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Vivian Gray
vagray@rcn.com

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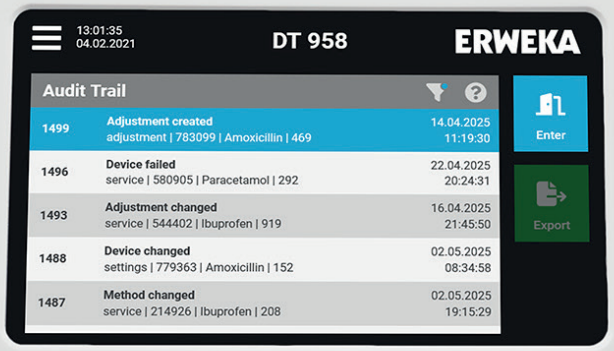

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Can Biorelevant Dissolution Testing Help Elucidate Salt Formulation Effects on Plasma Levels and Onset of Action? A Study of Ibuprofen and Its Salts

Niklas Rudolph[1#], Laurin Zöller[1#], Sandra Klein[2], Christoph Saal[3], and Jennifer Dressman[1*]
[1]*Fraunhofer Institute for Translational Medicine and Pharmacology, Frankfurt am Main, Germany.*
[2]*Department of Pharmacy, Institute of Biopharmaceutics and Pharmaceutical Technology, University of Greifswald, Greifswald, Germany.*
[3]*Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany.*

email: dressman@em.uni-frankfurt.de

ABSTRACT

Introduction: Salt formulations are widely used in the pharmaceutical industry to increase drug solubility and accelerate dissolution. In this work, the enhancing effect of salt formulations on the absorption rate of ibuprofen was investigated in both the fasted and fed states, using biorelevant dissolution testing. **Methods:** The dissolution behavior of two different ibuprofen salts was studied using two commercially available formulations: Dolormin, containing the lysinate salt of ibuprofen, and Spedifen, containing the arginate salt. Their dissolution was compared to that of the orodispersible tablet formulation containing the free acid (Nurofen) at different dose levels in both single and two-stage dissolution tests. **Results:** When administered in the fasted state, the rapid onset of action of the pharmaceutical salt formulations is directly related to their dissolution behavior, highlighting the importance of choosing suitable dissolution media and methodology to link in vitro with in vivo data. In the fed state, gastric emptying becomes rate-limiting to absorption, such that even fast-dissolving products are not able to provide a short onset of action. **Conclusion:** Biorelevant dissolution delivers key data for determining pharmaceutical salt formulation effects on dissolution in the fed and fasted states.

KEYWORDS: pharmaceutical salts, ibuprofen, biorelevant media, dissolution, fasted and fed state dissolution, two-stage testing

INTRODUCTION

Ibuprofen (IBU) is the non-steroidal anti-inflammatory drug (NSAID) most commonly prescribed to manage inflammatory diseases and pain modulation (*1*). The mechanism of action of NSAIDs involves the inhibition of cyclooxygenase (COX) enzymes, thereby reducing prostaglandin levels, which are pro-inflammatory signaling molecules (*2*). Consequently, NSAIDs represent a pivotal component in the treatment of inflammatory diseases (*3*). However, the influence of NSAIDs extends beyond inflammation, as they also function as analgesic and antipyretic agents, capable of reducing fever and providing relief from mild to moderate acute pain of diverse origins (*2*, *4*, *5*). Acute pain, which is defined as a physiological response to noxious stimuli that is intended to avert actual or potential tissue injuries, typically has a sudden onset (*6*). Consequently, achieving therapeutic drug levels as quickly as possible is imperative to pain relief.

Since the introduction of IBU in 1969, pharmaceutical companies have diligently sought to modify its release and absorption kinetics (*1*). Alongside the introduction of orodispersible formulations (e.g., Nurofen orodispersible tablets) as fast-dissolving alternatives, there has been a focus on formulations containing pharmaceutical salts to accelerate dissolution and consequently reduce the time to onset of action (*7*). This has resulted in the emergence of commercially available IBU products containing different pharmaceutical salts. A comprehensive understanding

*Corresponding author, #Equal first authors

of the extent to which these pharmaceutical salts can influence IBU release from the formulation, under both fasted and fed state conditions, is key to determining when a salt formulation will be advantageous over formulations of the free acid for the treatment of acute pain.

In the present study, the dissolution performance of three different formulations of IBU was investigated: Nurofen orodispersible tablets containing IBU free acid, Dolormin film-coated tablets containing the lysinate salt, and Spedifen granules containing the arginine salt. Single stage-dissolution was investigated in biorelevant media simulating the fasted state simulated gastric fluid (FaSSGF) and fed (FEDGAS) states to investigate the influence of formulation on the in vivo dissolution of IBU when given either on an empty stomach or after a meal.

The first objective of the studies was to determine whether the gastric pH at the time of ingestion has an effect of IBU release from the various formulations. When the dose is taken on an empty stomach, the volume of water swallowed with the dose dilutes any residual gastric acid, which in turn impacts the pH in the stomach. To standardize the volume ingested, the FDA Guidance on bioequivalence studies requires participating subjects to take tablets and capsules with a glass of water, a volume of approximately 8 oz (~240 mL) (*8*). Although patient information leaflets for most solid oral dosage forms also advise ingesting the dose with a glass of water, in practice, many patients take the dose with only a swallow or two of fluid. For orally disintegrating tablets, patients are advised that they can ingest the dose without water.

The biorelevant medium FaSSGF was designed to reflect the average pH in the stomach (pH 1.6) after ingestion of a glass of water in the fasted state (*9*). However, it has since been demonstrated that this results in a temporary increase in the pH of gastric fluids to a value of pH 3–4 (*10*). To more accurately replicate the conditions of release immediately after ingestion of a glass of water, Tsume et al. introduced a new medium, $FaSSGF_{DIL}$ ("diluted FaSSGF"), which has a higher pH of 2.34 (*11*). In these studies, dissolution of the products was tested in both media to reflect both the average (FaSSGF) and peak ($FaSSGF_{DIL}$) pH values in the fasted stomach. In addition to single-stage testing, two-stage testing was carried out using either FaSSGF and $FaSSGF_{DIL}$ as the donor phase, followed by conversion to fasted state simulating intestinal fluid-version 1 (FaSSIF-V1) as the acceptor phase (*12*).

The second objective was to ascertain the impact of the IBU dose on its release under fasted state conditions. To this end, dissolution tests were conducted for Nurofen, Dolormin, and Spedifen at various doses corresponding to 200, 400, and 600 mg of IBU as the free acid.

The third objective was to study release from the three formulations under fed state conditions to determine whether release from the dosage form or gastric emptying would be the rate-limiting step to absorption. For this purpose, the recently introduced FEDGAS media were used to assess the IBU products under fed gastric conditions (*13*). Three FEDGAS versions have been developed to mimic the composition of the gastric contents after ingestion of a high-fat meal in the early, middle, and late phases of digestion. Their compositions are specially tailored to represent the composition of gastric contents in the fed state over time, making FEDGAS media a valuable tool for forecasting formulation performance in product development (e.g., bioequivalence to originator product) (*13*).

As a last step, the results of the various dissolution studies were compared with the results of clinical studies, which have reported different pharmacokinetic behavior among the three products studied.

METHODS

Materials

Nurofen (200 mg) orodispersible tablets (lot KY216) were purchased commercially from Reckitt Benckiser Deutschland GmbH (Heidelberg, Germany). Dolormin (200 mg) film-coated tablets (lot KJL4L01) were obtained from Johnson & Johnson GmbH (Neuss, Germany). Spedifen (600 mg) granules (lot 380063) were obtained from Zambon Switzerland Ltd (Cadempino, Switzerland). The excipients in these three formulations are listed in Table 1.

IBU Pharmaceutical Secondary Standard (lot LRAC0253) was purchased from Sigma Aldrich (Taufkirchen, Germany).

Hydrochloric acid (1N HCl) (lot 22G134004), 1N sodium hydroxide (lot 21D204022), and acetonitrile ([CAN] lot 21l142132) as well as 0.45-µm Chromafil filters and 0.45-µm PTFE ReZist filters were purchased from VWR International GmbH (Darmstadt, Germany). Polyethylene prefilters (10 µm) were obtained from Quality Lab Accessories (Telford, PA, USA), and glass microfiber filters (diameter 0.47 mm; pore size 0.7 µm) (lot 9850840) were purchased from GE Healthcare (Chicago, IL, USA). Sodium chloride (lot: 52318748) was obtained from Carl Roth GmbH + Co. KG (Karlsruhe, Germany).

Table 1. Excipients in Nurofen Orodispersible Tablets, Dolormin Film-Coated Tablets, and Spedifen Granules

	Nurofen (*7*)	Dolormin (*43*)	Spedifen (*25*)
IBU Dose (Form)	200 mg (free acid)	200 mg (lysinate salt)	600 mg (arginate salt)
Excipients	Ethyl cellulose Silicon dioxide Hypromellose Mannitol Aspartame Croscarmellose sodium Magnesium stearate Lemon aroma	Microcrystalline cellulose Povidone (K30) Magnesium stearate Titanium dioxide Hyprolose Hypromellose	Arginine Sodium bicarbonate Sucrose Apricot aroma Aspartame Saccharin sodium

IBU: Ibuprofen.

$FEDGAS_{early}$ (lot FEDBUF6-0422), $FEDGAS_{middle}$ (lot FEDBUF45-0422-A), and $FEDGAS_{late}$ stage buffer concentrate (lot FEDBUF3-0422-A) as well as FEDGAS gel (lot FEDGAS-1222-A), FaSSIF/FeSSIF/FaSSGF powder (lot FFF-0222), and FeSSIF-V2 powder (lot V2FES-0322-A) were all purchased from biorelevant.com Ltd. (London, UK).

MilliQ (deionized) water was freshly obtained from a Milli Q Reference A+ system (Serial no. F7EA11088B) purchased from Merck KGaA (Darmstadt, Germany). Anhydrous sodium dihydrogen phosphate (lot A0430090) was obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Maleic acid (lot 8608238003B1W) and sodium hydroxide pellets (lot B183516204B1W) manufactured by Merck KGaA were purchased from VWR International GmbH.

Preparation of Biorelevant Dissolution Media

Biorelevant dissolution media were prepared according to the instructions on the biorelevant.com website (*13*, *14*). $FaSSGF_{DIL}$ and FaSSIF-V1 double concentrate media (for two-stage experiments) were prepared using FaSSIF/FeSSIF/FaSSGF powder (3F). The compositions of the media are shown in Tables 2 and 3.

The preparation of each individual FEDGAS medium ($FEDGAS_{early/middle/late}$) was performed using the corresponding buffer concentrate, which was then mixed with the FEDGAS gel.

This study utilized three distinct dissolution media, each of which represents a specific gastric residence time after a meal, because the composition and volume of the gastric contents undergoes changes with time postprandially (*15*). Accordingly, the volume and pH of the test medium was successively reduced from 900 mL in $FaSSGF_{early}$ (pH 6.0) to 500 mL in $FaSSGF_{middle}$ (pH 4.5) and $FaSSGF_{late}$ (pH 3.0), thereby mimicking the fed state in the stomach. Based on the time frames for FeSSGF suggested by Markopoulos et al., $FEDGAS_{early}$ corresponds to the first 75 minutes after meal ingestion, $FEDGAS_{middle}$ corresponds to 75–165 minutes post-ingestion, and $FEDGAS_{late}$ represents the period beyond 165 minutes (*16*). The dissolution of IBU from each product was tested in single stage experiments using each of the FEDGAS media.

Table 2. Compositions of Biorelevant Media Representing the Fasted Gastric State

	FaSSGF	$FaSSGF_{DIL}$	FaSSIF-V1 (double concentrated for two-stage experiments)
NaCl (mg)	499.75	99.95	2093
1 N HCl (mg)	7275	1455	-
NaOH pellets (mg)	-	-	210.0
NaH_2PO_4 (anhydrous) (mg)	-	-	1719
FaSSIF/FeSSIF/ FaSSGF powder (mg)	15	3	1090
Medium volume used in dissolution experiments (mL)	250	250	250
pH	1.60	2.34	6.55 / 7.50*

**Depending on the pH of the gastric medium, double concentrated FaSSIF-V1 was adjusted to either 6.55 ($FaSSGF_{DIL}$) or 7.50 (FaSSGF), with the aim of generating a final pH of 6.50 in the acceptor medium (FaSSIF-V1) in two-stage tests.*
FaSSGF: fasted state simulated gastric fluid; $FaSSGF_{DIL}$: diluted FaSSGF; FaSSIF-V1: FaSSIF version 1; FaSSIF: fasted state simulated intestinal fluid; FeSSIF: fed state simulated intestinal fluid.

Table 3. Compositions of Biorelevant Media Representing the Fed Gastric State (FEDGAS)

	$FEDGAS_{early}$	$FEDGAS_{middle}$	$FEDGAS_{late}$
FEDGAS buffer concentrate (g)	36.40	19.67	19.06
MilliQ (g)	732.20	406.78	406.72
FEDGAS gel (g)	152.90	84.94	84.94
Medium volume used in dissolution experiments (mL)	900	500	500
pH	6.0	4.5	3.0

Single-Stage Dissolution Testing

Under fasted gastric conditions, all three products were studied at doses of 200, 400, and 600 mg using single-stage dissolution tests. Under fed gastric conditions, 200 mg of Nurofen and Dolormin and 600 mg of Spedifen were studied.

Single-stage dissolution tests were performed in a calibrated USP paddle apparatus (type 2, PTWS 120S dissolution tester, Pharma Test Apparatebau AG, Hainburg, Germany) using standard vessels and paddles. Single-stage tests using FaSSGF at pH 1.6 or $FaSSGF_{DIL}$ were performed in 250 mL of dissolution medium. Tests using FEDGAS were conducted with 900 mL of $FEDGAS_{early}$ or 500 mL of $FEDGAS_{middle}$ and $FEDGAS_{late}$. All media were freshly prepared and maintained at 37 ± 0.5 °C. The paddle speed was set to 75 rpm, and all experiments were conducted at least in triplicate.

Samples from FaSSGF, $FaSSGF_{DIL}$, and FaSSIF-V1 were withdrawn using a 5-mL syringe, prefiltered with a 10-µm polyethylene filter. The first 4 mL were returned to the vessel, and 1 mL was filtered through a 0.45-µm PTFE filter (ReZist filter unit; GE Healthcare, Whatman Inc., NJ, USA). The filtrate sample was promptly diluted appropriately with mobile phase. The pH of each sample was measured using a 766 Calimatic pH meter (Knick Elektronische Messgeräte GmbH & Co. KG Berlin, Germany) with an Inlab Micro pH electrode (Mettler Toledo Berlin, Germany). The loss of media due to sampling was accounted for in the calculations.

Samples from FEDGAS were withdrawn using a 5-mL syringe, returning the first 3 mL to the vessel. The remaining 2 mL were collected in an Eppendorf vial, and 1 mL was withdrawn and diluted with acetonitrile in a ratio of 3:2 (v:v). The sample was then promptly vortexed, followed by centrifugation (MicroCL 21R centrifuge, Thermo Fisher Scientific, Karlsruhe, Germany) at 14,000 rpm and 4 °C for 20 minutes. After centrifugation, the supernatant was diluted in a ratio of 3:10 (v:v) with mobile phase for Nurofen and Dolormin and 3:40 for Spedifen, resulting in final dilutions of 1:5 (v:v) and 1:20 (v:v), respectively. The loss of media due to sampling was compensated for by adding fresh media to the vessel.

Two-Stage Dissolution Testing

Two-stage dissolution testing was performed at 200 mg for Nurofen; 200, 400, and 600 mg for Dolormin, and 200 and 400 mg for Spedifen.

Two-stage dissolution tests are typically used to characterize the supersaturation and subsequent precipitation of weakly basic drugs resulting from the pH shift between gastric and intestinal conditions. Another application is evaluation of the behavior of salts of weakly acidic drugs, for which precipitation of the free acid gastric conditions may lead to slower dissolution in the small intestine.

In this study, two slightly different two-stage dissolution protocols were compared. The first method was based on the approach by Mann et al., as implemented in the OrBiTo project (*12*). The second method was developed more recently as part of a Product Quality Research Institute (PQRI) ring study and was designed to better simulate the pH conditions immediately after ingestion of the dosage form with a glass of water (240 mL) (*11*, *17*).

