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RESEARCH PAPER

Design of Biorelevant Test Setups for the Prediction of Diclofenac *In Vivo* Features After Oral Administration

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ABSTRACT

Purpose Design of biorelevant test setups mimicking the physiological conditions experienced by drugs after oral administration along the passage through the mouth and the GI tract for the *in vitro* evaluation of diclofenac exhibiting multiple-peak phenomenon during absorption.

Methods The biorelevant models simulated successively saliva (SSF, pH6.2–6.75–7.4, 5 mL, 3 min), gastric (SGF-FaSSGF, pH 1.2–1.6, 50–250 mL, 30 min) and intestinal (FaSSIF, pH6.8, 250 mL, 60 min) fluids. Applying these models, diclofenac free acid and its sodium/potassium salt were comparatively evaluated for dissolution and further characterized by HPLC, optical morphogranulometry, DSC and PXRD to elucidate peculiar behaviors.

Results Diclofenac salts almost completely dissolved in SSF and showed a transitional dissolution pattern before complete precipitation in SGF/FaSSGF. This peculiar pattern correlated with simultaneous chemical modification and formation of agglomerates. With low dissolution in SSF and almost immediately complete precipitation, these behaviors were not observed with diclofenac free acid. Distinct diclofenac features were strongly determined by pH-modifications after oral administration.

Conclusions The multiple-peak phenomenon observed after administrating a solution, suspension or dispersible formulation of diclofenac salts are likely caused by drug precipitation and agglomeration in the stomach leading to irregular gastric-emptying. Diclofenac free acid may provide more reliable *in vivo* features.

KEY WORDS biorelevant test setups · diclofenac · mouth exposure · multiple absorption peaks · orodispersible tablets

ABBREVIATIONS

API	active pharmaceutical ingredient
D/S	dose/solubility
DR	delayed release
DSC	differential scanning calorimetry
FaSSGF	fasted state simulating gastric fluid
FaSSIF	fasted state simulating intestinal fluid
GI	gastrointestinal
HPLC	high performance liquid chromatography
IR	immediate release
NSAID	non-steroidal anti inflammatory drug
PXRD	powder x-ray diffraction
SGF	simulated gastric fluid
SR	sustained release
SSF	simulated salivary fluid

INTRODUCTION

Drug absorption following oral administration and subsequent clinical response are influenced by different variables including the physicochemical properties of the drug, physiological factors of the human body and features related to the drug formulation (1). The appearance of multiple peaks in plasma concentration-time

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curves due to drug erratic absorption from the gastrointestinal (GI) tract is a phenomenon occasionally encountered in pharmacokinetics (2). The potent non-steroidal anti-inflammatory drug (NSAID) diclofenac (weak acid pK_a 3.80; logP 4.4; logD 1.4 at pH6.8) is one well-described case for this phenomenon (3). As a representative of the phenylacetic acid derivatives group, diclofenac is widely used in the long-term treatment of inflammation and painful conditions of rheumatic and non-rheumatic origin (4). To date, most of the marketed oral dosage forms of diclofenac comprise its sodium or potassium salt. After oral administration of these formulations the appearance of two or more peaks of diclofenac in individual plasma concentration-time profiles are often reported (5–7).

Various mechanisms causing irregular absorption profiles of diclofenac, including formation of a poorly soluble tetrahydrate, bile-dependent intestinal absorption, enterohepatic recirculation or separated absorption windows along the GI tract, have been suggested so far (8–10). However, to date the occurrence of a multiple-peak phenomenon has been suggested to be dosage form-dependent (11). The administration of most common diclofenac sodium delayed-release (DR) preparations in fasted subjects is usually characterized by a single peak, i.e. one maximum in the plasma concentration-time curve (12). In contrast, the oral administration of diclofenac solutions, suspensions, effervescent or immediate-release (IR) preparations typically results in two or more peaks in the first hours after dosing. However, after a high initial peak concentration, multiple peaks are not further observed (6,7,13). To explain such phenomenon, alternative mechanisms, involving strong pH-dependent dissolution of diclofenac associated with fractionated gastric-emptying, have been proposed (5,11). According to such models, the initial peak in plasma concentration-time profiles would reflect rapid absorption of a part of the administered dose; the second and additional late peaks would be a result of the gastric-emptying of the remaining part of the dose that had precipitated under acidic conditions within the stomach where diclofenac solubility is poor (5,11). This hypothesis is supported by results from other published pharmacokinetic studies where diclofenac was administered as either an oral IR formulation or directly into the stomach (13,14). Interestingly, the multiple-peak phenomenon has not been observed with equivalent oral formulations comprising the free acid of diclofenac. This fact would suggest a contrasting performance of the drug free acid in the stomach, i.e. a contrasting precipitation behavior, compared to the salts. Increased interest has thus gained towards the unmodified form launched as a dispersible formulation (15,16).

It is proposed that a deeper understanding of the mechanisms involved in the heterogeneity of absorption observed from different drug forms of diclofenac and also other drugs could improve the selection of the most appropriate candidate for optimal bioavailability and patient treatment.

The correlation of *in vitro* drug dissolution data with *in vivo* drug profiles (*IVIVC*) is one of the prime challenges in pharmaceutical research (17). Many attempts have been made to design biorelevant *in vitro* tests taking into account relevant physiological data to improve the predictability of drug bioperformance (18). Since diclofenac is a poorly soluble and highly permeable weak acid, its bioavailability from solid oral dosage forms is mostly controlled by the rate and extent of dissolution in the GI tract (19). Therefore, adequate simulation of the relevant physiological conditions following oral diclofenac administration, in particular the strong pH-changes along the successive GI compartments, is a major prerequisite for estimating the *in vivo* behavior of the formulation studied. In the past, the *in vitro* dissolution characteristics of diclofenac salts from various formulations have been extensively studied under biorelevant conditions (11,14,20). The focus of these studies was to adequately simulate the composition of gastric and small intestinal fluids as well as the hydrodynamics in the respective GI segments. Even if diclofenac can be also administered by means of orodispersible formulations the oral cavity, and thus the contact of the drug with saliva, has never been addressed in detail. However, mouth exposure may be critical evaluating drug bioperformance from certain delivery systems especially solutions, suspensions as well as effervescent or dispersible formulations. Disregarding this compartment where *in vivo* the drug may already start to dissolve in the *in vitro* test design could lead to a poor predictive power of *in vitro* dissolution profiles (21).

In the literature few data are available on diclofenac sodium and potassium performances as active pharmaceutical ingredients (APIs); even fewer on the free acid. Moreover, besides dissolution screening, only little work has been focused on diclofenac features. A comparative screening of additional properties and performances of the three diclofenac APIs could therefore provide for a deeper insight into their peculiar biopharmaceutical features after oral administration.

The aim of the present study was therefore to develop biorelevant *in vitro* test setups for studying the behaviors of three different forms of diclofenac under the physiological conditions experienced by formulation after oral administration. Particular attention was given to adequately simulate drug exposure to the mouth

where the dosage form is already disintegrated or may disintegrate and where thus dissolution may initiate, as well as the stomach as the site where the drug has to rest until it is emptied into the small intestine, the site of absorption. The use of the novel test models is expected to provide a better understanding of the peculiar diclofenac *in vivo* features distinctly observed from the sodium and the potassium salts compared to the free acid.

MATERIALS AND METHODS

Materials

Commercially available grade of diclofenac free acid, diclofenac sodium and diclofenac potassium were used. Diclofenac free acid was purchased from Aarti Drugs Limited (India). Diclofenac sodium and diclofenac potassium were obtained from Unique Chemicals (India). Pepsin from porcine stomach mucosa (0.064 mg pepsin/mg solid) was purchased from Sigma Chemical (USA). Egg-lecithin (Lipoid® E PC S, > 98% phosphatidylcholine) was kindly donated from Lipoid GmbH (Germany). Sodium taurocholate (NaTC, PCA code 2012, > 99% pure) was purchased from Prodotti Chimici e Alimentari SpA (Italy). All other chemicals were of analytical or pharmaceutical grade and used as received.

