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Gastrointestinal and Systemic Disposition of Diclofenac under Fasted and Fed State Conditions Supporting the Evaluation of *in Vitro* Predictive Tools

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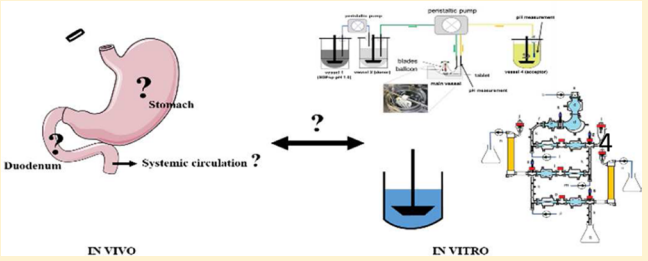
ABSTRACT: This study aimed to gain further insight into the gastrointestinal disposition of the weakly acidic BCS class II drug diclofenac and the implications for systemic drug exposure in humans under fasted and fed state conditions. For this purpose, gastrointestinal and blood samples were collected from healthy volunteers after oral intake of a commercially available tablet of the potassium salt of diclofenac (i.e., Cataflam) in different prandial states. Subsequently, these *in vivo* data served as a reference for the evaluation of *in vitro* tools with different levels of complexity, i.e., a conventional USP II dissolution apparatus, a modified version of the dynamic open flow through test apparatus, and the TNO gastrointestinal model equipped with the recently developed advanced gastric compartment (TIMagc). *In vivo* data suggested impaired drug dissolution and/or immediate precipitation in the fasted stomach, linked to the acidity of the gastric environment. Similarly, a vast presence of solid drug material in the stomach was observed under fed state conditions, which could be attributed to a marked delay in intragastric tablet disintegration after drug intake with a meal. Emptying of solid drug from the stomach into the duodenum generally resulted in rapid intestinal drug (re)dissolution in both test conditions, explaining the absence of a food effect on the extent of overall systemic exposure for diclofenac. *In vitro* tools were found to be capable of predicting *in vivo* intraluminal (and systemic) disposition of this compound, the extent of which depended on the degree to which the dynamic nature of the gastrointestinal process(es) to be investigated was simulated.



KEYWORDS: *oral drug delivery, biopharmaceutics, gastrointestinal tract, food effects, pharmacokinetics, USP II dissolution apparatus, dynamic open flow through test apparatus, TIMagc*

1. INTRODUCTION

In recent years, the use of *in vitro* and *in silico* tools in early stage evaluation of formulations for drug candidates has received increased attention. In theory, early stage comparison of formulations should aid in identifying the appropriate formulation strategy for a drug candidate of interest. In addition, the use of *in vitro* tools to evaluate dosage form performance may reduce the need for extensive animal testing in the nonclinical phase of drug development, in line with the 3R principle (i.e., replace, reduce, and refine). In the past, however, *in vitro* tools have mainly been used for quality control purposes, for which the biorelevant representation of gastrointestinal conditions (e.g., media composition, hydrodynamics, absorption) is not required.[1] As a result, these classical *in vitro* dissolution tools often lack predictive power, hereby limiting the added value of such tools in the drug development process. In the context of validating and improving biorelevant *in vitro* tools, the IMI-initiative OrBiTo (i.e., Oral Biopharmaceutics Tools) aims to (i) better understand what happens with a dosage form in the gastrointestinal tract after oral administration and (ii) translate these insights into *in vitro*/*in silico* tools that are predictive for *in vivo* drug behavior.[2]

High-quality *in vivo* data are essential to gain insight into gastrointestinal drug disposition. To this end, aspiration of

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intraluminal contents has been successfully performed to characterize both the gastrointestinal environment (e.g., fluid composition, regional intestinal permeability)[3−7] and drug disposition in the gastrointestinal tract after oral intake in humans.[8−11] In the past, these data have been utilized (i) to improve media simulating intraluminal fluid composition[12] and (ii) for the optimization and evaluation of new and/or existing *in vitro* tools and computational models.[13,14] In this study, the ability of *in vitro* tools to accurately predict *in vivo* drug disposition was further evaluated. First, the well-established intraluminal sampling technique was used to characterize the gastrointestinal behavior of a weakly acidic BCS class II drug in humans. Diclofenac, formulated as an immediate-release (IR) tablet of its potassium salt (Cataflam), was chosen as a model compound. In the past, diclofenac has been used to study the supersaturation/precipitation behavior of a weakly acidic compound in the upper gastrointestinal tract.[15] To allow the unambiguous assessment of these processes, this compound was administered as a solution of its potassium salt in water (i.e., predissolved tablet). Consequently, important processes that influence intraluminal drug disposition (e.g., dosage form disintegration, drug dissolution) were not investigated in this study. Therefore, the present study aimed to explore the intraluminal disposition of diclofenac under real-life dosing conditions (i.e., tablet as such). Emphasis was on investigating processes such as tablet disintegration, drug dissolution, and food-related changes in intraluminal drug disposition by collecting aspirates from healthy volunteers as a function of time after oral drug intake. In addition, blood samples were collected, in order to elucidate how gastrointestinal drug behavior may influence systemic drug disposition. Second, the *in vivo* data set was used as a reference for the evaluation of *in vitro* tools with different levels of complexity regarding their ability to predict trends observed *in vivo*. Selected tools comprised (i) the USP II dissolution apparatus, conventionally used in quality control testing, (ii) the modified dynamic open flow through test apparatus, and (iii) the TNO gastrointestinal model equipped with the recently developed advanced gastric compartment (TIMagc).

2. MATERIALS AND METHODS

2.1. Chemicals. *2.1.1. In Vivo Study and USP II Dissolution Experiments.* Acetonitrile (ACN) and methanol (MeOH) were purchased from Fisher Scientific (HPLC grade; Leicestershire, U.K.) and Acros Organics (HPLC grade; Geel, Belgium), respectively. Acetic acid (HOAc) and sodium chloride (NaCl) were ordered from VWR Belgium (99−100% p.a.; Haasrode, Belgium), and formic acid (HCOOH) was ordered from Biosolve (99%; Valkenswaard, The Netherlands). Sodium acetate trihydrate ($NaOAc{\cdot}3H_2O$) was purchased from Chem-Lab (Zedelgem, Belgium), while disodium monohydrogen phosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$) and sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$) were ordered from Sigma-Aldrich (St. Louis, MO). Geigy (Basel, Switzerland) supplied diclofenac free acid, and $^{13}C_6$-diclofenac was purchased from Alsachim (Illkirch Graffenstaden, France). Simulated gastrointestinal fluids for *in vitro* purposes were made using FaSSIF/FeSSIF/FaSSGF powder (Biorelevant.com, London, U.K.). Sodium hydroxide (NaOH) was purchased from Merck (pellets p.a.; Darmstadt, Germany). Water was purified using a Maxima system (Elga Ltd., High Wycombe Bucks, U.K.). Cataflam tablets (50 mg of diclofenac potassium; Novartis, Basel, Switzerland) were ordered from the hospital pharmacy of the University Hospitals Leuven (UZ Leuven, Leuven, Belgium).

2.1.2. Modified Dynamic Open Flow Through Test Apparatus Experiments. Potassium dihydrogen phosphate (KH_2PO_4, pure) and NaOH (pellets, pure) were purchased from Applichem (Darmstadt, Germany). Hydrochloric acid (HCl 37%, fuming) and sodium acetate (NaOAc, pure) were ordered from Merck (Darmstadt, Germany), while NaCl was purchased from Sigma-Aldrich (St. Louis, MO). Caelo (Hilden, Germany) and VWR (Darmstadt, Germany) supplied diclofenac sodium and MeOH (HPLC grade), respectively. Water was purified using a Milli-Q system (Merck, Darmstadt, Germany).

2.1.3. TNO Gastrointestinal Model−Advanced Gastric Compartment (TIMagc) Experiments. Biosolve (Valkenswaard, The Netherlands) supplied ACN (UPLC/MS grade) and HCOOH (99%, UPLC/MS grade). Bicarbonate, HCl (HPLC-S grade) and electrolytes (p.a.) were ordered from Merck (Schiphol-Rijk, The Netherlands). Enzymes as well as diclofenac sodium (100%, analytical standard) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Bile was ordered from Vion (Apeldoorn, The Netherlands). Milli-Q water was used for all experiments and for analytical purposes.

2.2. *In Vivo* Study. *2.2.1. Study Design.* Six healthy volunteers (age range: 20−26 years old; 4 males, 2 females) participated in a crossover study in which the following conditions were tested:

- Administration of one tablet of Cataflam (50 mg diclofenac potassium) with 240 mL of tap water under fasted state conditions.
- Administration of one tablet of Cataflam (50 mg diclofenac potassium) with 240 mL of tap water under fed state conditions.

