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An In Vitro Model for Release of Acetaminophen When an Overdose is Ingested Orally

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ABSTRACT

Acetaminophen (paracetamol) is an analgesic and antipyretic drug that is widely used across the globe due to its efficacy and safety within the therapeutic dose range. When acetaminophen is ingested in amounts higher than the recommended dosage over several days, severe hepatotoxic effects can result. Next to the opioid drugs, acetaminophen is often deliberately ingested as an overdose, in which case multiple dosage units may be ingested. In this study, a novel in vitro model aiming to understand the dissolution of acetaminophen in overdose situations is described and implemented to compare four commercially available formulations of acetaminophen. Increasing quantities of immediate-release and extended-release tablets as well as hard and soft capsule formulations were tested in the in vitro model using United States Pharmacopeia apparatus 3 (reciprocating cylinder). When a single dose was tested, acetaminophen dissolved rapidly from the immediate-release formulation and from the immediate release part of the extended-release formulation. At higher doses, acetaminophen was released more slowly and less extensively as the dose was increased. The results obtained with the in vitro model are in line with the literature data obtained in acetaminophen clinical trials. Additionally, the in vitro model was able to reproduce pharmacobezoar formation at very high doses, which has been observed in cases of deliberate overdose.

KEYWORDS: acetaminophen, paracetamol, overdose, dissolution, USP apparatus 3

INTRODUCTION

Acetaminophen (also known as paracetamol) is an analgesic and antipyretic drug, belonging to Biopharmaceutical Classification System class 3 (highly soluble, low permeability) (*1*). Acetaminophen was introduced in the early 1960s as an alternative to acetylsalicylic acid (aspirin), having lesser side effects, e.g. in the gastrointestinal tract. First popular in Europe, it gained popularity in the USA in the 1980s after Reyes syndrome in children was linked to aspirin (*2*). It is currently marketed throughout the world as tablets, capsules, syrups, or suspensions for oral administration, as well suppositories for rectal application.

After administration within its therapeutic dose range (recent recommendations are max 4 g daily), it is absorbed well, and its peak concentration in serum is observed after about an hour (*3-5*). Therapeutic doses are well tolerated by most patients and are safe in comparison to most non-steroid anti-inflammatory drugs. A few years after acetaminophen was marketed for the first time, it was reported that severe adverse effects could occur, especially concerning the liver (*2*). Nowadays, it is well known that in doses only slightly higher than recommended (so-called unintentional overdoses), acetaminophen ingestion may cause severe hepatotoxic effects within a few days and can lead to acute liver failure (*2*). On the other hand, much higher overdoses of acetaminophen (single doses even above 200 g) may be ingested deliberately in a suicidal attempt (*2*, *5*, *6*). Irrespective of whether the overdose is intentional or not, such cases present a severe burden to both patients and the public health care system in many countries. Several clinical trials of immediate-release (IR) dosage forms of acetaminophen in healthy volunteers, up to 2 g of a single dose, have been published (*5*, *7*). Yet, there is still a lack of in vitro studies aimed at understanding the dissolution of acetaminophen at these and higher levels of overdose.

The current study presents a novel in vitro dissolution method, based on United States Pharmacopeia (USP) apparatus 3 (reciprocating cylinder), for comparing various marketed acetaminophen oral dosage forms after overdose. This method is applied to IR tablets, extended

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(modified) release (ER) tablets, soft capsules, and hard capsules.

METHODS

Chemicals

The following acetaminophen (paracetamol) formulations were chosen for study: IR tablets (paracetamol STADA 500 mg, lot 14814, STADA Arzneimittel AG, Bad Vilbel, Germany), hard capsules (ben-u-ron 500 mg, lot 703M211, bene-Arzneimittel GmbH, Munich, Germany), soft capsules ($APAP_{caps}$ 500 mg, lot U1110771, US Pharmacia Sp. z o.o., Wroclaw, Poland), and modified release bi-layer (ER) tablets (Osteomol 665 mg, lot 22121357, Pharmacor Pty Ltd., Chatswood, Australia). All products were purchased commercially.

Hydrochloric acid 1 M solution (VWR Chemicals, lot 22C024019), maleic acid (Merck, lot S7667380943), sodium chloride (Carl Roth, lot 076235484), sodium hydroxide pellets (VWR Prolabo, lot 14A100027), sodium phosphate monobasic dihydrate (Merck, lot K91237142611), and tris base (Sigma-Aldrich, lot SLBP4240V) were used for preparation of the media buffers.

Acetonitrile, methanol, and trifluoroacetic acid (TFA), all analytical grade, were purchased from VWR International (Darmstadt, Germany). Paracetamol Chemical Reference Standard (CRS, Eur. Pharm., Batch 4.1), used as an analytical standard, was also purchased from VWR International.

Acetaminophen Solubility

The solubility of acetaminophen in the presence of excipients present in the IR tablets was tested in the buffers on which fasted state simulated gastric fluid (FaSSGF), fasted state simulated intestinal fluid version 2 (FaSSIF-V2), FaSSIF at midgut ($FaSSIF_{midgut}$), simulated intestinal fluid in the ileum (SIF_{ileum}), and fed state simulated colonic fluid (FeSSCoF) are based. The solubility experiments were performed in triplicate using a multi-position magnetic stirrer assembly (Variomag Poly 15, H+P Labortechnik GmbH, Oberschleißheim, Germany). The IR tablets were crushed and pulverized using a mortar and pestle. An excess of pulverized formulation (186 mg of powder, corresponding to 150 mg of acetaminophen) was weighed using an analytical balance (SECURA 225-D-1S, Sartorius AG, Göttingen, Germany) and transferred into a 20-mL glass container with a screw cap. A 15-mm stirring bar was placed into the vial, and 5 mL of media buffer was added. Subsequently, the vial was placed on the magnetic stirrer in a preheated incubator (IncuLine 68R, VWR International, Leuven, Belgium). The samples were incubated at 37 ± 1 °C for 24 hrs under stirring at 600 rpm. Samples (1.2 mL) were withdrawn after 24 hrs of incubation using 2 mL syringes (Injekt Luer Solo, lot 21G08C8, B.Braun, Melsungen, Germany) equipped with 21 G x 1.5-in. needles (FINE-JECT, lot 14-12300, HENKE SASS WOLF, Tuttlingen, Germany). The samples were filtered through 0.45-µm polytetrafluoroethylene (PTFE) membrane syringe filters (Acrodisc, LOT FC5752, PALL Corporation, Port Washington, NY, USA), discarding the first 0.8 mL and collecting the last 0.4 mL of filtrate for high-performance liquid chromatography (HPLC) analysis. HPLC analysis was performed after the samples were appropriately diluted with mobile phase.

Dissolution Testing

USP Apparatus 2 with Low Volume

In preliminary experiments, the dissolution of acetaminophen from the IR tablets was tested in a USP apparatus 2 (PTDT 820D, Pharma Test Apparatebau AG, Hainburg, Germany) equipped with scaled-down vessels (250 mL) and paddles. These experiments were performed in triplicate.