Mann et al. Protocol

For the Mann et al. protocol, in the first stage, 250 mL of FaSSGF (pH 1.6) was used to simulate average gastric conditions in the fasted state (*12*). Following a 30-minute period of dissolution testing in FaSSGF, the change in fluid composition due to gastric emptying was mimicked by adding 250 mL of prewarmed, double concentrated FaSSIF-V1 (pH 7.5). This resulted in a final pH of 6.5, which corresponds to the composition of FaSSIF-V1. Throughout the 3-hour test, the temperature was maintained at 37 ± 0.5 °C in the dissolution apparatus, and the paddle speed was set to 75 rpm. Sample preparation and analysis were conducted in the same manner as for the single-stage tests. Following filtration, the filtrates were promptly diluted with mobile phase in a ratio of 1:1, and the pH of each sample was measured. All studies were conducted at least in triplicate.

PQRI "More Restrictive" Protocol

For the PQRI ring study protocol, the gastric medium was $FaSSGF_{DIL}$, comprising 50 mL of FaSSGF (pH 1.60), to which 200 mL MilliQ water was added, yielding a final pH of 2.34 (*17*). The temperature was maintained at 37 ± 0.5 °C, and the paddle speed was set to 75 rpm. Following 30 minutes of dissolution testing in $FaSSGF_{DIL}$, a pH shift was induced by the introduction of 250 mL of prewarmed, double concentrated FaSSIF-V1 (pH 6.55) into the vessel. The utilization of a lower pH in the double concentrate was necessary, because $FaSSGF_{DIL}$ (pH 2.34) is less acidic than FaSSGF (pH 1.60). The preparation and analysis of the samples followed the same procedure as for the single-stage tests, and all studies were conducted at least in triplicate.

Analytical Methods

Quantitative analysis of samples using FaSSGF, $FaSSGF_{DIL}$, and FaSSIF-V1 (including samples from two-stage testing)

was carried out with an isocratic high-performance liquid chromatography (HPLC) method on a C18, 15-cm, 4.6-mm Purospher STAR, 5-µm LiChroCART column (Merck Millipore, Burlington, MA, USA) using a Hitachi Chromaster 5210 autosampler, 5110 pump, 5310 column oven, and 5410 ultraviolet (UV) detector (VWR Hitachi, Darmstadt, Germany). The mobile phase consisted of a mixture of ACN:MilliQ with 0.1% phosphoric acid (40:60) [v/v]. The column temperature was held at 30 °C, the injection volume was 20 µL, and the flow rate was set to 1.0 mL/min. The drug substance was quantified at 240 nm, with a retention time of 6.0 min. The calibration utilized the area under the curve (AUC) of IBU peaks over the concentration range 3–532 µg/mL and resulted in an $R^2 > 0.9999$, with a limit of detection (LOD) of 1.15 µg/mL and limit of quantification (LOQ) of 3.46 µg/mL.

Analysis of the FEDGAS samples was performed using a second HPLC-UV system, consisting of a VWR Hitachi Chromaster 5160 pump, 5310 oven, 5260 sampler, and a 5420 UV-Vis detector. An isocratic mobile phase consisting of a mixture of ACN:MilliQ with 0.1% trifluoroacetic acid (50:50) [v/v] was applied. A Gemini-NX 250 × 4.6 mm, 5-µm, 5 A column, equipped with a Gemini-NX 5-µm, 110-Å, 5 × 4.6-mm precolumn (Phenomenex, Aschaffenburg, Germany) was used. The temperature was set to 30 °C, the injection volume was 20 µL, and the flow rate was set to 1.0 mL/min. IBU achieved retention times of approximately 12.8 min using this method. The calibration utilized the AUC of IBU peaks over the concentration range 10–70 µg/mL and resulted in an $R^2 > 0.9999$, with an LOD of 1.18 µg/mL and a LOQ of 3.58 µg/mL.

Dissolution test results are expressed as mean ± SD percentage of cumulative release, along with the obtained pH of each sample.

RESULTS

Single-Stage Dissolution Testing Under Fasted Gastric Conditions

The results of single-stage dissolution testing under fasted state conditions are presented in Figures 1–3. The data underlying these figures are available from Zöller (*18*).

Nurofen Orodispersible Tablets

The dissolution profiles of Nurofen orodispersible tablets (free acid form of IBU) are presented in Figure 1. Using a single Nurofen orodispersible tablet (200 mg of IBU), slow and incomplete dissolution was observed in both FaSSGF and $FaSSGF_{DIL}$. After 50 minutes, a plateau concentration of approximately 53 µg/mL was attained in both media. The pH remained close to the initial pH throughout the tests. The dissolution profile was similar when higher doses were tested. For the 400 mg dose, concentrations reached a plateau of approximately 55 µg/mL after 40 minutes. For the 600 mg dose, a plateau of 53 µg/mL in FaSSGF and 56 µg/mL in $FaSSGF_{DIL}$ was reached after 20 minutes.

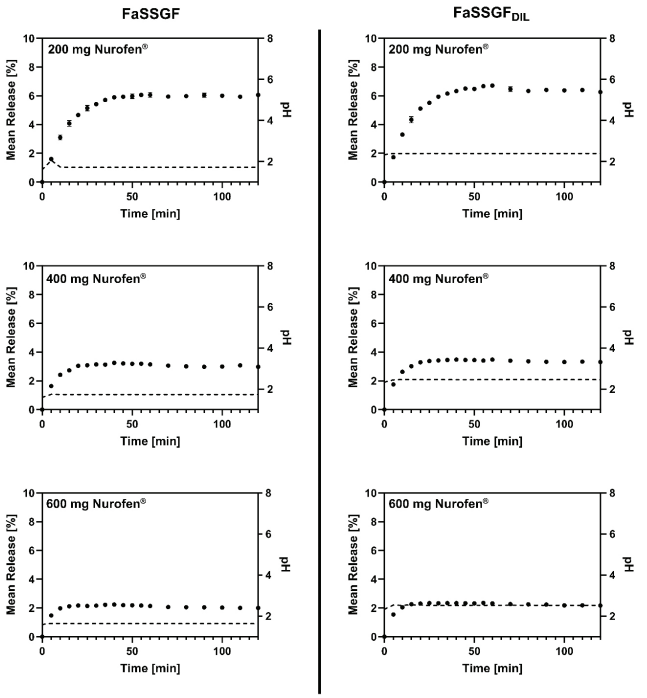

Figure 1. Mean ± SD percentage release of ibuprofen free acid from Nurofen orodispersible tablets (dots) and recorded pH values (dashed lines) in FaSSGF (left) and $FaSSGF_{DIL}$ (right). Most SD bars lie within the symbol. FaSSGF: fasted state simulated gastric fluid; $FaSSGF_{DIL}$: diluted FaSSGF.

Dolormin Film-Coated Tablets

The dissolution profiles of Dolormin film-coated tablets (lysinate salt form of IBU) are presented in Figure 2. The 200-mg single dose of Dolormin dissolved rapidly in both gastric media, initially reaching comparable concentrations of approximately 150 µg/mL. This resulted in supersaturation with respect to the free acid equilibrium solubility (25 µg/mL at pH 1.75 and 28 µg/mL at pH 3.51) (*19*). In FaSSGF, precipitation started after 50 minutes, whereas precipitation began after 25 minutes in $FaSSGF_{DIL}$. While only a marginal increase in pH was recorded for FaSSGF, an increase from 2.34 to 3.51 was measured in $FaSSGF_{DIL}$. However, in both media the final pH was still well below the pKa of IBU (4.5-4.6) (*20*).

More pronounced differences in release behavior of the formulation in the two gastric media were observed at higher doses. In FaSSGF, maximum concentrations of 159 µg/mL and 140 µg/mL were observed at 400 mg and 600 mg , respectively, which was similar to the behavior at 200 mg. Here, too, the maximum concentration was followed by precipitation to the free acid. In $FaSSGF_{DIL}$, by contrast,

the concentration of dissolved IBU reached 822 µg/mL at the 400-mg dose, followed by partial precipitation to a final concentration of 745 µg/mL after 60 minutes. No precipitation was observed for the 600-mg dose, which generated a concentration of 1674 µg/mL.

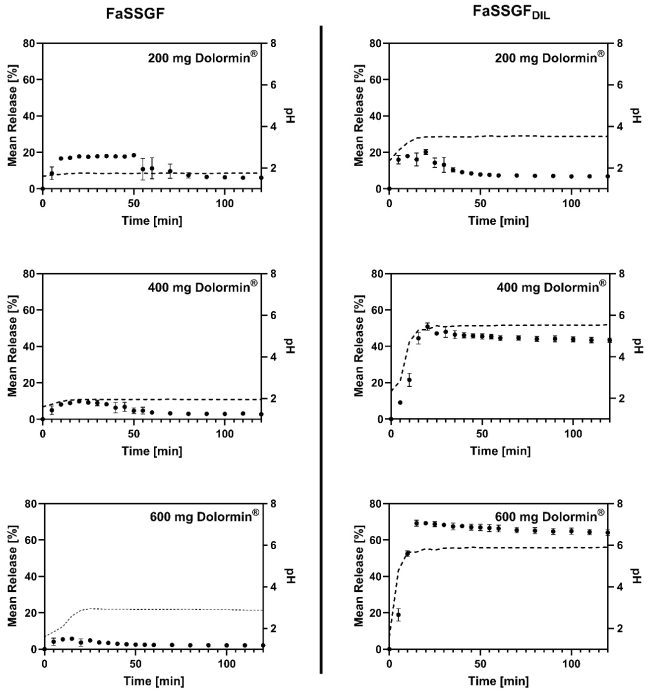

Figure 2. Mean ± SD percentage release of ibuprofen lysinate from Dolormin film-coated tablets (dots) and recorded pH values (dashed lines) in FaSSGF (left) and FaSSGF$_{DIL}$ (right). FaSSGF: fasted state simulated gastric fluid; FaSSGFDIL: diluted FaSSGF.

The differences in concentrations achieved in the two media align with the differences in the final pH of the medium. Final pH values averaged 3.51 for the 200-mg dose, 5.54 at the 400-mg dose and 5.90 at the 600-mg dose using FaSSGF$_{DIL}$. Only marginal increases in pH were observed in FaSSGF, in which final pH values were 1.75 at the 200-mg dose, 1.95 at the 400-mg dose, and 2.87 at the 600-mg dose.

Spedifen Granules

The dissolution profiles of IBU from the Spedifen granules (arginate salt form of IBU) are presented in Figure 3.

For Spedifen, significant variations in pH and the dissolution profile were observed between the two media, even at the lowest dose of 200 mg. Dissolution in FaSSGF reached only 14% release (111 µg/mL) of the dose, which was followed by precipitation to a plateau concentration of 10 µg/mL, and the pH increase was modest. In contrast, dissolution in FaSSGF$_{DIL}$ was almost complete within the first 10 minutes, with 92% release (787 µg/mL) and an increase in pH to nearly 8 by the end of the experiment. At the 400-mg dose, 36% (575 µg/mL) was released in FaSSGF after 15 minutes, with an attendant rise in pH to above 5. Some precipitation followed, to a plateau of around 31% release (533 µg/mL), during which the pH stabilized to a final value of 5.1. In contrast, the same dose in FaSSGF$_{DIL}$ 94% release (1611 µg/mL), comparable to the results with the 200-mg dose, and the final pH was 8.19.

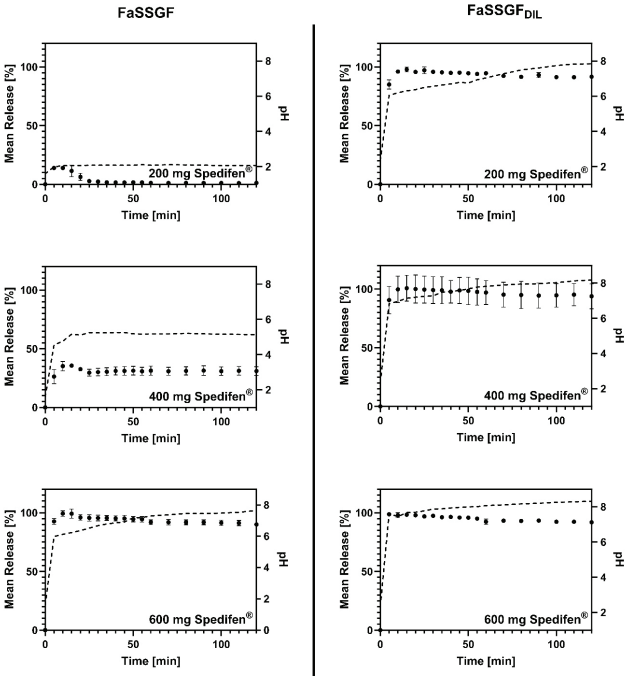

Figure 3. Mean ± SD percentage release of ibuprofen arginate from Spedifen granules (dots) and recorded pH values (dashed lines) in FaSSGF (left) and FaSSGF$_{DIL}$ (right). FaSSGF: fasted state simulated gastric fluid; FaSSGF$_{DIL}$: diluted FaSSGFin).

Interestingly, the dissolution profile for 600 mg of Spedifen was similar in both gastric media. In both cases, complete dissolution was achieved within the first 10 minutes of the test. The pH increased rapidly in the first 5 minutes of both tests to values of pH 5.98 in FaSSGF and pH 7.62 in FaSSGF$_{DIL}$, subsequently trending to a pH of around 8 by the end of the experiment.

Two-Stage Dissolution Testing

The results of two-stage dissolution testing under fasted state conditions using the Mann et al. and PQRI protocols are presented in Figures 4–6. The data underlying these figures are available from Zöller (*18*).

Nurofen Orodispersible Tablets

As shown in Figure 4, the dissolution profiles of the 200-mg Nurofen orodispersible tablets obtained with both protocols displayed a similar pattern. In the initial (gastric) phase, a maximum mean release of 6% (52 µg/mL) and 7% (58 µg/mL) was achieved after 30 minutes using the Mann et al. protocol and PQRI protocol, respectively.

These results were comparable to those in the single stage tests. The slightly higher pH of $FaSSGF_{DIL}$ had little effect on dissolution, as expected from the pKa of IBU (4.5–4.6) (*20*). Subsequent to the pH shift, induced by adding double concentrated FaSSIF-V1, complete dissolution was achieved in both cases. Due to the higher solubility of IBU in an almost pH-neutral environment (3172 µg/mL at pH 6.5), precipitation was neither expected nor observed in the intestinal phase of the two-stage tests (*19*).

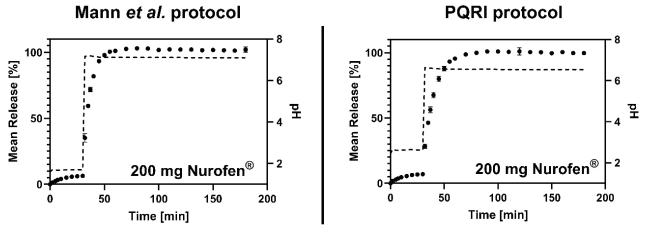

Figure 4. Mean ± SD percentage release of ibuprofen from Nurofen orodispersible tablets (dots) and recorded pH values (dashed lines) using FaSSGF (left) and $FaSSGF_{DIL}$ (right) in two-stage testing. Most SD bars lie within the symbols. PQRI: Product Quality Research Institute; FaSSGF: fasted state simulated gastric fluid; $FaSSGF_{DIL}$: diluted FaSSGF.

Dolormin Film-Coated Tablets

The changes observed in the single-stage tests for pH of the gastric medium were also observed in the gastric phase of the two-stage tests with Dolormin film-coated tablets (lysinate salt). As shown in Figure 5, at the 200-mg dose, the concentrations measured before initiating the intestinal stage were 141 µg/mL for FaSSGF and 164 µg/mL for $FaSSGF_{DIL}$. The pH in FaSSGF changed only marginally from 1.6 to 1.7, and the pH in $FaSSGF_{DIL}$ increased to an average value of pH 3.4.

Concentrations in the gastric phase using FaSSGF (Mann et al. protocol) at doses of 400- and 600 mg were also low, with only a minor increase in pH, in accordance with the results in single-stage testing. In contrast, using $FaSSGF_{DIL}$ (PQRI protocol) a pH of 5.13 and 5.41 were reached at the 400 and 600-mg doses, respectively, in the gastric phase. Higher pH values were associated with a much higher mean release of 52% and 62%, respectively.

After addition of double concentrated FaSSIF-V1, the pH increased to 6.4, resulting in instantaneous dissolution in both protocols at all three doses.

