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Research paper

# A novel technique for intraduodenal administration of drug suspensions/solutions with concurrent pH monitoring applied to ibuprofen formulations

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ABSTRACT

Characterization of dissolution of solid suspended drug particles in vivo is important for developing biopredictive in vitro tests. Therefore, methods to gain deeper insights into particle dissolution in vivo are needed. The soft Bioperm intubation method, a well established tool for investigation of permeability, absorption, metabolism, and drug interactions at predefined locations in the gastroinstinal tract, was modified. The novel intubation method involved pump-controlled infusion of pharmaceutical suspensions as well as simultaneous pH monitoring. This technique was used in a proof of concept study in healthy humans. Plasma sampling and non-compartmental analysis allowed comparison of three different ibuprofen drug products, a solution and two suspensions with different particle size distribution, as well as two different infusion rates. Both a particle size effect and an effect of altering infusion rates on pharmacokinetic parameters were shown. Moreover, it was possible to monitor intestinal pH changes after intestinal infusion. Infusion of ibuprofen resulted in a pH drop that was quantified by the concept of Area Between Curves (ABC).

## 1. Introduction

Intestinal dissolution and absorption of drugs are dependent on several physiological, drug substance and formulation variables that are generally difficult to control in a typical phase I study following oral administration of drug products. Consequently, an insightful analysis of the relevant factors determining in vivo dissolution and absorption requires more refined in vivo methods for monitoring and controlling physiological, physicochemical and drug formulation factors. Without careful delineation of the in vivo dissolution, transit, and absorption determining factors, drug product optimization becomes a trial and error approach. Furthermore, biopredictive in vitro dissolution tests are hard to establish in the absence of relevant in vivo dissolution data.

Ibuprofen, being a weak acidic low soluble / high permeable drug (BCS class IIa [1]), was chosen as model compound for this work. Its solubility is pH-dependent and rising with increasing pH [2–4]. Considering the pH-solubility profile, ibuprofen should dissolve in the small intestine (pH < 5). At pH-values < 3, as in the fasted stomach, dissolution is negligible. Thus, the small intestine is considered to be the main site of absorption of ibuprofen in healthy humans [5]. Previous work has shown that current dissolution test conditions might not be sufficiently biopredictive to reflect the in vivo relevance of different ibuprofen products and in vitro methods show differences in in vitro dissolution profiles that are not reflected in agreement with analysis of in vivo pharmacokinetic profiles [6]. At least three important parameters might influence the dissolution behavior of ibuprofen: 1. Particle size distribution (PSD), 2. Gastric emptying time, and 3. Intestinal pH and buffer capacity [7,8].

Commonly, methods to control gastric emptying rates and changing conditions in different gastrointestinal (GI) segments include various intubation procedures allowing local drug administration into defined GI segments through single, double and multi lumen catheters [8–16]. Some of these are uncomfortable for the subject, who may require tranquilizers that can affect gastrointestinal motility or the pharmacokinetics of the investigated drug in vivo [17,18]. Alternatives are noninvasive remote controlled capsules, which are swallowed, can move freely and can transmit information on local GI conditions while migrating through the GI tract [19–24]. These capsules can carry drug formulations of only limited volumes to be released only once.

Herein, a method is described that allows control of the variable

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gastric emptying [25–28] by direct infusion of different drug formulations into the duodenum of healthy subjects. Applying different first-order infusion rates, physiological gastric emptying can be precisely simulated and with it the degree of saturation of the intestinal juices by a drug of limited solubility, i.e. ibuprofen. Systemic plasma levels allow the estimation of drug input velocity and systemic exposure. Furthermore, the method introduced here for the first time allows simultaneous monitoring of duodenal pH to reflect the changes due to the dissolved acidic drug. A change of pH during infusion would indicate a depletion of the physiological buffer system and would affect the solubility and dissolution of ibuprofen itself in a negative feedback mechanism. Three formulations, two suspensions with different PSD and one solution, were developed and clinically tested in this study in order to address differences in in vivo dissolution due to different particle sizes of the suspended ibuprofen particles. It is expected that the results of this study can be used to improve in vitro methods in terms of biopredictivity and to improve computational models predicting in vivo dissolution and absorption as is addressed in the in vivo predictive dissolution approach (IPD) [1,29].

## 2. Materials and methods

### 2.1. Intestinal infusion technique

The technique used in this study is a modification of the Bioperm method for local administration of drugs in the human gut (Bioperm AB, Lund, Sweden). A small-bore, smooth tube is introduced through the nose, retrieved from the pharynx, equipped with a firm radio-opaque capsule, and swallowed. Peristalsis moves the capsule to the desired location in the gut where it is anchored before administration of drug in solution via the tube. An alternative capsule is designed for administration of solid formulations. This method has been used in several studies [30–33], involving over 350 intubations in more than 150 volunteers with drug administrations from the duodenum to the colon. There have been no serious adverse events.

### 2.2. Novel methodology

#### 2.2.1. Tubing and capsules

As pharmaceutical suspensions were to be given intraduodenal and the pH measured online 20 cm distally, modifications were necessary. The infusion tube was a soft polyethylene (PE) tube with an OD of 1.27 mm and an ID of 0.86 mm and a length of 200 cm, with the proximal end connected to a peristaltic pump. The tube was provided with colour marks for every 10 cm. Two outlet holes with diameter 0.8 mm were located opposite each other at the very end of the tube. The end of the tube was closed, but connected to a PE line with OD 0.61 mm and length 20 cm. The other end of this line was connected to a modified Bioperm capsule. Thus, the total length of the system was 220 cm. The capsule, made from polyoxymethylene (Delrin®), which was open-ended distally, had an OD of 9 mm and a length of 28 mm. A Heidelberg pH telemetric system (Heidelberg Medical, GA, USA) was placed within the modified Bioperm capsule and fixated, the pH electrode protruding 7 mm (Fig. 1). The pH capsule was activated and calibrated according to the manufacturers instructions.

#### 2.2.2. Pumping system

The proximal end of the PE tube was connected to a programmable peristaltic pump (Ismatec® Reglo ICC, IDEX Health & Science GmbH, Germany). Two Tygon® LFL pumping tubes (OD 4.47 mm, ID 2.79 mm, IDEX Health & Science GmbH, Germany) were connected with tube-connectors (Product no. CT34.1, Carl Roth GmbH + Co KG, Germany). The distal end of this system was connected to a Luer connector (Product no. CT59.1, Carl Roth GmbH + Co KG, Germany) to fit with the PE tube. A short segment of the pumping system was inserted into a cassette and positioned in the pump, which was computer controlled by remote (Fig. 2). The pumping tubes were primed with 200 ml distilled water at a speed of 10 ml/min for 15 min. After priming, the pump was calibrated by pumping 100 ml through a dummy infusion tube into a 250 ml graduated cylinder within 600 s and the value inserted into the computer program of the pump.

### 2.3. Subjects

Male and female healthy subjects who did not need any regular medication and had normal haemograms and physical status were recruited. Exclusion criteria were pregnancy, history of swallowing disorder, gastrointestinal disease and discomfort, prior abdominal surgery, pregnancy and lactation. Lupus erythematosus, mixed connective tissue disease, acute intermittent porphyria, asthma, hemorrhagic diathesis and diseases of liver and kidney were contraindications. Regular use of the following medications was forbidden: coumarin, glucocorticoid, probenecid, diuretics, antidiabetics, methotrexate, lithium, ACE-inhibitors, and proton pump inhibitors. The simultaneous administration of CYP2C9 inhibitors and inducers was prohibited as this enzyme was identified as primary isoform responsible for ibuprofen clearance [34]. In addition, hypersensitivity against ibuprofen and against any of the excipients in the study medication and the participation in another clinical study in parallel or within the last 90 days were also exclusion criteria. Informed consent was obtained following verbal and written information. Nine healthy subjects, 5 males and 4 females, aged 24–31 years, were included in the study. Their weights ranged from 55 to 92 kg (mean 75 kg) and their body mass index (BMI) from 21 to 27 kg/m$^2$ (mean 24 kg/m$^2$).

### 2.4. Intubation procedure

The throat of the subject was sprayed with lidocaine spray (Xylocain®, AstraZeneca GmbH, Germany). The nasal route as in the original method was omitted. The two combined capsules with the PE line was placed on the tongue and swallowed using a sucking motion; the pop-bottle method [35]. A test with a Dummy system was performed in order to verify ability to swallow the capsule. After swallowing, the distance from mouth to capsule was measured by the length of the tube. pH was measured continuously and a rising pH together with a distance of 65 cm to the mouth was indication of duodenal position of the capsule [36]. The capsule was then allowed to move 25 cm further distally, which positioned it proximal to the ligament of Treitz but distal to the papilla Vateri. The distal holes of the tube were then supposed to be positioned in the duodenum, distal to the pylorus but proximal to the papilla Vateri. The tube was fixated to the cheek of the subject with adhesive tape. X-ray was not employed. After termination of the study, the tube was cut and the system left the body the natural way.

