole using an in vitro transfer model (3). The transfer model mimicked the in vivo passage through the human GI tract by transferring a drug solution in simulated gastric medium into a simulated intestinal medium. Applying varying transfer rates in the range of 0.5–9 mL/min, Kostewicz et al. observed very small differences in the maximum degree of supersaturation achieved in the FaSSIF, indicating no clear dependence on the transfer rate (3). In the fed-state medium precipitation was not observed (3). The observations of the current in vitro dissolution study are in line with this, as the medium composition significantly affected the rate and extent of drug precipitation, whereas the transfer conditions (pH shift and transfer rate) had limited impact on the drug precipitation.

Biphasic dissolution studies were conducted to investigate the impact of incorporating an absorptive step into the dissolution model. In the present study, decanol was used as the lipophilic phase allowing dissolved drug to partition herein, simulating the in vivo drug removal by absorption into and across the intestinal membrane. The results displayed in Figure 3d–f show that dipyridamole distributes relatively fast into the decanol phase, thereby limiting drug precipitation. The fast drug partitioning into the organic phase, which limited drug precipitation in the aqueous phase, may appear to correlate with the in vivo data presented by Psachoulis et al. (24).

Overall, comparison of the dipyridamole dissolution profiles to in vivo data obtained following oral administration of dipyridamole showed that experiments I–III have limited relevance when estimating the in vivo