Spedifen Granules

Figure 6 shows that using the Mann et al protocol, during the gastric phase, the 200-mg dose of Spedifen had rapid initial dissolution during the gastric phase, resulting in a concentration of 370 µg/mL, followed by a decline in concentration, which was attended by a minor change in pH from 1.60 to 2.15. Conversely, following the PQRI protocol, dissolution of Spedifen in $FaSSGF_{DIL}$ reached 90% in the gastric phase, with a rapid increase to pH 6.7 at the start of the test. Carrying out the Mann et al. protocol with 400 mg of Spedifen resulted in a more pronounced pH increase to 5.33 in the gastric stage, with little precipitation. Using the PQRI protocol at the same dose, dissolution occurred rapidly without precipitation, with the pH climbing to an average value of 6.8 in the gastric phase. All results for the gastric phase were in concordance with the results of the single-stage tests of Spedifen.

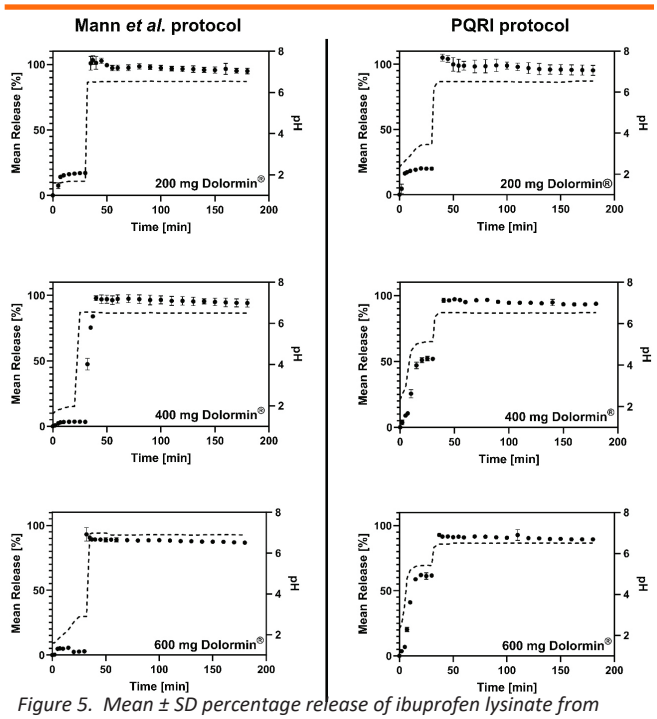

Figure 5. Mean ± SD percentage release of ibuprofen lysinate from Dolormin film-coated tablets (dots), and recorded pH values (dashed lines) using FaSSGF (left) and $FaSSGF_{DIL}$ (right) in two-stage testing. PQRI: Product Quality Research Institute; FaSSGF: fasted state simulated gastric fluid; $FaSSGF_{DIL}$: diluted FaSSGF.

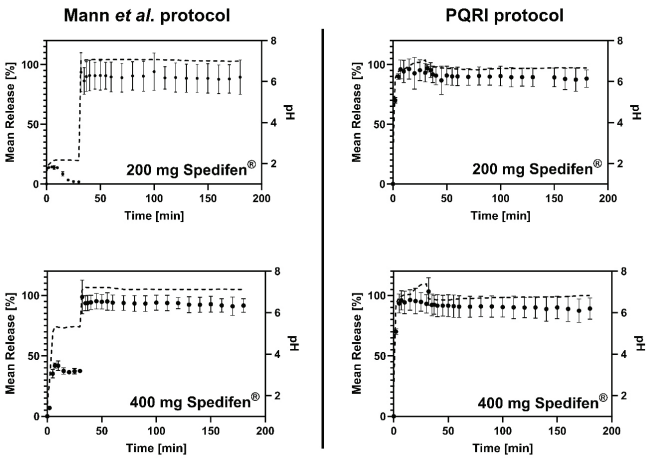

Figure 6. Mean ± SD percentage release of ibuprofen arginate from Spedifen granules (dots) and recorded pH values (dashed lines) using FaSSGF (left) and $FaSSGF_{DIL}$ (right) in two-stage testing. PQRI: Product Quality Research Institute; FaSSGF: fasted state simulated gastric fluid; $FaSSGF_{DIL}$: diluted FaSSGF.

A tendency for higher variability in the data for Spedifen compared to the Dolormin and Nurofen products was observed and may be attributable in part to the sample preparation. For Spedifen, only the appropriate mass of granules (corresponding to 200 or 400 mg of IBU) in each sachet was weighed out and used in the experiment.

Upon addition of FaSSIF-V1 double concentrate, dissolution was almost complete at both doses in both protocols, with final pH values ranging from 6.7–7.1.

Single-Stage Dissolution Testing Under Fed Gastric Conditions

The results of single-stage dissolution testing under fed gastric conditions are presented in Figure 7. The underlying data are available as supplemental material from the authors.

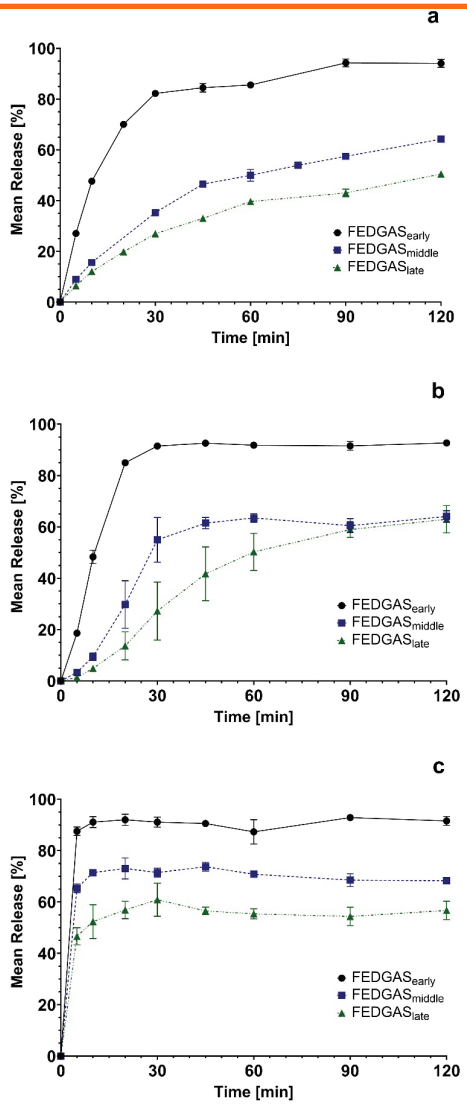

*Figure 7. Mean ± SD percentage release, under fed state simulated gastric conditions (FEDGAS), of (**a**) IBU free acid from 200-mg Nurofen orodispersible tablets, (**b**) IBU lysinate from 200-mg Dolormin film-coated tablets, and (**c**) IBU arginate from 600-mg Spedifen granules. Most SD bars lie within the symbols. IBU: ibuprofen.*

Nurofen Orodispersible Tablets

For Nurofen, the release of IBU was much faster in all three FEDGAS media than in FaSSGF, in which the percentage release was only about 2%; however, there were substantial differences in the release profiles using the fed state media. The highest release was observed in FEDGAS$_{early}$, in which almost 94% (209 µg/mL) of the dose was released within 120 minutes. In comparison, the release in FEDGAS$_{middle}$ was 64% (257 µg/mL), and just 50% (202 µg/mL) in FEDGAS$_{late}$ within the same period. The final pH was identical with the starting pH.

Dolormin Film-Coated Tablets

Here too, the release of IBU from the lysine salt formulation was much higher in FEDGAS media than in FaSSGF. Release was most rapid and extensive in FEDGAS$_{early}$, with 91% (203 µg/mL) of IBU released within 30 minutes, after which the release plateaued to 93% (206 µg/mL). More than 60% release was attained within 120 minutes in both FEDGAS$_{middle}$ and FEDGAS$_{late}$. The release was initially faster in FEDGAS$_{middle}$, with 55 % (220 µg/mL) in 30 minutes, compared to 27% (109 µg/mL) in FEDGAS$_{late}$. Again, the final pH was identical with the starting pH.

Spedifen Granules

Rapid IBU release from Spedifen was observed at the beginning of the dissolution test under fed gastric conditions. After just 5 minutes (first sampling time), a release of 88% (584 µg/mL) was measured in FEDGAS$_{early}$, with the concentration reaching 92% (610 µg/mL) by 120 min. In FEDGAS$_{middle}$ and FEDGAS$_{late}$, the release was lower, with 65% (784 µg/mL) and 47% (559 µg/mL) released within 5 minutes, respectively. By the end of the test, 68% IBU (819 µg/mL) had been released in FEDGAS$_{middle}$ and 57% (681 µg/mL) in FEDGAS$_{late}$. Here too, the final pH was identical with the starting pH.

DISCUSSION

Since the introduction of biorelevant media in 1998, they have become widely accepted to forecast drug performance in vivo (*16*, *21–24*). In this study, we investigated whether in vitro dissolution can be used to elucidate the clinical performance of three IBU products designed to facilitate fast release and thus fast absorption and a rapid onset of action of IBU: a) the free acid (Nurofen orodispersible tablets); b) the lysinate salt (Dolormin film-coated tablets); and c) the arginate salt (Spedifen granules). Three doses were tested under conditions simulating the fasted state, and selected doses were also tested in fed state media. As single-stage tests can be of limited predictive power, we also carried out two-stage

tests according to protocols suggested by Mann et al. and, more recently, by a PQRI working group (*11*, *12*, *17*).

Simulating Fasted State Administration

The higher solubility of the lysinate and arginate salts compared to the free acid, is expected to positively influence the dissolution behavior of their formulations (*19*, *25*). This was confirmed by our studies under fasted state conditions (single-stage tests in FaSSGF and $FaSSGF_{DIL}$), which showed more extensive dissolution of both salt formulations compared to the free acid. The positive effect of the salt form on IBU dissolution was especially pronounced at higher doses and in $FaSSGF_{DIL}$.

The higher solubility of the two salt forms initially leads to concentrations far higher than the solubility of the free acid. As the salt dissolves, the basic groups in the salt formers (lysine, containing a primary amino group with pKa 10, and arginine, containing a guanidium group with pKa 13.8) remain protonated, whereas the acidic group of IBU is initially deprotonated (*26*, *27*). During further dissolution in FaSSGF or $FaSSGF_{DIL}$, the protonated acidic group acts as a proton acceptor. IBU is therefore expected to precipitate as the more stable protonated (i.e., uncharged) form, as long as the pH of the medium remains below its pKa (*19*). As precipitation continues and more protons are removed from the medium by protonation of the IBU anion, the pH will shift to a higher value.

If the concentration of the salt former (i.e., the dose of the salt) is high enough, the buffer capacity of the dissolution medium can be exceeded. Thus, during dissolution of the salt and protonation of IBU, the pH may approach or even exceed the pKa of IBU, resulting in an attendant increase in its solubility (*28*).

For the lysinate salt (Dolormin), the choice of FaSSGF versus $FaSSGF_{DIL}$ does not impact dissolution at a 200-mg dose. Despite the low buffer capacity of FaSSGF, this dose is not sufficient to drive the pH over the pK_a of IBU (*29*). However, considering a more typical dosing for adults of 400-mg IBU, the choice of gastric simulating medium is crucial for dissolution from Dolormin (*30*). While the lower pH of FaSSGF suppressed the ability of the lysinate salt to shift the pH and thus led to low drug release, there was a marked increase in pH and thus faster and more extensive dissolution in $FaSSGF_{DIL}$. The results suggest that if Dolormin is ingested with a glass of water, dissolution in the stomach will be faster and more complete than if it is ingested away from water intake or with just a few sips.

For the arginate salt (Spedifen), the release was very fast and complete at all doses in $FaSSIF_{DIL}$. At a 600-mg dose, the salt also overwhelmed the pH of FaSSGF, driving the bulk pH up to a value of 8, resulting in fast and complete dissolution. As Spedifen is usually dosed at 600 mg, it is likely that it will facilitate fast dissolution in the stomach irrespective of the volume or timing of co-administered water. The difference in behavior between the two salt forms can be explained partly in terms of the salt formers' basicities. Compared to lysine with its primary amino group (pKa 10), the guanidinium group of arginine is more basic (pKa 13.8) and thus favors generation of a higher pH. A quantitative discussion of these effects can be found in Zöller et al. (*19*).

Comparison of the excipients in the two salt formulations (Table 1) suggests that these may also have an impact on dissolution. In Dolormin (lysinate salt), there are no excipients that could drive up the pH of the dissolution medium. In this case, the higher pH must therefore be attributed to the salt former. This finding is in line with the marked pH changes that have been observed when pure drug IBU sodium is dissolved in an HCl medium (*31*). On the other hand, Spedifen contains sodium bicarbonate and arginine in addition to the arginate salt of IBU. These two excipients are both basic and may partly explain the higher pH values observed during dissolution of Spedifen compared to Dolormin.

In contrast to the salt forms, dissolution of IBU from Nurofen orodispersible tablets containing the free acid form of IBU, was low in both fasted state gastric media. This was to be expected, given the pK_a of IBU of 4.5–4.6, as the acid group of IBU remains protonated at the pH of both media (pH 1.60 and 2.34) (*20*). Thus, little or no effect due to the volume or timing of water intake on dissolution in the stomach is expected.

In media simulating the intestinal fluids, release was (almost) complete for IBU formulations at all doses, with the free acid form taking slightly longer to achieve complete release.

Relationship Between In Vitro Dissolution and Pharmacokinetic Study Data - Fasted State

IBU free acid is poorly soluble in the fasted stomach. As a result, most of the dose is passed into the small intestine undissolved. As gastric emptying times for orodispersible tablets like Nurofen are short, (i.e., tablets disintegrate in the mouth and form a suspension-like mixture of gastric fluid and undissolved IBU particles in the stomach)

dissolution of the free acid in the small intestine becomes the rate-limiting step to absorption (*32*). For conventional tablets containing IBU free acid, this is also expected to be the case.

Administration of IBU as a salt formulation under fasted conditions has been shown to result in higher C_{max} and shorter t_{max} values compared to the free acid, which has been extensively studied for the lysinate salt (*33–36*). The faster onset of plasma concentrations represents a clinical advantage when rapid onset of drug concentrations and pain relief are desired. In a randomized cross-over study in healthy volunteers at a dose of 400 mg, the lysinate salt formulation achieved median C_{max} and t_{max} values of 44.9 µg/mL and 0.5 h, respectively, while the maximum plasma concentration for the free acid formulation was 31.8 µg/mL at 1.88 h. AUC values were similar to the free acid formulation. (*36*).

A 400-mg dose of IBU arginate oral formulation also resulted in a higher C_{max}, combined with shorter t_{max} values (0.25–0.5 h), while the AUC was similar to that of the free acid formulation (*37, 38*). Similarly, Shin et al. reported a rapid onset of IBU plasma levels under fasting conditions after ingestion of a 200-mg IBU arginate formulation, with a C_{max} of 30.2 µg/mL and a t_{max} of 0.42 h, compared with a C_{max} of 24.1 µg/mL at 1.25 h for the free acid formulation (39). Another clinical study of a 400-mg IBU arginate formulation reported a C_{max} of 56.4 µg/mL with a t_{max} of 0.4 h (*40*). Again, $AUC_{0-\infty}$ was similar for the salt and free acid formulations of IBU, indicating no differences in the extent of absorption (*39, 40*).

Comparing in vitro data with the clinical performance of the three products suggests that the pharmacokinetic data are closely tied to the gastric dissolution performance. Especially the results in $FaSSGF_{DIL}$ link well to the higher C_{max} and shorter t_{max} vales reported in the literature for both salts. Biorelevant dissolution testing combined with continual monitoring of pH revealed the mechanism behind the advantages of the salt forms in the fasted state. During dissolution of the salt forms, the bulk pH in the gastric media increases, especially at higher doses. This increase, which depends on the pK_a of the salt former, but may also be enhanced by basic excipients in the formulation, means that a greater amount of IBU is already present in the dissolved form when it leaves the stomach, leading to more rapid absorption.

The in vitro results for the free acid and pharmaceutical salt formulations of IBU line up well with the in vivo data: *IBU salts are particularly suitable for administration in the fasted state.*

Relationship Between In Vitro Dissolution and Pharmacokinetic Study Data - Fed State

The FEDGAS media were first introduced in 2021 (*13*). They were developed to simulate the composition of gastric contents after ingestion of a high-fat meal, representing different postprandial phases: $FEDGAS_{early}$ (pH 6.00), $FEDGAS_{middle}$ (pH 4.50) and $FEDGAS_{late}$ (pH 3.00) (*13*). Due to their specific composition and ability to reflect pH changes over time in the postprandial stomach, FEDGAS media serve as a valuable tool for predicting formulation performance during product development, including bioequivalence assessments (*13*).