### 2.5. Formulations and infusion

Three different drug formulations were developed and characterized: two suspensions with different particle size distributions and one solution (Table 1, Table 2). Drug products were manufactured individually for each subject within 4 days and 5 days before study day for suspension and for solutions, respectively, to ensure stability of the formulations which was experimentally verified by laser diffraction analysis. The product container was placed on a stirring magnetic plate with the primed and calibrated pumping tubes leading into the drug product (Fig. 3). With the system in correct position, the pump was started, simulating a 12 min (“fast”) or a 20 min (“slow”) gastric emptying half-life.

### 2.6. Clinical protocol

The study protocol was approved by the local ethics committee (Ref.

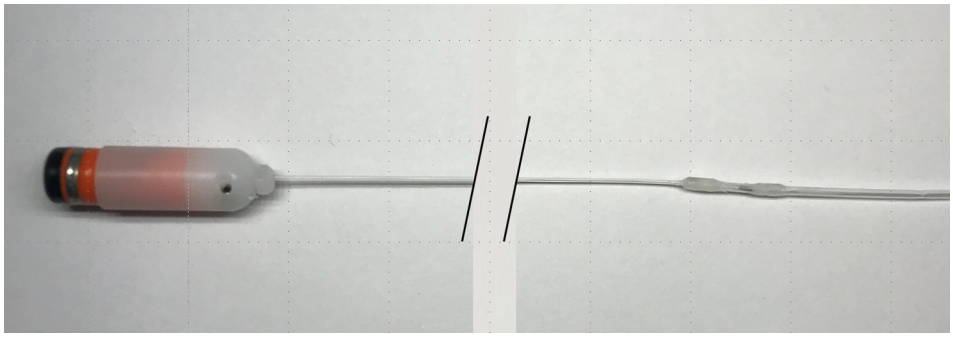

**Fig. 1.** Modified Bioperm capsule with pH capsule protruding. A 20 cm thin PE line connects the Bioperm capsule to the infusion tube.

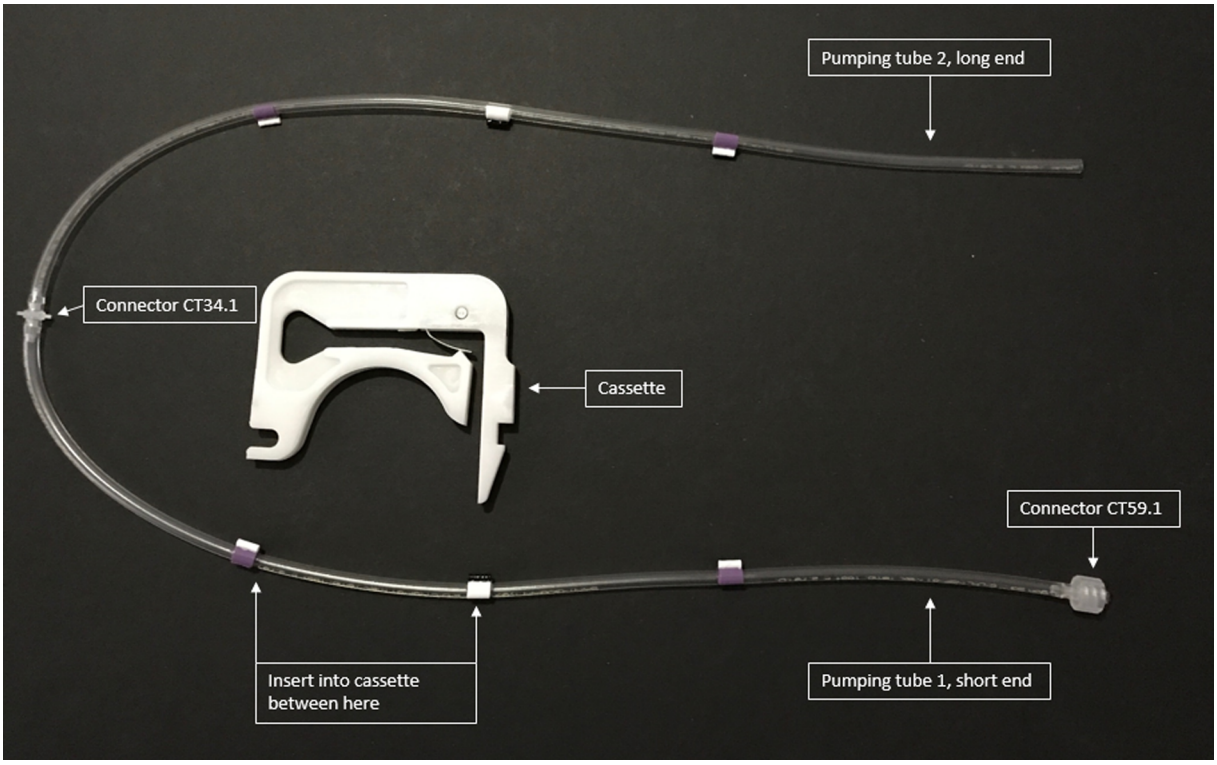


**Fig. 2.** Setup of pumping tubes and connectors.

837.481.15 (10249) Landesärztekammer Rheinland-Pfalz, Mainz, Germany). It was performed in accordance with the Declaration of Helsinki, with the International Conference of Harmonization Guidelines of Good Clinical Practice, the German Federal Data Protection Act and the professional law of medical doctors in Germany.

It involved testing of nine volunteers with the modified Bioperm capsule in a monocentric four-period crossover design. The schematic of the experimental setup is shown in Fig. 3. The study phases differed by the applied drug product and infusion rates: Phase A: Solution (SO) 400 mg/250 ml, infusion rate fast; Phase B: Suspension A (SA) 400 mg/

**Table 1**
Composition of the study formulations.

| Ingredient | Manufacturer* | Solution (SO) | Suspension A (SA) | Suspension B (SB) |
|---|---|---|---|---|
| Ibuprofen 25 | BASF | 0.400 g | 0.400 g | |
| Ibuprofen 70 | BASF | | | 0.400 g |
| Polysorbate 80 | Fagron | | 0.0625 g | 0.0625 g |
| Potassium Sorbate | Euro-OTC | | 0.250 g | 0.250 g |
| Sodium Chloride | Fagron | | 2.75 g | 2.75 g |
| Povidone K 90 | BASF | | 5.00 g | 5.00 g |
| Purified water | Fagron | | ad 250.00 g | ad 250.00 g |
| Disodium hydrogen phosphate dihydrate | Fagron | 0.450 g | | |
| Preserved water (DAC) | Fagron | ad 250.00 g | | |
| Glass container 250 ml (brown) | WEPA | X | X | X |

* BASF: BTC Europe GmbH, Germany; Fagron: Fagron GmbH & Co. KG, Germany; Euro OTC Pharma GmbH, Germany; WEPA Apothekenbedarf GmbH & Co KG, Germany.

**Table 2**
Particle sizes of the ibuprofen suspensions.

| | SA | SB |
|---|---|---|
| D10 [µm] | 16.80 | 35.50 |
| Median [µm] | 53.03 | 121.00 |
| D90 [µm] | 153.00 | 314.33 |

250 ml, infusion rate fast; Phase C: Suspension B (SB) 400 mg/250 ml, infusion rate fast; Phase D: Suspension B (SB), 400 mg/250 ml, infusion rate slow. Study design and series of phases were chosen after Williams [37]. The randomization was done using Microsoft® Excel® 2016 MSO. A minimum washout phase of 3 days between the study phases was maintained. Before the study, the practicability of the setup was tested with subject No. 1 in a pilot study. The study was performed at the I. Medical Department of the University Medical Center Mainz, Germany. Subjects were fasted overnight after 10 PM, the last evening meal being light. Coffee, tea, fruit juices and milk were not allowed. The volunteers were asked to avoid the use of toothpaste that consists of hydrogen carbonate/bicarbonate due to potential influences on local pH. No exhausting sport and use of alcohol were allowed within 24 h before the study day. The use of mobile phones was not permitted since its signal may interfere with the pH monitoring system. Study sessions started between 7 AM and 8 AM in the morning. Capsules were swallowed with non-carbonated water. Volunteers were asked to drink at least 1.4 L of water during the day. The subjects were allowed to move within the hospital and were instructed to keep notice of the length of the tube. They were not allowed to eat until 3 h after the first blood sample had been taken, to conduct exhausting activities or to leave the hospital. In case the capsule did not leave the stomach within 4 h, the subject was scheduled for another session. 15 min following termination of the infusion, the tube was cut and the pH measurement stopped. Times for swallowing the capsule, gastric emptying, time to reach the destination, start and stop of infusion, and cutting the tube were recorded.