Prior to enrolment in the study, all volunteers underwent a medical examination by a physician affiliated with the department of Gastroenterology (UZ Leuven). Volunteers suffering from hepatitis B/C and/or HIV infection were excluded from participation in order to guarantee the safety of the study personnel. Furthermore, illness at the time of the study, medication use, a history of acute/chronic gastrointestinal disease(s), (possible) pregnancy, and/or frequent exposure to radiation during the previous year were criteria for exclusion. The study was approved by the Federal Agency for Medicines and Health Products (FAHMP; EudraCT reference number 2013-004636-29) and the Medical Ethics Committee of the University Hospitals Leuven (ML10131) and was performed following the tenets of the Declaration of Helsinki. All volunteers provided written informed consent prior to the start of the study.

2.2.2. Study Protocol. Volunteers were asked to refrain from eating and to only drink water 12 h prior to the start of the study in order to ensure fasted state conditions. Upon arrival at the hospital, double-lumen catheters (Salem Sump PVC Gastroduodenal Tube, 14 Ch (4.7 mm) × 108 cm; Medtronic, Dublin, Ireland) were positioned in the participant's stomach (antrum) and duodenum (D2/D3 segment) via oral and/or nasal intubation to enable the aspiration of gastrointestinal fluids. The correct position of the aspiration catheters was guided and verified by fluoroscopic imaging. After a settling period of 10 min, volunteers were asked to ingest one tablet of Cataflam with 240 mL of tap water. In the case of drug administration under fed state conditions, 400 mL of Ensure

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Plus (liquid meal, 600 kcal; Sorgente, Houten, The Netherlands) was administered to fasted volunteers 20 min prior to oral drug administration. Subsequently, gastrointestinal aspirates and venous blood samples were collected at predetermined time points as a function of time. Under fasted state conditions, gastrointestinal contents were sampled for 3 h, i.e., 2, 7, 12, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 135, 150, 165, and 180 min after oral drug intake (t_0); under fed state conditions, samples were aspirated every 15 min during a 5 h period. In both test conditions, blood samples were collected in heparinized tubes (BD Vacutainer systems, Plymouth, U.K.) for 8 h, i.e., 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 210, 240, 270, 300, 360, 420, and 480 min after drug intake under fasted state conditions, and 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 255, 270, 285, 300, 330, 360, 390, 420, 450, and 480 min under fed state conditions.

2.2.3. Sample Preparation and Analysis. 2.2.3.1. Intraluminal Samples. Immediately after sampling, pH of the aspirate was measured to monitor changes in gastrointestinal pH as a function of time (Hamilton Knick Portamess, Bonaduz, Switzerland). Furthermore, part of the gastrointestinal aspirate was directly diluted to determine total drug content (i.e., diclofenac as solid and solute). A second part of the same aspirate was immediately centrifuged (20817*g*, 5 min; Microcentrifuge 5424, VWR International) to separate solid and liquid material, after which the supernatant was diluted to determine dissolved drug content (i.e., diclofenac as solute). Fasted and fed state aspirates were diluted in methanol:water (50:50 v/v) and methanol:0.1 M phosphate buffer pH 7 (50:50 v/v), respectively. Diluted samples were stored on ice pending analysis on the same day. Samples were analyzed using RP-HPLC with UV detection (279 nm; Chromaster 5410 UV detector, VWR International). Separations were performed on a Novapak C18 column under radial compression (4 μm, 8 × 100 mm, Waters, Milford, MA, USA). After injection of 100 μL of sample, diclofenac was isocratically eluted using a mixture of methanol and 25 mM sodium acetate buffer pH 3.5 (82:18 v/v) at a flow rate of 1.0 mL·min^{-1} for 10 min, resulting in a retention time of 6.5 min. After 10 min, a washing step was performed with acetonitrile:water (90:10 v/v) and water:25 mM sodium acetate buffer pH 3.5 (75:25 v/v) for 2 min and 1 min, respectively. Finally, the column was reconditioned with methanol:25 mM sodium acetate buffer pH 3.5 (82:18 v/v) for 2 min. Linearity was demonstrated over the range of 10,000 nM to 19.5 nM. For validation purposes, control samples were prepared in the corresponding relevant media containing 4000, 500, and 50 nM diclofenac. Concentrations of diclofenac could be precisely (intraday variability RSD ≤ 4.3% (n = 5) and interday variability RSD ≤ 10.3% (n = 5)) and accurately (range: 86.4−101.9%) determined in gastrointestinal samples. All criteria met the FDA requirements for bioanalytical method validation.

2.2.3.2. Plasma Samples. During the course of the experiment, venous blood samples were stored on ice. After finalizing the experiment, samples were centrifuged (1699*g*, 15 min, 37 °C; Centrifuge 5804R, Eppendorf, Hamburg, Germany) and the supernatant (plasma) was stored at −26 °C pending analysis. To quantify concentrations of diclofenac in plasma, 100 μL of plasma was added to 400 μL of methanol containing an internal standard (10 nM of $^{13}C_6$-diclofenac). After the mixture was vortexed for ten seconds, samples were centrifuged at 20238*g* (5 min, 4 °C). The supernatant was transferred to a microvial, followed by a 1 μL injection in an Acquity H-class UPLC system (Waters, Milford, MA, USA). Separation was performed using a Kinetex XB - C18 column (2.6 μm, 2.1 × 50 mm; Phenomenex, Utrecht, The Netherlands) held at 35 °C. The mobile phase consisted of a mixture of methanol (solvent A) and 0.05% formic acid in water (solvent B) at a flow rate of 500 μL·min^{-1}. Gradient elution was performed as follows: 65% of solvent A during 0.9 min, followed by 95% A for 1.6 min. After 2.5 min, solvent A decreased from 95% to 65%. Prior to the next injection, the column was re-equilibrated with 65% of solvent A and 35% of solvent B during 1.5 min. Diclofenac eluted after 1.07 min. An MS/MS positive ionization mode was carried out with an HESI source on a Xevo TQ-S micro mass detector (Waters, Milford, MA, USA) with the following parameters: 150 °C source temperature, 0.80 kV capillary voltage, 30 V cone voltage, 50 L N_2·h^{-1} cone flow, 800 L N_2·h^{-1} desolvation gas flow, 350 °C desolvation temperature. The mass transitions used for the detection of the different compounds were m/z diclofenac 296.11 → 214.00 (collision energy: 30 V) and m/z $^{13}C_6$-diclofenac 302.11 → 220.00 (collision energy: 30 V) with a dwell time of 65 ms for both compounds. The UPLC eluent was guided into the MS using a divert valve within the time frame of 0.7−1.6 min and into the waste for the remaining time. A calibration curve was made in plasma by serial dilution. Linearity was demonstrated over the range of 10,000 nM to 1.22 nM. The internal standard, $^{13}C_6$-diclofenac, was dissolved in methanol and spiked at a final concentration of 8 nM. For validation purposes, control plasma samples were prepared containing 5000, 500, 50, and 5 nM diclofenac. Concentrations of diclofenac could be precisely (intraday variability RSD ≤ 5.0% (n = 6) and interday variability RSD ≤ 19.7% (5 nM, n = 5)) and accurately (range: 88.5−100%) determined in plasma samples. All criteria met the FDA requirements for bioanalytical method validation.

2.3. *In Vitro* Studies. *2.3.1. USP II Dissolution Apparatus.* In a first set of *in vitro* experiments, dissolution testing was performed using a conventional USP II dissolution apparatus (SR8-PLUS dissolution test station, Hanson Research, Chatsworth, CA, USA). One tablet of Cataflam was added to a 500 mL dissolution vessel filled with 250 mL of fasted state simulated gastric fluid pH 1.6 (FaSSGF). The medium was kept at a constant temperature of 37 °C and was continuously stirred at a paddle speed of 75 rpm. 1 mL samples were collected 5, 10, 20, and 30 min after the start of the experiment. Subsequently, transfer of gastric contents to the small intestine was simulated by adding double-concentrated fasted state simulated intestinal fluid pH 7.5 (2×-FaSSIF) to the vessel to end up with regular FaSSIF pH 6.5. 2×-FaSSIF was made by adding twice the prescribed amount of FaSSIF/FeSSIF/FaSSGF powder to the phosphate buffer medium with twice the buffer strength of regular FaSSIF. Additional samples were collected after 35, 40, 50, 60, 90, and 120 min. Experiments were performed in triplicate. Sample preparation and determination of both total and dissolved drug concentrations in the collected samples were similar to the protocol for the *in vivo* samples (see section 2.2.3.1).