Doses of 1 or 10 acetaminophen IR tablets (500 mg and 5000 mg/vessel, respectively) were added to 100 mL of FaSSGF buffer or FaSSIF version 1 (FaSSIF-V1) buffer. The experiments in FaSSGF buffer were conducted for 2 hours and those in FaSSIF-V1 buffer for 6 hours. During the experiments, a temperature of 37 ± 0.5 °C was maintained.

Samples (5 mL) were withdrawn at 5, 10, 15, 20, 30, 45, 60, 90, and 120 min (FaSSGF buffer) and at 5, 10, 15, 20, 30, 45, 60, 90, and 120 min, and hourly thereafter (FaSSIF-V1 buffer) using a set of sampling cannulas equipped with Poroplast 10-µm cannula filters (ERWEKA, Langen, Germany) and 5-mL Omnifix Luer Lock Solo syringes (lot 22C21C8, B.Braun, Melsungen, Germany). The samples were filtered through 0.45-µm PTFE membrane syringe filters (Whatman Puradisc, lot A29640934, Cytiva, Marlborough, MA, USA), returning the first 4 mL of filtrate back into the vessel and collecting the last 1 mL for analysis. Collected samples were analyzed by HPLC after appropriate dilution with the mobile phase. After each sample was withdrawn, the media in the vessels was replenished by adding 1 mL of fresh, preheated medium.

To determine whether further dissolution would occur in more distal regions of the GI tract, it was necessary to switch to an apparatus that facilitates multiple media changes. For this reason, USP apparatus 3 was selected for further experiments.

USP Apparatus 3

The four acetaminophen formulations (i.e., IR tablets, ER tablets, hard capsules, soft capsules) were subjected to dissolution testing using USP apparatus 3 (reciprocating cylinder) (RRT 10 Caleva, ERWEKA, Langen, Germany). The dissolution tester was equipped with 250-mL flat-bottom vessels and inner glass cylinders fitted with two 420-nm nylon meshes, one at the upper and one at the lower cylinder opening. All experiments in USP apparatus 3 were performed in triplicate.

The dissolution media used for this assembly were the buffers contained in FaSSGF, FaSSIF-V2, $FaSSIF_{midgut}$, SIF_{ileum}, and FeSSCoF according to Markopoulos et al. (*8*). The compositions of these buffers are presented in Table 1. The vessels, each containing 220 mL of medium, were placed in a water bath in order of their sequence in the gastrointestinal (GI) tract, and a temperature of 37 ± 0.5 °C was maintained during the experiment. During the experiment, motility of the human GI tract was simulated by changing the dipping rate of the inner cylinders, as shown in Figure 1 and described in Table 2.

All formulations were tested for acetaminophen dissolution from single doses (500 mg for IR tablets and hard and soft capsules; 665 mg for ER tablets) and from two levels of overdosing: 1) 10 tablets or capsules per vessel (total dose of 5 g for IR and 6.65 g for ER dosage forms) and 2) 20 tablets or capsules per vessel (total dose of 10 g for IR and 13.3 g for ER dosage forms). Additionally, the IR tablets were tested for acetaminophen dissolution from 50 tablets per vessel, corresponding to a total dose of 25 g of acetaminophen.

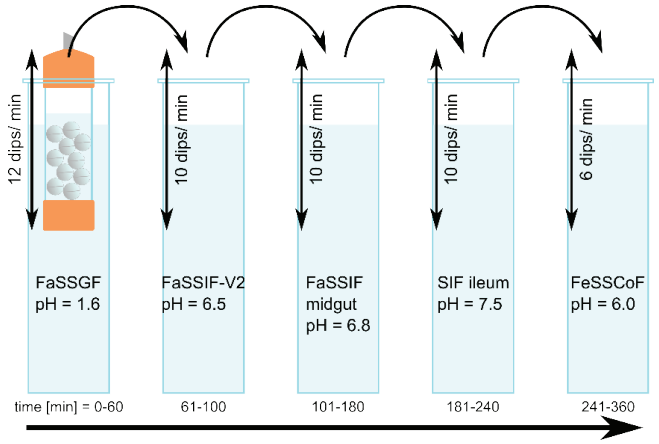

Figure 1. USP apparatus 3 setup for release of acetaminophen from different oral formulations. FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid; SIF: simulated intestinal fluid; FeSSCoF: fed state simulated colonic fluid. Note that in each case the buffer of the biorelevant medium was applied.

Samples (5 mL) were withdrawn after 10 and 20 min from the FaSSGF buffer and every 20 minutes thereafter from the FaSSGF, FaSSIF-V2, and $FaSSIF_{midgut}$ buffers. When

Table 1. Composition of Buffer Media Used in USP Apparatus 2 and 3 Experimental Setups According to Markopoulos et al. (8)*

Components	FaSSGF	FaSSIF-V1	FaSSIF-V2	$FaSSIF_{midgut}$	SIF_{ileum}	FeSSCoF
Tris base (mM)						30.5
Maleic acid (mM)			19.1	19.3	52.8	30.15
Sodium hydroxide (mM)		13.8	34.8	36.5	105	16.5
Sodium chloride (mM)	34.2	105.8	68.6	76.1	30.1	34.0
Sodium phosphate monobasic (mM)		28.7				
Hydrochloric acid	q.s. pH 1.6					
pH	1.6	6.5	6.5	6.8	7.5	6.0
Dissolution apparatus	USP 2 & 3	USP 2	USP 3	USP 3	USP 3	USP 3

**Buffers only (no bile components added).*
FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid; SIF: simulated intestinal fluid; FeSSCoF: fed state simulated colonic fluid.

Table 2. USP Apparatus 3 Testing Setup and Media Details

Row number	Media buffer*	Buffer pH	Duration (min)	Cumulative duration (min)	Dip rate (dips/min)
1	FaSSGF	1.6	60	60	12
2	FaSSIF-V2	6.5	40	100	10
3	$FaSSIF_{midgut}$	6.8	80	180	10
4	SIF_{ileum}	7.5	60	240	10
5	FeSSCoF	6.0	120	360	6

**Buffers only (no bile components added).*
FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid; SIF: simulated intestinal fluid; FeSSCoF: fed state simulated colonic fluid.

release in SIF_{ileum} and FeSSCoF buffers was tested, samples were withdrawn every 30 minutes. The samples were withdrawn using 5-mL Omnifix Luer Lock Solo syringes through sampling cannulas equipped with Poroplast 10-µm cannula filters. The samples were subsequently filtered through PTFE 0.45-µm membrane syringe filters, returning the first 4 mL of the filtrate into the vessel and collecting the last 1 mL for the HPLC analysis, which took place after appropriate sample dilution. In these experiments, the withdrawn media in the vessels was not replenished with fresh buffer after sampling, but the volume loss was accounted for when calculating the acetaminophen concentrations.

Analytical Method

Analysis of the samples collected in solubility and dissolution experiments was performed using reversed phase HPLC. The concentration of acetaminophen was measured using a Chromaster VWR/HITACHI HPLC system (VWR International) equipped with a 5110 pump, 5210 autosampler, 5310 column oven, 5410 UV-Detector, and Agilent OpenLab EZChrom software (version A.04.10).