To compare the behaviors of the three drug forms of diclofenac, the test dose was set at 23.25 mg of diclofenac free acid or at the free acid equivalent of its sodium/potassium salt; i.e. 23.25 mg of diclofenac free acid, 25 mg of diclofenac sodium and 26.25 mg of diclofenac potassium were used respectively in the study.

Solubility Studies

Diclofenac solubilities in several media with different pH values were assessed. The equilibrium solubility was determined using the saturation shake-flask method. Briefly, an excess amount of drug was added into the different media maintained at 37 ± 0.5°C and shaken at 50 rpm for at least 24 h, until equilibrium was evident. After equilibrium was reached, the excess of solid was removed by microfiltration using a 0.22 μm Whatman glass microfiber filter (Whatman, UK) and 1 mL of each sample was then transferred into a vial containing 0.4 mL of methanol for stabilization. After mixing the concentration of the substance in the supernatant solutions was determined by HPLC. All measurements were performed in triplicate. Subsequent to the solubility experiments, the dose/solubility ratios (D/S) were calculated according to the Biopharmaceutical Classification System (BCS) based on a dose of 23.25 mg of diclofenac (in equivalent free acid content). As diclofenac solubility is strongly pH-dependent, the pH of the media was confirmed at the end of the incubation period to ensure a sufficient buffer capacity of the test media.

Particle Size Analysis

The particle size distribution of diclofenac API powders was determined by laser diffraction in dry and wet dispersions. The methods used a Coulter LS 100 Q diffraction apparatus (Beckman Coulter, USA) equipped with a 750 nm wavelength laser beam. The scattered light was received on 126 detectors measuring particles from 0.375 to 948.2 μm using 84 size channels.

Dry dispersions of the APIs were obtained using a Dry Powder Module and a Powder Feeding System (vibrator and auger) adapted for free and non-free flowing samples (Beckman Coulter, USA). Powders were introduced into the hopper and circulated through the cell placed in the path of the laser beam. The powder feed rate was adjusted using appropriate auger and vibrator settings to obtain a suitable obscuration rate (4–7%) and a homogeneous powder flow.

Wet dispersions were prepared using a Small Volume Module Plus and a Fluid Transfer Pump (Beckman Coulter, USA). Small amounts of drugs were incorporated into 50 mL of 0.1 N HCl pH 1.1 and kinetic measurements were performed. Samples were withdrawn after 0, 30, 60, 90 and 120 min and introduced drop by drop into the system until the obscuration rate reached 8–12%.

Volumetric particle size distributions were calculated from the light energy distribution. According to the international standard for laser diffraction recommendations (ISO13320-1), Mie theory was used for the determination of particle size distribution. The optical model consisted of 1.0003 air refractive index, 1.431 $g.mL^{-1}$ sample density, 1.661 and 0.01 diclofenac real and imaginary components of the refractive index respectively (22,23). All measurements were performed in triplicate.

Dissolution Testing

A compendial dissolution method using apparatus II (paddle) and a biorelevant method using self-designed *in vitro* test setups were applied to evaluate diclofenac dissolution performances.

Compendial Dissolution Method

The compendial method using the Sotax apparatus II (paddle) model AT7 (Sotax, Switzerland) utilized 1,000 mL of phosphate buffer pH6.8 ± 0.05 as dissolution medium. The test

temperature was set at 37 ± 0.5°C and the paddle was driven at 50 rpm. The dose was set at 23.25 mg of diclofenac (in equivalent free acid content). The samples times were 5, 10, 15, 20, 30, 45 and 60 min. In addition, a pH-change method (official method for delayed-release articles, USP method "A") consisting of 2 h in 750 mL 0.1 N HCl pH1.1 before the phosphate buffer (1,000 mL) stage was investigated with sampling points at 30, 60, 90 and 120 min in the acidic stage and 5, 10, 15, 20, 30, 40, 50 and 60 min in the buffer stage. Samples of 5 mL were withdrawn automatically through the sampling device and fed back into the dissolution vessel after analysis. After filtration through a glass microfiber filter (Whatman GF/D) the cumulative drug release was monitored online using a UV spectrophotometer Perkin-Elmer Lambda 25 (Perkin-Elmer, USA) operating at 276 nm.

Biorelevant Dissolution Method

Biorelevant Conditions. *In vitro* diclofenac dissolution performance was evaluated mimicking oral administration in fasted state conditions. The biorelevant media and dissolution scheme are described in Tables I and II.

Drug exposure to the mouth (Table II step 1) was simulated by 3 min contact with 5 mL of simulated salivary fluid (SSF) as adapted from the literature (24–26). The pH values were adjusted according to the human physiological pH range of 6.2–7.4 (27).

Drug residence in the fasted stomach (Table II step 2) was then mimicked by 30 min contact with the standard compendial medium simulated gastric fluid *sine pepsin* (SGFsp) or the biorelevant dissolution medium fasted state simulating gastric fluid (FaSSGF) (18,28). Media pH values conformed to the average fasted gastric pH value usually lying in the range of pH1.5–1.9 (19). The fasted state gastric volume was set at 50 mL to represent the maximum resting volume in the fasted stomach (28). In an additional setup, a volume of 250 mL of simulated gastric fluid was used to mimic co-administration of the formulation with about 200 mL of fluid.

The contact with the intraluminal contents of the fasted small intestine (Table II step 3) was simulated by 60 min in 250 mL of fasted state simulating intestinal fluid (FaSSIF) pH6.8 adapted from the literature (19).

Dissolution Studies. The study design of the dissolution studies is summarized in Table III.

As the physiological volume of saliva is very small, a physiological fluid volume can hardly be used for dissolution studies. With the aim of simulating the *in vivo* conditions as close as possible, both the test dose of diclofenac and the test volume of SSF were therefore scaled up to the same extent; in detail 232.5 mg of diclofenac (in equivalent free acid content) were tested in 50 mL of SSF. The sampling times were 0.5, 1, 1.5, 2, 2.5, 3 and 5 min, respectively.

For studying gastric dissolution, after swallowing, a single dose of 23.25 mg of diclofenac (in equivalent free acid content) was added to a physiological volume of SSF (5 mL). After 3 min, the solution/suspension content was transferred into either 45 mL or 245 mL of simulated gastric fluid (SGF or FaSSGF) yielding to 50 mL or 250 mL of dissolution volume respectively. Subsequent to this media change, samples were taken after 1, 2, 3, 4, 5, 7.5, 10, 15 and 30 min.

To investigate the dissolution in the fasted upper small intestine, after swallowing and gastric-emptying, a single dose of 23.25 mg of diclofenac (in equivalent free acid content) was added to a physiological volume of SSF (5 mL) during 3 min. The resulting solution/suspension content was transferred into 45 mL of simulated gastric fluid (SGF or FaSSGF). After 30 min, this content was further added to 200 mL of a buffer for neutralization yielding to 250 mL of FaSSIF. Subsequently, samples were taken after 2.5, 5, 10, 15, 20, 30, 45 and 60 min.

Table I Biorelevant Dissolution Media Used for Simulation of the Conditions in the Oral Cavity and Upper Gastrointestinal Tract in the Fasted State

Composition/Medium	SSF I	SSF 2	SGFsp	FaSSGF	FaSSIF
Bile secretions					
Sodium taurocholate	–	–	–	80 μM	3 mM
Lecithin	–	–	–	20 μM	0.75 mM
Enzyme					
Pepsin	–	–	–	0.1 mg/mL	0.1 mg/mL
Sodium dihydrogenphosphate		2.38 g/L	–	–	3.438 g/l
Potassium dihydrogenphosphate	1.63 g/L	0.19 g/L	–	–	–
Sodium chloride	2.34 g/L	8.0 g/L	34.2 mM	34.2 mM	6.186 g/L
Calcium chloride	0.17 g/L	–	–	–	–
Hydrochloric acid conc.	–	–	7 mL/L	7 mL/L	–
Sodium hydroxide	ad pH6.2	ad pH6.75	ad pH1.2	ad pH1.6	ad pH6.8
Demineralized water	ad 1 L	ad 1 L	ad 1 L	ad 1 L	ad 1 L
pH	6.2–6.75–7.4	6.75	1.2–1.6	1.6	6.8

Table II Scheme for Biorelevant Dissolution Testing of the Three Diclofenac Drug Forms Assuming Intake in the Fasted State

Step	Segment of the GI tract	Dissolution media	Volume (mL)		Residence time (min)
			Intake without water	Intake with water (200 mL)	
1	Oral cavity	SSF1 or SSF 2	5	5	3
2	Stomach	SGFsp or FaSSGF	50	250	30
3	Small intestine	FaSSIF	250	–	60

In all dissolution experiments, the temperature was maintained at 37±0.5°C and the agitation rate was 50 rpm. All experiments were performed in triplicate.