### 2.7. Bioanalysis

Before, during and after the drug was infused, blood samples (7.5 ml) were taken through venous access into a heparinized blood collection system (S-Monovette® 7.5 ml, SARSTEDT AG & Co., Germany) after 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 75, 90, 105, 120, 180, 360, 540 min. Deviations were documented in the clinical report form. Plasma was separated by centrifugation for 10 min at 20 °C and 1500 rpm (Heraeus, Thermo Electron LED GmbH, Germany). The separated plasma was transferred into 2 containers (Cryo-vials, PP, 5 ml, Carl Roth GmbH + Co KG, Germany) and frozen/stored at −80 °C until bioanalysis. Ibuprofen was isolated from plasma by solid phase extraction (SPE) on Oasis HLB 1 cc Vac Cartridge, 30 mg Sorbent per Cartridge, 30 µm particle size (Waters GmbH, Germany). The columns were conditioned as per specification. The plasma sample was loaded onto the column followed by washing with 1 ml 5% methanol and eluted with 1 ml acetonitrile. Separation and quantification was done by HPLC/UV using a Shimadzu LC-2010A HT system (Shimadzu, Germany), a Luna® C18 column (150 mm × 3 mm, 5 µm) (Phenomenex LTD, Germany) at a wavelength of 224 nm. The mobile phase consisted of acetonitrile (HPLC grade, VWR International GmbH, Germany) and phosphoric acid, pH 2.6 (HPLC grade 85–90%, Fluka Analytical, Germany) at a ratio of 60:40 (v/v). Flow rate and injection volumes were 1 ml/min and 20 µl, respectively. The column was maintained at 30 °C. Retention times for ibuprofen and carbamazepine as internal standard were 2.952 and 1.143 min, respectively. Calibration curves were prepared ranging from 0.0375 to 60 µg/ml with acetonitrile using ibuprofen CRS (European Directorate for the Quality of Medicines & Health Care, France) and carbamazepine (Fluka Analytical, Germany).

### 2.8. Plasma pharmacokinetic parameters

Pharmacokinetic parameters were calculated using non-compartmental analysis with PKSolver [38]. The AUC was calculated with the linear trapezoidal method. Terminal half-lives were calculated following regression of the terminal 3, 4 and 5 time points, by selection based on highest $R^2$. The following parameters were observed and calculated for the individual plasma-time profile: Maximum plasma concentration ($C_{max}$), time to maximum concentration ($T_{max}$), terminal elimination rate constant ($\lambda_z$), elimination half-life ($t_{1/2}$), area under the concentration-time curve from 0 to last time point t ($AUC_{0\text{-}t}$), area under the concentration-time curve from 0 to infinity ($AUC_{0\text{-}inf}$), area under the first moment curve from 0 to infinity ($AUMC_{0\text{-}inf}$), mean residence time from 0 to infinity ($MRT_{0\text{-}inf}$), and mean dissolution time ($MDT_{0\text{-}inf}$) (1).

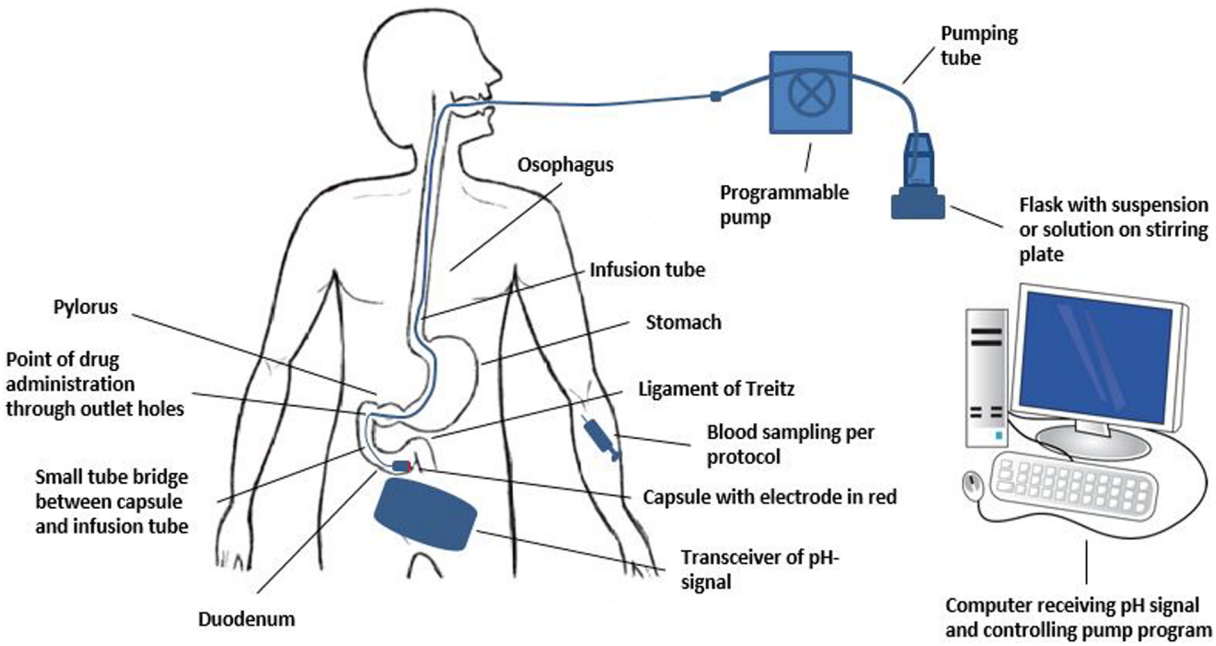


**Fig. 3.** Clinical setup of the modified Bioperm administration method.

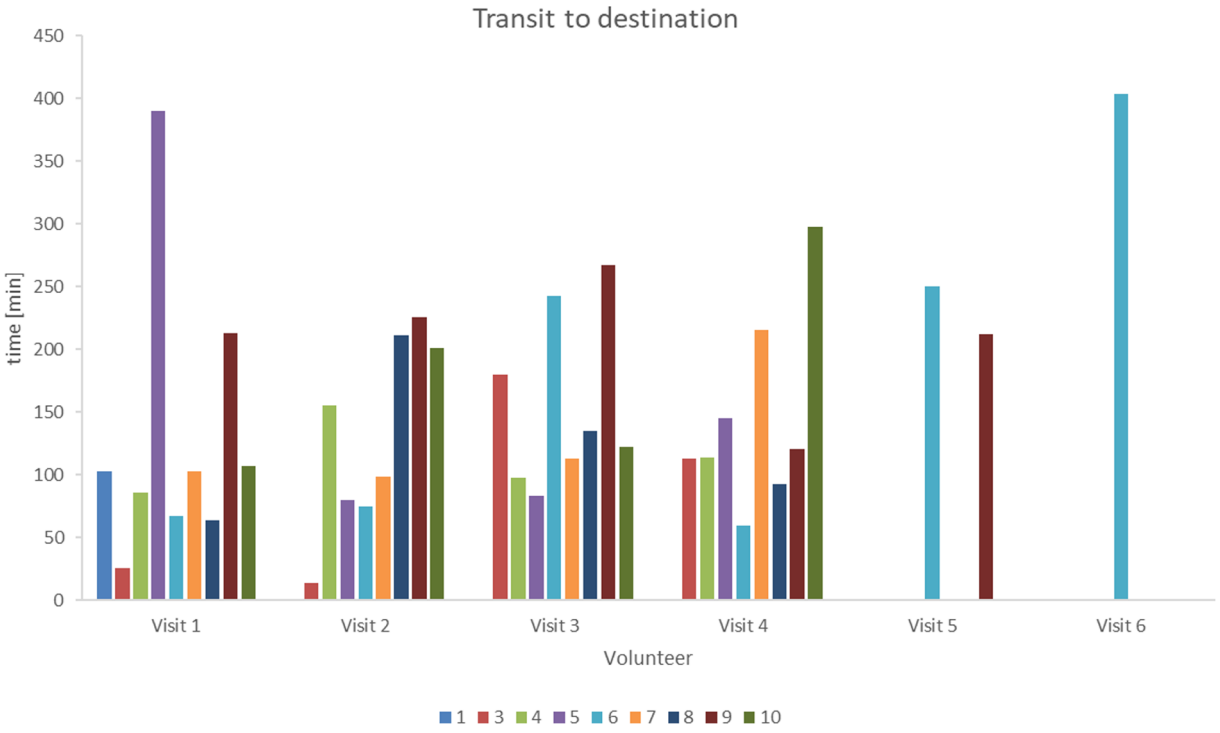


**Fig. 4.** Transit time from mouth to duodenum. Volunteers are distinguishable by colour. Note that volunteer 6 and 9 attended more than 4 times and the respective rows were left blank for volunteers 1, 3, 4, 5, 7, 8 and 10.