The analytical method was based on the method of Franeta et al., adjusted to shorten the elution time for acetaminophen while still obtaining well-defined peaks (*9*). The separation was performed using a LiChrospher 100 RP18 endcapped 5-µm 250-4 analytical column (Merck Millipore, Darmstadt, Germany) as the stationary phase. The mobile phase consisted of acetonitrile (VWR International) and MilliQ water (in-house filtration, high purity water system Ultra Clean GP UV UF, EVOQUA Water Technologies LLC, Günzburg, Germany) in a ratio of 15:85 v/v, to which 0.05% TFA was added. A flow rate of 0.8 mL/min resulted in a retention time of 5.1 min. Peak detection was performed at 240 nm. The limit of quantification (LOQ) of the analytical method was 1.46 µg/mL. For each sample set, a fresh calibration curve was prepared. The coefficient of determination (R^2) calculated for the calibration curves was always > 0.999.

Data Presentation and Statistical Analysis

Microsoft Excel (2016, Redmond, WA, USA) with the Data Analysis Tool Pack was used to calculate the acetaminophen concentration in each sample. For the statistical analysis of the data obtained during the experiments, SigmaPlot (version 12.5, SYSTAT Software Inc., Point Richmond, CA, USA) was used. All data are presented as mean values ± standard deviation. One-way analysis of variance (ANOVA) and post-hoc pairwise multiple comparison procedures using the Holm-Sidak method were performed. The acetaminophen formulations were compared with each other at the doses tested in the USP apparatus 3 assembly at the 10- and 60-min time points, and alpha was set to 0.05.

For the statistical comparison of two dissolution acetaminophen profiles, the similarity factor f_2 was calculated (without the optional weighting factor). The similarity factor is a statistical tool described by FDA to determine the equivalence of dissolution profiles from oral dosage forms (*10*). If f_2 is 50 or greater, this indicates that the two compared dissolution profiles differ by 10% or less from each other. The f_2 factors for each acetaminophen dissolution profile pair using data from USP apparatus 3 experiments were calculated using the first four sampling points in FaSSGF buffer. For dissolution profiles with more than four sampling points, the f_2 factor was calculated using all available time points.

RESULTS

Solubility

The solubility results for acetaminophen in the IR tablets are presented in Table 3. The solubility values after 24 hrs of incubation were consistent across all media tested, ranging from 23.60 ± 0.32 mg/mL in $FaSSIF_{midgut}$ buffer to 24.82 ± 0.96 mg/mL in FeSSCoF buffer.

*Table 3. Solubility (mg/mL) of Pulverized Paracetamol STADA (acetaminophen) 500-mg immediate-release tablets in USP 3 Dissolution Media Buffers**

FaSSGF	FaSSIF-V2	$FaSSIF_{midgut}$	SIF_{ileum}	FeSSCoF
23.98 ± 0.21	23.68 ± 0.95	23.60 ± 0.32	23.62 ± 0.31	24.82 ± 0.96

**Values are mean ± SD (n = 3)*
**Buffers only (no bile components added).*
FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid; SIF: simulated intestinal fluid; FeSSCoF: fed state simulated colonic fluid.

USP Apparatus 2 Dissolution

When one dose or 10 doses were tested in USP apparatus 2 with scaled-down vessels and paddles, the IR tablets fully disintegrated within the first minute after contact with FaSSGF buffer or FaSSIF-V1 buffer. After 30 min, more than 80% of the dose (i.e., 89.3 ± 1.6% in FaSSGF buffer and 82.9 ± 5.0% in FaSSIF-V1 buffer) had been released from a single tablet (Fig. 2). The profiles reached a plateau after 45 min in FaSSGF buffer and after 60 min in FaSSIF-V1 buffer. A plateau concentration of 4.74 ± 0.05 mg/mL (95.8 ± 1.1% of the total dose) was measured at 90 min in FaSSGF buffer. In FaSSIF-V1 buffer, a plateau concentration of 4.77 ± 0.05 mg/mL (96.4 ± 1.0%) was measured at 360 min.

When 10 tablets were tested, the percent release was lower than that of one dose in both FaSSGF and FaSSIF-V1 buffers. Similar to the dissolution of one dose, the profiles reached a plateau after 45 min in FaSSGF buffer and after