2.3.2. Modified Dynamic Open Flow Through Test Apparatus. The dynamic open flow through test apparatus has been developed by Garbacz et al. to simulate gastric fasting conditions during *in vitro* dissolution testing.[16] In this study, a modified version of the model was used to investigate intraluminal drug disposition. A schematic representation of

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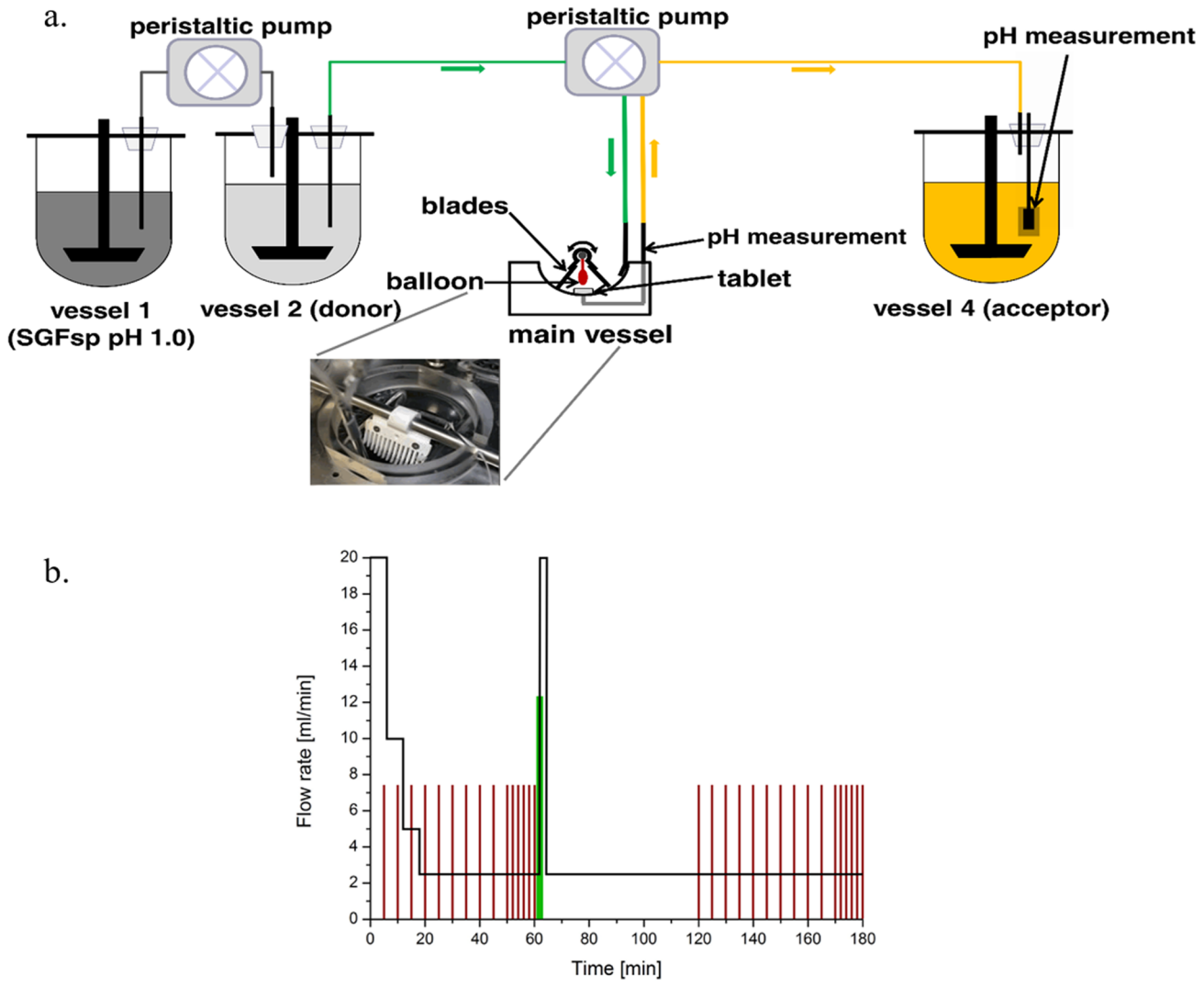

Figure 1. (a) Schematic representation of the modified dynamic open flow through test apparatus. (b) Operating protocol for the simulation of flow rate changes and pressure events in the gastric compartment as a function of time. Gray line represents flow rate as a function of time. Vertical lines represent single pressure events (6 s, 200 mbar) at a given time point. After 1 h, a brief period of intense pressure events (3 pressure events, 6 s each, 300 mbar) is simulated (bold vertical line) mimicking MMC phase III (i.e., gastric housekeeper wave).

the modified model is depicted in Figure 1a. The central part of the model is a pipelike bar that can be rotated by means of a programmable stepping motor. On the opposite side of the bar, a supply of compressed air is regulated, facilitating inflation and deflation of a balloon inside the main vessel (i.e., gastric compartment, total volume: ~80 mL). Hereby, time-dependent pressure forces exerted on a dosage form inside the stomach can be simulated. The balloon is mounted between two comb-shaped blades that define maximum balloon inflation. Upon rotation of the bar, the blades and the balloon perform an oscillatory movement inside the main vessel. During the experiment, the dosage form is placed inside the main vessel enabling its free floating. A programmable peristaltic pump (IPC 16, Ismatec, Germany) facilitates perfusion of the main vessel with medium stored in a donor vessel as well as transfer of main vessel contents to the acceptor vessel (i.e., duodenal compartment) at biorelevant flow rates (diameter perfusion tubes: 2.4 mm). Since the model has been specifically designed to simulate fasted state conditions, drug disposition under fed state conditions cannot be investigated using this particular setup.

Experiments in the modified dynamic flow through test apparatus were performed with 50 mL of demineralized water in the main vessel (n = 3). In the acceptor vessel, 300 mL of phosphate buffer pH 6.8 was used as the dissolution medium. During the experiments, the main vessel was perfused with a mixture of simulated gastric fluid without pepsin (SGFsp, pH 1.0) and demineralized water from the donor vessel. For this purpose, SGFsp was transferred to the donor vessel (start volume: 1 L of demineralized water) at a rate of 11.2 mL·min^{-1}. Initially, the flow from the donor to the main vessel and from the main to the acceptor vessel was set at 20 mL·min^{-1} and declined exponentially to reach a basal flow rate of 2.5 mL·min^{-1} after 18 min, simulating the emptying of 240 mL of water from the stomach within 30 min after ingestion (Figure 1b).[17] Given the fact that the flow rate into the main vessel equals the flow rate out of the vessel, its volume is kept constant (i.e., 50 mL) throughout the experiment; the volume of the acceptor vessel increased as a function of time. pH in the acceptor vessel remained constant during the experiments by ensuring adequate buffer capacity of the phosphate buffer. Pressure events inside the main vessel were programmed in

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order to simulate *in vivo* gastric contractility patterns (Figure 1b). Under fasted state conditions, gastrointestinal motility is characterized by a triphasic, cyclical pattern called the *migrating motor complex* (MMC): a period of contractile quiescence (i.e., MMC phase I) is followed by a period of irregular, moderate contractions (i.e., MMC phase II) and finally by a brief period of strong contractions (i.e., MMC phase III or gastric housekeeper wave), after which the cycle recommences.[18−20] At the start of the experiments, single pressure events (6 s, 200 mbar) were simulated every 5 min for 50 min and every 2 min for the next 10 min (i.e., early and late MMC phase II). After 1 h, a gastric housekeeper wave (i.e., MMC phase III) was simulated by means of three consecutive pressure events (6 s, 300 mbar each) accompanied by a 1 min oscillatory movement (90°, 100 rpm). For the next hour, no additional pressure events were programmed to mimic a period of contractile quiescence in the stomach (i.e., MMC phase I), after which a new cycle of phase II like contractions was simulated. During each pressure event, flow rate was briefly increased to 20 mL·min^{-1} to mimic the increased transpyloric flow of fluids during a peristaltic contraction wave.[21,22] 1 mL samples were collected from both the main and acceptor vessels 2, 7, 12, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 135, 150, 165, and 180 min after the start of the experiment. Prior to sampling, a single oscillatory movement was performed (90°, 100 rpm). Immediately after sampling, 1 mL of donor medium was added to the main vessel to make up for the aspirated volume. Sample preparation and determination of both total and dissolved drug concentrations in the collected samples were similar to the protocol for the *in vivo* samples (see section 2.2.3.1). pH was continuously recorded in both the main vessel (i.e., directly after medium outlet) and the acceptor vessel.

2.3.3. TNO Gastrointestinal Model−Advanced Gastric Compartment (TIMagc). The TNO gastrointestinal model-1 (TIM-1) is a computer-controlled, dynamic *in vitro* model simulating the successive dynamic conditions in the stomach and small intestine. The system consists of a stomach compartment and three compartments mimicking the small intestine (duodenum, jejunum, and ileum) connected by peristaltic valves, including a simulated ileocecal valve for ileal effluent (Figure 2). The reader is referred to earlier publications for a detailed description of the TIM-1 system.[23−25] Briefly, the system consists of a simulated pyloric sphincter for controlled gastric emptying of liquids and solids (particle size < 3−5 mm) with specific settings for the simulation of fasted and fed state conditions (see section 2.3.3.1). The conditions in the compartments are computer-controlled via pH, temperature, volume level, and pressure sensors. Gastric secretions consist of (i) artificial saliva with electrolytes and alpha-amylase and (ii) gastric juice with hydrochloric acid, pepsin, and lipase. In the small-intestinal compartments, secreted fluids consist of bicarbonate, electrolytes, pancreatic juice with digestive enzymes, and bile.[24,26] Jejunal and ileal compartments are connected to a polysulfone plasma filter (cutoff value, 50 nm; surface area, 0.3 m^2; Plasma Flux P1 dry, lot UID19100, Fresenius Medical Care, Bad Homburg, Germany) to remove released compound from the intraluminal environment. The amount of drug in the collected filtrate is considered the fraction available for absorption from the upper gastrointestinal tract, i.e., the bioaccessible amount. The bioaccessible amount, as measured in the TIM system, has been shown to correlate very well with the *in vivo* bioavailability of pharmaceutical compounds.[27] In this study, the recently developed "advanced gastric compartment" (TIMagc) was used to simulate the stomach.[28] This compartment has been designed based on anatomical and physiological characteristics of the stomach and is more suited for the investigation of specific research questions (e.g., the interaction between food and gastric behavior) than the standard TIM gastric compartment. The TIMagc consists of three different parts, each representing a specific part of the stomach: gastric body or corpus (Figure 2c), proximal antrum (Figure 2d), and distal antrum (Figure 2e). Within this compartment, physiological pressure and shear forces, gastric motility, gastric emptying, and secretion of gastric juice can be simulated.