The release of IBU in FEDGAS followed a pH-dependent trend in all the formulations investigated (Fig. 7). As expected for a weakly acidic compound, the highest release was observed at pH 6 in $FEDGAS_{early}$, followed by a lower release at pH 4.5 in $FEDGAS_{middle}$, and the lowest release at pH 3 in $FEDGAS_{late}$. In $FEDGAS_{early}$, all three formulations released 80% or more within 1 hour. So, if the dosage form is administered directly with a meal, dissolution in the stomach is expected to be comparable among formulations. A further consideration is the change in gut motility pattern that occurs upon feeding. Food ingestion disrupts the MMC cycle characteristic of fasted state gut motility, leading to a shift in the dynamics of gastric emptying (*32*). Previous studies have shown that, under fed conditions, gastric emptying is typically completed after more than 6 hours (*25*). During this time, the stomach contents are only gradually released into the small intestine. Thus, gastric emptying in the fed state is likely to be the rate-limiting step to absorption of IBU, irrespective of the formulation. Thus, for both dissolution and physiological reasons, any benefits of salt formulation with respect to fast onset of action will be attenuated if the dose is taken concordantly with a meal.

After oral administration of formulations containing the free acid form of IBU, relatively slow absorption has been reported in the literature (*37*). For the free acid, a negative effect of food on C_{max} and t_{max} has been reported in the literature (*41*). C_{max} was reduced from 33.5 µg/mL in the fasted state to 26.0 µg/mL in the fed state, while t_{max} was delayed by 0.25 h (1.38 h [fasted] to 1.63 h [fed]) (*41*). This limits the suitability of administering free acid formulations of IBU when a rapid onset of analgesia is required (*37*).

The in vitro dissolution profiles of the lysinate salt (Dolormin) and the free acid (Nurofen) in all three FEDGAS media were comparable. In accordance with the dissolution data, Weiser et al. reported that in the fed state, the plasma levels of the lysinate salt failed to show a significant improvement in C_{max} and t_{max} compared to the free acid at a dose of 400 mg (*36*). The C_{max} for the lysinate salt was 24.71 µg/mL compared to 27.34 µg/mL for the free acid, and the median t_{max} was reported to be 1.63 and 1.25 h, respectively (*36*).

According to the Swiss Prescribing Information for Spedifen, the absorption of IBU formulated as the arginate salt is also significantly slower in the fed state than in the fasted state, and lower plasma levels are achieved, despite its fast dissolution in $FEDGAS_{early}$ (*25*). Similarly, the results of the present study indicate that gastric emptying in the fed state is rate-limiting to IBU absorption.

In summary, the clinical results show that neither pharmaceutical salts nor free acid formulations of IBU are suitable for rapid pain relief if given with or after a meal. In general, IBU administered in fed state is more appropriate for the treatment of chronic conditions where prolonged pain relief is preferred to rapid onset of action. It is noted that for chronic therapy, it is considered advisable to take IBU in the fed state, as this may protect the stomach lining, so in this case it would be of little importance whether IBU is ingested as the free acid or in a pharmaceutical salt form (*42*).

CONCLUSION

Under fasted conditions, the improved dissolution behavior of IBU salts, compared to the free acid, is consistent with clinical performance. The faster onset of drug release observed in vivo appears to be dissolution-related and is mainly attributed to the ability of the salt form to increase gastric pH. The results highlight the need for biorelevant dissolution methods to investigate the role of dose, basiscity, and excipients in the behavior of pharmaceutical salts. Under fed state conditions, the dissolution of the lysinate salt showed no advantage over the free acid, which was confirmed by clinical studies. Although the arginate salt dissolves somewhat faster than the free acid in the fed state, this does not translate into clinical benefit, because slow gastric emptying of the meal becomes rate-limiting to absorption. Therefore, the benefits of pharmaceutical salt formulations of IBU with respect to rapid onset of action are primarily relevant for ingestion in the fasted state.

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DISCLOSURES

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SUPPLEMENTAL MATERIAL

Supplemental material is available for this article and may be requested by contacting the corresponding author.

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John Kiesewetter: 541-338-0022
jkiesewetter@drug-dev.com

Amy Nicklaus: 862-274-5872
anicklaus@drug-dev.com

Ralph Vitaro: 973-263-5476
rvitaro@drug-dev.com

drug-dev.com

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RKinetDS: A Flexible Open-Source Software for Modeling Dissolution Profiles

Natalia Obajtek[1], Jakub Szlęk[1*], Adam Pacławski[1], and Aleksander Mendyk[1]
[1]Chair of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland.

e-mail: j.szlek@uj.edu.pl

ABSTRACT

Introduction: Analysis of the dissolution process is becoming more prevalent, including fitting mathematical models to describe dissolution profiles. This paper presents the development of RKinetDS, an open-source software for fitting drug dissolution curves to mathematical models. **Methods:** The software was written in R (version 4.2.3), and the graphical user interface was developed using the Shiny R package. RKinetDS currently includes 36 dissolution models. The software uses various measures to evaluate the goodness of fit including RMSE, R^2, $R^2_{adjusted}$, and Akaike Information Criterion (AIC). To evaluate the reliability and practical applicability of the RKinetDS, its performance was assessed using real-world dissolution datasets sourced from published studies to reflect different experimental conditions and formulation types. RKinetDS was used to replicate the model fits. **Results:** RKinetDS produced results that were similar to those reported in published studies for tablets, oral suspension, and microspheres. For tablets, the best fit was the Weibull model (Castro et al.: β = 0.6238, R^2 = 0.9998; RKinetDS: β = 0.8146, R^2 = 0.9999). For oral suspension, Weibull model was the best fit as well (de Silva et al: β = 0.2900, R^2 = 0.9513; RKinetDS: β = 0.2101, R^2 = 0.9958). For microspheres, the Korsmeyer–Peppas model was most suitable (Murtaza et al.: K = 18.191, n = 0.487, R^2 = 0.9891; RKinetDS: K = 15.228, n = 0.5439, R^2 = 0.9841). **Conclusion:** RKinetDS is distinguished by advanced optimizers that enhance its ability to fit the most suitable models, a modern interface that simplifies navigation within the software, and extensive reporting options. RKinetDS supports the dosage form development process in academia and industry. RKinetDS is freely available on GitHub (github.com/AleksanderMendyk/RKinetDS_deploy) and the shinnyapps.io platform (jszlek.shinyapps.io/RKinetDS).

KEYWORDS: Drug dissolution, dissolution modeling, R software, Mathematical modeling

INTRODUCTION

Dissolution tests are an integral part of the pharmaceutical industry. They are essential in dosage form development and quality assurance to ensure appropriate release of the active pharmaceutical ingredient (API). Additionally, dissolution testing is used in developing in vitro-in vivo correlation models (IVIVC), and in certain instances, these tests may also be used directly to prove bioequivalence with a reference product (*1*, *2*). The significance of dissolution testing is supported by their inclusion in the regulatory requirements of the U.S. Food and Drug Administration and European Medicines Agency (*3*, *4*). In vitro dissolution testing provides valuable insight into the in vivo dissolution process, which is fundamental for the development of solid and semisolid dosage formulations. The dissolution profile is used to characterize and evaluate the release of API over time from drug products like tablets, capsules, gels, and novel drug delivery systems, including the most common oral route (e.g. tablets, suspensions) as well as parenteral (e.g., suspensions, implants), rectal (e.g., suppositories), topical (e.g., transdermal patches), sublingual, and buccal (*5*, *6*). Furthermore, dissolution tests are developed for locally acting products, including the vaginal (e.g., vaginal films), topical (e.g., ointments), and ophthalmic routes (*3*, *4*, *6*–*8*). Dissolution testing allows for quantitative evaluation and comparison of different formulations or batches, which can be performed using both model-dependent and model-independent methods (*9*).

Model-independent methods rely on statistical parameters used to quantitatively assess the rate and extent of drug release from a dosage form, such as

*Corresponding author

area under the curve (AUC), dissolution efficiency (DE), and mean dissolution time (MDT). Model-dependent approaches involve fitting the dissolution data to predefined mathematical models to describe the kinetics and mechanisms of drug release, offering insights into the formulation's behavior under specific conditions (*9, 10*).

Several dissolution models have been developed over the last few decades. Some are grounded on theoretical principles (e.g., Korsmeyer–Peppas model), and others are derived from experimental data (e.g., Weibull model) (*9, 11*). Among them, linear and nonlinear equations can be found. Linear equations are relatively easy to interpret, as each factor has a straightforward impact on the dissolution rate and contributes to the overall dissolution process; however, linear equations might lack flexibility to accurately describe the drug dissolution process. Nonlinear equations have greater flexibility and potential to represent the dissolution behavior more accurately than linear equations; however, nonlinear models might introduce numerical difficulties during the curve fitting process (*9*).

Fitting mathematical models to drug dissolution curves has become a common part of pharmaceutical research as a way to analyze and describe the relationship between experimental results and theoretical expectations. By applying curve fitting techniques, it is possible to gain insights into the dissolution mechanisms and compare the performance of various formulations. Such analysis can contribute to the optimization of drug formulations and support efforts to achieve more consistent and predictable drug dissolution (*12*). As a result, it enhances the reliability and predictability of dissolution models for regulatory and quality control purposes. Therefore, the significance of easy-to-use, time-efficient, and reliable tools for performing dissolution curve fitting is increasing.

So far, such opportunities remain relatively limited. It is possible to use spreadsheets (e.g., Microsoft Excel, LibreOffice Calc) and statistical software (e.g., Statistica) or packages (e.g., Systat) to fit dissolution data with the appropriate model equations (*13, 14*). Beyond requiring specific skills and specific input data from the user, these approaches are also time-consuming and prone to human errors. Hence, there is a demand for software to perform this type of calculation, though only a few are available. A commonly used and well-established tool is DDSolver, which was introduced in 2010 (*15*) as an add-in program for Excel with an extensive model library and the functionality of comparing drug dissolution profiles. Though it is widely used, its reliance on macros makes it sensitive to changes between different versions of Excel.

Another tool is KinetDS, a free open-source software published in 2010 for dissolution curve fitting written in Object Pascal with the graphical user interface (GUI) developed in the Lazarus (*16*). Although the software is fully functional, its development is hindered by the fact that Object Pascal is rarely used nowadays. Additionally, it is important to highlight that complex commercial tools exist to simulate dissolution studies (e.g., DDDPlus) or comprehensively analyze in vitro studies, including dissolution tests (e.g., Simcyp In Vitro Data Analysis) (*17, 18*).

The objective of this paper is to present the development of RKinetDS, an R-based flexible software for fitting mathematical models to dissolution data. The primary motivation for developing the software is to create a reliable tool that performs consistently across various systems, is easily configurable, and provides a user-friendly interface. RKinetDS, which is a descendant of KinetDS, was deliberately given a similar name by its authors to reflect this relationship.

METHODS

Computer Environment Specifications

RKinetDS was developed using a personal computer with the Microsoft Windows 10 (64-bit operating system). It was written in the R programming and statistical language (version 4.2.3), operating within an RStudio integrated development environment (version 2023.03.1+446) (*19, 20*). This enabled the use of statistical resources available in R, including powerful and state-of-the-art optimization methods, which are described further in this paper. The choice of R was dictated by its stability and reliability as well as the invariable importance of this statistical programming language.

Dissolution Models

Over the years, numerous models have been proposed to quantitatively describe dissolution behavior, such as the Polli dissolution equation (*21*). Therefore, efforts were undertaken to develop a solution that facilitates the straightforward implementation of new models. RKinetDS currently incorporates 36 models that were selected from the available literature with a focus on the most frequently used ones. Lag time was added as an option in every model. Importantly, the software is not restricted to this predefined set of models. Its open architecture, facilitated by the use of configuration files and the GNU license, allows for the addition of other models or modifications to existing ones, if needed.

Configuration Files

The configuration files define settings and parameters

that control the software's behavior. They allow users to customize and save preferred settings without modifying its core code, thereby reducing the risk of errors (*22*). In RKinetDS, the configuration file is divided into five sections: 1) model equations; 2) selected models; 3) optimization methods; 4) optimization parameters; and 5) data file format. The parameters specified in sections 2–5 are adjusted via the GUI depending on the user's individual preferences, which are linked to the type of data entered, preferred models, and available computational capabilities. The model equations can also be adjusted within the software; however, these changes must be made by directly editing the configuration file, as the modifications cannot be performed through the GUI.

Model-Fitting Algorithms

Model fitting algorithms are used to estimate the parameters of the dissolution models. In RKinetDS, several optimization methods are used including Broyden-Fletcher-Goldfarb-Shanno (BFGS), Nelder-Mead, simulated annealing (SANN), and genetic algorithms (Table 1).

Table 1. Optimization Methods Implemented in RKinetDS

Method	Algorithm	Function in R
BFGS	BFGS	optim( )
Nelder-Mead	Nelder-Mead	optim( )
SANN	SANN	optim( )
genSA	Generalized SANN	genSA( )
rgenoud	Genetic + BFGS	rgenoud( )
nloptr	Genetic (CRS2)	nloptr( )

BFGS: Broyden-Fletcher-Goldfarb-Shanno method; SANN, simulated annealing; CRS2: controlled random search v 2.0.

These optimization methods are either built-in with the R basic package called optim or require the installation of additional packages, namely genSA, rgenoud, and nloptr (*23–26*). When implemented in RKinetDS, the optimizers operate in a cascade manner. BFGS is always launched during optimization, while the remaining optimizers are optional. The optimization process starts with the execution of optional algorithms, then the BFGS algorithm is applied to achieve the final optimization result.

Optimization parameters are customized by the user, including tracing of optimization function evaluations, the value of the stop criterion, and the maximum number of iterations. As with most optimization tools, the necessity for careful consideration of the optimization settings should be kept in mind to ensure reliable results.

The strengths and weaknesses of each method should be carefully considered when selecting the most suitable approach. SANN is well-known for being effective in identifying optimal solutions; however, it has a slow convergence rate and may be computationally expensive (*27*). A more suitable option may be an expanded version of the simulated annealing algorithm presented by genSA for complex non-linear objective functions (*24*). The method implemented in the nloptr package relies on genetic search algorithms to efficiently explore a wide search space with no restrictions on the objective function's form. The rgenoud package implements a mix of evolutionary and derivative-based (BFGS) optimization methods, combining the ability to find the global minimum with efficient solution refinement (*25*).

Model Selection Criteria

RKinetDS provides various parameters to compare prediction results with observed data and evaluate curve fitting with mathematical models. These parameters include 1) root-mean-square error (RMSE); 2) coefficient of determination (R^2); 3) adjusted coefficient of determination ($R^2_{adjusted}$); and 4) Akaike Information Criterion (AIC) (*28*, *29*). The exact equations are available as supplementary material. RKinetDS generates a ranking of models based on their errors in an ascending or descending manner, making it easier to differentiate between models. Selection of the most suitable dissolution model should take into account not only the performance of the model itself, but also the dissolution data provided, the characteristics of the drug product, and obtained model parameters.

Graphical User Interface

During the development of RKinetDS, emphasis was placed on providing a user-friendly GUI (Fig. 1), which was built using the Shiny R package. The choice of Shiny was based on its integration with R's practical features, which allowed utilization of R's computational and statistical capabilities, including optimization techniques for modeling dissolution profiles, and facilitated the creation of a visually appealing GUI for integration into web-based applications. This not only improves user engagement but also simplifies the interaction with the software, making it more accessible to a broader audience.