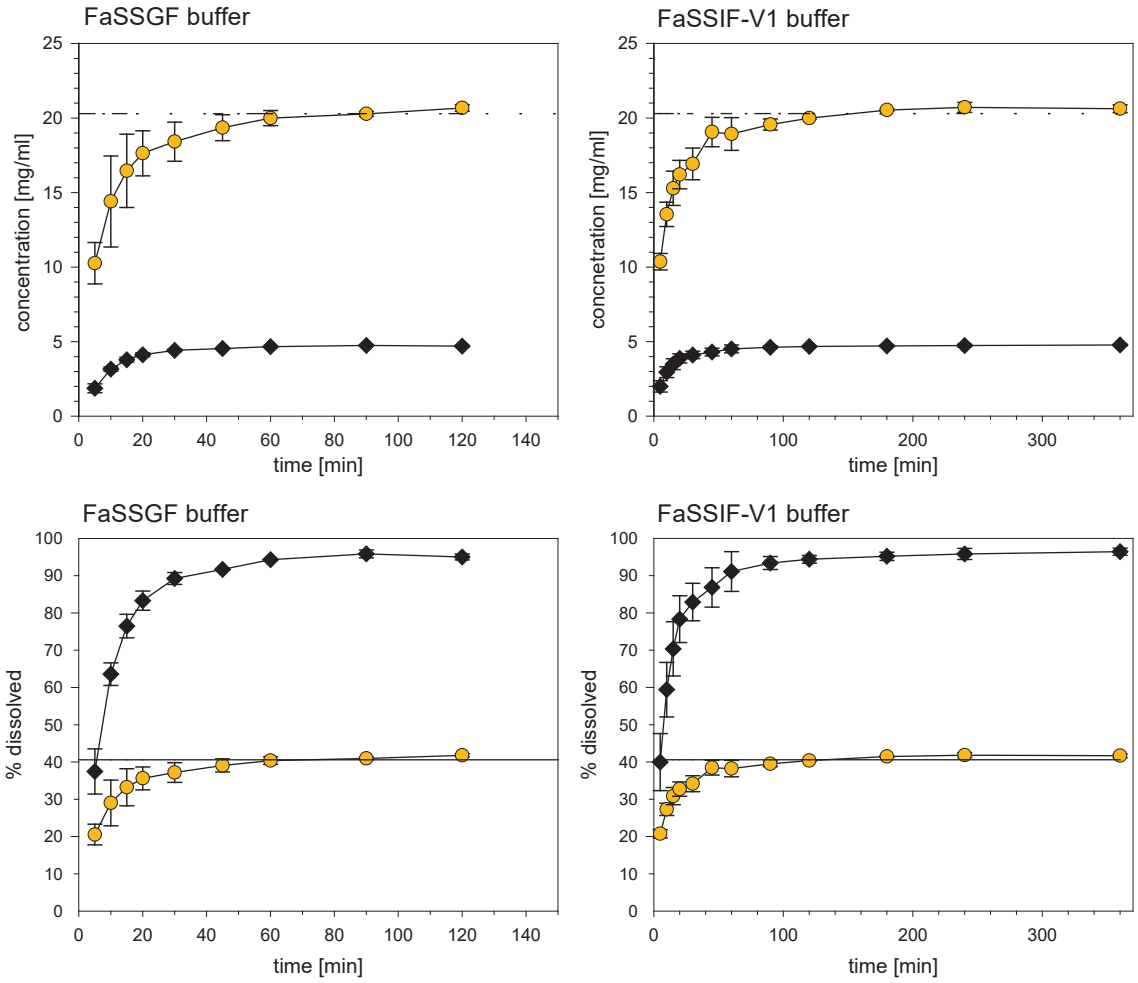

Figure 2. Paracetamol (acetaminophen) 500-mg IR tablets dissolution profiles tested using a low volume USP apparatus 2 method. Profiles are for one dose (diamonds) and 10 doses (circles). Top row shows mean acetaminophen concentration and solubility (dashed line) measured in various biorelevant buffers over the range of pH 1.2–9 based on Shaw et al. (11). Bottom row shows mean drug release and maximum dissolvable % for 10 tablets (solid line). IR: immediate release; FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid.

60 min in FaSSIF-V1 buffer. In FaSSGF buffer, a plateau concentration of 20.68 ± 0.22 mg/mL was reached by 2 hrs, which corresponds to 41.8 ± 0.4% of the total dose. In FaSSIF-V1 buffer, the plateau concentration at 240 minutes was 20.71 ± 0.34 mg/mL, which corresponds to 41.8 ± 0.7% of the total dose. In these experiments, acetaminophen dissolution from 10 IR tablets is limited by its solubility.

USP Apparatus 3 Dissolution

Dissolution profiles from all tests in USP 3 apparatus are shown in Figure 3. Photographs of the dosage forms at various stages of the tests are shown in Figures 4–6.

Immediate-Release Tablets

Acetaminophen dissolved rapidly from one IR tablet, reaching 89.8 ± 1.4% release (449 ± 7 mg) after 10 min in the first (FaSSGF buffer) compartment. After 60 min in FaSSGF buffer, some disintegrated tablet residues were still present in the inner cylinders. The experiment was therefore continued in the FaSSIF-V2 buffer compartment, where 100.9 ± 0.9% of the total dose was released (504 ± 4.5 mg) by 100 min. At this time, all tablet residues had passed through the bottom mesh into the outer vessel.

When 10 doses were tested, rapidly disintegrated IR tablets formed a dense clump (Fig. 4B.1), resulting in some disruption of the flow pattern inside the inner cylinder. The clumping slowed the acetaminophen dissolution profile in comparison to that of one tablet. At the last time point (60 min) in FaSSGF buffer, 72.0 ± 7.7% of the total dose had been released. The experiment was therefore continued into the FaSSIF-V2 buffer, where 94.8 ± 2.9% of the total dose was released, and then in $FaSSIF_{midgut}$, where the tablet residues penetrated through the nylon

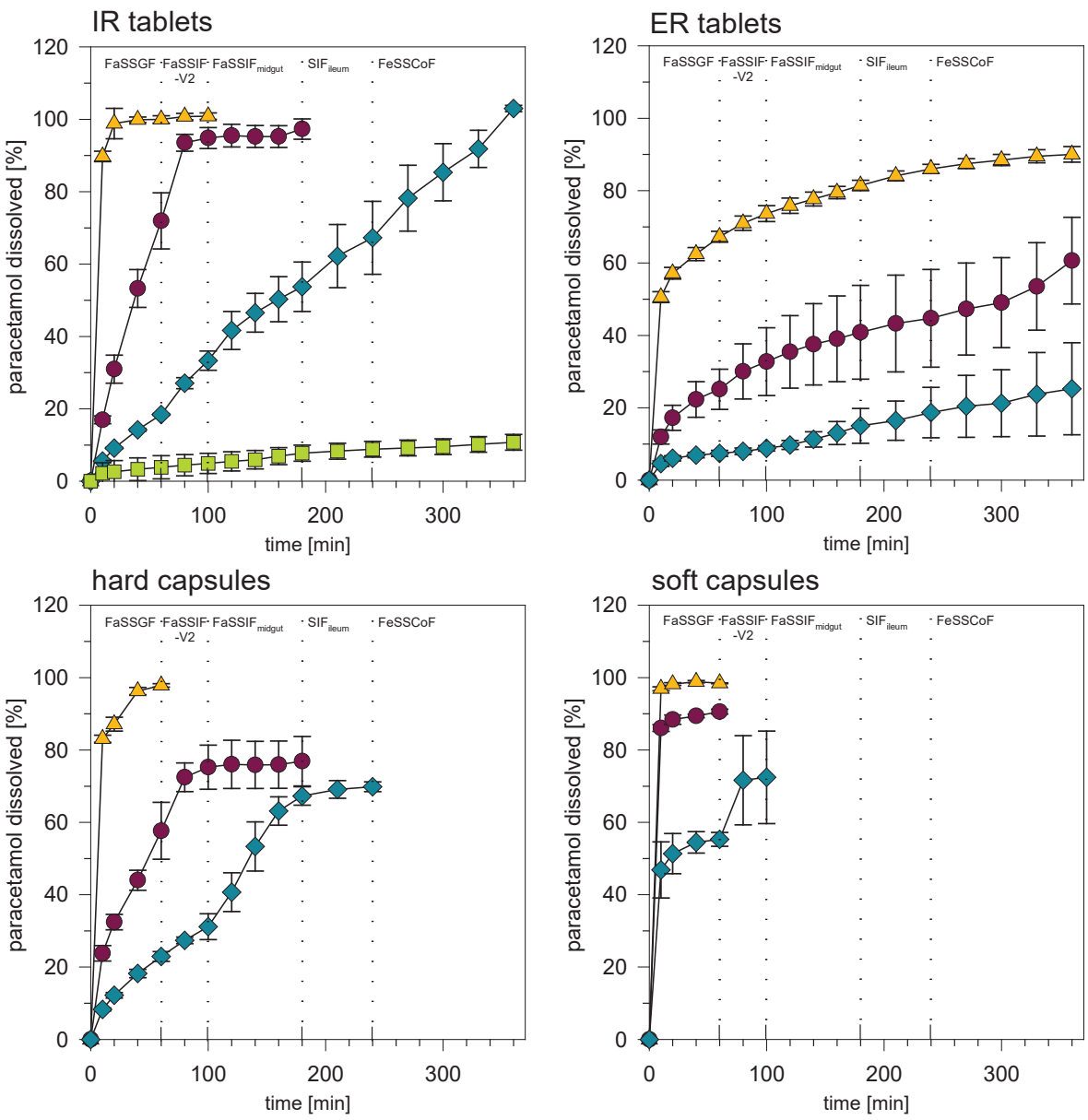

Figure 3. Dissolution profiles of four acetaminophen formulations tested using USP apparatus 3, expressed as mean ± SD percent of the tested dose (n = 3). Profiles show one dose (triangles), 10 (circles), 20 (diamonds), and 50 doses (squares, IR tablets only). FaSSGF: fasted state simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid; SIF: simulated intestinal fluid; FeSSCoF: fed state simulated colonic fluid; IR: immediate release; ER: extended release. Note that in each case the buffer of the biorelevant medium was applied.

mesh into the outer vessel, releasing 97.3 ± 2.8% of the dose, corresponding to 4866 ± 140 mg of acetaminophen.