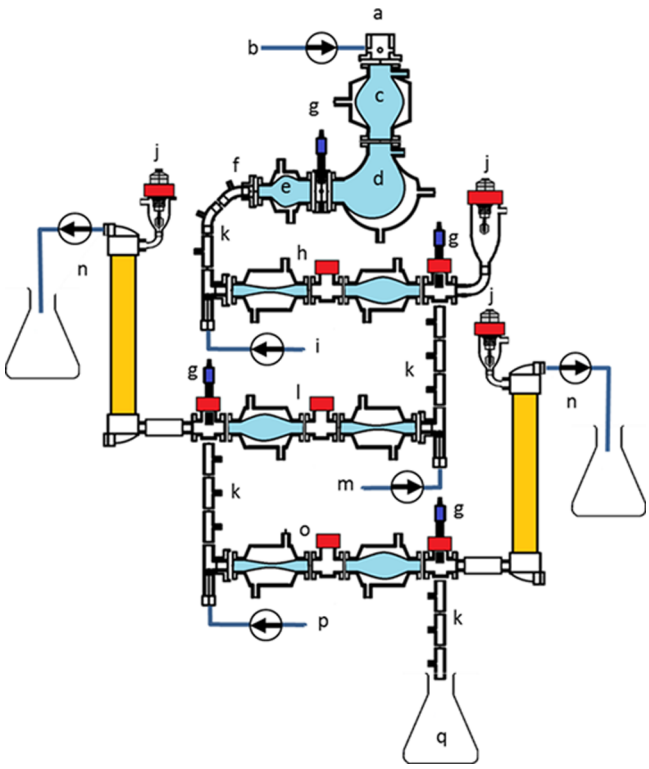

Figure 2. Schematic representation of the TIM-1 system with the advanced gastric compartment (TIMagc): a, meal inlet; b, gastric secretion; c, corpus; d, proximal antrum; e, distal antrum; f, pyloric valve; g, pH electrode; h, duodenum; i, duodenal secretion; j, level sensor; k, peristaltic valve pump; l, jejunum; m, jejunal secretion; n, filtration system; o, ileal compartment; p, ileal secretion; q, ileal effluent.

2.3.3.1. Simulation of Fasted and Fed State Conditions. In this study, TIMagc experiments were performed under fasted and fed state conditions in duplicate (n = 2). Under fasted state conditions, one tablet of Cataflam was introduced in the gastric compartment (containing 30 mL of gastric fluid pH 2), together with 240 mL of water. Under fed state conditions, the dosage form was introduced in the gastric compartment (containing 10 mL of gastric fluid pH 2) with 200 mL of Ensure Plus and 100 mL of water (mixed prior to administration).

Average conditions in the upper gastrointestinal tract of healthy human adults were simulated. Under fasted state conditions, gastric emptying was simulated using first order kinetics (gastric emptying $t_{1/2}$: 10 min).[29] Gastric pH dropped from 3.0 to 1.8 ± 0.2 within 30 min by simulated secretion of HCl. Under fed state conditions, gastric emptying followed a power exponential curve, simulating an initial lag phase in the

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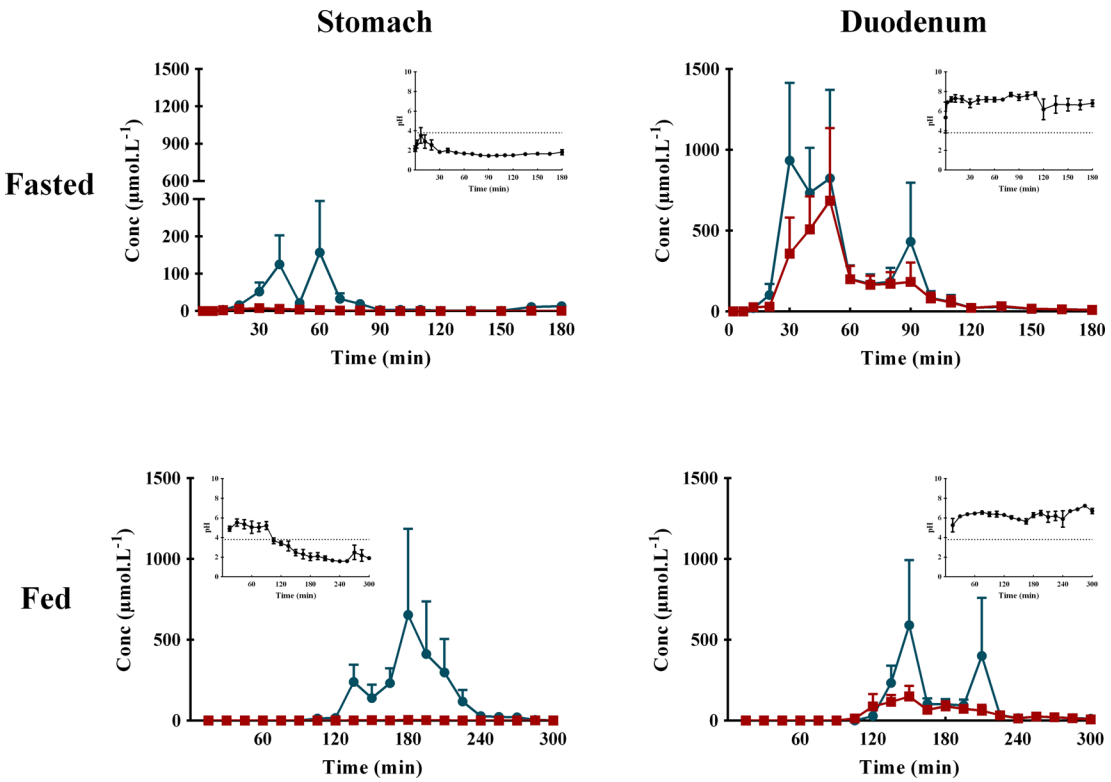

Figure 3. Mean (+ SEM) intraluminal drug concentration−time profiles in healthy volunteers (n = 6) after oral intake of one tablet of Cataflam (50 mg diclofenac potassium) with 240 mL of water under fasted (top) and fed state (bottom) conditions. (■) Dissolved drug concentration. (●) Total drug concentration (solid + solute). (Left) Drug concentration in the stomach as a function of time. (Right) Drug concentration in the duodenum as a function of time. Insets depict mean (± SEM) pH profile in the corresponding section of the gastrointestinal tract as a function of time.

emptying pattern of the meal (gastric emptying $t_{1/2}$: 80 min).[29] Gastric pH dropped from 6.5 to 4.1 ± 0.2 during the first 120 min and to 2.1 ± 0.2 within the next hour. Duodenal pH under fasted and fed state conditions was 6.2 ± 0.3 and 6.5 ± 1.1, respectively, while pH in the jejunal and ileal compartments was kept at 6.5 ± 0.2 and 7.3 ± 0.2, respectively. pH in the intestinal compartments was controlled by secretion of sodium bicarbonate. A gastric housekeeper wave (i.e., MMC phase III) was simulated after 1 h for the fasted state and after 3 h for the fed state by opening the pyloric valves to allow mixing of duodenal with gastric contents. While gastric volume was programmed to change as a function of time (i.e., simulation of gastric emptying), volume levels inside the intestinal compartments were kept constant via the continuous secretion of fluids and filtration through the semipermeable filter membranes. Bile secretion under fasted state conditions was five times less concentrated than under fed state conditions, resulting in final bile salt concentrations of approximately 2 and 10 mM, respectively, similar to physiological intraluminal concentrations after taking a drug with water or a meal.[4,30]

Luminal samples were collected from the antrum region of the stomach 2, 7, 12, 20, 30, 40, 50, and 60 min after the start of the experiment under fasted state conditions and 2, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 min after the start of the experiment under fed state conditions. Simultaneously, duodenal samples were collected under fasted state conditions after 2, 7, 12, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 min, and after 2, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, and 240 min under fed state conditions. Filtrates from the jejunal and ileal compartments as well as ileal effluent were collected in 0.5 h aliquots (0−0.5 h, 0.5−1 h etc.) for five (fasted state) or six (fed state) hours. At the end of the experiment, the residues in the gastric and intestinal compartments were collected. The volumes of the collected fluids were measured, and subsamples were stored in duplicate at 2−10 °C pending analysis. Secretion and filtrate fluid volumes were documented before the start and at the end of the experiments. During the experiments, all settings were continuously monitored and logged. These data were used to assess the technical quality of each experiment based on predetermined quality criteria.