Sample Treatment and Assay. Samples of 3 mL were withdrawn manually (with sample replacement by fresh media) using 5 mL Fortuna Optima syringes (Sigma-Aldrich, USA) fitted with stainless tubings connected to 10 μm poroplast filters (Erweka, Germany). The samples were then filtered through 0.22 μm PVDF membrane filters (Whatman, UK) discarding the first 1 mL. Drug adsorption onto the filters was checked by HPLC and confirmed to be negligible. The amount of diclofenac release was analyzed by HPLC. Except for the dissolution tests in SGF/FaSSGF, the samples collected from the experiments were immediately injected into the HPLC system after filtration. For dissolution tests in SGF/FaSSGF, 1 mL of the collected samples was first stabilized by addition to a vial containing 0.4 mL of methanol then injected into the HPLC system after mixing. The HPLC analysis was performed on a Perkin Elmer (Perkin-Elmer, USA) equipped with a binary pump 200, a diode array detector 235C, an autosampler ISS 200 and a thermostated column. Diclofenac samples were analyzed using a Xterra MS C8 5 μm column (length 150 mm, internal diameter 3.9 mm) (Waters, USA) and a C8 pre-column Security Guard Cartridge System kit (Phenomenex, Switzerland). The mobile phase comprised a mixture of methanol and phosphate buffer solution pH2.5±0.05 70:30 with a flow rate of 1 mL/min (isocratic mode). The injection volume was 30 μl for assay determination, except for the dissolution tests in SSF which used 3 μl. The column temperature was 25°C and the detection wavelength was set at 254 nm. Drug elution could be observed after about 6 min.

As the exposure to the successive media could lead to the degradation of the drug, dissolution patterns might be subsequently affected (29). To monitor the chemical stability of the three diclofenac forms along the dissolution tests, the main impurity as described in the diclofenac USP monograph, also known as diclofenac Related Compound A (diclofenac RC A) or (*N*-(2,6-dichlorophenyl)indolin-2-one), was quantified using the same detection system.

Particle Counting

The counting of drug particles was performed applying an optical morphogranulometer Occhio FlowCell FC200S+ (Occhio, Belgium) based on image analysis technology. The system was equipped with a high precision syringe pump, a core cell of 200 μm width and a telecentric lens of a magnification of 4. The samples were evaluated by blue backlighting illumination using a 440 nm collimated monochromatic light beam to analyze particles in the range of 400 nm to 1 mm. CountCal standards (ThermoScientific, USA) were used to calibrate the equipment at 0.86 μm/pixel. A single dose of 23.25 mg of diclofenac (in equivalent free acid content) was

Table III Simulated Conditions Applied for the Dissolution Study of the Three Diclofenac Drug Forms

	Dissolution test conditions		
	Oral cavity	Stomach	Intestine
Dissolution media	SSF I pH6.2	SSF I pH6.2 → SGF pH1.2	
		SSF I pH6.2 → SGF pH1.6	
	SSF I pH6.75	SSF I pH6.75 → SGF pH1.2	
	SSF 2 pH6.75	SSF 2 pH6.75 → SGF pH1.2	
	SSF I pH7.4	SSF I pH7.4 → SGF pH1.2	
		SSF I pH7.4 → SGF pH1.6	SSF I pH7.4 → SGF pH1.6 → FaSSIF pH6.8
		SSF I pH7.4 → FaSSGF pH1.6	SSF I pH7.4 → FaSSGF pH1.6 → FaSSIF pH6.8
Residence time (min)	3	30	60
Sampling points (min)	0.5, 1, 1.5, 2, 2.5, 3, 5	1, 2, 3, 4, 5, 7.5, 10, 15, 30	2.5, 5, 10, 15, 20, 30, 45, 60

introduced into 5 mL of SSF 1 pH7.4. After 3 min, the resulting suspension/solution was transferred into 45 mL of SGF pH1.6 or FaSSGF pH1.6 yielding to 50 mL of test volume. During all experiments, the temperature was maintained at 37±0.5°C and the agitation rate was 50 rpm. Measurements were performed after 3 min in SSF and after 2.5, 5, 7.5, 10, 15 and 30 min in SGF/FaSSGF after the media change. The software Callisto 2012 5.38 was used for image acquisition.

Particle Image Analysis

Microphotographs of drug particles were captured with a Zeiss AxioCam MRc5 Digital Camera mounted on a Zeiss AxioScope A1 microscope (Carl Zeiss, Germany) using an 25–1000 amplification range. Image analyses of diclofenac particles were performed in the dry state, i.e. on start powder materials, and in suspension. In order to visualize suspended particles avoiding any sampling, experiments were scaled down and performed directly onto glass plates. Therefore, a single dose of 2.325 mg of diclofenac (in equivalent free acid content) was introduced into 0.5 mL of SSF 1 pH7.4. After 3 min, the resulting suspension/solution was transferred into 4.5 mL of FaSSGF pH1.6 yielding to 5 mL of test volume. The temperature of the media was 37±0.5°C. Pictures were taken in FaSSGF after 1, 2.5, 5, 7.5, 10, 15, and 30 min. Images acquisition was driven by AxioVision 4.8 software (Carl Zeiss, Germany).

Particle Thermal and Structural Analysis

The particle state of the three diclofenac drug forms was evaluated *in vitro* under gastric conditions after swallowing. In order to provide sufficient drug particle quantities for thermal and structural analyses, the test dose of diclofenac as well as the test volumes of SSF and SGF/FaSSGF were scaled up to the same extent. A test dose of 465 mg of diclofenac (in equivalent free acid content) was added into 100 mL of SSF 1 pH7.4 for 3 min. The resulting solution/suspension was then transferred into 900 mL of SGF pH1.6 or FaSSGF pH1.6 yielding to 1,000 mL of test volume. During the experiments, the temperature was maintained at 25±0.5°C and the agitation rate was 50 rpm. After 30 min in SGF/FaSSGF, the content was filtered through 2.5 μm paper filters (Whatmann, UK) and dried at 40°C in an oven overnight. The residual moisture content of the samples was evaluated using a Mettler-Toledo halogen moisture analyzer HB 43 (Mettler-Toledo, Germany) at 105°C and 1 mg/30 s. With all samples, a loss on drying (LOD) of value < 1% was confirmed. The states of the particles obtained and pure drug particles were comparatively analyzed by Differential Scanning Calorimetry (DSC) and X-Ray Powder Diffraction (XRPD). Experiments were performed in duplicate.

The phase state of the samples was acquired by DSC using a calorimeter DSC 1 (Mettler Toledo, Germany). The samples (3–5 mg) were sealed in 40 μl pierced aluminum pans (an empty pan as reference). Analyses were carried out under nitrogen flow (70 mL/min) in a temperature range of 3 to 300°C using a heating rate of 10°C/min. The heat flow was recorded and thermograms were generated by Star^e Software version 9.20 (Mettler-Toledo, Germany).

The structural state of the samples was evaluated by X-Ray Powder Diffraction (XRPD). Diffraction patterns were obtained using a diffractometer PANalytical X'Pert MDP PW3040/00 DY 653 (Philips, The Netherlands) with Cu-Kα radiation (λ = 1.5406 Å) at 40 kV and 40 mA. Data were collected in the reflection mode from 15 to 35 ° 2 Theta with the following parameters: 0,0167113 ° step size, 50.165 s/step and 0.042307 °/s scan speed.