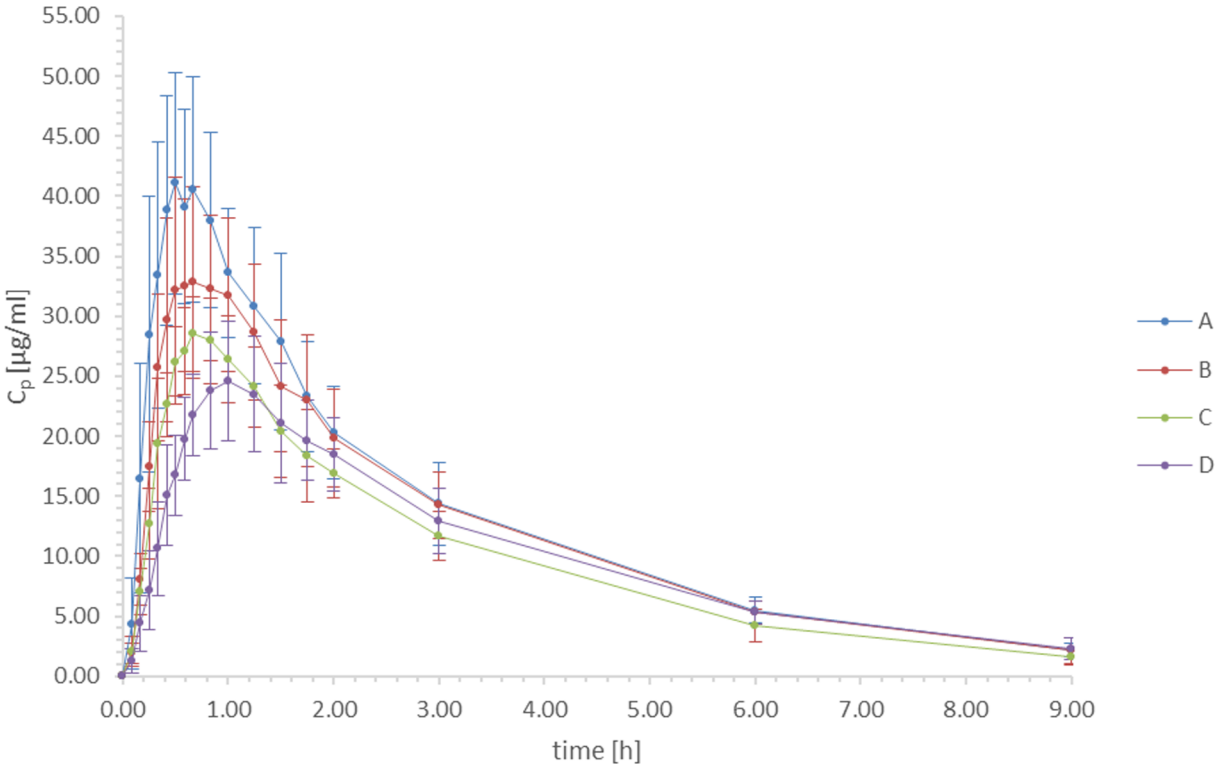


**Fig. 5.** Mean plasma concentration of ibuprofen versus time. Curves depict arithmetic mean and standard deviation: A (8 subjects): 400 mg/250 ml solution infused with 12 min half-life of simulated gastric emptying, B (9 subjects): 400 mg/250 ml suspension small particles infused with 12 min half-life of simulated gastric emptying, C (5 subjects): 400 mg/250 ml suspension large particles infused with 12 min half-life of simulated gastric emptying, D (6 subjects): 400 mg/250 ml suspension large particles infused with 20 min half-life of simulated gastric emptying.

$$MDT_{0-inf\,(B,C,D)} = MRT_{0-inf\,(B,C,D)} - MRT_{0-inf\,(A)} \quad (1)$$

Following determination of individual parameters arithmetic and geometric mean, standard deviations, and medians were calculated. Individual plasma concentrations were linear interpolated in case actual sampling times deviated from the predefined times, and for the last plasma concentration by extrapolating from the last measured value.

**Table 3**
Noncompartmental PK Analysis.

| Observation | | $C_{max}$ [µg/ml] | | $AUC_{0\text{-}inf}$ [µg/ml h] | | $T_{max}$ [h] | | MRT [h] | MDT [h] |
|---|---|---|---|---|---|---|---|---|---|
| Phase | Number | GM | GSD | GM | GSD | AM | ASD | AM | AM |
| A | 8 | 42.41 | 1.21 | 120.90 | 1.23 | 0.58 | 0.10 | 3.03 | – |
| B | 9 | 35.48 | 1.21 | 112.93 | 1.23 | 0.70 | 0.14 | 3.29 | 0.27 |
| C | 5 | 30.21 | 1.08 | 95.13 | 1.13 | 0.69 | 0.13 | 3.33 | 0.30 |
| D | 6 | 25.52 | 1.19 | 97.86 | 1.18 | 1.01 | 0.13 | 3.70 | 0.67 |

**Table 4**
Statistical evaluation: ratio, p-values in parentheses.

| Ratio | $C_{max}$ (A, B, C, D) | $AUC_{0\text{-}inf}$ (A, B, C, D) |
|---|---|---|
| B:A | 0.84 (0.0714) | 0.93 (0.4936) |
| C:A | 0.71 (0.0012) | 0.79 (0.0235) |
| D:A | 0.60 (0.0003) | 0.81 (0.0556) |
| C:B | 0.85 (0.0448) | 0.84 (0.0644) |
| D:C | 0.84 (0.0710) | 1.03 (0.7527) |

### 2.9. pH analysis

pH values were measured by the pH remote capsule and sent to a transceiver that was located on the abdomen of the volunteer. The accuracy of the capsule was ± 0.4 at pH 1 and ± 0.5 at pH 8.0. pH values were extracted and plotted in intervals of one second after start of the infusion. Whenever possible, time intervals between reaching destination and starting the infusion as well as between the end of infusion and cutting the tube were plotted for the individual subject. Arithmetic means and standard deviations were calculated and plotted within a range of 0–75 min for phase A, B and C and 0–105 min for phase D, respectively. If necessary, the individual plots were smoothed by running median over a range of ± 30 s. Higher ranges (up to ± 1000 s) had to be chosen in cases of severe scattering and connection loss. The area under individual baseline (AUIB) was calculated as shown in Eq. (2). The area under individual pH profile (AUIP) was obtained by the trapezoidal rule in intervals of 1 s. Area between curves (ABC) is given by Eq. (3) and introduced as metric for the effect of the drug on the physiological duodenal pH.

$$AUIB = individual\ \ 5\ \ min\ \ pH\ \ median\ \ before\ \ start\ \ of\ \ the\ \ infusion * 4500\ \ s(B\ \ and\ \ C)\ \ and\ \ 6300\ \ s(D) \quad (2)$$

$$ABC = AUIB - AUIP \quad (3)$$

### 2.10. Statistical analysis

Statistical comparisons of plasma data were done assuming two-tailed distributions by two-sample unequal variance *t*-test. P-values < 0.05 were considered statistically significant. The analysis was done using Microsoft® Excel® 2016 MSO using the build-in formula for *t*-test (TTEST(array1; array 2; 2; 3)).

## 3. Results

### 3.1. Compliance with protocol

Out of 38 attempted drug administrations, 28 were successful. Four subjects completed all 3 phases, two phases A, B and D, one phases A, B and C, one phases A and B and one phase B. The subjectś compliance was excellent. Swallowing the capsule could be a problem initially before introducing correct technique. Ten attempts were not successful for various reasons. On two occasions, gastric emptying took very long and the study day had to be terminated due to stress for the volunteer. Once, the capsule flipped back into the stomach while the drug was infused making the data unusable. In one case blood sampling was impossible in an acceptable timeframe. Six times the drug product could not be administered accurately and safely due to clogging of the tube. One subject reported moderate abdominal discomfort and pain on the following day, not requiring medical attention or analgesics.

### 3.2. Transit times of capsule

Transit times of the capsules to the designated location are illustrated in Fig. 4. The variability is large, with no correlation to subject or study session. The majority reached the destination within 250 min (mean 152 ± 92 min; median 117.5 min).

### 3.3. Pharmacokinetics in plasma

Fig. 5 shows mean plasma concentration time profiles and pharmacokinetic parameters are shown in Table 3. In study phase A (ibuprofen solution) the highest $C_{max}$ was reached followed by phases B (suspension small particles, fast infusion), C (suspension large particles, fast infusion) and D (suspension large particles, slow infusion). In phase A also the highest AUC was reached followed by B, D and C with D having a higher AUC than C. As expected, $T_{max}$ was lowest for phase A followed by B and C, which were almost superimposable, and D. Table 4 displays ratios of pharmacokinetic parameters. The following phases were paired in order to address the scientific question: B:A, C:A, D:A, C:B and D:C. Differences in $C_{max}$ and AUC were found in all five pairs with $C_{max}$. C:A, D:A and C:B showing significant ($p < 0.05$) and B:A and D:C showing borderline-significant ($0.05 < p < 0.10$) differences. Comparison of AUC values showed significant deviations ($p < 0.05$) for C:A, while D:A, and C:B were borderline-significant ($0.05 < p < 0.10$). B:A and D:C were non-significant ($p > 0.10$). After plotting individual $C_{max}$ and AUC as a function of the phase, a negative trend for $C_{max}$ can be seen in order of A-B-C-D while AUC values tend to be stable or slightly reduced (Fig. 6).

### 3.4. pH values

Fig. 7 shows a full pH profile for one volunteer. The profile is divided into different phases. A preparation phase (1), which covers the time between calibration and swallowing of the system is followed by a short esophageal phase (2). The rapid drop of the pH signals the beginning of a gastric phase (3), with low pH levels and varying duration. A short emptying phase (4) is followed by further movement into the duodenum (5). The registration ends with the infusion phase (6), which covers the interval after start of the pump until end of the administration. Mean pH profiles and mean baseline pH after start of infusion are shown in Fig. 8 and sorted by respective study phase. The calculated individual baseline pH values and respective areas AUIB, AUIP and ABC are given in Table 5. Interestingly, the effect of ibuprofen dissolution (phases B, C and D) on the measured pH-drop in intestinal fluids correlates with intestinal baseline pH. A higher baseline pH leads to a larger pH reduction (ABC) (Fig. 9).