2.3.3.2. Mechanistic Study of Tablet Disintegration under Simulated Fed State Conditions. Additional TIMagc experiments were performed to further investigate factors affecting dosage form disintegration under simulated fed state conditions. Three different scenarios were tested in singular experiments. In these experiments, only total drug concentrations were measured, as this suffices for the investigation of dosage form disintegration behavior. Apart from changes in dosage form administration and/or gastric acid secretion, experiments were performed under simulated fed state conditions as described in section 2.3.3.1. In a first scenario, the dosage form was coadministered with 300 mL of Ensure Plus (i.e., Ensure Plus only) as opposed to coadministration with a mixture of 200 mL of Ensure Plus and 100 mL of water. In a second scenario, the pH inside the gastric compartment was kept at a constant, high level by secreting water instead of hydrochloric acid (i.e., high pH). Finally, a combination of the above-mentioned scenarios (Ensure Plus only + high pH) was tested.

2.3.3.3. Sample Analysis. Diclofenac concentration in the collected samples was determined using UPLC with UV

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detection (Waters, Milford, MA, USA) at 276 nm. After injection of 5 μL of sample, separations were performed at a flow rate of 0.5 mL·min^{-1} using a Kinetex phenyl-hexyl column (1.7 μm, 2.1 × 100 mm; Phenomenex, Utrecht, The Netherlands) held at 40 °C. The mobile phase consisted of a mixture of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The percentage of solvent A decreased from 75 to 0% within a time frame of 1.3 min. After 3 min, the column was re-equilibrated for 1 min using the initial starting conditions.

2.4. Data Presentation. Data in text are reported as mean ± SD unless stated otherwise. The mean fraction of drug present as solid material in the stomach or the *in vitro* simulated gastric compartment was calculated using following equation:

$$\text{mean solid fraction} = \frac{1}{n}\sum_{t=1}^{n}\frac{(\text{drug}_{\text{total}})_t - (\text{drug}_{\text{dissolved}})_t}{(\text{drug}_{\text{total}})_t}$$

where $(\text{drug}_{\text{total}})_t$ is total drug content (solid + solute) at time point t and $(\text{drug}_{\text{dissolved}})_t$ is dissolved drug content at time point t.

A nonparametric Wilcoxon test was used for statistical hypothesis testing on the obtained *in vivo* data. Differences between test conditions were considered statistically significant at $p < 0.05$.

In vivo data are graphically presented as mean + SEM ($n = 6$) to improve the comprehensibility of the graphs. Data from the USP II two-stage dissolution experiments ($n = 3$) and the experiments with the modified dynamic open flow through test apparatus ($n = 3$) are presented as mean + SD. With regard to the TIMagc experiments, drug content in the collected gastric and duodenal samples as a function of time is presented as mean ± range ($n = 2$). The mean amount of diclofenac present in the filtrate (30 min aliquots) was calculated by multiplying the drug concentration in the filtrate with the volume of the filtrate (i.e., bioaccessible amount) and was expressed relative to the total drug content recovered from all compartments (i.e., recovery). Subsequently, the cumulative amount of diclofenac available for absorption over a 5 h (fasted state conditions) or a 6 h (fed state conditions) period was calculated.

3. RESULTS AND DISCUSSION

3.1. *In Vivo* Study. *3.1.1. Gastrointestinal Drug Disposition. 3.1.1.1. Fasted State.* Under fasted state conditions, one tablet of Cataflam (50 mg of diclofenac potassium) was administered to healthy volunteers ($n = 6$) with 240 mL of tap water. In this test condition, tablet disintegration was initiated within 7 to 12 min after oral drug intake, as indicated by the presence of drug material (solid and/or solute) released from the dosage form in the aspirated gastric fluids. Coadministration of water did in general not result in an increase in gastric pH (mean pH: 1.9 ± 0.9) above the pK_a of diclofenac (i.e., 3.8; Figure 3 inset). This particular situation was only briefly observed for two out of six volunteers, in which gastric pH returned to values below the pK_a of diclofenac before disintegration of the dosage form was initiated. During its residence in the stomach, diclofenac was mainly present as a solid for all volunteers, as can be deduced from the difference between total (i.e., diclofenac as solid and solute) and dissolved (i.e., diclofenac as solute) drug concentrations (Figure 3) and the calculated mean solid fraction (0.77 ± 0.23). Most likely, the acidity of the gastric environment (Figure 3a inset) causes incomplete dissolution and/or immediate precipitation of diclofenac following dissolution of its potassium salt.

Drug transfer from the stomach to the duodenum started as soon as tablet disintegration was initiated (Figure 3). Upon transfer, diclofenac was generally found to be fully dissolved in the duodenum, as evidenced by the similarity between total and dissolved drug concentrations measured in duodenal aspirates. Again, this finding is most likely linked to the pH of the luminal fluids. Mean pH in fasted state duodenal aspirates amounted to 7.1 ± 1.0, promoting the rapid (re)dissolution of solid drug particles being emptied from the stomach. This statement is corroborated by results from an earlier study, in which rapid redissolution of gastric drug precipitate upon entry in the duodenum was observed after a solution of diclofenac potassium in water was administered to healthy volunteers.[15] In the present study, however, solid drug material was retrieved from the duodenum at certain time points for three out of six volunteers. As fast duodenal drug (re)dissolution is expected, a more likely hypothesis might be the occasional rapid emptying of not-yet-disintegrated tablet fragments from the stomach at an early stage. Emptying of such a tablet fragment in the duodenum may initially result in the presence of a large amount of solid material. As the tablet fragment disintegrates, drug would then be able to subsequently dissolve in duodenal fluids. This hypothesis is supported by the observation that solid material was mainly present in the duodenum early after the start of the experiment, except for one volunteer, as total and dissolved duodenal drug concentrations were generally identical from 45 min onward (Figure 3).

3.1.1.2. Fed State. Ingestion of a liquid meal (400 mL of Ensure Plus, 600 kcal) 20 min prior to intake of the dosage form resulted in a marked delay in tablet disintegration in the stomach (Figure 3). Based on the absence of drug in gastric aspirates, tablet disintegration only started, on average, after 2 h of gastric residence. These findings were in line with earlier observations by Brouwers et al., who reported a mean delay in disintegration of a fosamprenavir tablet of more than 150 min when administered under fed state conditions compared to fasted state conditions.[8] The authors attributed this finding to the impaired ingression of water into the tablet surface due to the formation of a meal component dependent layer around the dosage form.[23] Similarly to the observations under fasted state conditions, mainly solid drug material was present in the fed stomach (mean solid fraction: 0.88 ± 0.21; Figure 3). This finding can again be explained on the basis of the pH of the gastric aspirates. Ingestion of a liquid meal (pH 6.6) initially resulted in an elevation of gastric pH above the pK_a of diclofenac for all volunteers (Figure 3 inset). However, by the time tablet disintegration in the fed stomach was initiated, gastric pH had already declined to values close to or below pH 3.8, resulting in incomplete drug dissolution and/or immediate precipitation in the stomach. As a result, similar trends as for the administration under fasted state conditions were observed upon drug transfer from the stomach to the duodenum, being (i) the overall rapid (re)dissolution of solid drug material and (ii) the occasional presence of solid material in the duodenum (two out of six volunteers; Figure 3).

3.1.2. Systemic Exposure. Mean systemic concentration−time profiles ($n = 6$) after administration of one tablet of Cataflam under fasted and fed state conditions are depicted in Figure 4. When administered under fasted state conditions, maximal systemic drug concentrations were reached within 1 h after administration for all volunteers (mean t_{max}: 42.5 ± 11.3

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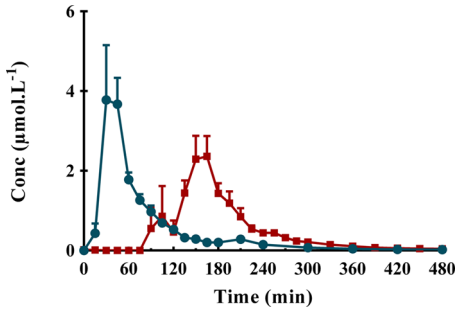

Figure 4. Mean (+ SEM) systemic drug concentration−time profiles in healthy volunteers (n = 6) after oral intake of one tablet of Cataflam (50 mg of diclofenac potassium) with 240 mL of water. (●) Fasted state data. (■) Fed state data.