RESULTS

Solubility Studies

The solubility values and corresponding dose/solubility ratio (D/S) of diclofenac free acid, its sodium and potassium salt are reported in Table IV. The results confirm the pH-dependent solubility of diclofenac with very low solubilities in media of pH≤4.5 (close or lower than the drug pK_a) but much higher solubilities in media of pH≥6. Overall, a clear distinction between the free acid and its sparingly soluble salts can be noticed. However, in all acidic media, extremely low solubilities and high D/S ratios being similar for all three forms can be observed. Even the small concentration of bile compounds contained in FaSSGF does not have a significant impact on diclofenac solubility. However, an improved drug wettability was observed. In neutral/slightly alkaline media, diclofenac free acid shows a markedly lower solubility than its salt counterparts. As can be seen from Table IV, the solubility of the salts increases more with increasing SSF pH value than that of the free acid. It can also be observed that at a pH of 6.75, diclofenac free acid solubility in SSF 2 is higher than in SSF 1. The salts in contrast are oppositely influenced by SSF composition; this effect is even more pronounced for the potassium salt the solubility of which decreases of about 60% in SSF 2. As expected, as a result of the presence of physiological concentrations of bile salts, enhanced solubility of all compounds could be observed in FaSSIF when compared with that in the compendial phosphate buffer of the same pH value.

Particle Size Analysis

Figure 1 shows the size distribution patterns of diclofenac particles in the dry state and dispersed in acidic media.

Table IV Solubility Values and Dose/Solubility Ratios (D/S) of Diclofenac Free Acid and its Sodium and Potassium Salt in Different Media Determined Experimentally; Mean ± SD ($n=3$)

Solvent/media	Solubility at 37°C (μg/ml) – D/S (ml)					
	Diclofenac acid		Diclofenac sodium		Diclofenac potassium	
	Solubility	D/S	Solubility	D/S	Solubility	D/S
Water (purified)	9.61 ± 0.10	2417	89237 ± 0.15	0	94318 ± 0.08	0
0.1 N HCl pH1.1	2.49 ± 0.15	9328	2.71 ± 0.06	9239	2.40 ± 0.05	10943
SGF pH1.2	1.84 ± 0.01	12619	1.88 ± 0.76	13301	1.78 ± 0.82	14939
SGF pH1.6	1.84 ± 0.01	12665	1.81 ± 0.00	13814	1.67 ± 0.00	15705
FaSSGF pH1.6	1.72 ± 0.01	13516	1.63 ± 0.76	15387	1.58 ± 0.82	16569
SSF 1 pH6.2	179 ± 0.00	130	7521 ± 0.43	3	9398 ± 0.55	3
SSF 1 pH6.75	457 ± 0.00	51	10438 ± 0.61	2	11511 ± 0.66	2
SSF 1 pH7.4	1005 ± 1.45	23	20349 ± 2.42	1	19431 ± 3.76	1
SSF 2 pH6.75	615 ± 0.01	38	9652 ± 0.56	3	4834 ± 0.28	5
Phosphate buffer pH6.8	637 ± 0.05	36	1529 ± 0.06	16	1147 ± 0.06	23
FaSSIF pH6.8	805 ± 0.01	29	13044 ± 0.76	2	13321 ± 0.82	2

Diclofenac free acid particle size distribution (Fig. 1a) is narrow and ranging from about 1 to 20 µm; some particles can be observed in the 0.2–1 µm and in the 20–60 µm ranges. Upon contact with acidic media, the particle size distribution shows a pattern similar to that in the dry state but with a slight shift to larger particle sizes. As a result, the mean particle size is increased from about 7 to 14 µm and particles can be detected in the 20–100 µm size group. Similar patterns are observed with kinetic measurements within 120 min.

Diclofenac sodium particles (Fig. 1b) are distributed between about 10 and 300 µm with particles also present in the 300–600 µm range. The contact with acidic dispersant particularly affects the particle size distribution pattern within the 50–350 µm size range. The mean particle size is increased to about 100 µm (30% higher than in the dry state) and particles are also observed in the 350–1,000 µm range. Kinetic measurements reveal strong modifications of the particle size distribution pattern with progressively less fines and large particles within 120 min. Until 60 min of exposure to acidic media, the mean particle size decreases from about 100 to 73 µm and remains like this until the end of the experiments.

The particle size distribution of diclofenac potassium (Fig. 1c) extends from about 0.6 to 60 µm. The particle size is markedly influenced by the acidic media contact resulting in a distribution pattern from 2 to 100 µm and a high proportion of particles in the 100–400 µm range. After dispersion in acidic media, an increased mean particle size of 26 µm is observed in comparison to a mean diameter of 14 µm measured for the dry dispersion. Kinetic measurements show a rapid decrease of the proportion of fines and large particles with similar mean particle size around 25 µm, but particles in the 250–600 µm size group can be observed.

Dissolution Testing

Compendial Dissolution Method

In Fig. 2 the dissolution profiles of the three diclofenac drug forms using compendial dissolution methods are shown. The salts are completely dissolved within 60 min in phosphate buffer pH6.8 in all test conditions, but dissolution rates in this buffer stage are significantly slower subsequent to the pretreatment with the acidic phase (Fig. 2b). In that later case, dissolution data are decreased after 5 min and 15 min to about 40% and 60% for the sodium and 50% and 75% for the potassium. In contrast, diclofenac free acid displays similar dissolution performances irrespective of the test method with about 40% cumulative release after 15 min and about 80% after 60 min. So it seems that an acidic pretreatment affects the diclofenac salts but not the free acid.

Biorelevant Dissolution Method

The dissolution profiles of diclofenac compounds in different combinations of SSF and SGF/FaSSGF media are depicted in Fig. 3.

Diclofenac performance in SSF depends on both the composition and the pH of the medium. The resulting dissolution profiles clearly distinguish the free acid from its salt counterparts with significantly slower dissolution rates. Cumulative drug release of diclofenac free acid (Fig. 3a) ranges from 3% in SSF 1 pH6.2 to 18% in SSF 1 pH7.4 after 3 min. About 50% and 100% of the drug is released in these respective media from the salt forms (Fig. 3b and c). The free acid shows similar drug releases in SSF 1 pH7.4 and SSF 2 pH6.75 (data not shown).

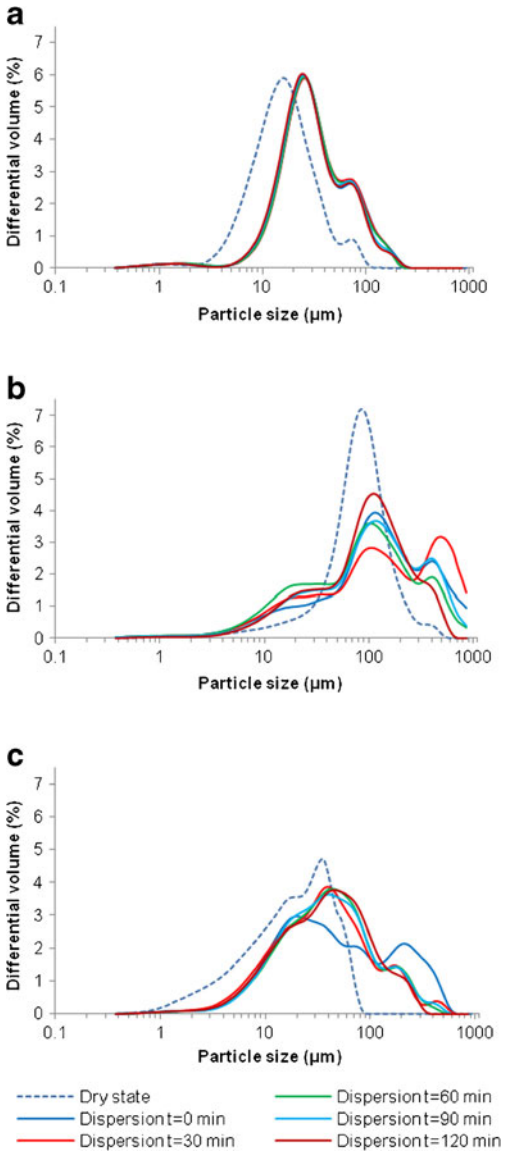

Fig. 1 Particle size distribution of diclofenac free acid (**a**), sodium (**b**) and potassium (**c**) in differential volume measured by light scattering; mean ($n = 3$).