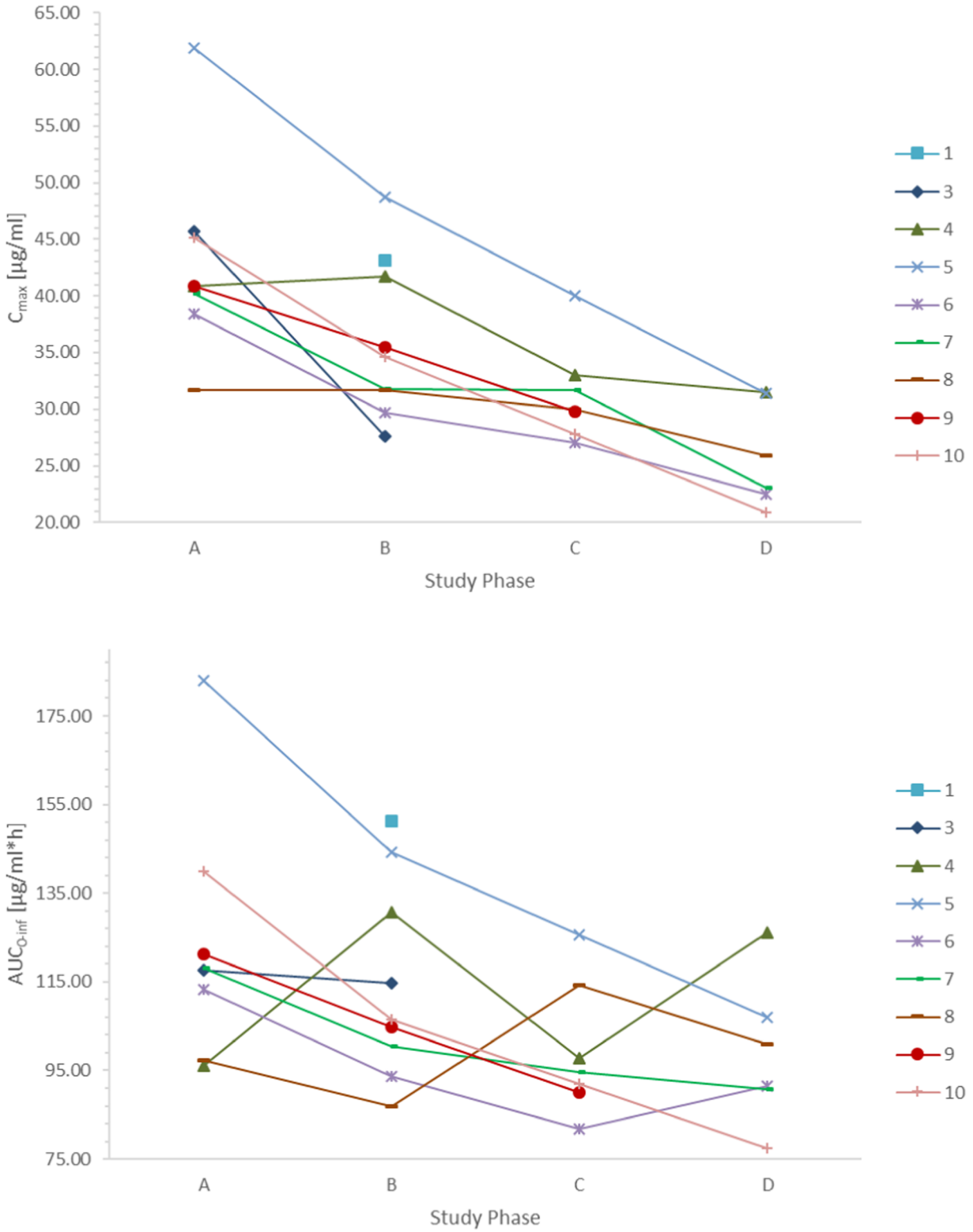


**Fig. 6.** $C_{max}$ and AUC plotted as function of study phase for nine subjects. Note that $C_{max}$ is applied from 20 to 65 µg/ml and $AUC_{0\text{-}inf}$ from 75 to 190 µg/ml h.

## 4. Discussion

The purpose with this study was to get increased knowledge about in vivo dissolution and thus absorption of ibuprofen under controlled conditions. The parameters under investigation which influence these processes are the particle size distribution of ibuprofen, the large inter- and intraindividual variation of gastric emptying times and the pH and buffer capacity of the intestine.

We encountered mainly two problems. One was difficulties in swallowing noted initially. We attributed this to the larger tube size and to the 20 cm long bridging line that possibly created a skein in the throat interfering with the swallowing reflex. It was solved by adopting the pop-bottle technique [35]. More problematic was clogging of the tube. As the setup was tested in vitro for the different drug products and

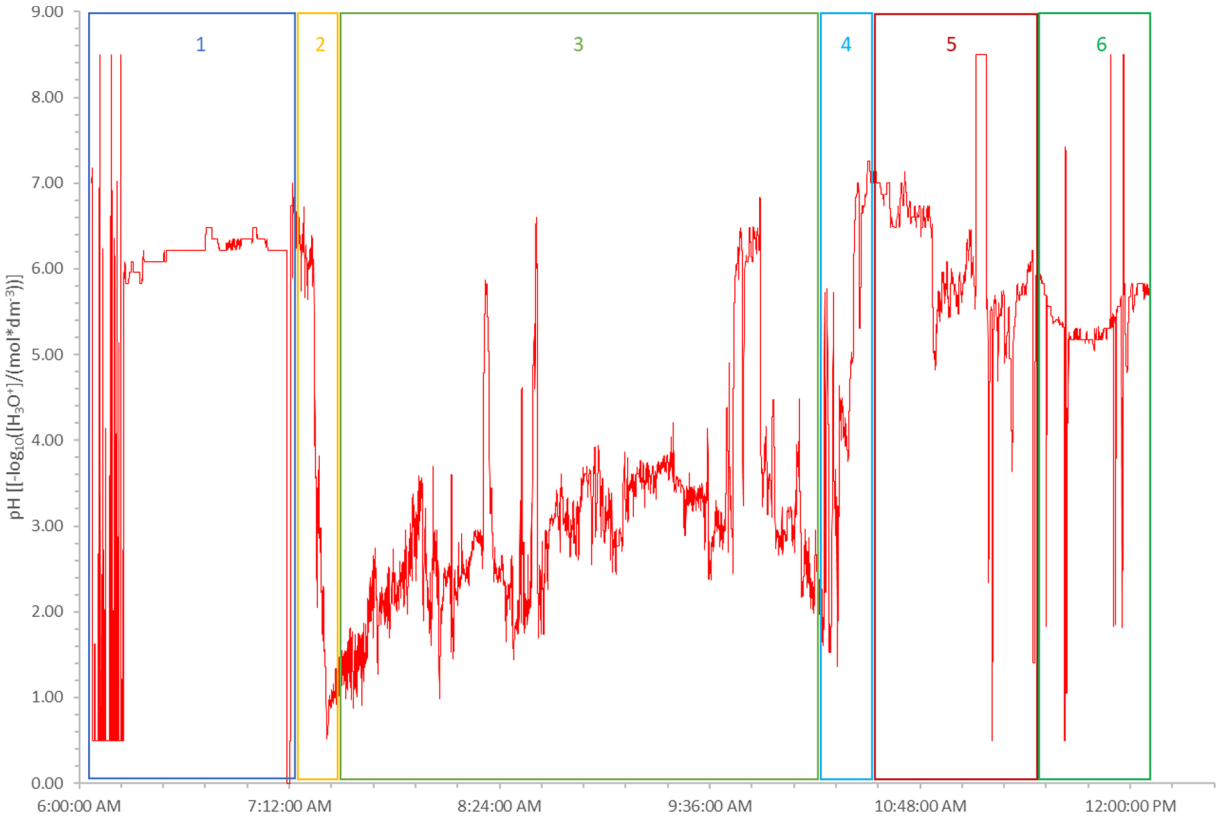


**Fig. 7.** Entire pH time profile of volunteer 7 in study phase B: Block 1 marks preparation, block 2 marks swallowing and transit into the stomach through esophagus, block 3 marks the period in the stomach, block 4 marks gastric emptying, block 5 marks further migration into small intestine and block 6 marks the infusion.