min). Not surprisingly, t_{max} was markedly delayed when administered with a liquid meal compared to administration of the dosage form under fasted state conditions (3.6-fold delay, mean t_{max}: 152.5 ± 30.6 vs 42.5 ± 11.3 min, p = 0.03). Given the generally rapid (re)dissolution of solid drug material in the duodenum, this finding can most likely be attributed to the delay in intragastric tablet disintegration under fed state conditions. Furthermore, systemic t_{max} correlated nicely with the time at which maximal drug concentrations were measured in the duodenum (r: 0.98; Figure 5a), illustrating the lack of permeability restrictions for this compound ($P_{appCaco\text{-}2} \approx 20 \times 10^{-6}$ cm·s^{-1}).[31] In three out of 12 cases, a second peak in the systemic concentration−time profile was observed (Figure 5b), which could be traced back to the occurrence of a second peak in duodenal drug concentrations. These findings may support the hypothesis that systemic second peak phenomena may in certain cases be related to the fractionated emptying of solid drug material from the stomach.[32] Dosage form administration under fed state conditions resulted in a limited decrease in C_{max}, compared to administration to fasted volunteers (mean C_{max}: 3.5 ± 1.0 vs 4.2 ± 2.6 μM, respectively; p > 0.05). Total systemic drug exposure did not differ between test conditions (mean AUC_{0-8h}: 230.1 ± 74.4 vs 232.5 ± 46.7 μmol L^{-1}·h, ns), indicating that, for this compound, coadministration of food does not affect the extent of systemic exposure in humans, in line with earlier reports.[33]

3.2. *In Vitro* Studies. *3.2.1. USP II Dissolution Apparatus.* A first series of *in vitro* experiments was performed using a simple USP II dissolution apparatus (Figure 6). Gastric residence was simulated for 30 min, after which an equal volume of 2×-FaSSIF was added to the dissolution vessel, mimicking the emptying of gastric contents in the duodenum as a single bolus (i.e., "dumping" scenario). During the first 30 min of the experiment, dissolved drug content was markedly lower compared to the total amount of drug recovered from the dissolution vessel (mean solid fraction: 0.81 ± 0.25). These findings suggest incomplete dissolution and/or immediate precipitation of diclofenac due to the acidity of the gastric environment, which is in agreement with the observed *in vivo* drug behavior (mean solid fraction: 0.77 ± 0.23). Furthermore, with respect to the fate of solid drug material upon transfer to the duodenum, USP II dissolution experiments predicted rapid drug (re)dissolution, again in line with the observed *in vivo* trend (Table 1). Although this model reduces the differences between gastrointestinal segments to a constant difference in pH and surfactant concentrations, it succeeded in capturing the general dissolution behavior observed *in vivo* under fasted state conditions. This is due to the observation that the behavior of this weakly acidic BCS class II compound is dominated by the pH in the gastrointestinal tract (see section 3.1.1). Although gastric and duodenal pH were shown to fluctuate in the *in vivo* study as a function of time, pH generally remained consistently below (mean pH: 1.9 ± 0.9) or above (mean pH: 7.1 ± 1.0) the pK_a of diclofenac (i.e., 3.8) in the respective regions of the gastrointestinal tract, similarly to the USP II experiments. However, the static nature of a one-compartmental USP II dissolution apparatus limits its application for the prediction of *in vivo* drug disposition to pH-dependent drug behavior under fasted state conditions, as dynamic (food-related) changes in gastrointestinal physiology and fluid composition (e.g., volume, pH, hydrodynamics) cannot be simulated using this model.

3.2.2. Modified Dynamic Open Flow Through Test Apparatus. The modified version of the dynamic open flow through test apparatus aims to better reflect the dynamic nature of the upper gastrointestinal tract under fasted state conditions through the incorporation of (i) gastric motility, (ii) dynamic changes in gastric pH, and (iii) biorelevant transfer rates.[16] In the context of further optimizing the model, experiments were performed simulating drug intake with water under fasted state conditions. In this model, gastric contractions can be simulated by means of computer-controlled inflation of a small balloon in the compartment representing the stomach as a function of

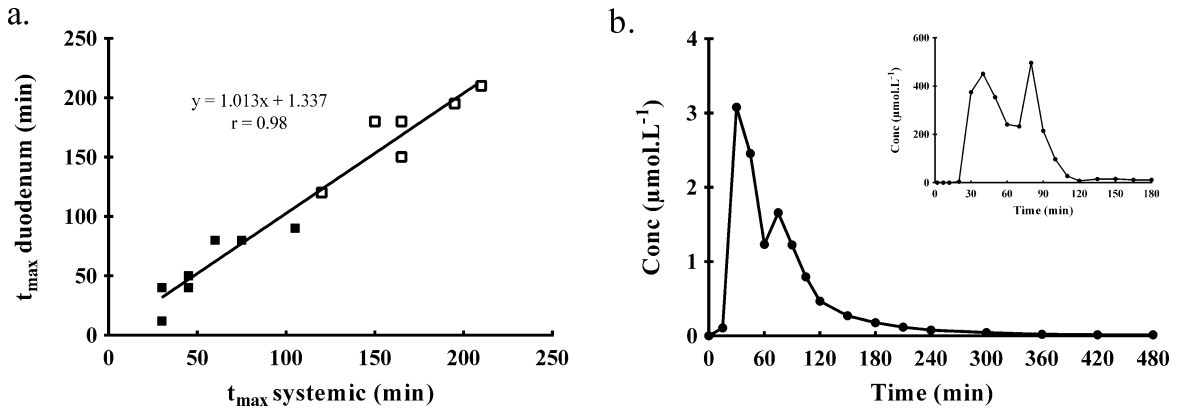

Figure 5. (a) Correlation between time to maximal dissolved drug concentration in duodenum and time needed to achieve maximal systemic drug concentration (r = 0.98). (■) Fasted state data. (□) Fed state data. (b) Typical example of a second peak in the systemic concentration−time profile for one volunteer (fasted state). Inset depicts the corresponding dissolved drug concentration in the duodenum as a function of time.

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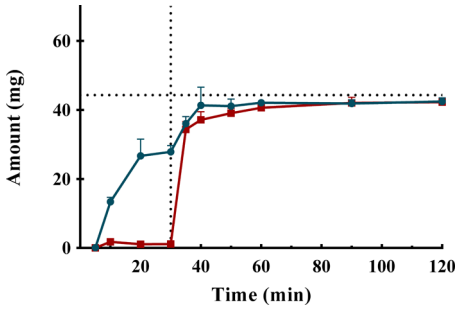

Figure 6. Mean (+SD) drug content as a function of time obtained using the USP II dissolution apparatus (n = 3). (■) Dissolved drug content. (●) Total drug content (solid + solute). The vertical dotted line represents the time at which 250 mL of 2×-FaSSIF pH 7.5 was added to 250 mL of FaSSGF pH 1.6. The horizontal dotted line represents the amount of diclofenac present in one tablet of Cataflam (i.e., 44.3 mg).

time, allowing for the investigation of motility-dependent effects on intraluminal drug disposition. However, as *in vivo* reference data regarding the effect of drug intake during different phases of gastric contractile activity could not be collected in the current study design, experiments with the modified dynamic open flow through apparatus focused on simulating drug intake during a period of contractile activity in the stomach (i.e., MMC phase II). In this scenario, tablet disintegration in the gastric compartment occurred rapidly (Figure 7a). Although this was at first thought to be related to the simulation of contractions during the first hour of the experiment, preliminary experiments simulating drug administration in the absence of contractions revealed similar tablet disintegration behavior (data not shown). Rather than the simulation of gastric motility, the oscillatory movement of the blades inside the gastric compartment at each sampling time point may have initiated rapid disintegration of the dosage form in both scenarios. This suggests a pressure-independent disintegration behavior, as is desirable for IR tablets, in line with the rapid initiation of tablet disintegration observed for all volunteers under fasted state conditions (see section 3.1.1.1).

Second, dynamic changes in gastric pH, observed *in vivo* after drug intake with water, can be incorporated in this model. By starting with demineralized water in the gastric compartment and subsequently perfusing this vessel with acidic medium, an initial rise and a subsequent rapid decrease in pH inside the gastric compartment could be mimicked (Figure 7a inset), similar to the short rise in gastric pH often observed *in vivo* after drug intake with water. However, it should be noted that gastric pH displayed marked fluctuations during the first 30 min of the experiment, coinciding with each sampling time point. These fluctuations may reflect poor mixing of acidic perfusate with main vessel contents in the absence of hydrodynamics, as fluctuations occurred after introducing mixing inside the gastric compartment by means of an oscillatory movement prior to sampling. Despite these initial pH fluctuations, drug was almost exclusively present as a solid inside the gastric compartment, reflecting the presence of vast amounts of solid drug content recovered from the stomach in fasted healthy volunteers (Figure 7a; mean solid fraction: 0.82 ± 0.20 vs 0.77 ± 0.23, respectively).

Third, dynamic changes in transfer rate between compartments as a function of time can be simulated by means of programmable peristaltic pumps. Initially, flow at the start of the experiment was set at a high rate and subsequently declined exponentially to baseline, simulating rapid transfer of coadministered water from the stomach to the duodenum as observed *in vivo* by Mudie et al.[17] Furthermore, each simulated contraction was accompanied by a brief increase in fluid flow rate, similar to increases in *in vivo* transfer rates in the presence of contractile activity.[21,22] In this configuration, transfer of drug from the gastric to the duodenal compartment started as soon as tablet disintegration was initiated (Figure 7). Moreover, approximately all drug was transferred to the duodenal compartment after 110 min (mean dissolved duodenal drug content: 45.22 ± 10.66 mg; Figure 7b), in line with *in vivo* observations. Upon transfer, drug was present in the duodenal compartment as solute, illustrating the pH-dependent dissolution behavior of diclofenac. Furthermore, fast transfer and intestinal (re)dissolution of solid drug material from the gastric compartment suggest a rapid onset of intestinal absorption, as was observed in the *in vivo* study under fasted state conditions (mean t_{max}: 42.5 ± 11.3 min, see section 3.1.2).