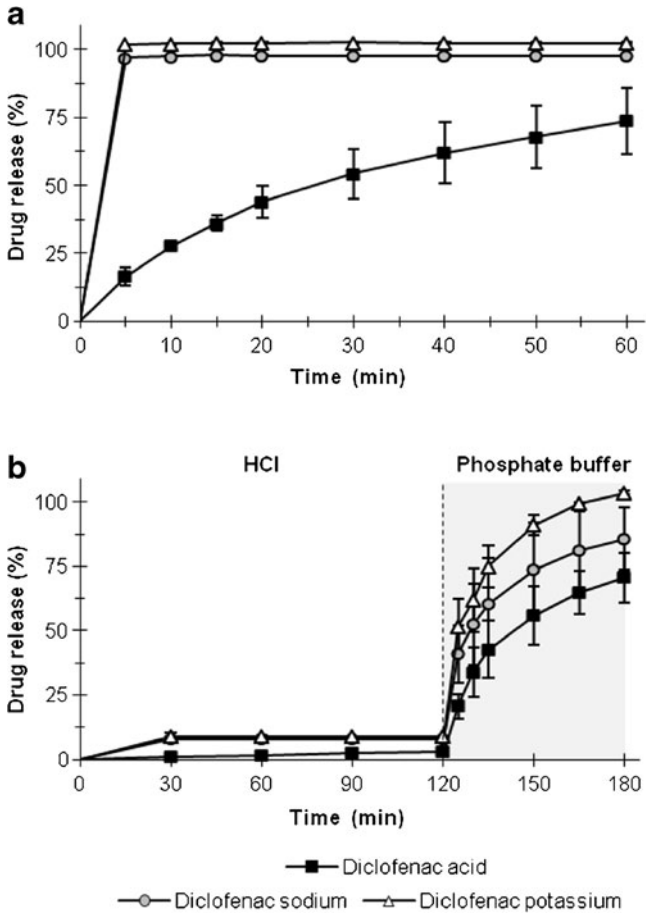

Fig. 2 Comparison of the dissolution profiles from diclofenac free acid, sodium and potassium using compendial dissolution methods: phosphate buffer pH6.8 (**a**) and a pH change method 0.1 N HCl pH1.1—phosphate buffer pH6.8 (**b**).

Upon contact with SGF or FaSSGF media, immediate precipitation is observed from the three compounds in all media combinations as highlighted by a strong decrease of cumulative drug release after media change. Diclofenac dissolution performances are influenced by the volume of SGF/FaSSGF as well as the drug form. Drug precipitation is more pronounced in 50 mL than in 250 mL of SGF/FaSSGF, with respectively about 1% and 4% of the dose dissolved within 30 min for all three drug forms. In all test conditions, the concentration of dissolved diclofenac free acid (Fig. 3a) reaches almost immediately an equilibrium after media change. In contrast, the dissolution profiles of diclofenac sodium (Fig. 3b) and potassium (Fig. 3c) show a transitional state, characterized by an increase and subsequent decrease of the concentration of dissolved drug. This behavior is independent on the SSF used to simulate residence in the oral cavity. This state lasts about 4 and 10 min for 50 mL and 250 mL of SGF/FaSSGF volume respectively.

Figure 4 shows the dissolution profiles resulting from a simulated passage through the oral cavity, the fasted stomach and the fasted upper small intestine. The release of diclofenac salts in FaSSIF is strongly influenced by the composition of the media used to simulate the fasted gastric fluid. However, this is not the case for the free acid. Using FaSSGF pH1.6 to simulate fasted gastric conditions (Fig. 4b) results in a complete dissolution of the two salts in FaSSIF within 5 min. In contrast, when the fasted gastric environment is simulated with SGF pH1.6 complete dissolution in the upper small intestine requires 20 min (Fig. 4a). Comparing dissolution of the two salts, this effect is more pronounced for diclofenac sodium than potassium. After the medium change from SGF pH1.6 to FaSSIF pH6.8, approximately 35% and 70% of diclofenac sodium are

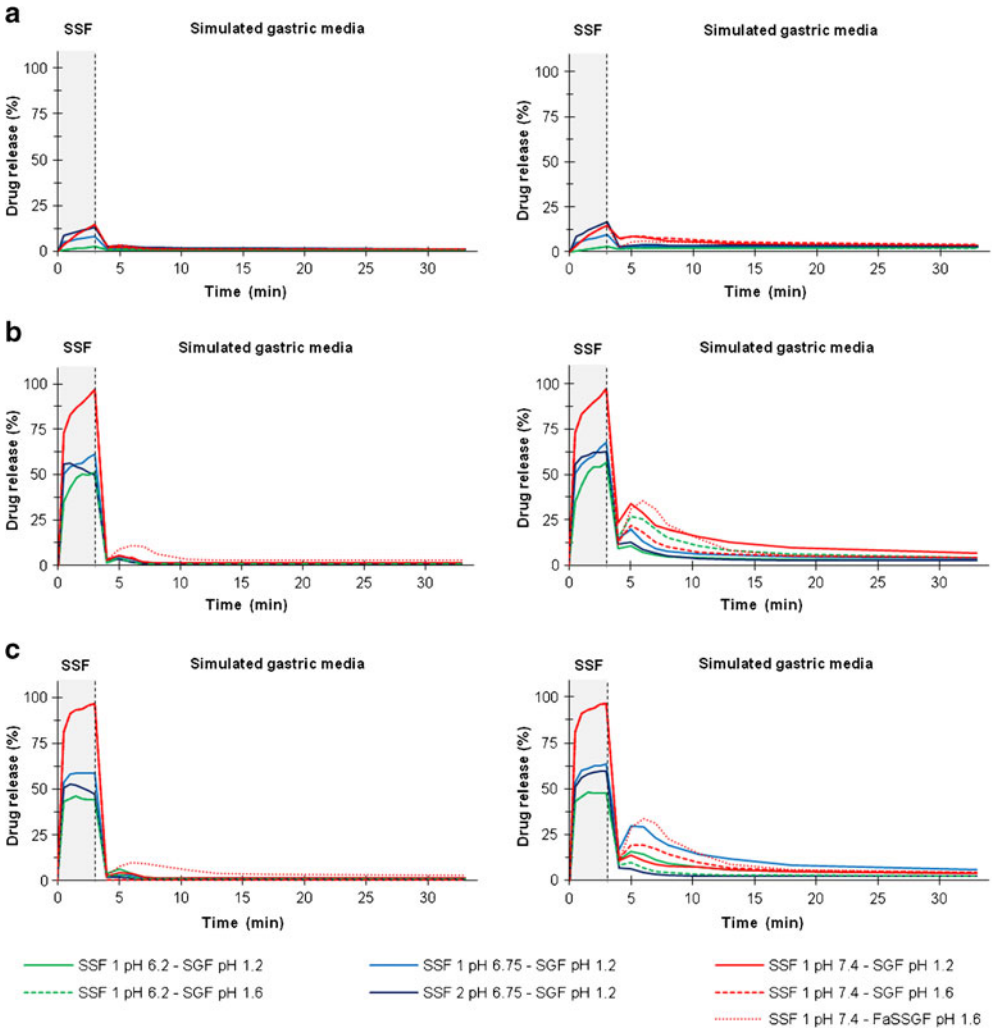

Fig. 3 Comparison of the dissolution profiles from diclofenac free acid (**a**), sodium (**b**) and potassium (**c**) in different combinations of simulated salivary fluid (SSF) and simulated gastric contents (SGF or FaSSGF) using a biorelevant dissolution method. The stomach resting volume is 50 mL in the left panel and 250 mL in the right panel.

dissolved after 2.5 and 10 min; whereas the dissolution rate of the potassium salt is significantly higher resulting in 60% and 78% of the dose dissolved at the corresponding time points. The amount of diclofenac free acid is similar in both test conditions with about 75% drug release after 5 min and 90% after 15 min.