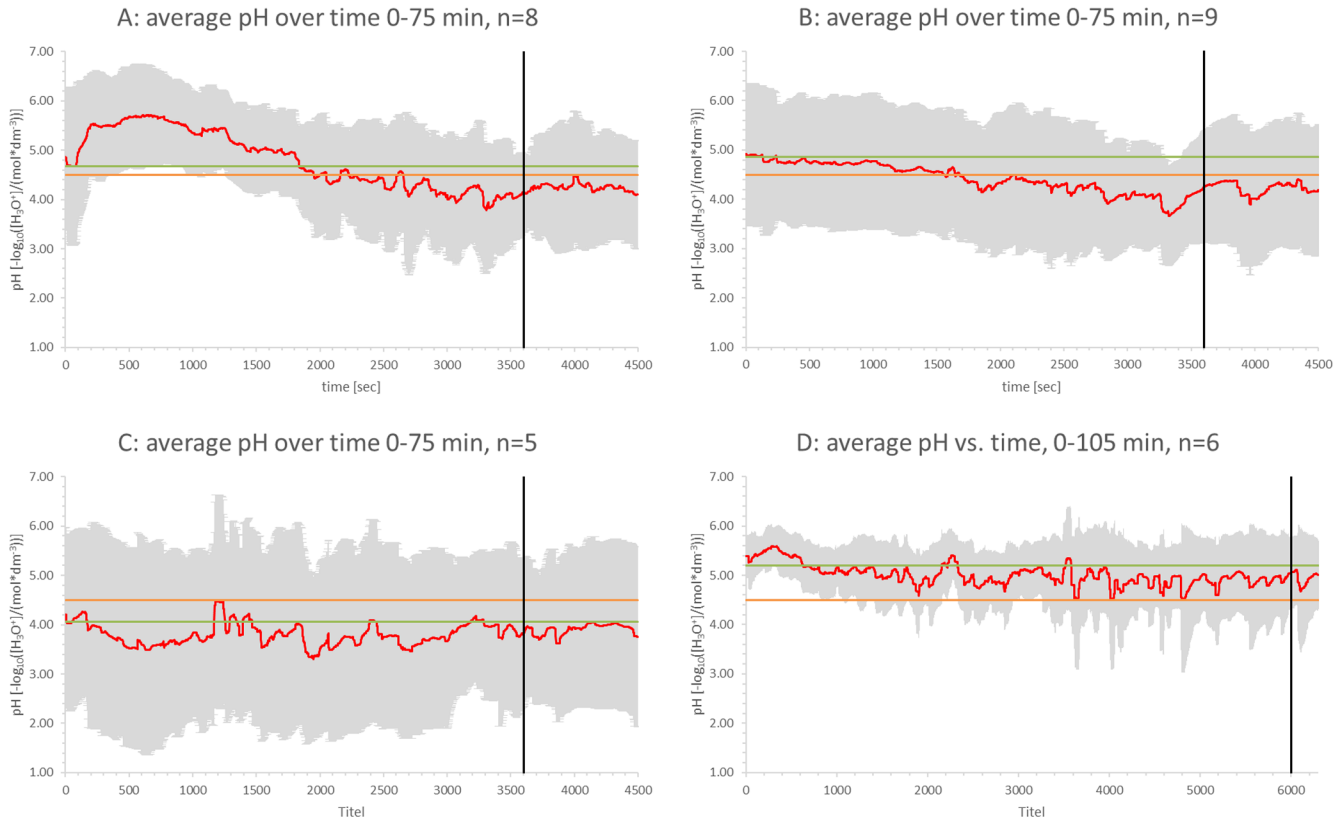


**Fig. 8.** pH vs. time recordings. Red average pH, grey: standard deviation, green: average baseline pH, orange: $pK_a$ Ibuprofen (4.5) [50]. Infusion start was at time 0. Infusion time ended as indicated by black vertical line.

**Table 5**
Evaluation of individual pH data.

| Phase A | 1 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | AM | ASD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Baseline pH | | 5.18 | 4.05 | 4.50 | 6.45 | 5.23 | 5.60 | 2.15 | 4.24 | 4.67 | 1.20 |
| AUIB | | 21,775 | 18,215 | 20,236 | 29,045 | 23,523 | 25,181 | 9680 | 19,080 | 20,842 | 5352 |
| AUIP A | | 20,304 | 17,987 | 20,763 | 29,105 | 22,059 | 24,643 | 14,703 | 18,085 | 20,956 | 4151 |
| ABC | | 1471 | 228 | −527 | −60 | 1463 | 538 | −5023 | 995 | −114 | 1970 |
| Phase B | 1 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | AM | ASD |
| Baseline pH | 5.18 | 5.08 | 5.62 | 5.75 | 5.39 | 6.61 | 5.75 | 2.68 | 1.62 | 4.85 | 1.52 |
| AUIB | 23,328 | 22,850 | 25,269 | 25,896 | 24,268 | 29,739 | 25,875 | 12,060 | 7278 | 21,840 | 6862 |
| AUIP B | 22,011 | 20,168 | 20,691 | 23,428 | 21,795 | 25,108 | 23,075 | 14,827 | 6518 | 19,736 | 5405 |
| ABC | 1317 | 2682 | 4579 | 2468 | 2473 | 4632 | 2800 | −2767 | 760 | 2105 | 2102 |
| Phase C | 1 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | AM | ASD |
| Baseline pH | | | 4.30 | | 1.31 | 5.71 | 6.01 | 2.95 | | 4.05 | 1.75 |
| AUIB | | | 19,340 | | 5878 | 25,673 | 27,027 | 13,263 | | 18,236 | 7890 |
| AUIP C | | | 18,135 | | 4184 | 26,819 | 22,620 | 14,200 | | 17,191 | 7761 |
| ABC | | | 1204 | | 1694 | −1146 | 4408 | −937 | | 1045 | 2024 |
| Phase D | 1 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | AM | ASD |
| Baseline pH | | | 4.41 | 4.84 | 5.74 | 4.32 | 5.65 | | 6.18 | 5.19 | 0.70 |
| AUIB | | | 27,792 | 30,514 | 36,188 | 27,243 | 35,588 | | 38,912 | 32,706 | 4430 |
| AUIP D | | | 25,240 | 28,112 | 32,241 | 26,898 | 31,553 | | 36,233 | 30,046 | 3699 |
| ABC | | | 2553 | 2402 | 3948 | 345 | 4036 | | 2679 | 2660 | 1224 |

infusion speeds without problems, it is likely that blockage has occurred distally at the two holes of the tube. A longer fasting period might reduce the risk of clogging but also decrease compliance.

One restricting factor in typical pharmacokinetic studies is the poor distinction between several relevant and possibly interacting factors. With our new methodology, it is possible to investigate two important aspects of in vivo drug dissolution, i.e. particle size distribution and simulated gastric emptying by infusion into the small intestine under controlled conditions.

The pharmacokinetic parameters of ibuprofen (Table 3) were in accordance with literature data for 400 mg doses [39–43]. More specifically, pharmacokinetic parameters of 400 mg solutions dosed orally in a human bioavailability study were comparable to parameters obtained in this study [44]. Notable differences between A, B, C and D were seen for $C_{max}$ (Table 4). This may be due to different dissolution rates of suspended ibuprofen particles affecting the rate of drug uptake due to the different surface area [45]. The calculated ratio between C and B was 0.85 ($p = 0.0448$) and strengthens the importance of particle size for in vivo dissolution of ibuprofen while it was found to be negligible elsewhere [6]. In the latter study, the particle size effect was detected in vitro but not in vivo. In our opinion, this could have been caused by too similar particle size distributions. Study phase D was designed to investigate an influence of gastric emptying rate on ibuprofen systemic uptake. As expected, slower infusion resulted in lower $C_{max}$ while the difference was borderline significant (D:C: 0.85, $p = 0.0710$). Slower gastric emptying may cause lower $C_{max}$ compared to fast emptying and may be a reason for discrepancies in bioequivalence studies especially if the rate of gastric emptying is very slow [16]. Phase B, C and D show somewhat diminished AUC values compared to Phase A while significance reduction can be shown for Phase C (C:A: 0.79 $p = 0.0235$). Incomplete drug dissolution for doses of 800 mg was reported by Koenigsknecht et al. [16] and might be the reason for reduced bioavailability also in our study. Since systemic availability for large particles was diminished some large particles might have remained undissolved and exit the body undissolved.

Localization of the infusion system based on tube length and pH proved to be robust. Gastric emptying was clearly visible on the real time pH graph (Fig. 7). Running median smoothing did reduce noise and scattering of the signal. Several reasons for scattering were identified. (1) The pH capsule needs incubation in gastrointestinal juices to assure contact with the built-in zinc ring in order to be fully functional. The absence of liquid prevents the generation of a measurable signal. Since liquid in the GI-tract might be separated into pockets of different volume [46], this phenomenon might have contributed to the generation of noise in the pH determinations. (2) It is hypothesized that the tube might be locally disturbing the GI-system to cause abnormal peristalsis or emptying of gastric juice. The latter was identified as risk of altering gastric emptying by changing the function of the pylorus to open and close. Unusual amounts of gastric fluids would bias results and risk the result of the study [16]. No effect on gastric emptying was visible by using a catheter of larger dimension and it was concluded that results with and without catheter should be similar [16,47]. In this work, a tube with an outer diameter of 1.27 mm was used. The line was almost 3-fold smaller than in studies cited above (3.0 mm and 3.3 mm, respectively). (3) Fluctuations in duodenal pH were reported elsewhere [48] and possibly caused by emptying of acidic contents into the small intestine with variation of pH being higher in the proximal segment [27]. The pH values tend to be more stable distal to the ligament of Treitz while fluctuations in proximal segments may also be induced and amplified by the very limited luminal buffer capacity [8].