RKinetDS supports reactive programming, ensuring that the GUI is automatically and instantly updated in response to data changes, thus increasing application interactivity with relatively little code, which would be more complex in environments like C#. The GUI provides intuitive and

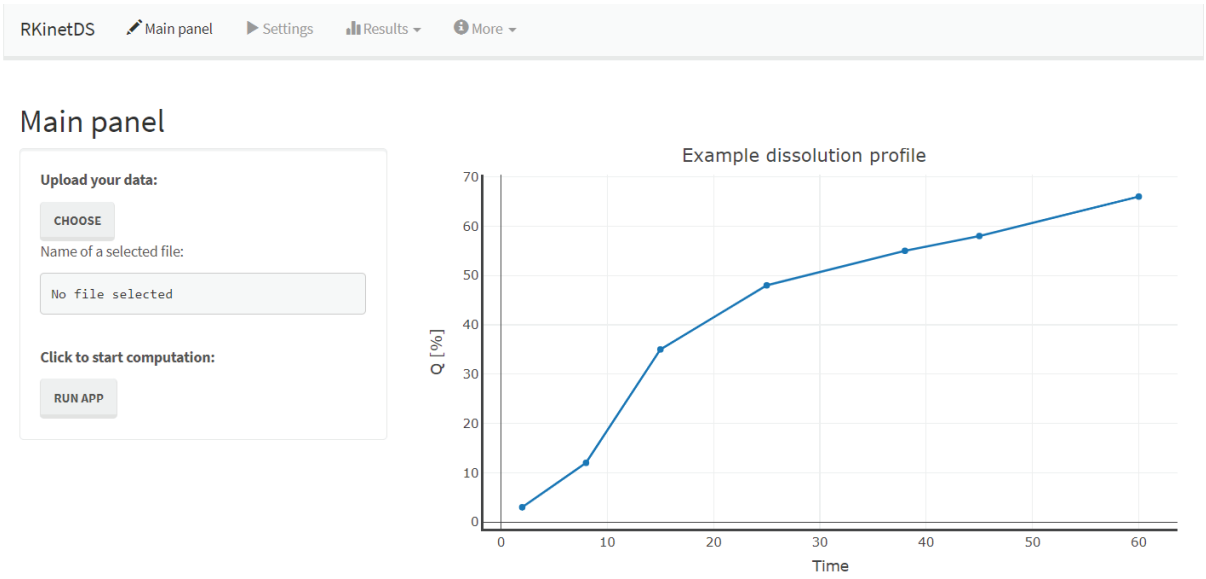

Figure 1. Screenshot of the graphical user interface of RKinetDS (main panel).

customizable data visualization options, allowing for tailored graphical representations of data (*30*). These interactive visualizations enhance the clarity of data insights and facilitate more effective data exploration. This is essential, as the initial assessment of model fitting is typically conducted visually and subsequently validated through measures of the model's fit with the data. Additionally, the software provides extensive reports including system information (operating system, installed packages with versions), settings, and seed values, enabling users to replicate results.

RKinetDS can be run locally with only R and the necessary packages installed, which are freely available for all major operating systems. Alternatively, the application can be deployed to a server and accessed through a web browser, with no additional software required on the client side, making it easy to share applications with users across different platforms and locations. This ease of use is especially beneficial for users without a deep knowledge of the R programming language, with the option of serving solutions as SaaS (Software as a Service). To demonstrate this solution, RKinetDS was deployed on the cloud-based application shinyapps.io to provide wide and easy access to the software for users (*31*).

Software Testing

To evaluate the reliability and practical applicability of the RKinetDS, its performance was assessed using real-world dissolution datasets sourced from published studies to reflect different experimental conditions and formulation types. RKinetDS was used to replicate the model fits and compare with the published results (i.e., obtained with DDSolver, which uses Nelder-Mead optimization algorithms) for each dosage form. Because some publications lacked tabulated dissolution data (i.e., only presented as graphs), WebPlotDigitizer was used to extract the numerical dissolution data (*32*). Thus, small differences in values for coefficients and model selection criteria may result.

In addition, the software was tested on Windows and Linux to ensure its independence from the type of operating system used.

RESULTS

The outcome of this work is a functional software capable of fitting various mathematical models to the provided dissolution data. Results of testing demonstrate the software's capability to analyze the dissolution processes of traditional dosage forms, such as tablets, and more complex systems, such as microspheres.

Tablets

Castro et al. evaluated four different albendazole tablets available in the Mexican market using DDSolver (*33*). The in vitro dissolution data were fitted to the first-order and Weibull models based on R^2 and AIC values to select the optimal model. For product A (reference) and C, the best-fitting model was Weibull (R^2 = 0.9998 and 0.9986, respectively).

The extracted data were used to run RKinetDS with Nelder-Mead and nloptr set as optimizers using default factory

parameters. DDSolver has several different versions of the Weibull equation. In RKinetDS, the analogous equation to the one being tested is called "Weibull with lag time." Satisfactory similarity was demonstrated between the results reported by Castro et al. and those calculated by RKinetDS (Table 2) (*33*). In RKinetDS, the Weibull equation also emerged as the most suitable model for product A and C (R^2 = 0.9999 and 0.9945, respectively) (Table 3). After running all available models in RKinetDS, the best fit was provided by the "double Weibull with lag time" model (not available in DDSolver) for both products (R^2 = 0.9999 and 0.9988, respectively).

Table 2. Amount of Dissolved Drug from Tablets: Castro et al. vs RKinetDS

Time (min)	Dissolved amount (%)	
	Castro et al. (*33*) – Reference[a]	RKinetDS (model: Weibull)
0	0	0
10	77.6057	77.9128
15	88.2992	87.7699
20	93.1652	92.9787
30	96.9130	97.5175
45	98.9131	99.4157
60	99.9686	99.8497
90	100.9758	99.9882

[a]*Product A*

Table 3. Dissolution Modeling Results for Tablets: Castro et al. vs RKinetDS

Model	Castro et al. (*33*) - Reference	RKinetDS
Reference (Product A)		
First order	K_1 = 0.143	K_1 = 0.144
	R^2 = 0.9991	R^2 = 0.9990
	AIC = 17.465	AIC = 2.073
Weibull	α = 2.1505	α = 4.3215
	β = 0.6238	β = 0.8146
	T_i = 3.6676	T_i = 0.0000
	R^2 = 0.9998	R^2 = 0.9999
	AIC = 7.9686	AIC = –5.0236
Test (Product C)		
First order	K_1 = 0.085	K_1 = 0.079
	R^2 = 0.9738	R^2 = 0.9661
	AIC = 23.914	AIC = 28.882
Weibull	α = 1.3421	α = 3.6672
	β = 0.3161	β = 0.5606
	T_i = 7.8703	T_i = 0.0000
	R^2 = 0.9986	R^2 = 0.9945
	AIC = 43.518	AIC = 18.295

AIC: Akaike information criterion.

Bilayer Tablets

Crisan et al. evaluated the dissolution profiles of various formulations of diclofenac sodium bilayer tablets with immediate and sustained dose (*34*). They applied dissolution models to better understand the mechanisms of the behavior of these tablets by evaluating the fit with the zero-order, first-order, Korsmeyer–Peppas, Hixon–Crowell, Baker Lonsdale, and Higuchi models (software used was not specified). The best fit was the Korsmeyer–Peppas model, with suggested Fickian diffusion mechanism, as the n value was below 0.45. For the formulation composed of 19% (w/w) Kollidon SR, Crisan et al reported K = 37.41, n = 0.134, and AIC = 50.5 (*34*). RKinetDS results were similar, with K = 38.50, n = 0.124, and AIC = 41.85.

Oral Suspension

For oral suspension, data from da Silva et al. were used to evaluate RKinetDS (*35*). The Weibull model was considered appropriate for describing the drug's kinetic profile with β < 1, indicating parabolic curves with initial inflection. For product A, de Silva reported β = 0.2900, and R^2 = 0.9513. RKinetDS confirmed these results, obtaining β = 0.2101, and R^2 = 0.9958.

Microspheres

Murtaza et al. evaluated the dissolution process of different formulation cefixime-loaded chitosan microspheres for prolonged drug release (*36*). They performed model analysis of dissolution data using DDSolver, and tested three models: zero order with Q_0, Higuchi, and Korsmeyer–Peppas. For formulation 3, Murtaza et al found that the most suitable one was Korsmeyer–Peppas, with K = 27.328, n = 0.412, and R_2 = 0.9708, suggesting a Fickian diffusion mechanism (*36*). Similar results were obtained using RKinetDS (K = 21.364, n = 0.4556, R^2 = 0.9642). For formulation 5, the appropriate model was also Korsmeyer–Peppas, with K = 18.191, n = 0.487, and R^2 = 0.9891, suggesting anomalous diffusion of drug (*36*). RKinetDS results were similar, with K = 15.228, n = 0.5439, and R^2 = 0.9841.

DISCUSSION

In drug dissolution studies, it is becoming increasingly common to apply suitable mathematical models to the data to better understand the mechanisms underlying the process and to compare dissolution profiles. Model-fitting techniques can be particularly beneficial in drug discovery and development by predicting bioavailability, optimizing formulations, supporting regulatory submissions with robust data about the dissolution mechanism, and comparing dissolution curves. These techniques allow

for better understanding of drug dissolution profiles, which can lead to faster and more efficient formulation development.

RKinetDS was designed to modernize the available free-to-use solutions for model-fitting that have been in use for the past 15 years. In this publication, details of its development, functionality, and effectiveness were presented. The software is fully functional and provides reliable results similar to those obtained by other methods. It is distinguished by its simplicity of use with a modern GUI, which eases navigation and simplifies analysis, and by providing extensive reports of the fitting procedure. RKinetDS features an open architecture, meaning that its source code is freely available for use, allowing for modification and expansion. Users can customize software features based on their specific needs and preferences (e.g., model equations, optimization parameters). In this way, RKinetDS differs from other tools available for free for fitting dissolution models to specific data, such as DDSolver or KinetDS.

RKinetDS is an ongoing project, with plans for future releases that include more advanced analyses using model-independent measures, such as dissolution efficiency (DE), mean dissolution such as dissolution efficiency (DE) and mean dissolution time (MDT). Additionally, future updates will include comparing dissolution profiles using various methods (e.g., similarity factor analysis).

RKinetDS is freely available on GitHub (github.com/AleksanderMendyk/RKinetDS_deploy) and the shinnyapps.io platform (jszlek.shinyapps.io/RKinetDS).

CONCLUSIONS

RKinetDS is designed to fit drug dissolution profiles to various models. This open-source software is fully functional and comprises a wide range of dissolution models, in which the best-fitting parameters are found using up-to-date optimization techniques available in R environment. RKinetDS is freely available under the GNU General Public License v3 license.

SUPPLEMENTAL MATERIAL

Supplementary materials are available upon request by contacting the corresponding author.

DISCLOSURES

The authors of the publication are the developers of RKinetDS software. They received no financial support for this work.

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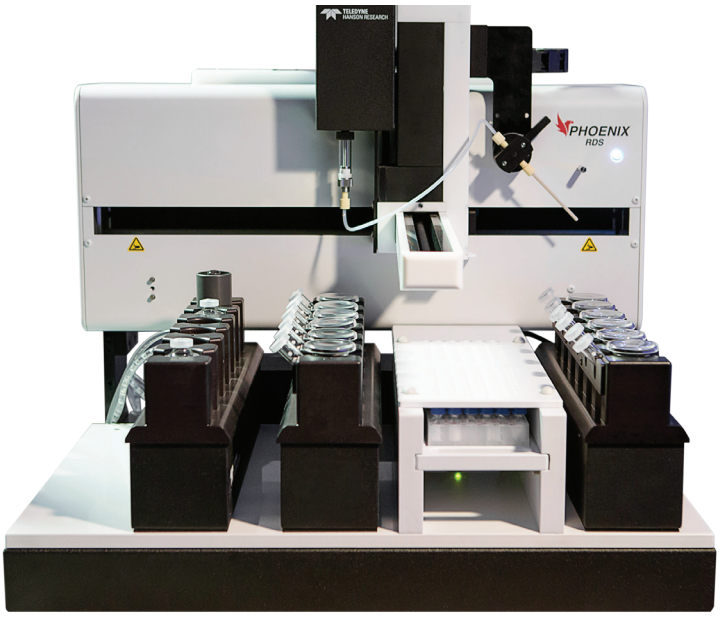

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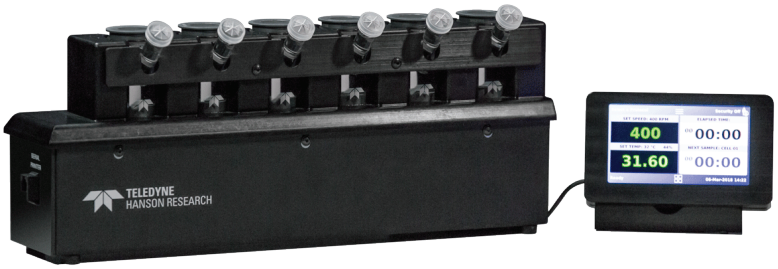

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Validation of Spectrophotometric Method for Quantification of Folic Acid in Capsules

Mariana Alves Souza, Angelo Elias Meri Junior*, Whocely Victor de Castro, André Oliveira Baldoni, Cristina Sanches, and Ana Julia Pereira Santinho Gomes
Federal University of São João del-Rei, Divinópolis, MG, Brazil.

e-mail: angeloemj@gmail.com

ABSTRACT

Introduction: Folic acid (FA) deficiency can be associated with various pathophysiological conditions, altering the homeostasis of the human organism. This study addresses the unique analytical challenges of FA quantification in hard capsules, a less-studied oral solid dosage form compared to tablets. The evaluation of quality, safety, and efficacy is essential to ensure that this product meets the characteristics described in the pharmacopoeia to guarantee biopharmaceutical and pharmacotherapeutic performance. This study aimed to develop and validate an analytical method for the quantification of FA in capsules using ultraviolet-visible absorption spectrophotometry. **Methods:** Starch and microcrystalline cellulose were selected as excipients for the preparation of capsules containing 5 mg of FA. Parameters such as selectivity and matrix effect, linearity, precision, accuracy, limit of detection (LD), limit of quantification (LQ), and robustness were evaluated according to RDC no. 166/2017. Later, weight determination, assay, content uniformity, and dissolution tests were conducted. **Results:** The method displayed high selectivity in pH 7.2 at 280 nm, with no matrix effect. Statistical treatment of the linearity (r = 0.9998, from 1.0–15 µg/mL) confirmed homoscedasticity of the data, showing a normal distribution (p > 0.05) and independence of residues. Precision, accuracy, LD (0.249 µg/mL), LQ (0.755 µg/mL), and robustness to wavelength and temperature variations were suitable. The capsules showed satisfactory results for weight determination (limits of variation of ± 10.0%, RSD (3%), and variation of theoretical content (96–101%). The validated analytical method demonstrated applicability in the quantification of FA encapsulated for the assay (96.3%), content uniformity (AV = 7.0), and dissolution tests (103%) using basket as apparatus, and phosphate buffer as dissolution medium. **Conclusion:** This method offers a cost-effective alternative for routine quality control of FA capsules, particularly in resource-limited settings. Though it is essential to validate the conditions used in the dissolution test.

KEYWORDS: Analytical method, spectrophotometry, quality control, folate, dissolution

INTRODUCTION

Folic acid (FA), also known as vitamin B9 or folate or vitamin M, can be naturally found in various foods such as spinach, beans, kale, oranges, soy, and beef liver, or it can be added to grain-derived foods (*1*). FA exhibits a fundamental neurotrophic property, playing a significant role in the differentiation, growth, and regeneration of the central nervous system. It has the potential to repair injuries and reduce the risk of neurological diseases, such as neural tube defects, spinal cord regression, Alzheimer's disease, and other neuropathies (*2*, *3*). Moreover, it is notably involved in the recovery of peripheral nerves, likely inducing Schwann cell proliferation, as well as migration and secretion of neural growth factors (*2*–*5*). It may also influence DNA synthesis and cell replication, particularly during embryogenesis and intrauterine growth, potentially impacting the gestation process (*5*, *6*).

FA deficiency can be associated with various patho-physiological conditions, such as congenital defects, impaired neonatal growth, cardiovascular diseases, inflammatory bowel disease, and even certain types of

*Corresponding author

cancer (*7*). This association may be linked to poor vitamin absorption, increased catabolism, or heightened folate requirements, thereby altering the homeostatic state of FA in the body (*6*). Furthermore, it can lead to conditions such as megaloblastic anemia, mucositis, recurrent infections, hereditary folate malabsorption (HFM), and neurological deficits (*1, 6, 8, 9*).

In 2023, there was still no consensus regarding the exact Biopharmaceutics Classification System (BCS) classification of FA (*10*). According to Wu et al., 1 mg of FA was soluble in approximately 500 mL of aqueous medium at pH 3.0 (*11*). Considering the solubility criteria of *Brazilian Pharmacopeia* (FB7), FA can therefore be considered poorly soluble in aqueous medium adjusted to pH 3.0 (*12*). Hofsäss et al. evaluated the possibility of biowaivers for bioequivalence testing of generic immediate-release FA tablet formulations for registration purposes (*13*). According to these authors, FA absorption appears to be dose-dependent, with absorption reaching approximately 90% for doses below 320 µg, which can be achieved through a balanced diet, whereas for formulations containing 5 mg of the drug, absorption is significantly reduced and can drop to less than 50% (*13*). This classification underscores the need for sensitive analytical methods to detect low concentrations of dissolved FA, as well as robust dissolution testing to ensure adequate release, posing unique challenges for capsule formulations where shell dissolution may further influence bioavailability. Based on this, it was considered in this study that FA falls under BCS class 4, i.e., low solubility and low permeability.