The cyclic compound diclofenac RC A is detected in the scheme SSF pH7.4 (5 mL), SGF or FaSSGF pH1.6 (50 mL) and FaSSIF pH6.8 (250 mL) of the dissolution test (data not shown). Its appearance is dependent on the drug form tested. During the dissolution studies of diclofenac salts, only negligible amounts of diclofenac RC A are found in SSF. This amount increases in FaSSGF for the sodium and the potassium salt in a higher extend than in SGF. Switching in FaSSIF, diclofenac RC A almost disappears. As observed with the salt drugs, these dissolution patterns of diclofenac RC A show a transitional state, defined by an increase and subsequent decrease of the concentration of dissolved substance. In contrast, almost no diclofenac RC A is generated during the entire dissolution test of the free acid.

Particle Counting

In Fig. 5 the count of diclofenac particles dispersed in SGF pH1.6 or FaSSGF pH1.6 following 3 min contact in SSF is shown as a function of particle size.

After 5 min in SGF (Fig. 5a left) and FaSSGF (Fig. 5a right), counting patterns from diclofenac free acid particles are different from those from the diclofenac salt particles which are very similar. Analyzing the free acid, the particles detected are markedly more numerous and distributed in smaller size ranges in comparison to the salts. In SGF, the particle concentration from the free acid is approximately 30 times higher with about 5,500,000 particles/mL than from the sodium and potassium reaching 180,000 particles/mL. Particles are mainly distributed between 0.5 and 5 μm for the free acid compared to 10 and 100 μm for the salts. More abundant large particles in the 75–100 μm range are detected for the potassium than for the sodium salt in SGF. However, this is not observed in FaSSGF. The counting patterns from the salts show higher particle concentration in FaSSGF than in SGF with about 900,000 particles/mL (5 times

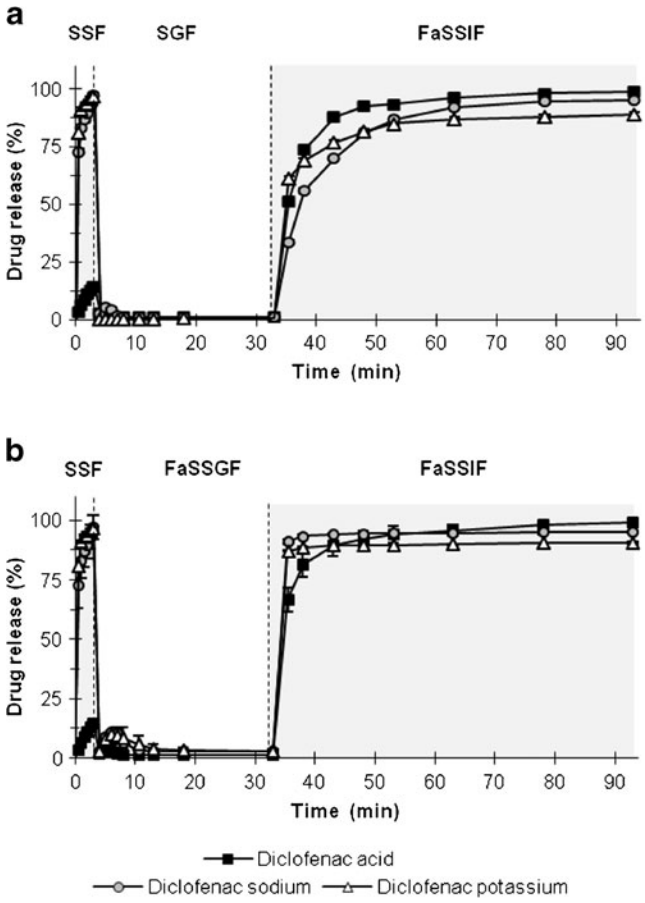

Fig. 4 Comparison of the dissolution profiles from diclofenac free acid, sodium and potassium simulating a passage through the oral cavity, the fasted stomach and the fasted upper small intestine by using a biorelevant dissolution method utilizing simulated salivary fluid (SSF), simulated fasted gastric fluid and fasted state simulating intestinal fluid (FaSSIF). The simulated fasted gastric fluid was SGF in (**a**) and FaSSGF in (**b**).

higher); the counting pattern from the free acid is similar in both media.

Figure 5b focuses on kinetic counting patterns of diclofenac sodium particles in SGF and FaSSGF. Clear influence of the exposure to these media is observed, mostly during the first 5 min of the experiment. The particle concentration strongly decreases from about 13,000,000 to 180,000 particles/mL between 1 and 5 min in SGF (approximately 70 times); this decrease is lower in FaSSGF where about 1,100,000 particles/mL can be counted after 5 min (approximately 12 times the SGF concentration). For diclofenac sodium, subsequently, a trend towards the generation of larger particles can be observed. The average particle size is in the range of 0.5–10 μm after 1 min and increases to 1–50 μm after 5 min in both media. Further modifications of particle concentration patterns are not observed within 30 min, but the fraction of larger particles in the 10–100 μm size group represents 15% of the particle number directly after 5 min in SGF and gradually within 30 min in FaSSGF. Applying the same method, similar behaviors are observed from the potassium salt. The free acid shows in contrast almost no modification of counting patterns during the testing period (data not shown).

Particle Image Analysis

Figure 6 discloses diclofenac particles as start materials, i.e. powders, and dispersed in FaSSGF pH1.6 following 3 min of contact in SSF.

In the dry state, diclofenac free acid has needle-like particles in the 1–100 μm size range. Pretreated with SSF, and after 5 min in FaSSGF, diclofenac free acid particles are mainly of about 50 μm; some very small particles (of about 1 μm) are observed. Particles of very similar shape and size can be seen in images taken after 15 and 30 min.

Diclofenac sodium particles range from about 10 to 300 μm with a spherical shape. Small particles in the 1–5 μm range are observed after 5 min in FaSSGF which reduce in number but increase in size upon time; particle size are about 10–80 μm after 15 min and 10–120 μm after 30 min. It seems, from these microscopic pictures that, after 15 min in FaSSGF, diclofenac sodium particles are rather multiple agglomerate units of particles with a needle-like shape than single units. These agglomerates show to increase in size between 15 and 30 min.

The particles of diclofenac potassium are in the size of about 1–60 μm and have a squared/rectangular shape. 5 min after the change from SSF to FaSSGF, very numerous small particles (of 1–5 μm) are observed together with very large "lumps" in the 50–250 μm size range. Upon contact in FaSSGF, less fines and very large lumps can be seen; it seems that the size of the suspended material is getting more homogeneous. Particles of about 1–10 μm present after 15 min can no longer be observed after 30 min, and large agglomerates (of 50–150 μm) are replaced by more numerous agglomerates but of smaller sizes (50–100 μm).

From these data, it is clear that diclofenac sodium and potassium show a tendency to form agglomerates in FaSSGF when pretreated with SSF. This tendency is not seen for diclofenac free acid in identical conditions, the particles of which remain of similar shape and size.

Particle Thermal Analysis

Thermograms of diclofenac particles were determined by DSC before and after contact with simulated fasted gastric conditions (Fig. 7). Results reveal unique features for each drug form. The thermogram of diclofenac free acid (DH) shows a characteristic strong and sharp endothermic peak at about 180°C corresponding to the melting point of diclofenac (30). The DSC scan of diclofenac sodium (DNa) displays an inflection at approximately 285°C relating to the start of the