The solubility of ibuprofen ($pK_a$ 4.5) in acidic form is altered by any dynamic change of pH in the proximal duodenum. As ionizable compound it can be either promoted or handicapped by the surrounding media and therefore change in vivo dissolution rates [2,49,50]. Dissolving ibuprofen may decrease the media pH as previously postulated by in vivo experiments and in silico simulations [7,8]. Fig. 8 shows arithmetic mean pH vs. time profiles over a time range of 0–75 min (phases A, B, and C) and 0–105 min (phase D) with minute 0 being the start of the infusion revealing an in vivo pH-decreasing effect of dissolving ibuprofen particles. Furthermore, this effect on individual intestinal pH was quantified by manual integration of pH profiles (Table 5). Small particles undergoing fast infusion rates led to a more pronounced pH-drop compared to large particles under same flow conditions, as demonstrated by a larger integrated pH effect (ABC). Fast dissolution may more readily deplete the local buffer system (i.e. bicarbonate concentration in small intestine). Due to the lack of bicarbonate, free hydrogen ions generated from dissociation of free ibuprofen acid will not be buffered and thus lower the local intestinal pH. Interestingly, a slower infusion rate (gastric emptying rate) for identical suspensions led to a larger ABC compared to fast infusion. Slower infusion rates will lead to longer transit times of the intestinal aqueous content and thus more time is allowed for dissolution of the drug within the given segment.

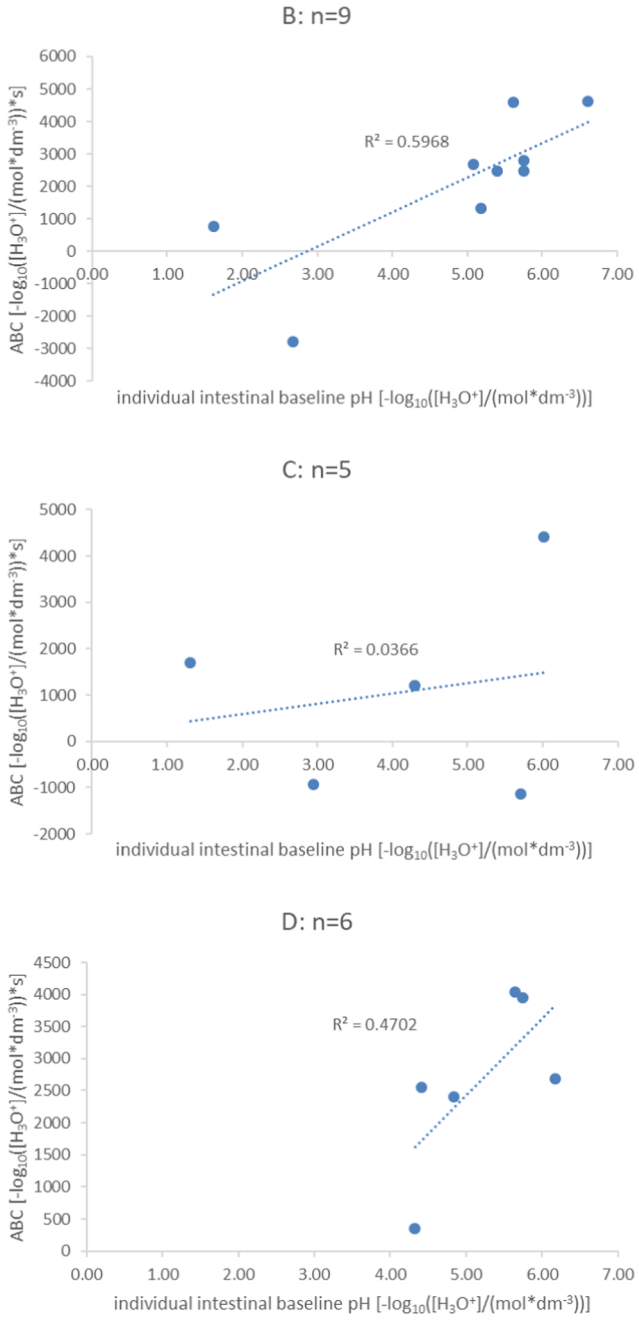


**Fig. 9.** Correlation between area between curves and individual intestinal baseline pH.

## 5. Conclusions

A new in vivo method is introduced allowing the infusion of pharmaceutical suspensions with a defined and characterized particle size to a predefined site of the gastrointestinal tract under a pump-controlled flow rate. The pH was monitored while the system was migrating and the drug product was infused. A relationship between particle size and rate of ibuprofen systemic input was shown. Moreover, different gastric emptying rates were mimicked by changing intestinal infusion rates. The dissolution of ibuprofen from a suspension resulted in a drop of duodenal pH. The latter was characterized and quantified by introducing the "Area Between Curves" concept (ABC). The Bioperm method synergizes the approach of a site-specific administration under avoidance of adverse events and stress to the subjects. Absorption and concurrent intestinal pH monitoring might be helpful for investigations of compounds that show limited, and/or slow dissolution and a pH sensitive dissolution pattern. An extension to other active pharmaceutical ingredients, such as weak bases is obvious. For example, it would be possible to infuse a solution of a basic drug into the intestine and monitor pH changes when the base is precipitating under increasing pH conditions.

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## References

[1] Y. Tsume, D.M. Mudie, P. Langguth, G.E. Amidon, G.L. Amidon, The biopharmaceutics classification system: subclasses for in vivo predictive dissolution (IPD) methodology and IVIVC, Eur. J. Pharm. Sci. 57 (2014) 152–163.
[2] C.D. Herzfeldt, R. Kümmel, Dissociation-constants, solubilities and dissolution rates of some selected non-steroidal antiinflammatories, Drug. Dev. Ind. Pharm. 9 (1983) 767–793.
[3] H. Potthast, J.B. Dressman, H.E. Junginger, K.K. Midha, H. Oeser, V.P. Shah, H. Vogelpoel, D.M. Barends, Biowaiver monographs for immediate release solid oral dosage forms: ibuprofen, J. Pharm. Sci.-US 94 (2005) 2121–2131.
[4] R.M. Watkinson, C. Herkenne, R.H. Guy, J. Hadgraft, G. Oliveira, M.E. Lane, Influence of ethanol on the solubility, Ionization and permeation characteristics of ibuprofen in silicone and human skin, Skin. Pharmacol. Phys. 22 (2009) 15–21.
[5] S.S. Adams, R.G. Bough, E.E. Cliffe, B. Lessel, R.F. Mills, Absorption, distribution and toxicity of ibuprofen, Toxicol. Appl. Pharmacol. 15 (1969) 310–330.
[6] C. Alvarez, I. Núñez, J.J. Torrado, J. Gordon, H. Potthast, A. García-Arieta, Investigation on the possibility of biowaivers for ibuprofen, J. Pharm. Sci.-Us. 100 (2011) 2343–2349.
[7] Y. Tsume, P. Langguth, A. García-Arieta, G.L. Amidon, In silico prediction of drug dissolution and absorption with variation in intestinal pH for BCS class II weak acid drugs: ibuprofen and ketoprofen, Biopharm. Drug. Dispos. 33 (2012) 366–377.
[8] B. Hens, Y. Tsume, M. Bermejo, P. Paixao, M.J. Koenigsknecht, J.R. Baker, W.L. Hasler, R. Lionberger, J.H. Fan, J. Dickens, K. Shedden, B. Wen, J. Wysocki, R. Loebenberg, A. Lee, A. Frances, G. Amidon, A. Yu, G. Benninghoff, N. Salehi, A. Talattof, D.X. Sun, G.L. Amidon, Low buffer capacity and alternating motility along the human gastrointestinal tract: implications for in vivo dissolution and absorption of ionizable drugs, Mol. Pharmaceut. 14 (2017) 4281–4294.
[9] G. Jobin, A. Cortot, J. Godbillon, M. Duval, J.P. Schoeller, J. Hirtz, J.J. Bernier, Investigation of drug absorption from the gastrointestinal-tract of man. 1. Metoprolol in the Stomach, Duodenum and Jejunum, Brit. J. Clin. Pharmacol. 19 (1985) S97–S105.
[10] H. Lennernäs, O. Ahrenstedt, R. Hallgren, L. Knutson, M. Ryde, L.K. Paalzow, Regional jejunal perfusion, a new invivo approach to study oral-drug absorption in man, Pharmaceut. Res. 9 (1992) 1243–1251.
[11] W.H. Barr, E.M. Zola, E.L. Candler, S.M. Hwang, A.V. Tendolkar, R. Shamburek, B. Parker, M.D. Hilty, Differential absorption of amoxicillin from the human small and large-intestine, Clin. Pharmacol. Ther. 56 (1994) 279–285.
[12] K.K.H. Chan, A. Buch, R.D. Glazer, V.A. John, W.H. Barr, Site-differential gastrointestinal absorption of benazepril hydrochloride in healthy-volunteers, Pharmaceut. Res. 11 (1994) 432–437.
[13] T. Gramatté, E. Eldesoky, U. Klotz, Site-dependent small-intestinal absorption of ranitidine, Eur. J. Clin. Pharmacol. 46 (1994) 253–259.
[14] L. Bønløkke, F.N. Christensen, L. Knutson, H.G. Kristensen, H. Lennernäs, A new approach for direct in vivo dissolution studies of poorly soluble drugs, Pharmaceut. Res. 14 (1997) 1490–1492.
[15] S.C. Sutton, The use of gastrointestinal intubation studies for controlled release development, Brit. J. Clin. Pharmacol. 68 (2009) 342–354.
[16] M.J. Koenigsknecht, J.R. Baker, B. Wen, A. Frances, H.X. Zhang, A. Yu, T. Zhao, Y. Tsume, M.P. Pai, B.E. Bleske, X.Y. Zhang, R. Lionberger, A. Lee, G.L. Amidon, W.L. Hasler, D.X. Sun, In vivo dissolution and systemic absorption of immediate release ibuprofen in human gastrointestinal tract under fed and fasted conditions,