When 20 IR tablets were tested, they also disintegrated quickly (within the first 10 sec after contact with FaSSGF buffer), but then formed a dense clump (Fig. 5B.1), obstructing media flow between the inner and outer cylinders. After 60 min in FaSSGF buffer, only 18.5 ± 0.2% of the total dose (1845 ± 24 mg) was released. The experiment continued in the FaSSIF-V2, FaSSIF$_{midgut}$, and SIF$_{ileum}$ buffer compartments, in which 33.3 ± 2.7% (3335 ± 265 mg), 53.7 ± 6.8% (5374 ± 684 mg), and 67.3 ± 10.1% (6727 ± 1007 mg) of the total acetaminophen dose was released, respectively. Because some tablet residues were still present in the inner cylinder, testing in FeSSCoF buffer was also conducted. During this part of the experiment, the rest of the tablet material penetrated through the nylon mesh into the vessel. After 6 hrs of testing (in all five compartments), 20 IR tablets released 103.0 ± 0.9%, corresponding to 10,300 ± 87 mg of acetaminophen.

When 50 IR tablets were tested, rapid tablet disintegration followed by clumping was again observed (Fig. 6). At the final time point of the FaSSGF buffer compartment, only 3.9 ± 3.2% of acetaminophen total dose had been released. In further compartments, the percent release

rose to 5.0 ± 2.8% in FaSSIF-V2, 7.8 ± 2.2% in $FaSSIF_{midgut}$, 8.9 ± 2.2% in SIF_{ileum}, and 10.8 ± 2.2% in FeSSCoF. This final percent release corresponds to 2695 ± 547 mg released acetaminophen in total, considerably less than values for either 10 or 20 tablets.

Extended-Release Tablets

In all experiments, the outer layer of the ER tablets disintegrated shortly after coming in contact with FaSSGF buffer, and an initial dose of acetaminophen was released.

When one ER tablet was subjected to dissolution, 50.6 ± 1.5% (336 ± 10 mg) was released within 10 min in FaSSGF buffer. This corresponds to the IR fraction of the tablet. Subsequent acetaminophen dissolution was slower, reaching 67.3 ± 1.5% (447 ± 10 mg) at the end of the test period (60 min) in FaSSGF buffer. On the lower mesh of the inner cylinder, a moist and slightly swollen tablet core was observed after finishing the FaSSGF buffer part of the test. This remnant persisted throughout the subsequent compartments, including FeSSCoF buffer. In FaSSIF-V2, release reached 73.7 ± 2.2% (490 ± 15 mg), 81.4 ± 1.5% (542 ± 10 mg) in $FaSSIF_{midgut}$, 86.0 ± 1.3% (572 ± 8 mg) in SIF_{ileum}, and 92.2 ± 2.0% (613 ± 13 mg) in FeSSCoF, in accord with the ER properties of the tablet.

When 10 doses were tested, the rapidly disintegrated outer layer of the ER tablets formed a dense clump of residual material inside the inner cylinder (Fig. 4B.2), which impeded media flow. After 60 min in FaSSGF buffer, 25.1 ± 5.6% (1671 ± 370 mg) of acetaminophen had been released. Subsequently, acetaminophen release reached 32.8 ± 9.4% (2179 ± 624 mg) in FaSSIF-V2, 40.9 ± 13% (2719 ± 862 mg) in $FaSSIF_{midgut}$, 44.7 ± 13.5% (2975 ± 897 mg) in SIF_{ileum}, and 60.7 ± 12% (4034 ± 796 mg) at the end of the test (6 hrs).

When 20 ER tablets were tested, disintegration of the

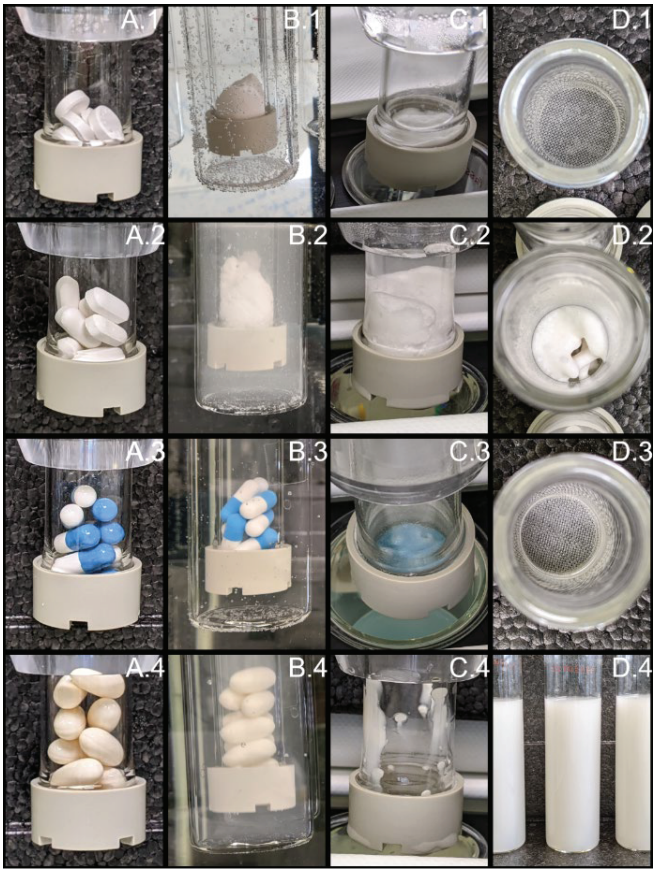

Figure 4. Photographs of 10 doses of acetaminophen during USP apparatus 3 testing. Row 1 shows IR tablets, 2 shows ER tablets, 3 shows hard capsules, and 4 shows soft capsules. A.1–A.4 depicts the dosage forms in the inner cylinder shortly before test start; B.1–B.4 is within first 2 minutes in FaSSGF; C.1–C.3 is after 60 minutes in FaSSGF, C.4 shows 10 soft capsules after 15 minutes in FaSSGF; D.1–D.3 shows inner cylinder content after finishing the test, and D.4 shows strongly colored FaSSGF buffer due to penetration of all soft capsule residuals into the outer vessel.
USP: United States Pharmacopiea; IR: immediate release; ER: extended release; FaSSGF: fasted state simulated gastric fluid.