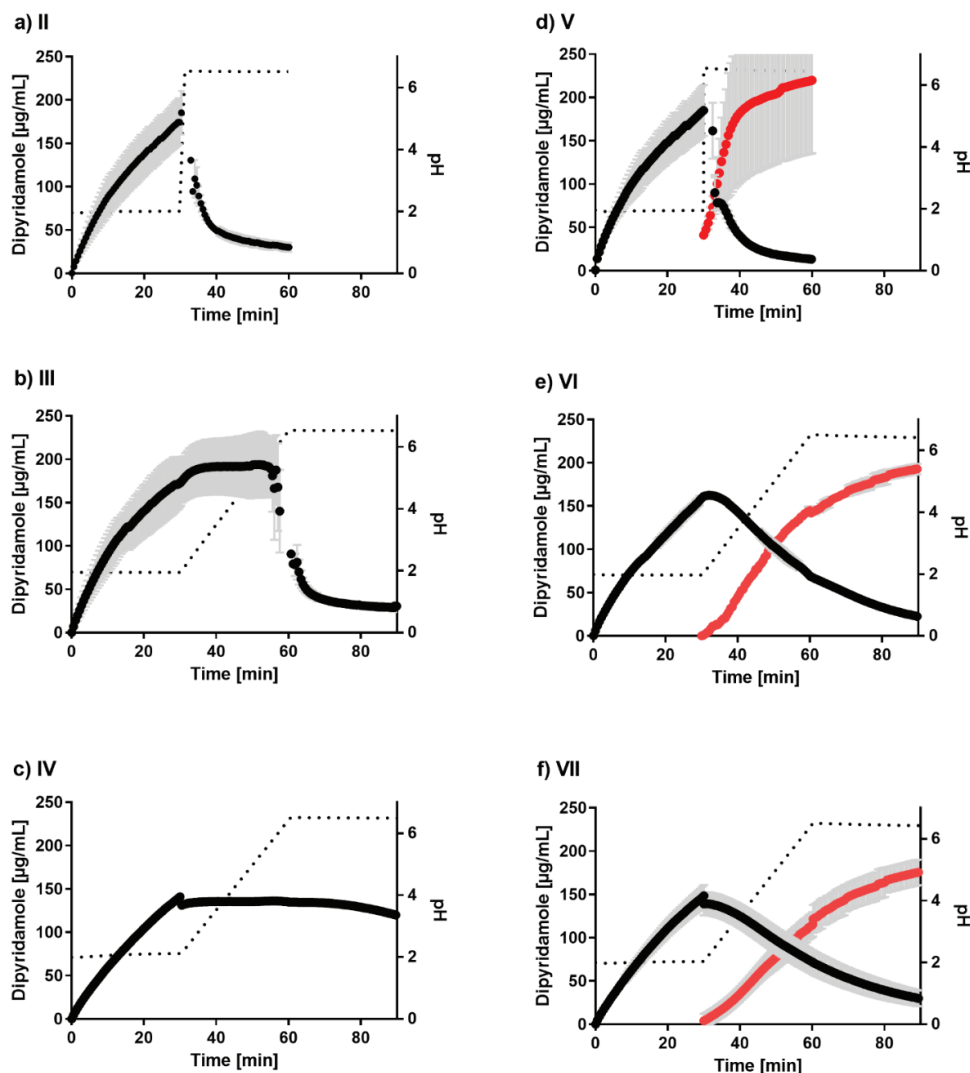


Figure 3. Dipyridamole dissolution profiles obtained at 37 °C at the conditions described in Table 1 (experiments II–VII in a–f, respectively). Monophasic dissolution in aqueous buffers at pH 2.0 and pH 6.5 with a fast pH shift (a: II), slow pH shift (b: III), and slow pH shift from buffer pH 2.0 to FaSSIFv2 pH 6.5 (c: IV). Biphasic dissolution with similar aqueous media and pH shifts (d–f: V–VII). Dotted lines indicate the pH, black circles represent the amount of dipyridamole dissolved in the aqueous solutions, and red circles represent dipyridamole dissolved in decanol (mean $\pm$ SD, $n = 3–4$).

performance of dipyridamole, as these single-phase settings all led to very low drug concentrations in the SIF following 30 min of dissolution ($< 50 \mu\text{g/mL}$, Figs. 2, 3a, and 3b). Based on the dissolution profiles depicted in Figure 3, it appears that experiments IV–VII produced in vivo-relevant results, as very small amounts of drug precipitation were observed using those settings (Fig. 3c–f), i.e., using biorelevant media to simulate the intestinal fluid or using a biphasic dissolution setup. A recent study by Klumpp and Dressman demonstrated how physiologically based pharmacokinetic (PBPK) model output is dependent on dissolution data using glibenclamide and dipyridamole as case examples (25).

The authors found that dissolution input from one-step dissolution testing in simulated gastric medium led to a close simulation of the pharmacokinetic profile of dipyridamole (25). Using a two-step dissolution model, with immediate transfer from gastric to intestinal medium (FaSSIF v2), the authors observed immediate precipitation to a drug concentration of $36 \pm 1 \mu\text{g/mL}$ (25). The resultant PBPK model showed that the simulated pharmacokinetic profiles are very sensitive to calculated precipitation rate constants. Based on actual human plasma data, little precipitation occurs in vivo, especially as dipyridamole is a highly permeable drug. Therefore, biphasic dissolution (or a combined dissolution permeation model as described

by Mizoguchi et al.) is recommended when estimating the in vivo performance of weak bases resembling dipyridamole (44).

Piroxicam Dissolution

Figure 2b and 4 show the dissolution profiles for piroxicam obtained from the experimental conditions in Table 1 (experiments I–VII). The effects of changing the medium during dissolution experiments were less pronounced for piroxicam as compared to dipyridamole and opposite in terms of pH-dependence of the dissolution rate (Fig. 4 vs Fig. 3). Upon shifting the pH, instantaneously or gradually applying the linear gradient over 30 min (Fig. 4a and b), an increase in piroxicam dissolution rate was observed. The intrinsic dissolution rates of piroxicam were 0.11

$\pm 0.02 \text{ mg/min cm}^{-2}$ and $0.29 \pm 0.03 \text{ mg/min cm}^{-2}$ at pH 2.0 and 6.5, respectively. The observed lower dissolution rate of piroxicam at pH 2 vs pH 6.5, is consistent with the increase in degree of ionization, which in turn increases the solubility and dissolution rate of piroxicam in the given dissolution medium. When comparing Figure 2b and Figure 4, it is apparent that including a gastric step simply delayed the dissolution process. Following 30 min of dissolution at pH 6.5, irrespective of whether gastric dissolution was included or not, the same amount of piroxicam was dissolved, i.e., 311 ± 39 , 315 ± 15 , and $321 \pm 32 \text{ } \mu\text{g/mL}$ for experiments I, II, and III, respectively.