Compared to the conventional USP II dissolution apparatus, the modified dynamic open flow through apparatus is a more biorelevant representation of the upper gastrointestinal tract under fasted state conditions. In addition to the pH-dependent dissolution behavior, the model was able to accurately capture the transfer of drug from the gastric to the duodenal compartment as a function of time. However, further work regarding the optimization of the model should focus on investigating the mixing behavior inside the gastric compartment in order to improve the strength of the model (Table 1).

3.2.3. TIMagc. 3.2.3.1. Gastrointestinal Drug Disposition. The TIMagc model provides the opportunity to closely mimic the *in vivo* study design as well as the dynamic changes in the gastrointestinal tract as a function of time (e.g., pH, motility, volume). In a first set of experiments, the dosage form was coadministered with 240 mL of water, identical to the fasted state administration protocol in the clinical study (Figure 8). Under the simulated fasted state conditions, the dosage form started to disintegrate within 20 min after the start of the

Table 1. Capacity of *in Vitro* Models To Predict *in Vivo* Disposition of Diclofenac under Fasted and Fed State Conditions[a]

	in vivo observation			
	fasted state conditions			fed state conditions
	solid drug material in the fasted stomach	dynamic gastric dissolution reflecting time-dependent changes in pH	(re)dissolution of solid drug material in the small intestine	intraluminal drug behavior under fed state conditions
USP II dissolution apparatus	++	−	++	−
modified dynamic open flow through test apparatus	++	+	++	−
TIMagc	++	++	++	++

[a]−: not captured in the *in vitro* setup. +: captured in the *in vitro* setup but requires optimization. ++: adequately captured in the *in vitro* setup.

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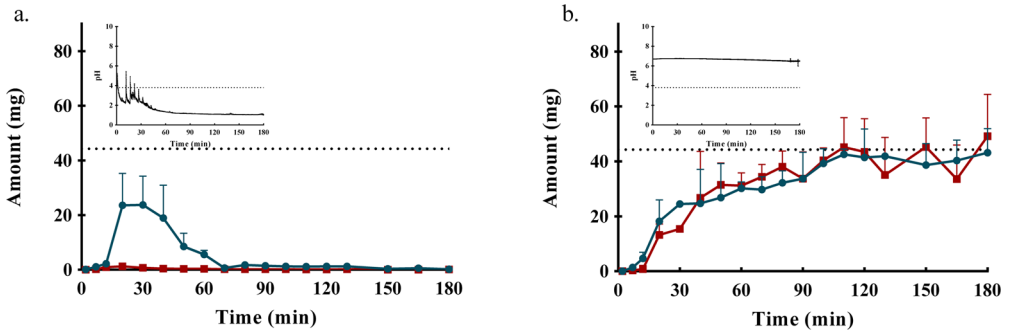

Figure 7. Mean (+SD) drug content as a function of time obtained using the modified dynamic open flow through test apparatus (n = 3). (■) Dissolved drug content. (●) Total drug content (solid + solute). (a) Drug content present in the gastric compartment as a function of time. (b) Drug content present in the duodenal compartment as a function of time. The dotted line represents the amount of diclofenac present in one tablet of Cataflam (i.e., 44.3 mg). Insets depict mean (±SD) pH profile in the corresponding compartment as a function of time.

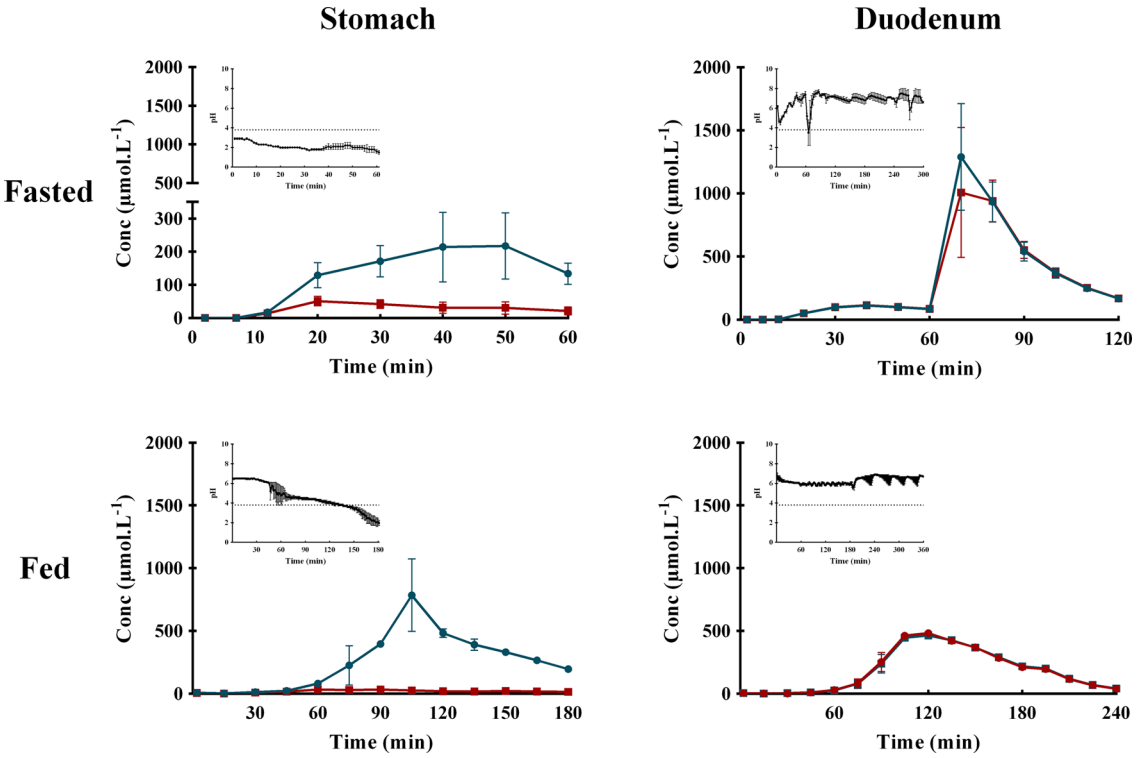

Figure 8. Mean (±range) drug concentration−time profiles as a function of time obtained using the TIMagc (n = 2). (Top) Experiments performed under simulated fasted state conditions. (Bottom) Experiments performed under simulated fed state conditions. (■) Dissolved drug concentration. (●) Total drug concentration (solid + solute). (Left) Drug concentration in the gastric compartment as a function of time. (Right) Drug concentration in the duodenal compartment as a function of time. Insets depict mean (±range) pH profile in the corresponding compartment as a function of time.

experiment. Total drug concentrations in the gastric compartment were similar to mean concentrations measured *in vivo* (C_{max}: 217.3 ± 99.4 vs 157.2 ± 138.4 μM, respectively). Although dissolved concentrations of diclofenac were higher compared to those observed *in vivo*, *in vitro* experiments predicted the presence of solid material in the stomach well (mean solid fraction: 0.68 ± 0.26 vs 0.77 ± 0.23, respectively). Moreover, drug transfer from stomach to duodenum was accurately predicted to start as soon as dosage form disintegration was initiated. After 1 h, a gastric housekeeper wave was simulated, transferring all gastric contents from the gastric to the duodenal compartment. This transfer resulted in a drop in duodenal pH, explaining the initial presence of solid material in the duodenal compartment 70 min after the start of the experiment (Figure 8). However, duodenal pH rapidly returned to baseline values, promoting drug (re)dissolution in the duodenal compartment as observed in humans (Table 1).

In addition to the simulation of fasted state conditions, the TIMagc model is capable of simulating physiological changes along the gastrointestinal tract in response to food intake, providing the opportunity to investigate intraluminal drug behavior under fed state conditions (see section 2.3.3.1, Table 1). In this study, fed state administration was simulated by coadministering the dosage form with a mixture of Ensure Plus and water. Under these conditions, similar pH-dependent dissolution behavior as that reported in the *in vivo* study was observed (Figure 8). For instance, the presence of solid material in the fed stomach, due to reacidification of gastric

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contents by the time dosage form disintegration is initiated, was clearly reflected in this set of experiments (mean solid fraction: 0.70 ± 0.32). In addition, the rapid transfer of solid material from the stomach to the duodenum and the initiation of drug (re)dissolution after transfer were accurately captured.