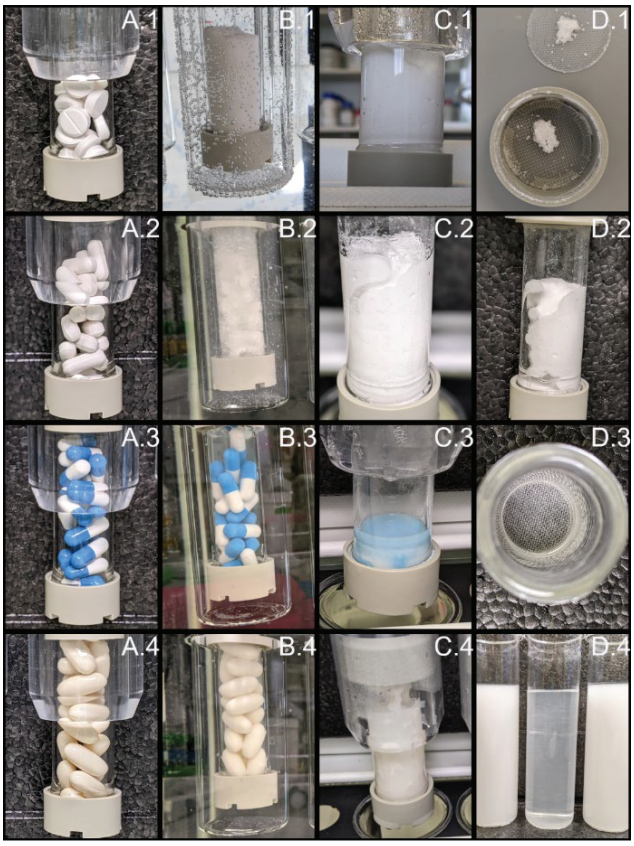

Figure 5. Photographs of 20 doses of acetaminophen during USP apparatus 3 testing. Row 1 shows IR tablets, 2 shows ER tablets, 3 shows hard capsules, and 4 shows soft capsules. A.1–A.4 depicts the dosage forms in the inner cylinder shortly before test start; B.1–B.4 is within first 2 minutes in FaSSGF; C.1–C.3 is after 60 minutes in FaSSGF, and C.4 is after 20 minutes in FaSSGF, with media present in the inner cylinder due to clumped capsule material on the bottom mesh; D.1–D.3 shows inner cylinder content after finishing the test, and D.4 shows strongly colored FaSSGF buffer due to penetration of all soft capsule residuals into outer vessels 1 and 3 (not observed in vessel 2).
IR: immediate release; ER: extended release; FaSSGF: fasted state simulated gastric fluid

outer layer again caused obstruction of the media flow due to the dense clump of residual material (Fig. 5B.2). In FaSSGF buffer, release amounted to just 7.4 ± 0.8% of the total dose (989 ± 111 mg). The experiment continued through the FaSSIF-V2, $FaSSIF_{midgut}$, SIF_{ileum}, and FeSSCoF buffer compartments, where 8.8 ± 0.7% (1172 ± 98 mg), 15.0 ± 4.8% (1998 ± 641 mg), 18.7 ± 7.0% (2487 ± 928 mg), and 25.3 ± 12.7% (3364 ± 1691 mg) of the total acetaminophen dose was released, respectively. After the test was finished, some tablet residues were still present on the lower mesh of the inner cylinder (Fig. 5D.2).

Hard Capsules

The ben-u-ron gelatin capsule shells started to dissolve within 2 minutes after the test was started, releasing granular acetaminophen and talc. Single hard capsules released acetaminophen rapidly, reaching 83.1 ± 1.0% release (416 ± 5 mg) after 10 minutes in FaSSGF buffer. After 1 hr, 97.9 ± 0.5% (489 ± 2 mg) of acetaminophen had been released and no residual capsule material was present in the inner cylinders. Therefore, the experiment was not continued.

When 10 doses were tested, the residual material formed a clump after the capsule shells started to dissolve (Fig. 4C.3), impeding media flow. After 60 min, 57.7 ± 7.9% (2884 ± 393 mg) of the total dose had been released. The experiment continued in the FaSSIF-V2 buffer, where release reached 75.3 ± 6.1% (3763 ± 306 mg). In the $FaSSIF_{midgut}$ buffer compartment, all capsule material residues penetrated through the nylon mesh into the outer vessel, reaching 76.9 ± 6.5% drug release, which corresponds to 3846 ± 343 mg of acetaminophen.

When 20 hard capsules were tested, the residual material again formed a dense clump after dissolution of the capsule shells (Fig. 5C.3). This caused obstruction of the media flow. After 60 min in FaSSGF buffer, only 22.9 ± 1.3% (2292 ± 134 mg) of the total dose had been released. The experiment was continued through the FaSSIF-V2, $FaSSIF_{midgut}$, and SIF_{ileum} buffers, releasing 31.2 ± 3.6% (3116 ± 358 mg), 67.3 ± 2.6% (6730 ± 255 mg), and 71.6 ± 2.0% (7162 ± 199 mg) of the total acetaminophen dose, respectively. At that point, no residues were present in the inner cylinder, so the experiment was not continued.

Soft Capsules

Similar to the hard capsules, the gelatin capsule shell started to rupture and release acetaminophen into the test medium within 2 minutes, forming a suspension.

Acetaminophen was released very rapidly when one capsule was tested: after 10 minutes, 96.9 ± 0.6% (485 ± 3 mg) of the dose had been released. In the FaSSGF buffer, 98.5 ± 0.1% (492 ± 0.5 mg) of acetaminophen was released, and no capsule residues were present in the inner cylinders.

When 10 capsules were tested, acetaminophen release was still very rapid. After 10 minutes in FaSSGF buffer, 86.1 ± 0.9% (4304 ± 47 mg) of acetaminophen was released. After 60 minutes, 90.6 ± 0.7% (4529 ± 34 mg) of the total dose had been released. Similar to the single dose test, no residual material was observed in the inner cylinder, so the experiment was not continued. The remaining 10% of the dose did not dissolve within 60 minutes of the experiment, even though the whole capsule content was exposed to the medium.

When 20 soft capsules were tested, dissolution was considerably slower than for one or 10 capsules. After 10 min, 46.9 ± 7.8% (4685 ± 778 mg) of the total dose was released. After 1 hr in FaSSGF buffer, 55.3 ± 1.9% (5533 ± 192 mg) of the drug had been released. Contrary to the lower doses, some residual material was present on the inner cylinder mesh, so the experiment continued in the FaSSIF-V2 buffer compartment where 72.5 ± 12.8% (7247 ± 1277 mg) of acetaminophen was released. No capsule residue was present in the inner cylinders, so the experiment was not continued. The remaining 27.5% of the total dose did not dissolve in FaSSGF buffer or FaSSIF-V2 buffer sections (100 min in total) despite the capsule content being completely exposed to the medium.

Similarity Factor f_2 Calculations

The f_2 results indicate that the profiles of one, 10, 20, and, for IR tablets, 50 dosage units of the same formulation differ from each other significantly. The only exception applies to the profiles of one vs. 10 soft capsules, where the f_2 value was 50.85, indicating that these two profiles differ from each other by less than 10% and can therefore be regarded as similar.