The use of FaSSiF v2 as compared to neat aqueous buffer at pH 6.5 had a negligible effect on the dissolution rate of

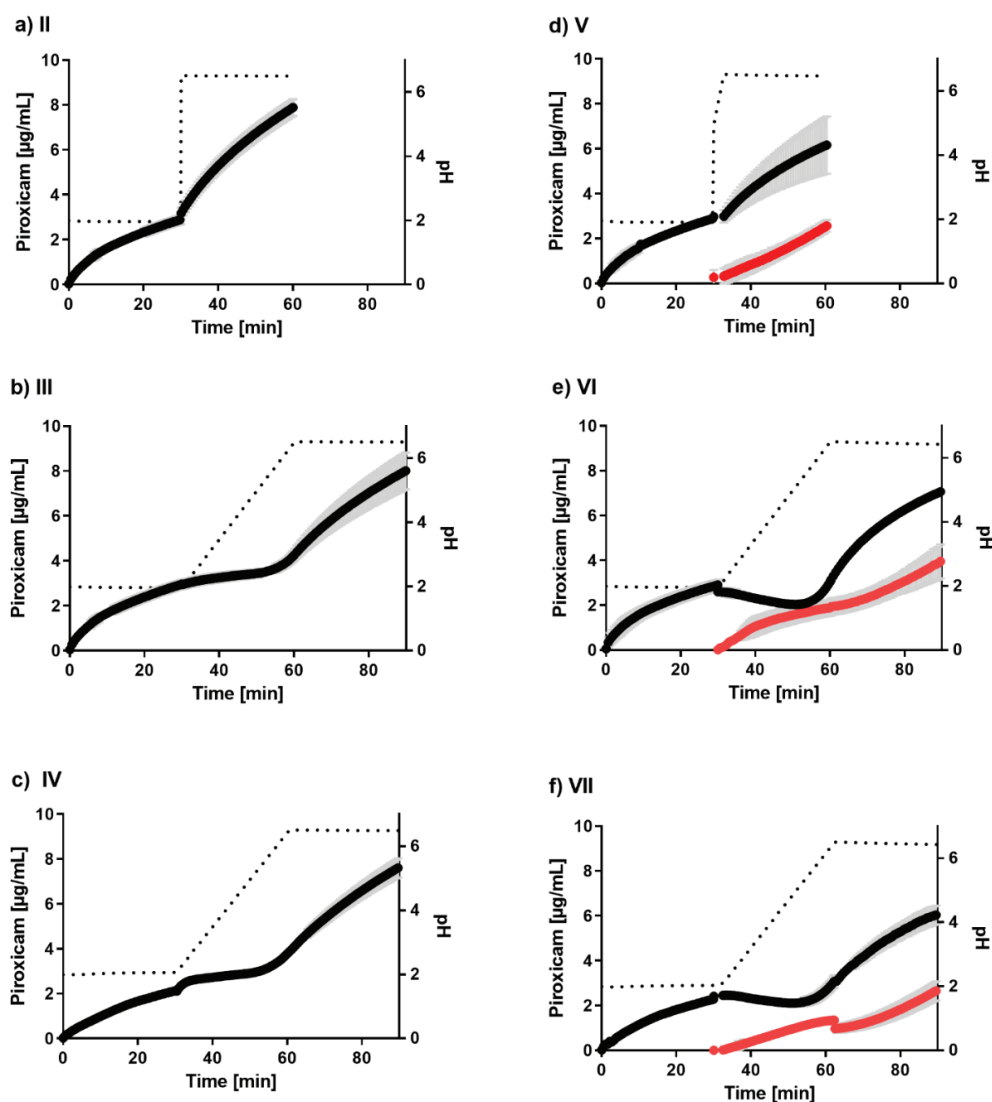


Figure 4. Piroxicam dissolution profiles obtained at 37°C with the conditions described in Table 1 (experiments II–VII in a–f, respectively). Monophasic dissolution in aqueous buffers at pH 2.0 and pH 6.5 with a fast pH shift (a: II), slow pH shift (b: III), and slow pH shift from buffer pH 2.0 to FaSSiFv2 pH 6.5 (c: IV). Biphasic dissolution with similar aqueous media (d–f: V–VII). Dotted lines indicate pH, black circles represent the amount of piroxicam dissolved in the aqueous solutions, and red circles represent piroxicam dissolved in decanol (mean $\pm$ SD, $n = 3\text{--}4$).

piroxicam (experiment III and IV, Fig. 4b and c). At pH 6.5, 94% of the piroxicam is negatively charged (calculated based on the Hendersson-Hasselbalch equation) and the remainder is present as a zwitter-ion/neutral species. The piroxicam anion has been shown to have very little affinity for the micelles formed by surfactants, so the lack of difference observed when comparing the dissolution profile of piroxicam in phosphate buffer pH 6.5 and FaSSIF v2 pH 6.5 (Fig. 4b and c) is not surprising (45). The present results also correlate well with results presented by Khadra et al. in a study, where the effects of composition of SIF on equilibrium solubility for BCS class II compounds were investigated (46). The authors found that pH was the most important factor leading to increased solubility; none of the other investigated SIF parameters (i.e., content of sodium oleate, bile salts, and buffer concentrations) had a significant effect on the solubility of piroxicam. Therefore, pH is the single most important factor affecting the solubility and dissolution rate of piroxicam in simulated gastric and intestinal fluids.

Biphasic dissolution studies, with decanol as the lipophilic phase, were also conducted with piroxicam. As apparent from Figure 4d and f, piroxicam partitioned into the decanol phase only to a limited extent. In line with expectations, the predominantly net negatively charged molecule did not interact appreciably with a lipophilic partitioning phase nor with the bile salts and phospholipids of the biorelevant medium (FaSSIFv2).