FA is characterized as a yellow-orange crystalline powder; soluble in dilute solutions of alkaline hydroxides, carbonates, hydrochloric acid, and sulfuric acid; slightly soluble in cold water; and insoluble in ethyl alcohol, acetone, benzene, chloroform, and ether (*12, 14*). The assay of FA in tablets is performed using high-performance liquid chromatography equipped with an ultraviolet detector at 283 nm (HPLC-UV) (*12, 15*).

On the other hand, the literature includes studies on various active pharmaceutical ingredients quantified through previously validated spectrophotometric methods, applied to pharmaceuticals, foods, or plant-based products (*16–23*). There are also reports of FA quantification using UV-visible spectrophotometry in different matrices, such as salt, flours, and tablets (*24–28*). Despite these advancements, the application of UV-visible spectrophotometry to FA in hard capsules remains underexplored, particularly regarding excipient interactions and the dissolution behavior of FA in this dosage form, which may differ from tablets due to capsule shell disintegration dynamics. Added to this is the fact that this method is simple, fast, highly reproducible and accessible, with low operational costs, selectivity, sensitivity, satisfactory accuracy and precision (*26, 28–30*). Furthermore, it allows for the use of safer solvents, minimizing the production of waste from organic solvents and their environmental impacts, thereby complying with the 12 principles of green analytical chemistry proposed by Gałuszka et al. (*31*).

Compared to HPLC, the standard method for FA assay, UV-visible spectrophotometry offers lower operational costs and simpler instrumentation, making it accessible for small-scale laboratories. However, careful optimization is required to mitigate risks of interference, i.e., working range, pH, and temperature, and to ensure sensitivity for low-dose formulations like FA capsules (*23*). In this regard, a validation step is essential before applying the method.

This study aimed to develop and validate an analytical method for the quantification of FA in hard capsules using UV-visible absorption spectrophotometry, according to parameters by RDC no. 166, of July 24, 2017 (*32*). In addition, the validated method was applied to assess FA capsules via assay, content uniformity, and dissolution tests (*12, 33*).

METHODS

Materials and Reagents

The FA (batch 202009006) and microcrystalline cellulose (MCC) (PH-101 batch 210236) used in this study were obtained from Gemini Indústria de Insumos Farmacêuticos Ltda. (Anápolis, GO, Brazil). Corn starch (batch SE0449) was acquired from Fagron do Brasil Farmacêutica Ltda. (São Paulo, SP, Brazil). The hard gelatin capsules (no. 3, colorless, batch 7174903I) were purchased from Capsugel (Rio de Janeiro, RJ, Brazil). Purified water was supplied by the Gehaka reverse osmosis system (model OS10LXE, São Paulo, SP, Brazil). Buffer solutions were prepared at the time of use and according to the *Brazilian Pharmacopeia* (FB7) (*12*). All other reagents were of analytical grade.

Equipment

The following equipment was used in this study: FA2004C analytical balance (Jugo, Shanghai, China), A3 Tepron manual encapsulator (São Paulo, SP, Brazil), UDT-812GS dissolution apparatus (Logan Instruments Corp., Somerset, NJ, USA), and Nova Instruments UV-Vis 1600 spectrophotometer (Piracicaba, SP, Brazil). Thermo Scientific automatic pipettes (São Paulo, Brazil) and previously calibrated analytical glassware were also used.

Excipients Selection and Capsules Preparation

Considering the excipients present in solid oral formulations containing FA registered by Anvisa, corn starch and MCC (1:10) were selected as suitable diluents for filling the hard capsules (*34*).

Substances such as sodium lauryl sulfate (SLS) and lactose were excluded due to their potential to interfere with FA selectivity by altering its spectral properties and causing adverse events like gastrointestinal intolerance, respectively. Additionally, SLS may enhance FA solubility in a way that masks true dissolution behavior, while lactose could contribute to degradation in humid conditions (*35*, *36*).

Progressive dilution was carried out to obtain a random mixture, which was used to fill volumetrically 60 capsules (FA 5 mg plus excipients: 10.3 mg of starch, and 93.2 mg of MCC) (*26*, *37*). All products (n = 3) were stored in opaque high-density polyethylene containers, labeled, and kept at controlled ambient temperature (15–30 °C) and relative humidity (40–65%).

Analytical Validation

The analytical method was validated considering the parameters of selectivity and matrix effect, linearity, precision and accuracy, limit of detection, limit of quantification, and robustness (*32*).

Selectivity and Matrix Effect

FA can be spectrophotometrically detected in alkaline medium (*14*). In this regard, the stock solution (1000 µg/mL) was prepared using 0.1 mol/L sodium hydroxide (NaOH) as the solvent. Subsequently, three distinct solutions were prepared in triplicate at a concentration of 10 µg/mL using phosphate buffer pH 7.2 (PB 7.2), borate buffer pH 8.0, and purified water, which were subjected to scanning in the spectral region between 230 and 310 nm. Based on the highest intensity of the analytical response, PB 7.2 was chosen as the working solution.

Solutions containing corn starch, MCC, SLS, and lactose were prepared individually in PB 7.2, reaching 10 µg/mL, due to the nominal concentration of sample. After filtration using qualitative paper, the samples were subjected to spectral reading between 230 and 310 nm.

The matrix effect was evaluated by comparing the angular coefficients of the analytical curves constructed with FA Reference Standard (RS) in PB 7.2 and with the sample fortified with excipients (corn starch and MCC). The curves were established in the same manner as in the linearity for the same concentration levels in triplicate for FA concentrations of 1, 2.5, 5, 10, and 15 µg/mL. The measurements were taken at 280 nm. Parallelism of the lines indicates the absence of interference from the matrix components (*32*).

Linearity

The linearity of the method was evaluated at 280 nm on three distinct days using solutions prepared independently in triplicate. Initially, a stock solution of FA RS in 0.1 mol/L NaOH (1000 µg/mL) was prepared, which was then used to prepare solutions at concentrations of 1, 2.5, 5, 10, and 15 µg/mL using PB 7.2 as the solvent. PB 7.2 was used as the blank.

Precision and Accuracy

The precision and accuracy were evaluated using three points of the analytical curve: 1, 5, and 15 µg/mL. The FA solutions were prepared in triplicate on three distinct days, using PB 7.2 as the solvent. Relative standard deviation (RSD) and percentage recovery values were used to assess precision and accuracy, respectively.

Limit of detection (LD) and limit of quantification (LQ)

The LD and LQ values were determined according to RDC No. 166, of July 24, 2017 (*32*). In this study, LD and LQ were tested on three distinct days, in triplicate.

Robustness

The impact of variations in the analytical signal was evaluated to check the robustness of the proposed method. For this purpose, three variables were considered: wavelength (280 vs 283 nm), solvent (PB 7.2 vs water), and temperature (25 vs 30 °C). All solutions were prepared in triplicate (*32*).

Quality Assessment of Folic Acid (FA) Capsules

According to the *National Formulary of the Brazilian Pharmacopoeia*, three criteria are considered for approval: limits of variation (± 10.0% to mean < 300 mg), RSD (< 4%), and variation of theoretical content (VTC) within the range of 90–110% (*33*). Capsules (n = 10) were individually weighed, providing mean, standard deviation and RSD values. VTC values were determined from theoretical weight (156.5 mg) (*33*).

Assay

The sampling was based on the assay method as described in the monograph for FA tablets in accordance with FB7 (*12*). Twenty (n = 20) capsules were weighed individually and then emptied. The content of the capsules was homogenized, and an amount of powder equivalent to 20 mg of FA was transferred to a 100-mL volumetric flask, with the aid of 50 mL of PB 7.2. The mixture was homogenized, and the volume was completed with the same solvent, to obtain a solution of FA at 200 µg/mL.

After agitation and filtration through qualitative paper, 5 mL of the filtrate was transferred to a 100-mL volumetric flask using PB 7.2 as the solvent to obtain an FA working solution of 10.0 µg/mL. Subsequently, the readings were taken at 280 nm, using PB 7.2 as the blank. The amount of FA in the capsules was calculated from the obtained analytical curve. The test was performed in triplicate. It is expected to find no less than 90.0% and no more than 110.0% of the labeled amount of FA (*12*).

Content Uniformity

The content uniformity test was conducted with 10 units, individually weighed. The content of each capsule was transferred to separate 50-mL volumetric flasks (100 µg/mL), and the volume was completed with PB 7.2. After homogenization and filtration through qualitative paper, 1 mL of the filtrate was transferred to a 10-mL volumetric flask using PB 7.2 as the solvent to obtain the working solution of FA at 10 µg/mL. The measurements and the amount of FA in each tested unit were determined as described above. The acceptance value (AV) was calculated according to the *United States Pharmacopeia* (USP) (*15*).

Dissolution

In the present study, the dissolution test was conducted to evaluate the possibility of quantifying FA in capsules (n = 6) by spectrophotometry. The conditions described in FB7 for tablets were used with some adaptations (*12*).

A basket apparatus (apparatus type 1) was selected to accommodate capsule dissolution, i.e., to prevent floating or sticking issues that are common with paddles. PB 7.2 was chosen as the dissolution medium to align with the validated analytical method and because FA exhibits higher solubility in slightly alkaline media due to deprotonation of its carboxylic groups, thereby enhancing detection sensitivity compared with water, where solubility is limited.

For the dissolution test, 500 mL of PB 7.2 was used with light-protected vessels, and the apparatus was set to 50 rpm for 45 minutes. At the end of the specified time, 5 mL was collected from the middle zone, estimating a concentration of 10 µg/mL, after filtration. The absorbances were measured based on the spectrophotometric method developed and previously validated. The amount of dissolved FA in the medium was determined using the analytical curve. The allowed tolerance is at least 80% of the cumulative percentage of FA dissolved in relation to the declared dose (*12*, *14*).

Statistical Analysis

The Student's t-test was employed to assess the matrix effect by comparing the angular coefficients of analytical curves with and without excipients, with a significance level of 5% ($p < 0.05$).

Statistical treatment of linearity was performed by removing outliers using the Jackknife test, followed by verification of normality (Ryan-Joiner test) and independence of residuals (Durbin-Watson test).

Homoscedasticity of the data was verified by analyzing the residuals using the Kolmogorov-Smirnov test, where normal distribution was considered when $p > 0.05$. One-way analysis of variance (ANOVA) was applied to evaluate inter-day precision and robustness across variable conditions (wavelength, solvent, and temperature).

All values were expressed as arithmetic mean ± SD. Statistical data were obtained using GraphPadPrism (version 8.0.1, GraphPad Software Inc., CA, USA) and IBM SPSS (version 2.6.1) statistical software.

RESULTS AND DISCUSSION

Analytical Validation

In the present study, the proposed spectrophotometric method proved to be suitable for the quantification of FA in hard capsules, being selective, precise, accurate, and with adequate linearity in the established working range.

It is known that FA molecule exhibits solubility in alkaline hydroxide solutions and may show absorption peaks at 256nm (A_1^1 = 549a) and 283 nm (A_1^1 = 539a) (*12*, *14*, *15*). Based on this information, the spectral behavior of FA was verified in different media (Fig. 1). After spectral scanning, phosphate buffer pH 7.2 (PB 7.2) and wavelength of 280 nm were selected for detection and analysis, as the analytical response showed greater intensity.

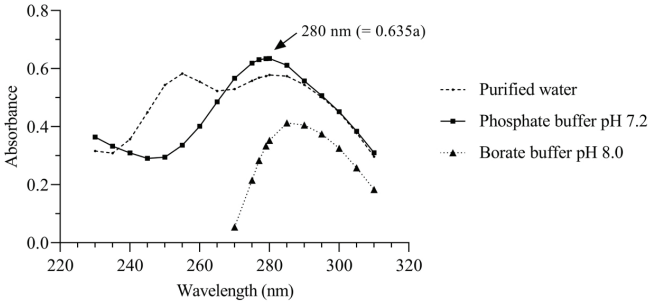


Figure 1. Ultraviolet spectrum of folic acid at 10 µg/mL (n = 3) obtained in purified water, phosphate buffer pH 7.2, and borate buffer pH 8.0 in the range of 230–310 nm.

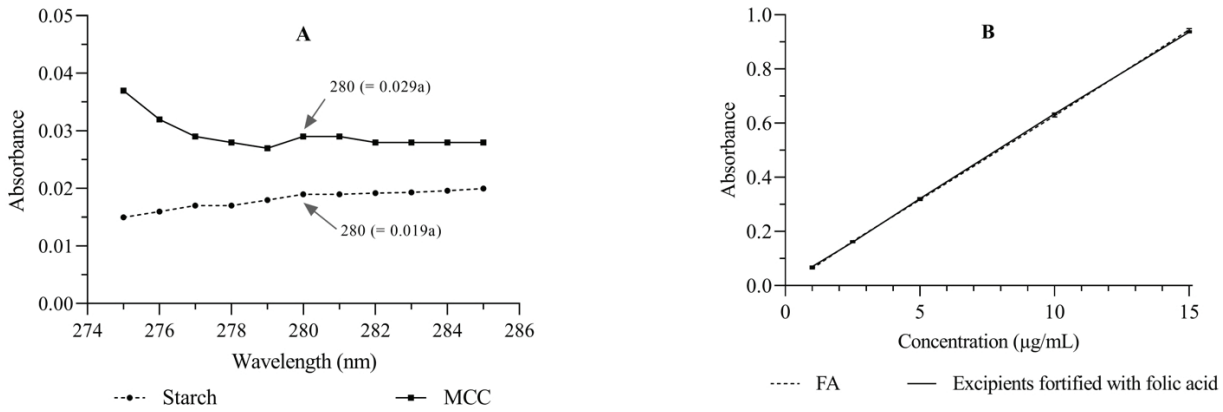


*Figure 2. Evaluation of selectivity and matrix effect verification. (**A**) Spectral scanning of excipients (corn starch and microcrystalline cellulose [MCC]) diluted in phosphate buffer pH 7.2, within the range of 275–285 nm. (**B**) Analytical curves of folic acid (FA) with or without excipients at 280 nm.*

Other studies of FA in different matrices using UV detection have reported working with solutions in the pH range of 7.0–8.0 and similar wavelengths (280–290 nm) (*25*, *26*, *38–40*). Modupe et al. evaluated the selectivity of a spectrophotometric method to quantify FA in fortified salt using three different wavelengths (256, 283, and 366 nm). Readings at 283 nm showed less interference from FA degradation products (p-amino benzoic acid glutamic acid and pterin-6-carboxylic acid) and other components of the selected matrix (*24*).

The minimal absorbance of corn starch (3.0%) and MCC (4.6%) at 280 nm can be attributed to their lack of chromophores absorbing in the UV range, unlike FA, which has a pteridine ring structure that contributes to its strong absorption. This ensures high selectivity for FA in the presence of these excipients (Fig. 2A). Data presented in Figure 2B reveal no matrix effect, because there is significant parallelism between the analytical curves ($p = 0.9892$).

As shown in Figure 3, the method demonstrated linearity within the established working range (1–15 µg/mL). The linear correlation coefficient (r) of the analytical curve was greater than 0.990, and its slope (a) in the one-way ANOVA test was significantly different from zero, with $p < 0.0001$ (*32*).

After statistical treatment of the linearity data, homoscedasticity was confirmed, showing a normal distribution ($p > 0.05$) and independence of residues (data not shown). Based on this, the ordinary least squares (OLS) model was adopted to fit and obtain the linear regression equation; i.e., to evaluate the linear association between variables using r and determination coefficient (R^2).

The proposed method showed adequate precision and accuracy, as the RSD was below 7%, and the recovery was between 80% and 120% in all analyses (Table 1) (*41*,*42*). Similar values were obtained in other studies using different analytical techniques (*38*).