For all doses tested, the f_2 values of ER tablets compared to the IR tablets, hard capsules, and soft capsules are lower than 50, as might be expected when an ER formulation is compared with its IR counterparts. Among the IR formulations, f_2 results indicated similarity of the dissolution profiles when one dosage unit was tested. All other comparisons (except 20 IR tablets vs. 20 hard capsules) were well below the cut-off for similarity (f_2 = 50).

The f_2 analysis was supported by the ANOVA results, where the same doses were compared among the formulations.

Among one, 10, and 20 doses, the dissolution data showed statistically significant differences ($p < 0.001$) at the 10- and 60-min time points.

DISCUSSION

Solubility Testing

The aqueous solubility of acetaminophen was reported by Kalantzi et al. to be 14.7 mg/mL at 20 °C, 14.3 mg/mL at 25 °C, and 23.7 mg/mL at 37 °C (*1*). Shaw et al. tested the solubility of acetaminophen in various buffers over the pH range 1.2–9 and reported values of 18.7 ± 0.2–24.8 ± 0.3 mg/mL (*11*). Our results (approximately 24 mg/mL at 37 °C) are in line with these data. Although polyvinylpyrrolidone (Povidone), which is listed among the excipients contained in the IR tablets, may positively influence the solubility of acetaminophen (*12*), it did not appear to influence the solubility of acetaminophen in the STADA tablets.

Dissolution Testing

USP Apparatus 2

Preliminary experiments were performed in FaSSGF and FaSSIF-V1 buffers at a reduced volume (100 mL). The volume chosen is lower than the 250 mL specified for co-ingestion of water in bioequivalence studies, because patients often ingest drugs with just a few swallows of water. In the USP apparatus 2 setup, the experiments with a single IR tablet were performed under sink conditions, whereas dissolution from 10 IR tablets was limited by the solubility of acetaminophen. Similar limitations may also occur in vivo. Because dissolution in the FaSSGF and FaSSIF-V1 buffers was not complete in USP apparatus 2 at higher doses, the experiments were extended to better understand whether dissolution could be completed in more distal regions of the GI tract. To facilitate simulation of the changes in pH all the way along the GI tract, USP apparatus 3 was selected for further studies.

USP Apparatus 3

The rapid release of acetaminophen from three IR formulations (tablets, hard capsules, and soft capsules) when tested as a single dose is commensurate with the objective of providing rapid pain relief. Likewise, part of the dose in ER tablet is released rapidly to guarantee a rapid analgesic effect, which is then maintained for up to 8 hours by the ER part of the tablet to reduce the dosing frequency.

When higher doses were tested, acetaminophen was released more slowly and less extensively as the dose was increased. This tendency was consistent among all four acetaminophen formulations. In an extreme case, when 50 doses of the IR tablet were tested, the release slowed dramatically and only a small fraction (less than 10%) was released even when the tablets were subjected to conditions representative of passage along a major part of the GI tract. The failure of release can be traced back to extensive clumping of the tablets (Fig. 6).

Figure 6. Photograph of 50 IR tablets after 60 minutes in FaSSGF buffer in USP apparatus 3. Clumped tablet material filled nearly the entire volume of the inner cylinder. This was observed in all five compartments (up to 6 hours).
IR: immediate release; FaSSGF: fasted state simulated gastric fluid; USP: United States Pharmacopeia.

This observation might be linked to the clinically observed formation of a pharmacobezoar. Acetaminophen tablets have been reported to form a bezoar, e.g., in a case report of 70 tablets taken by a male patient (7 g acetaminophen in total, administered as a fixed dose combination with dihydrocodeine phosphate) (*13*). Pharmacobezoar formation was also demonstrated by Li et al. ex vivo (*14*). In stomachs removed from pigs, 75 or 100 IR tablets (37.5 or 50 g of acetaminophen) formed a bezoar when they were brought in contact with 28 mL of simulated gastric fluid in a water bath for 4 hrs, but 50 tablets (25 g) did not form the bezoar (*14*). Although pigs are often used as the model animal for in vivo drug absorption, it is important to note that there are some anatomical differences between pig and human stomachs, i.e. the pig stomach is two to three times bigger than the human stomach (*15*). In other work, Hoegberg et al. reported that 30 ER tablets formed a bezoar in contact with 1000 mL of simulated gastric fluid, but 30 IR tablets did not (*16*). The amounts of the four dosage forms, tested within our study, are lower than those studied in the ex vivo and in vitro reports, except for the test with 50 IR tablets. However, we used more physiologically relevant volumes in USP apparatus 3 than Hoegberg et al.

The rate and extent of release in our acetaminophen dissolution model is in line with clinical data. In the

late 1970s, Rawlins et al. studied acetaminophen pharmacokinetics after intravenous (IV) and oral administration in six volunteers (*5*). A dose of 1000 mg IV, as well as doses of 500, 1000, and 2000 mg orally, was administered in a crossover regimen with a 1-week washout period. The study showed incomplete bioavailability of acetaminophen after oral administration of these doses (all of which are under the recommended total daily dose) (*5*). The maximum plasma concentration after oral administration of 500 or 1000 mg acetaminophen was reached within 1 hour, but after oral administration of 2000 mg, the maximum plasma concentration was not reached until 2 hours, suggesting that the acetaminophen absorption was slower when a higher dose was administered.

Spyker et al. recently published a population pharmacokinetic (PK) model for acetaminophen based on data from randomized clinical trials of supratherapeutic IR doses and ER and modified release (MR) formulations as well as overdose case reports (*17*). The ER and MR formulations contained 650 mg acetaminophen; ER tablets had a 50:50 ratio of IR to ER whereas MR tablets had a 69:31 ratio, and the mechanism of release from the ER portion differed (*17*). Using the model, simulations of various acetaminophen doses (up to 100 g) for IR, ER, and MR formulations were performed. It was shown that increasing the acetaminophen dose would result in reduction of the absorption rate and bioavailability for all formulations. The model also suggested differences among formulations. After overdose, bioavailability values were lowest for MR formulations, greater for ER, and highest for IR (*17*). In the present study, data from the USP apparatus 3 method agree with the population PK model, with just 20% of the dose released from ER tablets within 6 hrs compared to 60% (hard capsules) or 100% (IR tablets) when 20 doses were tested. Thus, our in vitro model may be helpful in predicting clinical PK data after acetaminophen overdose, as only released drug can be absorbed. However, it is difficult to test intentional overdoses greater than 25 g acetaminophen in the in vitro model due to the size limitation of USP apparatus 3.

Most studies reported in the literature are patient cases and clinical trials involving IR or ER tablets. This study with USP apparatus 3 also included soft and hard capsules. These formulations had similar (hard) or faster (soft) dissolution rates than that of IR tablets. This might be important information regarding safety precautions for the marketed soft capsule formulations, because they are expected to be absorbed faster and more completely at higher doses than the tablets.