Collectively, the utilization of GI biorelevant media and biphasic dissolution conditions is of larger significance for the dissolution behavior of the basic molecule dipyrindamole as compared to a weakly acidic compound such as piroxicam (Figs. 3 and 4). For piroxicam, the simplest dissolution setup (experiment I) produced similar results as the most complicated setup (experiment VII), i.e., 311 ± 39 and 324 ± 10 $\mu\text{g/mL}$, respectively. Therefore, a simple dissolution setup may be recommended for evaluating the oral performance of piroxicam (20).

CONCLUSIONS

Using the automated instrument platform, inForm, the dissolution behavior of dipyrindamole and piroxicam was investigated while varying testing conditions in terms of pH, dissolution medium, and the presence of a partitioning phase. The weak base dipyrindamole dissolved rapidly at pH 2.0; shifting the pH to 6.5 during dissolution testing brought dipyrindamole into a supersaturated state, from which it precipitated. Upon addition of FaSSIF v2, dipyrindamole precipitation was slower, and a higher concentration was maintained in solution. Utilizing the biphasic dissolution assay, rapid

distribution of dipyrindamole into the decanol phase minimized precipitation. For piroxicam, the dissolution rate increased with increasing pH. The inclusion of FaSSIF v2 and the introduction of a partition phase had a limited effect on the dissolution behavior of piroxicam consistent with ionization properties of the drug. The automated system allows for tailoring of the dissolution assays in an efficient manner, enabled detailed drug characterization, and possibly increased biorelevance and in vivo predictability. The incorporation of an absorptive sink into dissolution experiments may be important for unraveling the supersaturation and dissolution behavior of weakly basic drug compounds.

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CONFLICT OF INTEREST

Karl Box is an employee of Pion Inc. The other authors disclosed no conflicts of interest related to this article.

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