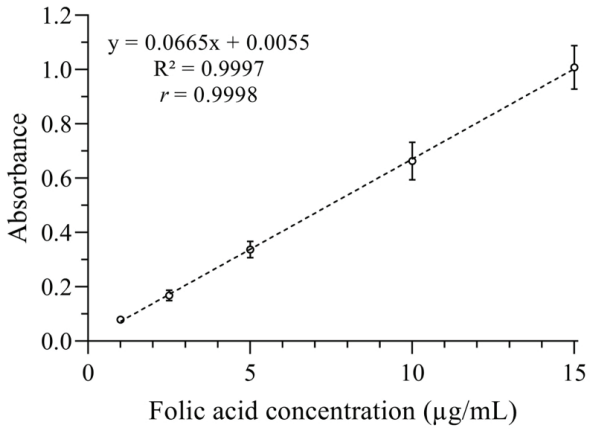


Figure 3. Analytical curve (n = 9) obtained from the method validation using phosphate buffer pH 7.2 at 280 nm. Data expressed as mean ± SD

Compared to HPLC, the standard method for FA assay, the proposed spectrophotometric method offers advantages in cost and simplicity (*12*, *15*). HPLC typically requires expensive equipment and organic solvents, with reported LD and LQ values for FA tablets around 0.5–1.0 µg/mL and 1.5–3.0 µg/mL, respectively (*39*). In contrast, our method achieved a lower LD (0.249 µg/mL) and LQ (0.755 µg/mL), indicating higher sensitivity, while reducing operational costs and solvent waste. However, HPLC may offer better specificity for complex matrices, suggesting that our method is best suited for routine quality control of capsules with minimal impurities. Furthermore, LD and LQ found are below the lowest concentration used in the

Table 1. Precision and Accuracy of Analytical Method for Quantification of Folic Acid in 280 nm Using Phosphate Buffer pH 7.2

Theoretical Concentration, µg/mL	Precision (Repeatability)											
	Day 1 (*n* = 3)			Day 2 (*n* = 3)			Day 3 (*n* = 3)					
	Conc. Obtained, µg/mL	RE %	RSD %	Conc. Obtained, µg/mL	RE %	RSD %	Conc. Obtained, µg/mL	RE %	RSD %	Conc. Obtained, µg/mL	RE %	RSD %
1	0.83	82.85	1.66	0.94	94.07	1.62	0.86	86.07	4.81	0.88	87.67	6.29
5	4.43	88.63	0.18	4.91	98.25	1.70	5.14	102.85	1.36	4.83	95.74	6.59
15	13.38	89.20	0.10	14.53	96.85	0.10	15.66	104.42	0.45	14.52	95.86	6.81

Conc.: concentration; RE: recovery; RSD: relative standard deviation.

analytical curve (1 µg/mL). Higher LD and LQ values for FA have been reported in different matrices, such as fortified salt (0.64–0.85 and 1.80–2.85 mg/L, respectively) and tablets (2.73 and 8.27 µg/mL, respectively) (*26*, *27*).

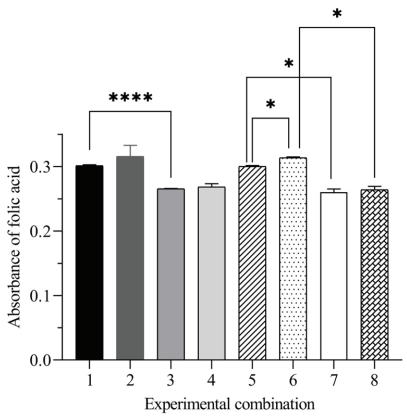
Recently, Omer et al. developed and validated two spectrophotometric methods for quantification of FA in tablets with satisfactory linearity (*43*). In the first method, an aqueous solution with sodium bicarbonate (0.1 mol/L) and three wavelengths (256, 283, and 366 nm) was used; LD was 1.46–2.44 µg/mL, LQ was 4.45–7.38 µg/mL, and the assay was approximately 104% FA. In the second method, a solution of sodium bicarbonate (0.1 mol/L) with hydrochloric acid (0.1 mol/L) at a wavelength of 295 nm was used, finding LD and LQ values of 1.53 and 4.66 µg/mL, respectively, and approximately 106% of FA for the assay (*43*). The authors reported that both methods can be used as a simple alternative to more complex methods for the assay of FA tablets (*43*).

Additionally, the proposed method showed robustness in two of three evaluated parameters: wavelength and temperature. When using water as solvent, a significant reduction in the analytical response was observed (Fig. 4). The reduced response in water highlights a key limitation, as FA's solubility decreases in neutral media, leading to aggregation or precipitation that lowers absorbance. This suggests that the method's applicability may be restricted to laboratories with access to pH-controlled buffers, potentially limiting its use in resource-constrained settings. Future studies could explore alternative buffers or pH adjustments to enhance robustness across solvents.

Ribeiro et al. evaluated other robustness parameters in different pharmaceutical formulations containing FA, including capsules (*26*). These authors concluded that derivative spectrophotometry is also robust in terms of stability and the effects of different sodium hydroxide brands and solvent concentrations (*26*).

Quality Assessment

Table 2 shows that the hard capsules obtained by the compounding process complied with the criteria of determination of weight, as well as content uniformity, dissolution, and assay tests (*12*, *33*). Cumulative release of FA was 103.1 ± 2.1%, which meets the pharmacopeial specification (*12*). This result corroborates with a study by Younis et al, who evaluated the dissolution of products containing FA in simulated intestinal fluid (*44*). These authors suggest that PB 7.2 likely enhances FA solubility compared to physiological conditions, where FA's solubility is limited due to protonation (e.g., gastric fluid, pH 1.2–3.0). This might overestimate the dissolution rate in vivo, highlighting the need for validation across a range of pH conditions to better predict bioavailability.



Variable	Nominal condition	Alternative condition	Combination							
			1	2	3	4	5	6	7	8
Wavelength (nm)	280 (A)	283 (a)	A	A	A	A	a	a	a	a
Solvent	Phosphate buffer pH 7.2 (B)	Water (b)	B	B	b	b	B	B	b	b
Temperature (ºC)	25 (C)	30 (c)	C	c	C	c	C	c	C	c

Figure 4. Assessment of the robustness in the analytical signal, considering three variables: wavelength (280 vs 283 nm), solvent (phosphate buffer pH 7.2 vs water), and temperature (25 vs 30 °C). Capital letters (A, B, C) correspond to the nominal conditions, and lowercase letters (a, b, c) correspond to the alternative conditions.
**Significant difference with p < 0.1; ****significant difference with p < 0.0001.*

Other studies in literature have also evaluated the dissolution of products containing FA. Đuriš et al. tested 17 mono and multi-component products with FA in various pharmaceutical forms (*45*). Among these, only 10 (59%) met the dissolution test requirements, including all hard capsules, whereas tablets and soft capsules (41%) failed to meet pharmacopeial criteria, which establishes a

Table 2. Results of Weight Determination, Content Uniformity, Dissolution, and Assay Tests of Folic Acid Capsules

	Weight Determination Test (*n* = 10)			Content Uniformity, % (*n* = 10)	Dissolution, % yield (*n* = 6)	Assay, mean % ± SD (*n* = 3)
	Limits of Variation* (mg)	RSD, %	VTC, %			
Specifications (*12*, *33*)	139.4–170.4	< 4	90–110	AV ≤ 15.0, n = 10 AV ≤ 25.0, n = 30	> 80	90.0–110.0
Sample	151.0–158.8	3	96–101	7.0%	101–106	96.3 ± 0.1

**Values from mean and standard deviation (154.9 ± 3.9 mg).*
AV: acceptance value; RSD: relative standard deviation; VTC: variation of the theoretical content based on theoretical weight of 156.5 mg.

tolerance of at least 80% (Q = 75% + 5%) of the stated FA amount at the end of the specified time (*45*).

Also, an increase in the release rate of FA was observed when subjected to dissolution testing in a basic medium compared with a more acid medium. This indicates a variation in FA solubility across different media, further supporting its solubility in slightly alkaline media (*14*, *45*). Some studies have suggested that FA solubility is limited in acidic pH due to the protonation of carboxylic groups. Conversely, in alkaline pH, FA becomes highly soluble. In summary, this occurs due to the deprotonation of carboxylic acid groups in the glutamic acid portion, resulting in the formation of carboxylic salts (*46*, *47*).

Matias et al. developed and validated a spectrophotometric method to quantify FA in tablets. The authors also evaluated the possibility of quantifying FA in the dissolution test using phosphate buffer pH 9.0 as a medium and compared this technique with the reference method (HPLC). Statistically, the proposed method has the same performance and can be applied for FA quantification, including the dissolution test, compared to HPLC (*25*). Several studies in the literature have found that the spectrophotometric method is equivalent to HPLC for the quantification of other therapeutic agents, yielding results comparable to the reference method with acceptable and satisfactory requirements. Thus, the method represents an alternative method for quality control (*18*).

Limitations of the current study include the lack of validation for the dissolution method employed, and PB 7.2 was used in all quality tests of hard capsules. Tests for FA tablets present different specifications than those used in this study (*12*, *15*). Our study is suitable for the spectrophotometric quantification of FA in hard capsules. Therefore, the quality of the hard capsules submitted to the validated method was satisfactory.

This study fills a critical gap in the literature by validating a spectrophotometric method for FA in hard capsules, a dosage form where capsule shell disintegration and excipient interactions (e.g., with corn starch and MCC) may uniquely affect analytical performance. Unlike tablets, capsules require careful consideration of shell dissolution, which our method accounts for by optimizing detection at pH 7.2, where FA solubility is maximized, ensuring accurate quantification despite these challenges.

CONCLUSION

The developed spectrophotometric method, using PB 7.2 as solvent and a wavelength of 280 nm for detection, proved to be suitable for quantification of FA in hard capsules. The method demonstrated selectivity, precision, accuracy, adequate linearity within the established working range, and robustness to wavelength and temperature variations. However, a lack of robustness to solvent changes (e.g., water) may limit the method's applicability in diverse settings, and the dissolution method requires validation to ensure reliable bioavailability assessments. The FA capsules analyzed by the validated method presented satisfactory quality and met the pharmacopoeial specifications required for immediate-release oral solid dosage forms. Future studies could also investigate the method's applicability to other FA dosage forms, such as tablets or soft capsules, and assess scalability for industrial quality control, potentially broadening its impact in pharmaceutical manufacturing. This validated method provides a cost-effective and environmentally friendly alternative to HPLC, with potential to enhance quality control of FA capsules in resource-limited settings, ensuring therapeutic efficacy for a critical nutrient.

DISCLOSURES

The authors thank the Institutional Program of Scientific Initiation Scholarships (PIBIC/FAPEMIG) at the Federal University of São João del-Rei (UFSJ), the Research and Graduate Studies Office of UFSJ, and the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001. The authors have no conflicting interests.

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Q+A Question & Answer Section

*The following questions have been submitted by readers of Dissolution Technologies. Margareth R. Marques, Ph.D. and Mark Liddell, Ph.D., United States Pharmacopeia (USP), authored responses to each of the questions. *Note: These are opinions and interpretations of the authors and are not necessarily the official viewpoints of the USP. E-mail for correspondence: mrm@usp.org.*

Q In the USP general chapter <1092> The Dissolution Procedure – Development and Validation, under 5.1 Specificity/Placebo interference, it is stated that any interference should not exceed 2.0%. Is this requirement applicable for degradants or any other component, and if it is applicable to placebo/blank interference?

A The requirement that the interference should not exceed 2.0% is applicable to interference from any of the components specific to the dissolution method and formulation, including:

- Test components/accessories: filter, surfactant, medium, antifoam agent, buffer salts, etc.
- Formulation/Product components: capsule, excipients, coating, colorant, drug substance degradation products, impurities, etc.
- Dissolution equipment: plastic from sampling tubing/syringes, metals, polymer coatings, filter components, etc.

Q Is it acceptable to have an assay value of 95% and then the dissolution test gives 105%. The Q value is 70%, knowing that the acceptable range for the assay is 90-110%?

A Yes, it is possible to have dissolution results above 100%, but the source of the high dissolution value should be investigated. As only one dosage unit is introduced in each dissolution vessel/cell, this unit may have a drug content close to the upper limit of the uniformity of dose. An investigation would typically be required to rule out other sources for the high value. Possible sources of interference that may result in dissolution results above 100% are listed above. Other reasons for high values may include method-related issues such as inappropriate filter selection or sampling procedure.

Q If a validated method with manual sampling is being used, and the procedure is optimized using an autosampler, keeping the same test conditions except for the sampling volume (with medium replacement) because the equipment requires a larger volume, is it necessary to perform a new validation? Or is the qualification of the equipment enough?

A In addition to proper qualification of the sampling system, additional validation steps must be completed to demonstrate that there is no sample adsorption in the system and that the sampling system is not contributing other components (e.g., leachables and extractables) that may interfere with the quantitative step. This evaluation needs to be done for each product where the automated/semi-automated system is implemented.

Q We would like clarification regarding the degassing procedure described in USP <711> Dissolution. The chapter specifies that the medium should be filtered under vacuum while mixing vigorously, then continue mixing under vacuum for approximately 5 minutes. The General Notices state that "vacuum" denotes exposure to a pressure of less than 20 mmHg (2.67 kPa), unless otherwise indicated. On the other hand, USP <1092> The Dissolution Procedure – Development and Validation, states that "The extent of deaeration can be evaluated by measuring the total dissolved gas pressure or by measuring the concentration of dissolved oxygen in water. For example, an oxygen concentration below 6 mg/L has been found effective as a marker for adequate deaeration of water for Dissolution <711>, Apparatus, Apparatus Suitability, Performance verification test with USP Prednisone Tablets RS." However, the certificate for the Dissolution Performance Verification Standard – Prednisone states the recommended degassing procedure as follows: "Measured vacuum should be less than 100 mbar."

A Please note, the general chapters use the term "deaeration" while the USP reference standard certificate uses the term "degassing." In the context of removing dissolved gases from dissolution media, these two terms are used interchangeably. Please also note that the definition of "vacuum" provided in the General Notices states, "*unless otherwise indicated*." The information in <1092> regarding the performance verification test with USP Prednisone Tablets RS is provided as an example of a specific deaeration technique for a specific product. In this example, a specific dissolved oxygen limit of 6 mg/L was found to be effective to ensure consistent dissolution results. The recommended degassing technique in the certificate for the

Dissolution Performance Verification Standard – Prednisone (i.e., measured vacuum should be less than 100 mbar) provides sufficient reduction in dissolved oxygen and other dissolved gas(es) that may be present in dissolution media; however, consider that this method was developed and validated for a specific product as well. For general dissolution purposes, the degassing methods and dissolved gas limits may vary based on the specific formulation and media degassing equipment used. The degassing method and dissolved gas limits should be considered as part of the dissolution method validation procedure when it can be shown that dissolved gas has an impact on the dissolution results for a specific product.

Q USP general chapter <711> Dissolution states that sinkers can be used if the dosage form floats in the dissolution medium. Is the use of sinkers allowed with USP apparatus 2 (paddle) even if the compendial monograph for a particular drug product does not mention its use?

A Sinkers can be used if the dosage form floats or if it sticks to the equipment walls. In many cases the use of sinkers is product-dependent. If sinkers are needed, they can be used even if the compendial monograph does not mention its use. If it is determined that a sinker is needed for a specific product, the appropriate design and size should be selected on a case-by-case basis and should be described in the final dissolution method because the use of an inappropriate sinker may compromise the dissolution results.

Q I am working on a project for a dosage form containing a polymeric nanomaterial. I found information in the literature that N-methyl-2- pyrrolidone can be used as co-solvent in dissolution media. What co-solvent concentration is allowable for dissolution method development?

A One of the main objectives of the dissolution test is to be discriminative for the critical quality attributes. Consequently, if the use of co-solvents is deemed appropriate, then the type and concentration should be selected to maintain the discriminatory power of the dissolution test. The type, concentration, and purity of any co-solvent used in a dissolution method needs to be determined using a case-by-case approach, utilizing data obtained from the sample under evaluation, and should be scientifically justified.

Q In the USP general chapter <711> Dissolution, under Procedure, Apparatus 1 and 2, Time, it states, "Specimens are to be withdrawn only at the stated times, within a tolerance of ± 2%." For immediate-release products, this tolerance should be fine, but is the tolerance of ± 2% applicable for extended-release products, wherein the time points will be 8, 12, or even 18 hours?

A This tolerance level is applicable to the time to initiate the sampling procedure. It does not account for the entire duration of the sampling procedure. The sampling should start at the time specified in the method within the tolerance of ± 2% of the specified sampling time. This tolerance is applicable to all types of dosage forms, to all types of sampling procedures (manual, automated, or semi-automated), and to all types of dissolution testing including dissolution profiles.

Q Can the dissolution test replace the disintegration test for immediate-release solid dosage forms? If yes, are there published regulations that discuss this issue and clarify the conditions in which this case can be applied.

A USP general chapter <1711> Oral Dosage Forms – Performance Tests provides instruction for cases where a disintegration test is appropriate based on different types of solid oral dosage forms. Generally, you should always start with the development of a dissolution test whenever possible. Then, depending on the physical-chemical properties of the drug substance and on the dissolution profile, the dissolution test may be replaced with a disintegration test with appropriate justification based on the data obtained from the samples being evaluated.

Q+A

Every issue of *Dissolution Technologies* features a Question and Answer section. This section is designed to address general dissolution questions submitted by our readers.