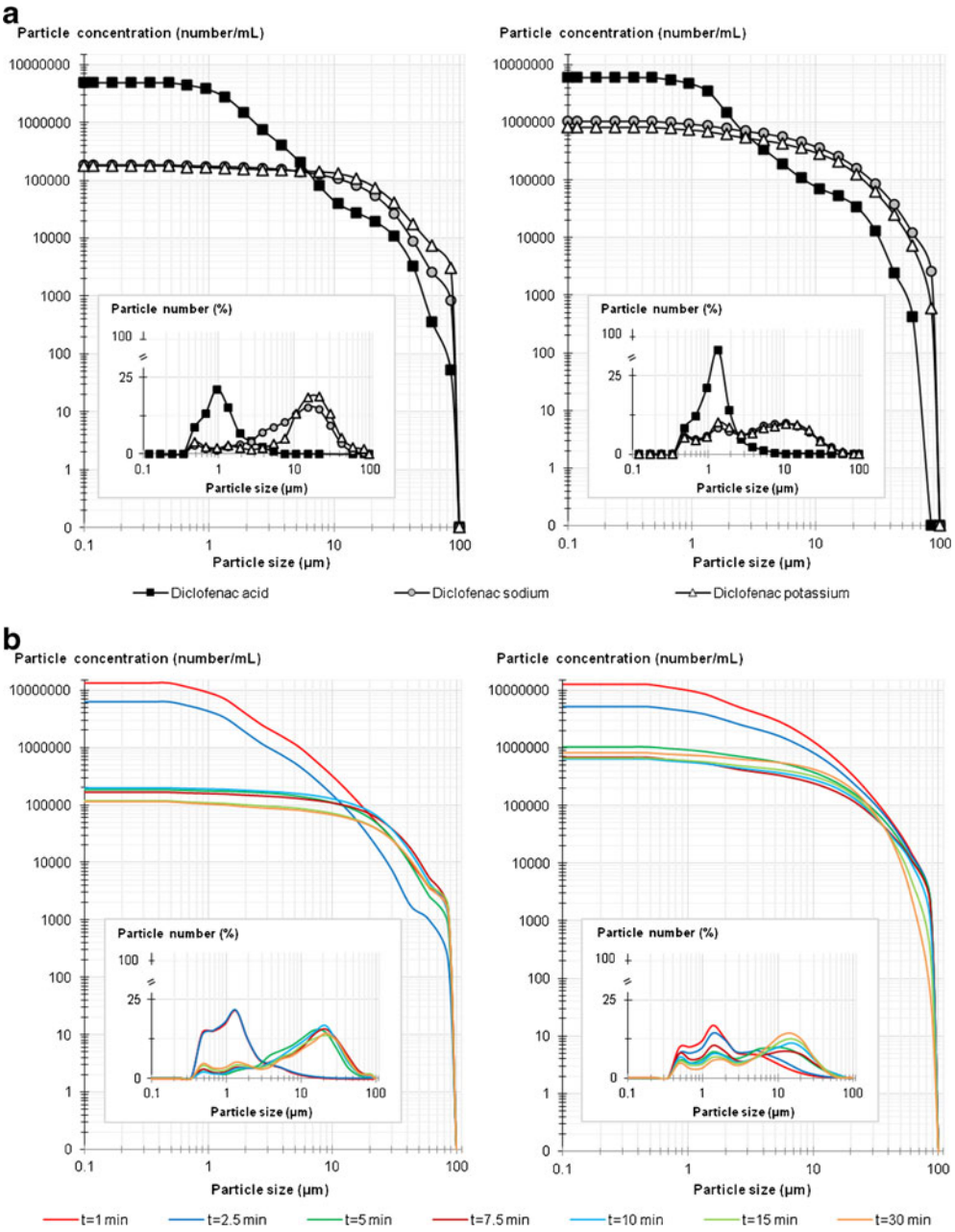

Fig. 5 Particle counting of diclofenac free acid, sodium and potassium obtained from optical morphogranulometry 5 min after a media change to simulated gastric fluid subsequent to a 3 min residence time in SSF I pH7.4 (**a**). Particle counts of diclofenac sodium after 1, 2.5, 5, 7.5, 10, 15 and 30 min are shown in (**b**). *Left panel*: data obtained in SGF, *right panel*: data obtained in FaSSGF.

drug melting which is in good agreement with that described in the literature (31). This endotherm is immediately followed by an exothermic event consistent with other published data which could be attributed to a complex decomposition and/or a conversion of diclofenac sodium (30,32). For diclofenac potassium (DK), no evidence of drug melting is observed. The exothermic peak at about 309°C could refer to the salt decomposition stated in available literature reports (23,33). After contact with SGF or FaSSGF, endotherms with peak maximum at about 180°C are similarly observed for the three diclofenac substances and are characteristic of the free acid form of the drug. The shift in melting points observed in the thermograms of the salts in SGF and FaSSGF are most probably the result of their protonation in these acidic media forming the free acid compound. After the contact with SGF or FaSSGF, no modification of diclofenac free acid melting points is observed which correspond to sharp endothermic peaks (DH-SGF and DH-FaSSGF) with similar onsets (179°C) and maximum (180°C). When have been in contact with these media, markedly reduced melting points of diclofenac sodium and potassium with peak maxima of 180°C are found. For both salts, the endothermic peaks, indicating the melting points, are broader when the diclofenac salts have been incubated in SGF rather than in FaSSGF. DSC peak

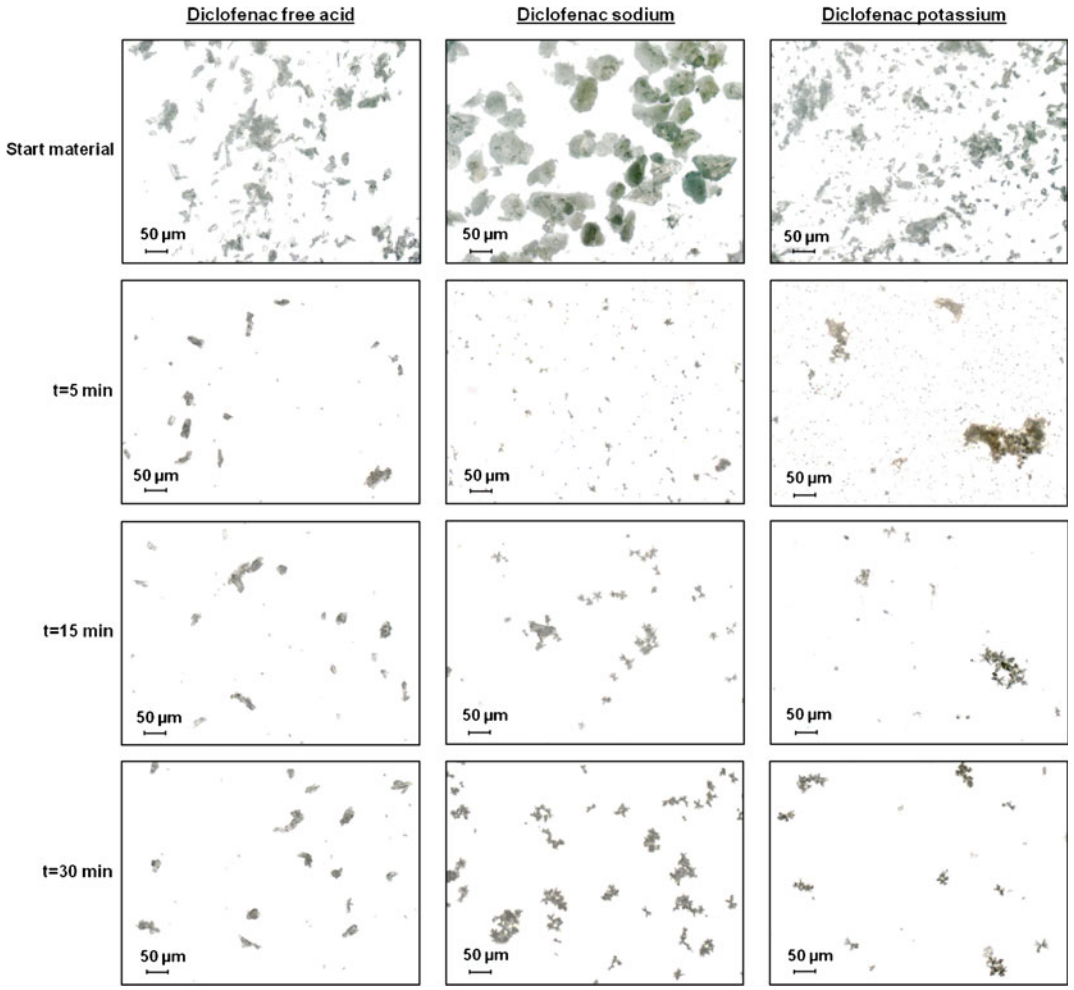

Fig. 6 Microphotographs of diclofenac free acid, sodium and potassium particles as start materials (powders) and dispersed in FaSSGF pH1.6 following 3 min of contact in SSF. In dispersion, the pictures are taken 5, 15 and 30 min after the media change SSF-FaSSGF.