Mol. Pharmaceut. 14 (2017) 4295–4304.
[17] W. Behrendt, R. Kuhlen, Effects of anesthesia and analgosedation on gastrointestinal functioning, Chir. Gastroenterol. 16 (2000) 1–6.
[18] T. Inada, T. Asai, M. Yamada, K. Shingu, Propofol and midazolam inhibit gastric emptying and gastrointestinal transit in mice, Anesth. Analg. 99 (2004) 1102–1106.
[19] A.H. Staib, D. Loew, S. Harder, E.H. Graul, R. Pfab, Measurement of theophylline absorption from different regions of the gastrointestinal-tract using a remote controlled drug delivery device, Eur. J. Clin. Pharmacol. 30 (1986) 691–697.
[20] A. Lambert, F. Vaxman, F. Crenner, T. Wittmann, J.F. Grenier, Autonomous telemetric capsule to explore the small-bowel, Med. Biol. Eng. Comput. 29 (1991) 191–196.
[21] Y.K. Pithavala, W.D. Heizer, A.F. Parr, R.L. O'Connor-Semmes, K.L.R. Brouwer, Use of the InteliSite (R) capsule to study ranitidine absorption from various sites within the human intestinal tract, Pharmaceut. Res 15 (1998) 1869–1875.
[22] I. Wilding, Site-specific drug delivery in the gastrointestinal tract, Crit. Rev. Ther. Drug Carrier Syst. 17 (2000) 557–620.
[23] I.R. Wilding, A.L. Connor, P. Carpenter, C. Rordorf, J. Branson, S. Milosavljev, G. Scott, Assessment of lumiracoxib bioavailability from targeted sites in the human intestine using remotely activated capsules and gamma scintigraphy, Pharmaceut. Res. 21 (2004) 443–446.
[24] T. Zhu, J.C. Ansquer, M.T. Kelly, D.J. Sleep, R.S. Pradhan, Comparison of the gastrointestinal absorption and bioavailability of fenofibrate and fenofibric acid in humans, J. Clin. Pharmacol. 50 (2010) 914–921.
[25] W. Weitschies, H. Blume, H. Mönnikes, Magnetic marker monitoring: high resolution real-time tracking of oral solid dosage forms in the gastrointestinal tract, Eur. J. Pharm. Biopharm. 74 (2010) 93–101.
[26] B. Hens, J. Brouwers, B. Anneveld, M. Corsetti, M. Symillides, M. Vertzoni, C. Reppas, D.B. Turner, P. Augustijns, Gastrointestinal transfer: In vivo evaluation and implementation in in vitro and in silico predictive tools, Eur. J. Pharm. Sci. 63 (2014) 233–242.
[27] M. Koziolek, M. Grimm, D. Becker, V. Iordanov, H. Zou, J. Shimizu, C. Wanke, G. Garbacz, W. Weitschies, Investigation of pH and temperature profiles in the GI tract of fasted human subjects using the intellicap((R)) system, J. Pharm. Sci.-US 104 (2015) 2855–2863.
[28] B. Hens, M. Corsetti, R. Spiller, L. Marciani, T. Vanuytsel, J. Tack, A. Talattof, G.L. Amidon, M. Koziolek, W. Weitschies, C.G. Wilson, R.J. Bennink, J. Brouwers, P. Augustijns, Exploring gastrointestinal variables affecting drug and formulation behavior: methodologies, challenges and opportunities, Int. J. Pharm. 519 (2017) 79–97.
[29] H. Lennernäs, A. Lindahl, A. Van Peer, C. Ollier, T. Flanagan, R. Lionberger, A. Nordmark, S. Yamashita, L. Yu, G.L. Amidon, V. Fischer, E. Sjögren, P. Zane, M. McAllister, B. Abrahamsson, In vivo predictive dissolution (IPD) and biopharmaceutical modeling and simulation: future use of modern approaches and methodologies in a regulatory context, Mol. Pharmaceut. 14 (2017) 1307–1314.
[30] L. Nyberg, W. Månsson, B. Abrahamsson, J. Seidegård, O. Borgå, A convenient method for local drug administration at predefined sites in the entire gastrointestinal tract: experiences from 13 phase I studies, Eur. J. Pharm. Sci. 30 (2007) 432–440.
[31] J. Seidegård, L. Nyberg, O. Borgå, Presystemic elimination of budesonide in man when administered locally at different levels in the gut, with and without local inhibition by ketoconazole, Eur. J. Pharm. Sci. 35 (2008) 264–270.
[32] J. Seidegård, L. Nyberg, O. Borgå, Differentiating mucosal and hepatic metabolism of budesonide by local pretreatment with increasing doses of ketoconazole in the proximal jejunum, Eur. J. Pharm. Sci. 46 (2012) 530–536.
[33] D. Dahlgren, C. Roos, A. Lundqvist, B. Abrahamsson, C. Tannergren, P.M. Hellström, E. Sjögren, H. Lennernäs, Regional intestinal permeability of three model drugs in human, Mol. Pharmaceut. 13 (2016) 3013–3021.
[34] L.L. Mazaleuskaya, K.N. Theken, L. Gong, C.F. Thorn, G.A. FitzGerald, R.B. Altman, T.E. Klein, PharmGKB summary: ibuprofen pathways, Pharmacogenet. Genomics 25 (2015) 96–106.
[35] J.T. Schiele, H. Schneider, R. Quinzler, G. Reich, W.E. Haefeli, Two techniques to make swallowing pills easier, Ann. Fam. Med. 12 (2014) 550–552.
[36] T.T. Kararli, Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory-animals, Biopharm. Drug. Dispos. 16 (1995) 351–380.
[37] E.J. Williams, Experimental designs balanced for the estimation of residual effects of treatments, Aust. J. Scientific Res. Ser. A 2 (1949) 149–169.
[38] Y. Zhang, M.R. Huo, J.P. Zhou, S.F. Xie, PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel, Comput. Methods Programs Biomed. 99 (2010) 306–314.
[39] K.S. Albert, C.M. Gernaat, Pharmacokinetics of Ibuprofen, Am. J. Med. 77 (1984) 40–46.
[40] N.M. Davies, Clinical pharmacokinetics of ibuprofen. The first 30 years, Clin Pharmacokinet 34 (1998) 101–154.
[41] P. Bramlage, A. Goldis, Bioequivalence study of three ibuprofen formulations after single dose administration in healthy volunteers, BMC Pharmacol. 8 (2008) 18.
[42] P.M. Dewland, S. Reader, P. Berry, Bioavailability of ibuprofen following oral administration of standard ibuprofen, sodium ibuprofen or ibuprofen acid incorporating poloxamer in healthy volunteers, BMC Clin. Pharmacol. 9 (2009) 19.
[43] T. Tanner, S. Aspley, A. Munn, T. Thomas, The pharmacokinetic profile of a novel fixed-dose combination tablet of ibuprofen and paracetamol, BMC Clin. Pharmacol. 10 (2010) 10.
[44] W.R. Gillespie, A.R. DiSanto, R.E. Monovich, K.S. Albert, Relative bioavailability of commercially available ibuprofen oral dosage forms in humans, J. Pharm. Sci. 71 (1982) 1034–1038.
[45] K.R. Chu, E. Lee, S.H. Jeong, E.S. Park, Effect of particle size on the dissolution behaviors of poorly water-soluble drugs, Arch. Pharm. Res. 35 (2012) 1187–1195.
[46] D.M. Mudie, K. Murray, C.L. Hoad, S.E. Pritchard, M.C. Garnett, G.L. Amidon, P.A. Gowland, R.C. Spiller, G.E. Amidon, L. Marciani, Quantification of gastrointestinal liquid volumes and distribution following a 240 mL dose of water in the fasted state, Mol. Pharm. 11 (2014) 3039–3047.
[47] S.A. Müller-Lissner, C.J. Fimmel, N. Will, W. Müller-Duysing, F. Heinzel, A.L. Blum, Effect of gastric and transpyloric tubes on gastric emptying and duodenogastric reflux, Gastroenterology 83 (1982) 1276–1279.
[48] D. Riethorst, R. Mols, G. Duchateau, J. Tack, J. Brouwers, P. Augustijns, Characterization of human duodenal fluids in fasted and fed state conditions, J Pharm. Sci.-Us 105 (2016) 673–681.
[49] W.I. Higuchi, E. Nelson, J.G. Wagner, Solubility + dissolution rates in reactive media, J. Pharm. Sci.-US 53 (1964) 333.
[50] A. Avdeef, K.J. Box, J.E.A. Comer, C. Hibbert, K.Y. Tam, pH-metric logP 10. Determination of liposomal membrane-water partition coefficients of ionizable drugs, Pharmaceut. Res. 15 (1998) 209–215.