Please send your questions to:
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Correction to "Evaluation of In Vitro Dissolution Behavior of Ibuprofen Suspensions Based on the Flow-Through Cell Method"

In the August 2025 issue of *Dissolution Technologies*, in the article entitled "Evaluation of In Vitro Dissolution Behavior of Ibuprofen Suspensions Based on the Flow-Through Cell Method" by Li Xie et al (DOI: 10.14227/DT320325P140), there was a typographical error on page 2.

In the section, "Dissolution Profile Determination Based on the Flow-Through Cell Method," the second sentence has been corrected as shown in bold: "The **open**-loop configuration was used, with a pump pulse of 120 r/min." In the same paragraph, the details of the open-loop configuration are described correctly. Therefore, this error does not affect the interpretation or validity of the study's results.

To ensure accuracy and avoid confusion, the online version of the article has been corrected.

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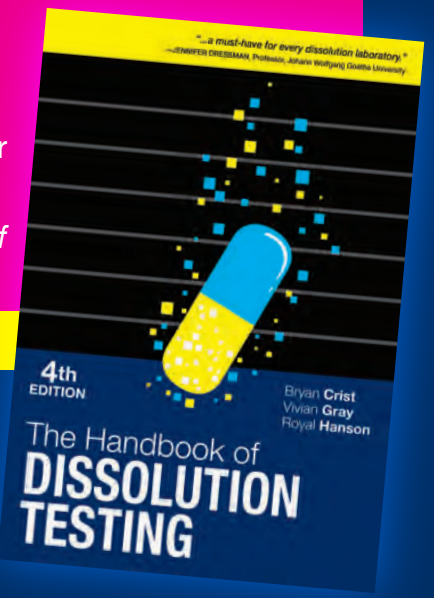

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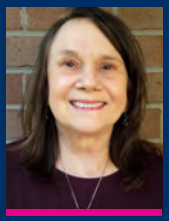

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Dissolution Technologies, Incorporated
9 Yorkridge Trail • Hockessin, DE 19707 USA

Calendar of Events

April 30– May 1, 2026

M-CERSI Workshop: "Role of In Vitro Dissolution Studies for Predictive Insight into In Vivo Performance and Biopharmaceutics Risk Mitigation"

Location: Universities at Shady Grove, Rockville, MD, Building II

Registration: www.pharmacy.umaryland.edu/centers/cersievents/2025dissolution

May 5– 6, 2026

Bioequivalence Innovations for Oral Generic Products: Biowaivers, Bridging, and Generic Development for Oncology and Discontinued Products

Location: Universities at Shady Grove, Rockville, MD

Registration: https://www.complexgenerics.org/education-training/bioequivalence-innovations-for-oral-generic-products-biowaivers-bridging-and-generic-development-for-oncology-and-discontinued-products/

July 6– 9, 2026

Controlled Release Society 2026 Annual Meeting

Location: Lisbon, Portugal

For information, visit https://www.controlledreleasesociety.org/events/crs-2026-annual-meeting-exposition

October 25– 28, 2026

PharmSci 360 AAPS Meeting

Location: Ernest N. Morial Convention Center, New Orleans, LA

For information, visit https://www.aaps.org/pharmsci/annual-meeting

November 16–18, 2026

Eastern Analytical Symposium and Exhibition

Location: Crowne Plaza Princeton-Conference Center, Plainsboro, NJ, USA

For information, visit eas.org

December 1– 2, 2026

Long-Acting Injectable (LAI) Generics: Navigating Technical Hurdles in Product Development and Regulatory Assessment

Location: Universities at Shady Grove, Rockville, MD

Registration: https://www.complexgenerics.org/education-training/long-acting-injectable-lai-generics-navigating-technical-hurdles-in-product-development-and-regulatory-assessment/

On Demand Events

- ***Powder Flow Testing***
  https://www.copleyscientific.com/events/webinar-foundations-of-powder-flow-testing/
- ***dissoLab Software: Predictive Dissolution Simulated from Microscopic Images***
  https://vimeo.com/1054617734?share=copy
- ***Fiber Optic UV: Better Dissolution Testing On Demand***
  https://www.distekinc.com/watch/fiber-optic-uv-better-dissolution-testing/
- ***Advances in In Vitro Bioequivalence Assessment for Topical Products Part 2***
  https://youtu.be/iqphypToHZ0?si=mn9FJLDhm-VBoWMm
- ***Ocular Administration (OCAT™) in GastroPlus® On Demand***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-ocular-administration-ocat-virtual/

- ***Oral Cavity Administration (OCCAT™) in GastroPlus® On Demand***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-oral-cavity-administration-occat-virtual/

- ***Pulmonary Administration (PCAT™) in GastroPlus® On Demand***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-pulmonary-administration-pcat-virtual/

- ***GastroPlus® ADR – 4 Course Bundle (TCAT™ / OCAT™ / OCCAT™ / PCAT™)***
  https://www.simulations-plus.com/events/gastroplus-adr-4-course-bundle-tcat-ocat-occat-pcat/

- ***GastroPlus® ADR – 5 Course Bundle (TCAT™ / OCAT™ / OCCAT™ / PCAT™ / Injectables)***
  https://www.simulations-plus.com/events/gastroplus-adr-5-course-bundle-tcat-ocat-occat-pcat-injectables/

- ***Transdermal Administration (TCAT™) in GastroPlus®***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-transdermal-administration-tcat-virtual/

- ***Injectables (IM, SQ, IA) in GastroPlus® Including Biologics and LAIs***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-injectables-incl-lai-biologics-virtual/

- ***GastroPlus® X Tutorial Series***
  https://www.simulations-plus.com/events/gastroplus-x-tutorial-series/

- ***GastroPlus® X Tutorial Series I – Panels and Start-up Essentials***
  https://www.simulations-plus.com/courses/gastroplus-x-tutorial-series1/

- ***GastroPlus® X Tutorial Series II – Modules***
  https://www.simulations-plus.com/courses/gastroplus-x-tutorial-series-ii-modules/

- ***GastroPlus® X Tutorial Series III – New Features in GastroPlus X.2***
  https://www.simulations-plus.com/courses/gastroplus-x-tutorial-series-iii-new-features-in-gastroplus-x-2/

- ***Complimentary Introduction to GastroPlus® for up to v.9.9***
  https://www.simulations-plus.com/events/complimentary-introduction-to-gastroplus-v-9-9/

- ***Complimentary Introduction to GPX™***
  https://www.simulations-plus.com/events/complimentary-introduction-to-gpx/

Free Tutorials (AI Language Translation)

- ***GastroPlus® Tutorial in French***
  https://www.youtube.com/watch?v=h-_4m8xpDg8&list=PLQuCii74Pfd327VslFvlp_lRxqJlfzEF0

- ***GastroPlus® Tutorial in Japanese***
  https://www.youtube.com/watch?v=IkTsLRPVIms&list=PLQuCii74Pfd317she0RsVTFVCyl2yvz9X

- ***GastroPlus® Tutorial in Mandarin***
  https://www.youtube.com/watch?v=XabV2jiAsVU&list=PLQuCii74Pfd3LojzBlatK7mnbLxPvqFDa

- ***GastroPlus® Tutorial in Portuguese***
  https://www.youtube.com/watch?v=idkaPR6av9M&list=PLQuCii74Pfd0zttMQdhy6sv97OlK9MyE5

- ***GastroPlus® Tutorial in Spanish***
  https://www.youtube.com/watch?v=kEmj98nnpx8&list=PLQuCii74Pfd2oVl6tsQNvxZs6sX0Ww9FT

Simulations Plus Positioned to Capitalize on FDA's Streamlined Nonclinical Safety Guidance with Advanced Mechanistic and Model-Informed Solutions

Validated engines and AI-orchestrated ecosystem align with the emerging regulatory framework

Simulations Plus, Inc., a global leader in model-informed and AI-accelerated drug development that advances biopharma innovation, responded to the U.S. Food and Drug Administration's draft guidance on streamlined nonclinical safety studies for monospecific monoclonal antibodies. The guidance encourages reduced reliance on animal studies and elevates mechanistic understanding, pharmacokinetics, and integrated weight-of-evidence (WoE) assessments for nonclinical decision-making.

"The FDA's draft guidance signals a clear regulatory mandate towards mechanistic, model-informed science, and Simulations Plus is uniquely positioned to help clients respond with confidence," said Shawn O'Connor, Chief Executive Officer of Simulations Plus. "Our scientific engines, workflows, and cross-disciplinary capabilities have supported these integrated approaches for years."

Mechanistic WoE Approaches Already Embedded in Simulations Plus' Practice

Simulations Plus enables clients to integrate physiologically based pharmacokinetics (PBPK), quantitative systems toxicology/pharmacology (QST/QSP), clinical pharmacokinetics (PK), and mechanistic insights to support WoE assessments aligned with FDA-recommended approaches. This includes PBPK modeling in GastroPlus® to project human exposure and guide dose selection, mechanistic toxicity assessment in BIOLOGXsym™, and population modeling in MonolixSuite® to connect preclinical and clinical understanding.

Case studies published in the *International Journal of Molecular Sciences, Pharmaceutics*, and elsewhere show how combining PBPK and QST can strengthen the scientific rationale for streamlined nonclinical strategies for monospecific antibodies, including determining when extended non-human primate toxicology studies may not be warranted (*1*, *2*).

Ecosystem-Level Alignment with the New Regulatory Paradigm

The Simulations Plus ecosystem aligns with the FDA's focus on mechanistic, model-informed evidence. Its validated scientific engines—including GastroPlus, MonolixSuite, ADMET Predictor®, and QSP platforms—provide the transparency, traceability and cross-domain mechanistic reasoning regulators expect. Supporting this scientific core is an artificial intelligence (AI)-orchestrated framework that enables reproducible multi-engine workflows and guided decision support through AI copilots, all grounded in validated methods.

Continued investment in biologics modeling, including planned enhancements to GastroPlus and BIOLOGXsym, will expand support for monoclonal antibodies, antibody-drug conjugates, and immune-related pathways highlighted in recent FDA guidances.

"The draft guidance gives the industry a clear direction, and as model-informed safety and toxicology become essential disciplines, a new growth frontier opens for Simulations Plus in areas of our customer organization that have historically been underpenetrated," said O'Connor. "Our mission is to help innovators navigate this transition with clarity, rigor, and confidence."

References

1. Beaudoin, J. J.; Clemens, L.; Miedel, M. T.; Gough, A.; Zaidi, F.; Ramamoorthy, P.; Wong, K. E.; Sarangarajan, R.; Battista, C.; Shoda, L. K. M.; Siler, S. Q.; Taylor, D. L.; Howell, B. A.; Vernetti, L. A.; Yang, K. The combination of a human biomimetic liver microphysiology system with biologxsym, a quantitative systems toxicology (QST) modeling platform for macromolecules, provides mechanistic understanding of tocilizumab- and GGF2-induced liver injury. *Int J Mol Sci.* **2023**, *24* (11), 9692. DOI: 10.3390/ijms24119692

2. Vallejo, C.; Meaney, C.; Clemens, L.; Yang, K.; Lukacova, V.; Zhou, H. physiologically based pharmacokinetic models for infliximab, ipilimumab, and nivolumab developed with GastroPlus to predict hepatic concentrations. *Pharmaceutics.* **2025**, *17* (3), 372. DOI: 10.3390/pharmaceutics17030372.

Introducing the Copley Inhaled Dissolution Apparatus: A First-of-a-Kind Commercially Available Solution for Inhaled Product Dissolution Testing

Addressing a critical gap in the in vitro testing toolkit to meet growing demand for the discriminating assessment of dissolution behavior

Nottingham, UK: The innovative Inhaled Dissolution Apparatus (IDA) from Copley Scientific introduces discriminating dissolution testing to the commercially available in vitro toolkit for inhaled drug products. Addressing a longstanding and evolving industrial need, the IDA brings new insights into in vivo behavior, enabling faster and more effective development.

The IDA is a complete, integrated solution comprising a dedicated sample collection and transfer system and a purpose-designed dissolution tester. Robust and easy-to-use, it supports confident differentiation of formulation and device performance in early-stage development, while reducing reliance on more complex, expensive, and time-consuming in vivo methods. The IDA is suitable for all types of inhaled drug products but will be of particular interest to generic developers working towards regulatory submission in accordance with FDA Product Specific Guidances (PSGs). A growing number of these now specify dissolution for the demonstration of bioequivalence (BE).

"The launch of the IDA breaks new ground in inhaled product testing, reinforcing Copley's commitment to advancing industry standards," said Mr Jamie Clayton, CEO. "Its rigorously optimized design reflects our in-depth understanding of emerging challenges in inhaled drug development and builds on our unrivalled record of delivering reliable and relevant testing solutions. By supporting the development of standardized, easily transferable dissolution test methods, the IDA has the potential to de-risk development and strengthen regulatory submissions – both of which are major gains."

Despite specific references to dissolution testing in the FDA PSGs, there are currently no standardized methods, leaving many researchers little alternative but to develop in-house solutions. Too often, the result is suboptimal, labor-intensive methods that are difficult to validate and transfer, and prone to significant lab-to-lab variability, which compromises their value.

The IDA (patent pending) has been specially engineered to integrate sample collection, sample transfer, and dissolution testing into a coherent, three-step workflow. Enabling clinically relevant assessment of the respirable fraction of the delivered dose, the IDA:

- Works in combination with established inertial impaction tools to isolate the sub-5-µm fraction of the delivered dose
- Enables undisturbed sample transfer from the dose collector to the dissolution tester using the unique IDA filter holder, maintaining sample integrity and simplifying the workflow
- Includes a dedicated dissolution tester, the IDA-specific DIS 600i-ID or DIS 800i-ID, built on established, trusted Copley technology.

Suitable for multiple device types, the IDA offers the flexibility to tailor dose collection test conditions such as flow rate and inlet type for compatibility with established methods. The result is data that integrate directly with compendial results to build a more complete picture of in vivo fate and how formulation and device characteristics influence product performance. Early IDA studies,

presented at Drug Delivery to the Lung 2025, demonstrate the ability of the apparatus to generate reproducible discriminatory data and its potential value for the nuanced comparison of reference and test products in generic development.

“The IDA offers developers a solution that dovetails with existing workflows to make routine dissolution testing highly accessible.” said Clayton. “Customer response to the IDA has been extremely positive, and we are confident it will deliver real value by supporting stronger in vitro-in vivo relationships and reducing reliance on in vivo studies.”

To find out more about the IDA contact us (sales@copleyscientific.co.uk) or visit our website.

About Copley Scientific

Copley Scientific is widely recognised as the world’s leading manufacturer and supplier of inhaled product testing equipment and is a major provider of testing systems for other pharmaceutical dosage forms. The company also supplies equipment for detergent testing.

Copley’s pharmaceutical product range includes testing equipment for all types of orally inhaled and nasal drug products - metered-dose inhalers, dry powder inhalers, nebulisers and nasal products - with a particular focus on solutions for delivered dose uniformity and aerodynamic particle size distribution measurement. It also includes testers for tablets (dissolution, disintegration, friability and hardness) capsules, powders, suppositories, semisolids and transdermals.

Used from R&D through to QC, this extensive range of equipment is supported by a full validation and aftersales service. Copley works in partnership with specialist distributors, extending localised support across the world. This network provides expert help and training to every customer, directly enhancing the application of all Copley products.

www.copleyscientific.com

For further information

Press information/Company contact:
Rosanna Kelly, Marketing Manager, Copley Scientific Limited
Colwick Quays Business Park, Road No. 2, Nottingham, NG4 2JY, UK
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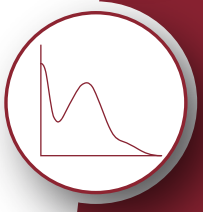

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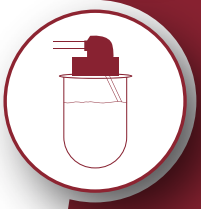

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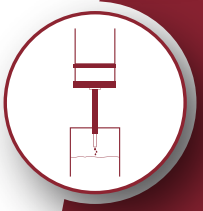

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- Jennifer, Sr. Scientist

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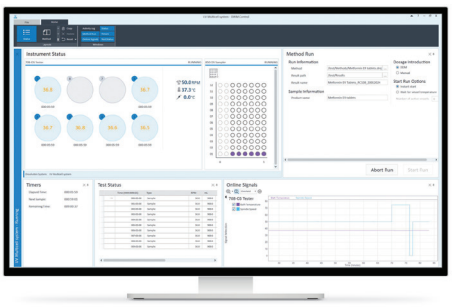

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