However, tablet disintegration under fed state conditions was observed to differ markedly when comparing *in vitro* and *in vivo* data sets. Using the TIMagc, tablet disintegration started after 1 h of gastric residence, while a delay in disintegration for up to 2 h was observed *in vivo*. A first possible explanation for this apparent discrepancy may be related to the way in which the dosage form was administered to either the healthy volunteer or the *in vitro* model. While, in the simulated fed state experiments, the liquid meal was mixed with water and administered as such, the *in vivo* study design comprised the administration of the dosage form with water 20 min after ingestion of the liquid meal. By premixing water and the liquid meal *in vitro*, the rate of water ingression into the tablet surface may be increased compared to the *in vivo* situation, in which mixing of the liquid meal with the ingested water may be hampered.[34] A lack of mixing of water with the meal was simulated *in vitro* by administering the dosage form in the absence of water (Figure 9, Ensure Plus only). In this scenario, tablet disintegration was found to be markedly delayed compared to the initial administration protocol (onset of disintegration: 105 vs 60 min, respectively), in line with the above-mentioned hypothesis that fluid ingression rate influences dosage form disintegration behavior under fed state conditions. A second factor contributing to the discrepancy between *in vitro* and *in vivo* disintegration may be related to the pH dependency of pepsin activity. Previous studies have reported maximal activity of this proteolytic enzyme at pH 2 and almost complete inactivation at pH 5−6 or higher.[35−37] As discussed earlier, food components such as proteins may form a layer around the dosage form resulting in delayed dosage form disintegration (see section 3.1.1.2). Upon reacidification of gastric contents, pepsin-mediated proteolysis will aid in breaking down the meal component coating surrounding the dosage form, leading to dosage form disintegration by facilitating fluid ingression into the tablet surface. Under simulated fed state conditions, an important drop in gastric pH was recorded after 50 min, whereas this was not observed until 90 min after the start of the *in vivo* experiments. This may have resulted in the faster *in vitro* activation of pepsin compared to the *in vivo* situation. Therefore, premature pepsin activation was hypothesized to contribute to the faster initiation of dosage form disintegration observed *in vitro*. To test this hypothesis, an additional fed state experiment was performed in which gastric pH was kept at a high level in order to inhibit the enzyme's proteolytic activity for the entire duration of the experiment (Figure 9, high pH). Compared to the initial simulations, this scenario resulted in a marked delay in tablet disintegration (onset of disintegration: 60 vs 105 min, respectively), indicating that pepsin activation is a factor to be taken into consideration in the dosage form disintegration process under fed state conditions. Lastly, when combining both test scenarios, the onset of disintegration observed *in vivo* is adequately predicted, suggesting a dual effect on *in vivo* dosage form disintegration (Figure 9, Ensure Plus only + high pH).

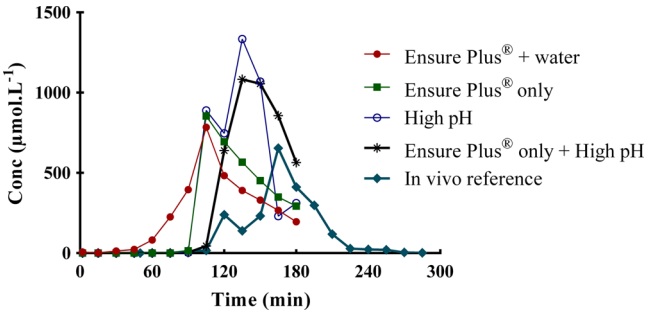

Figure 9. TIMagc experiments investigating the mechanisms involved in gastric tablet disintegration under simulated fed state conditions. Different scenarios were tested in single experiments, except for the initial dosing scenario (i.e., Ensure Plus + water; $n = 2$). Total drug concentration (solid + solute) measured as a function of time in the gastric compartment for the different scenarios is compared to the mean *in vivo* reference data ($n = 6$).

3.2.3.2. Bioaccessible Amount. By determining drug concentration in filtrates collected from the jejunal and ileal compartments every 30 min, the amount of drug available for absorption (i.e., the bioaccessible amount) could be calculated (see section 2.4). Figure 10 depicts the cumulative bioaccessible amount of diclofenac as a function of time under both test conditions. Based on the limited cumulative amount of diclofenac available for absorption after 1 h, systemic t_{max} would not be expected to be reached within the first hour after drug intake under fasted state conditions (cumulative bioaccessible amount$_{0-60min}$: 4.1 ± 0.2%). However, mean t_{max} *in vivo* was reached after 42.5 ± 11.3 min, suggesting faster emptying of solid drug material from the stomach *in vivo* compared to the *in vitro* simulated conditions (i.e., housekeeper wave simulated 1 h after the start of the experiment, see section 2.3.3.1). Furthermore, it should be noted that duodenal absorption is not taken into account *in vitro*, as the bioaccessible drug amount is calculated based on the presence of drug in filtrates collected from the jejunal and ileal compartments (see section 2.3.3).

Under simulated fed state conditions, a marked delay in cumulative drug bioaccessibility compared to simulated fasted state conditions (e.g., cumulative bioaccessible amount$_{0-120min}$: 10.2 ± 0.8 vs 55.9 ± 1.2%, respectively) could be observed, suggesting a delay in systemic t_{max} after drug intake with food. These findings correlate well with the observations from the *in vivo* study that showed a shift in systemic t_{max} under fed state conditions (mean t_{max} fed state, 152.5 ± 30.6 min, vs mean t_{max} fasted state, 42.5 ± 11.3 min, see section 3.1.2). However, given the more rapid onset of intragastric tablet disintegration in the TIMagc setup (see section 3.2.3.1), the estimated delay in systemic t_{max} is expected to be less extensive than observed *in vivo*. At the end of the experiments, cumulative bioaccessible amount amounted to 83.6 ± 3.6% and 87.3 ± 1.1% under simulated fasted and fed state conditions, respectively. Based on these *in vitro* experiments, one would therefore not expect a food effect regarding overall systemic drug exposure, in line with *in vivo* observations in this study as well as earlier literature reports.[33]

4. CONCLUSIONS

In this study, the intraluminal behavior of the weakly acidic BCS class II drug diclofenac was investigated in healthy volunteers under both fasted and fed state conditions. In the fasted stomach, the vast presence of solid drug material is most likely linked to incomplete dissolution and/or immediate precipitation of drug given the acidity of gastric contents. Due

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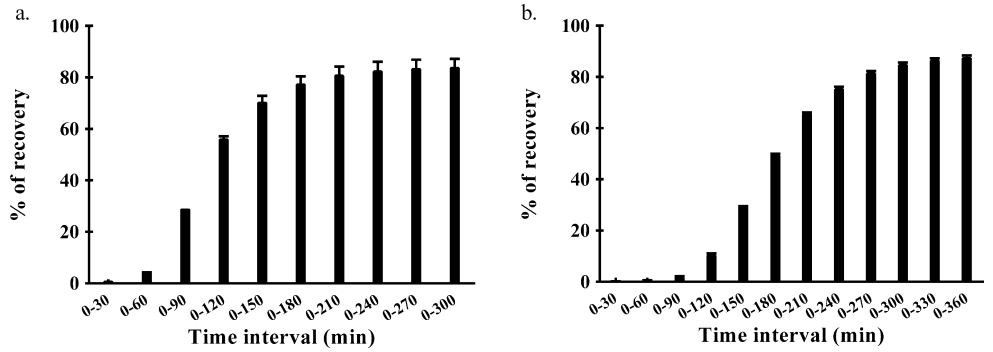

Figure 10. Mean (±range) cumulative drug amount present in small intestinal filtrates in TIMagc at specific time intervals (n = 2) expressed relative to the total drug content recovered from all compartments (i.e., recovery). (a) Experiments performed under simulated fasted state conditions. (b) Experiments performed under simulated fed state conditions.

to reacidification of gastric contents by the time tablet disintegration was initiated, drug disposition in the fed stomach was similar to that in the fasted stomach. For both test conditions, emptying of solid drug material from the stomach resulted in an overall rapid drug (re)dissolution in the duodenum. As the intraluminal behavior of this compound did not differ between test conditions, except for a delay in tablet disintegration in the fed stomach, no effect of drug intake with a liquid meal on overall systemic drug exposure was observed. *In vitro* tools were found to be capable of predicting *in vivo* intraluminal (and systemic) disposition of this compound to some extent, depending on the degree to which the complexity and the dynamic nature of the processes to be investigated were simulated. While the use of static models may suffice for the prediction of pH-dependent drug behavior, the investigation of dynamic processes affecting drug disposition requires more dynamic *in vitro* models. Although the complexity of controlling numerous physiological factors may sometimes be considered a limiting factor in the widespread use of dynamic models, it is this complexity that may enable the identification of specific factors potentially contributing to crucial steps in intraluminal drug disposition (e.g., dosage form disintegration). For instance, the modified dynamic open flow through test apparatus was able to mimic transfer of drug content from the stomach to the duodenum under fasted state conditions, while the dynamic TIMagc could also be used to investigate the effect of food on intraluminal (and systemic) drug disposition. Therefore, it is concluded that the dynamic nature of the process(es) to be investigated should be taken into account when selecting an appropriate *in vitro* tool to predict intraluminal drug disposition in human.

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Notes

The authors declare no competing financial interest.

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