Dissolution testing performed in USP apparatus 3 discriminated well between the four formulations. The ER tablet appears to be less prone to toxicity after overdosing. Importantly, the release and absorption of acetaminophen after intentional overdose (usually doses of 12–50 g) is shifted in time (*2*). Our results potentially link this shift to bezoar formation. So-called "double peak" (or "double hump") behavior has also been reported in the literature for acetaminophen pharmacokinetics in overdose cases. In the course of acetaminophen poisoning, a reduction of plasma concentration followed later by another rise in concentration was observed in some patients, regardless of the administration of an N-Acetyl cysteine antidote (*18*, *19*). The second peak might possibly be caused by disruption of a bezoar.

CONCLUSION

A novel in vitro dissolution model was established for the release of acetaminophen when ingested in supratherapeutic quantities. The experiments were designed to reflect the physiology of the human GI tract in a fasted state and to simulate the ingestion of a single standard dose of acetaminophen as well as various degrees of overdose. Four acetaminophen products were compared: IR tablets, ER tablets, hard capsules, and soft capsules. The results obtained with the model are in line with published data obtained in acetaminophen clinical trials and showed important differences among the various formulations. Additionally, the in vitro model was able to model pharmacobezoar formation at very high doses, which has also been reported in the literature. This in vitro model for studying acetaminophen overdose may also be useful for testing other drugs, such as opioid analgesics.

SUPPLEMENTAL MATERIAL

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

DISCLOSURES

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REFERENCES

1. Kalantzi, L.; Reppas, C.; Dressman, J. B.; Amidon, G. L.; Junginger, H. E.; Midha, K. K.; Shah, V. P.; Stavchansky, S. A.; Barends, D. M. Biowaiver monographs for immediate release solid oral dosage forms: acetaminophen (paracetamol). *J. Pharm. Sci*. **2006**, *95* (1), 4–14. DOI: 10.1002/jps.20477
2. Lee, W. M. Acetaminophen (APAP) hepatotoxicity-Isn't it time for APAP to go away? *J. Hepatol*. **2017**, *67* (6), 1324–1331. DOI: 10.1016/j.jhep.2017.07.005

3. Don't Double Up on Acetaminophen. U. S. Food & Drug Administration website. September 10, 2018. https://www.fda.gov/consumers/consumer-updates/dont-double-acetaminophen (accessed March 7, 2023).
4. Albert, K. S.; Sedman, A. J.; Wilkinson, P.; Stoll, R. G.; Murray, W. J.; Wagner, J. G. Bioavailability studies of acetaminophen and nitrofurantoin. *J. Clin. Pharmacol*. **1974**, *14* (5-6), 264–270. DOI: 10.1002/j.1552-4604.1974.tb02312.x
5. Rawlins, M. D.; Henderson, D. B.; Hijab, A. R. Pharmacokinetics of paracetamol (acetaminophen) after intravenous and oral administration. *Eur. J. Clin. Pharmacol*. **1977**, *11* (4), 283–286. DOI: 10.1007/BF00607678
6. Gunnell, D.; Murray, V.; Hawton, K. Use of paracetamol (acetaminophen) for suicide and nonfatal poisoning: worldwide patterns of use and misuse. *Suicide Life Threat. Behav*. **2000**, *30* (4), 313–326. DOI: 10.1111/j.1943-278X.2000.tb01098.x
7. Gelotte, C. K.; Auiler, J. F.; Lynch, J. M.; Temple, A. R.; Slattery, J. T. Disposition of acetaminophen at 4, 6, and 8 g/day for 3 days in healthy young adults. *Clin. Pharmacol. Ther*. **2007**, *81* (6), 840–848. DOI: 10.1038/sj.clpt.6100121
8. Markopoulos, C.; Andreas, C. J.; Vertzoni, M.; Dressman, J.; Reppas, C. In-vitro simulation of luminal conditions for evaluation of performance of oral drug products: Choosing the appropriate test media. *Eur. J. Pharm. Biopharm*. **2015**, *93*, 173–182. DOI: 10.1016/j.ejpb.2015.03.009
9. Franeta, J. T.; Agbaba, D.; Eric, S.; Pavkov, S.; Aleksic, M.; Vladimirov, S. HPLC assay of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets. *Farmaco* **2002**, *57* (9), 709–713. DOI: 10.1016/S0014-827X(02)01265-X
10. O'Hara, T.; Dunne, A.; Butler, J.; Devane, J. A review of methods used to compare dissolution profile data. *Pharm. Sci. Technol. Today* **1998**, *1* (5), 214–223. DOI: 10.1016/S1461-5347(98)00053-4
11. Shaw, L. R.; Irwin, W. J.; Grattan, T. J.; Conway, B. R. The effect of selected water-soluble excipients on the dissolution of paracetamol and Ibuprofen. *Drug Dev. Ind. Pharm*. **2005**, *31* (6), 515–525. DOI: 10.1080/03639040500215784
12. Garekani, H. A.; Sadeghi, F.; Ghazi, A. Increasing the aqueous solubility of acetaminophen in the presence of polyvinylpyrrolidone and investigation of the mechanisms involved. *Drug Dev. Ind. Pharm*. **2003**, *29* (2), 173–179. DOI: 10.1081/DDC-120016725
13. Yamaguchi, J.; Kinoshita, K.; Noda, A.; Furukawa, M.; Sakurai, A. Delayed increase in serum acetaminophen concentration after ingestion of a combination medications: a case report. *J. Int. Med. Res*. **2018**, *46* (8), 3435–3439. DOI: 10.1177/0300060518777860
14. Li, Y. K.; Lam, K. F.; Wong, C. L. W.; Wong, A. In vitro study of pharmacobezoar formation in simulated acetaminophen overdose. *Clin. Toxicol*. **2020**, *58* (9), 900–906. DOI: 10.1080/15563650.2019.1705971
15. Kararli, T. T. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm. Drug Dispos*. **1995**, *16* (5), 351–380. DOI: 10.1002/bdd.2510160502
16. Hoegberg, L. C. G.; Refsgaard, F.; Pedersen, S. H.; Personne, M.; Ullah, S.; Panagiotidis, G.; Petersen, T. S.; Annas, A. Potential pharmacobezoar formation of large size extended-release tablets and their dissolution – an in vitro study. *Clin. Toxicol. (Phila.)* **2019**, *57* (4), 271–281. DOI: 10.1080/15563650.2018.1513138
17. Spyker, D. A.; Dart, R. C.; Yip, L.; Reynolds, K.; Brittain, S.; Yarema, M. Population pharmacokinetic analysis of acetaminophen overdose with immediate release, extended release and modified release formulations. *Clin. Toxicol*. **2022**, *60* (10), 1113–1121. DOI: 10.1080/15563650.2022.2114361
18. Hendrickson, R. G.; McKeown, N. J.; West, P. L.; Burke, C. R. Bactrian ("double hump") acetaminophen pharmacokinetics: a case series and review of the literature. *J. Med. Toxicol*. **2010**, *6* (3), 337–344. DOI: 10.1007/s13181-010-0083-9
19. Tan, H.; Stathakis, P.; Varghese, B.; Buckley, N. A.; Chiew, A. L. Delayed acetaminophen absorption resulting in acute liver failure. *Case Rep. Crit. Care* **2022**, *2022* (3672248), 3672248. DOI: 10.1155/2022/3672248