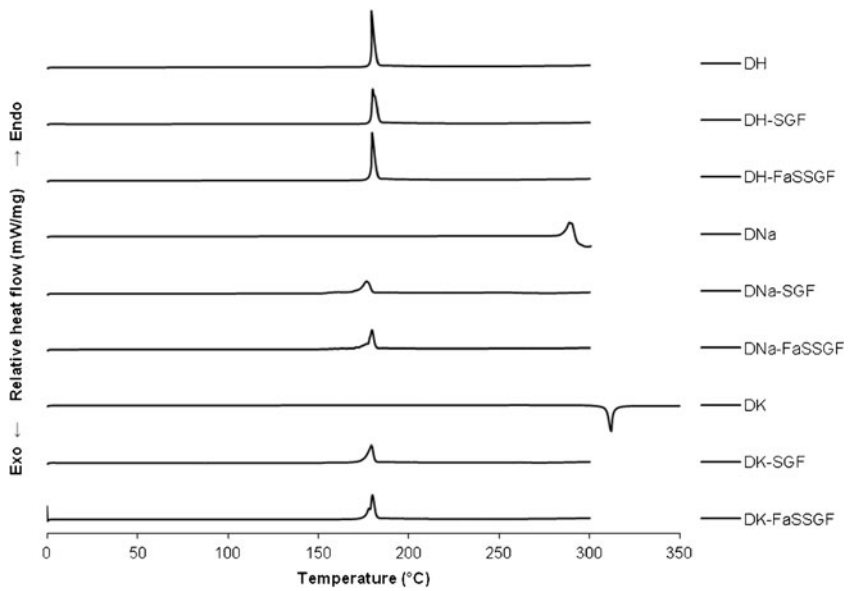

Fig. 7 Differential Scanning Calorimetry (DSC) curves of diclofenac free acid (DH), sodium salt (DNa) and potassium salt (DK) as pure powders and after 30 min contact in SGF or FaSSGF.

onsets of about 172°C (DNa-SGF) and 176°C (DK-SGF) in SGF compared to 177°C (DNa-FaSSGF) and 178°C (DK-FaSSGF) in FaSSGF were determined.

Particle Structural Analysis

XRPD patterns of diclofenac particles are displayed in Fig. 8. Crystalline peak 2θ values observed from 15 to 35 ° indicate a crystalline nature of the compounds. The three drug forms show distinct characteristic peaks after XRPD analysis. After the pretreatment with SSF, diclofenac free acid particles in simulated fasted gastric conditions (DH-SGF and DH-FaSSGF) show diffraction patterns identical to those of the pure free acid form (DH). Subsequent to the contact with SSF, the diffractograms from the sodium and potassium salts in SGF (DNa-SGF and DK-SGF) reveal structural modifications of the particles when compared to their respective pure substances; some peaks are similarly observed in the diffractogram of the pure free acid, but have smaller heights. In contrast, in FaSSGF, the XRPD patterns of diclofenac sodium and potassium particles pretreated with SSF (DNa-FaSSGF and DK-FaSSGF) disclose very similar peaks to the XRPD pattern of free acid particles.

DISCUSSION

Many attempts have been made to enhance the dissolution properties, and thus the oral bioavailability, of very poorly soluble or lipophilic compounds. The most common and effective alternative technique is the use of the salt of the drug (34). For acidic compounds, the salt forms have generally higher solubilities than the corresponding unmodified form, although solubilities of different salts of a particular acid can differ (34). For solid dosage forms, much higher dissolution rates of several salts of weakly acid compounds have been observed under pH-conditions of the GI tract than from their respective free acid forms (35).

Diclofenac, a phenylacetic acid derivative, is mainly available as the free acid and its sodium/potassium salt. To date, most of the marketed oral dosage forms of diclofenac comprise the salt forms showing enhanced solubility properties, although multiple peaks are commonly observed in patient plasma profiles (5–7). Typical oral preparations of diclofenac are delayed-release (DR) or sustained-release (SR) tablets comprising the sodium salt. To provide faster pain relief, immediate-release (IR) tablets have been launched using the potassium form claimed to dissolve under the acidic conditions of the stomach (36). With the aims of faster dissolution and earlier absorption in the upper digestive tract, novel dosage forms have been developed. Currently, there is an increased interest in solutions, suspensions as well as effervescent or dispersible formulations. The multiple-peak phenomenon in plasma profiles after oral administration of diclofenac products has been extensively studied and was found to be formulation-dependent (11).

The multiple peaks observed after oral administration of DR and SR formulations could be explained by extended gastric retention of the formulation, pH-changes during the GI transit time or the breakup of the dosage form resulting in dose dumping (11,37,38). The susceptibility of diclofenac sodium DR tablets to physical stress events that occur during the GI transit has been demonstrated *in vitro* by Garbacz *et al.*, most probably explaining irregular *in vivo* drug releases (11). After administration of diclofenac solutions, effervescent, dispersible or IR preparations, multiple peaks are

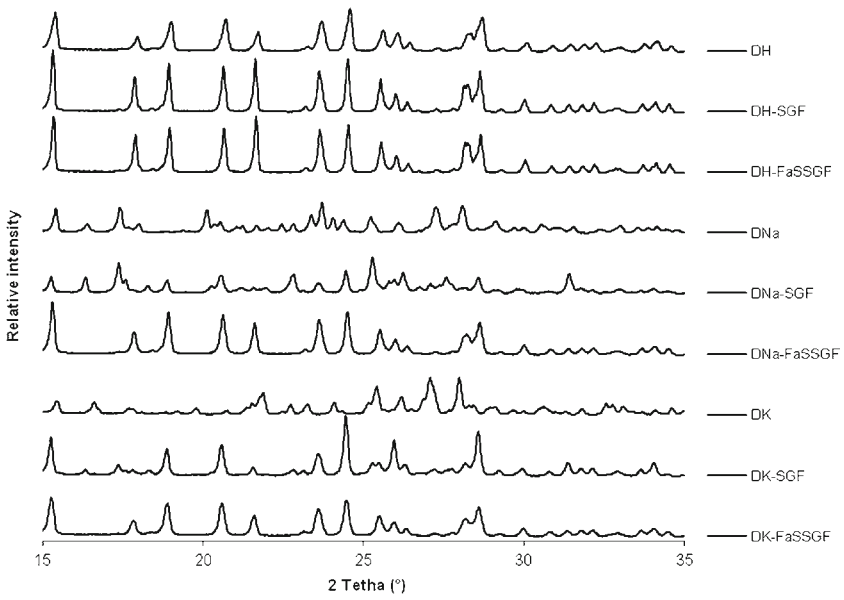

Fig. 8 X-Ray Powder Diffraction (XRPD) patterns of diclofenac free acid (DH), sodium salt (DNa) and potassium salt (DK) as pure powders and after 30 min contact in SGF or FaSSGF.

usually erratically detected *in vivo* within the first hours after dosing. Proposed mechanisms involve the strong pH-dependent dissolution of diclofenac associated with fractionated gastric-emptying (5,11). In contrast to the multiple peaks observed from the diclofenac salt formulations, such phenomena are not observed for equivalent formulations comprising the free acid, suggesting this phenomenon to be also dependent on the drug form (15). Thus, it is likely that the differences in the biopharmaceutical properties of diclofenac free acid and its salt counterparts may explain the contrary behaviors of the three drug forms after oral absorption. Based on this hypothesis, essential features of the three forms of diclofenac were evaluated *in vitro* in this comparative study.

As a BCS class II drug, the rate-limiting steps in diclofenac absorption from oral formulations are i) the *in vivo* disintegration of the dosage form, ii) gastric-emptying and iii) the rate and extent of *in vivo* dissolution (3). Due to its weakly acidic properties with a very low solubility under acidic conditions, the drug shows a highly variable solubility along the GI tract. The fasted stomach does certainly represent the least favorable place for diclofenac to dissolve. The small intestine generally corresponds to the region where drugs are the best absorbed. For poorly soluble, weakly acidic and lipophilic compounds like diclofenac, the dissolution would be even more favored in this segment (17).

Initial investigations using compendial dissolution methods tend to differentiate the free acid from the salts of diclofenac. Evaluated in standard phosphate buffer, diclofenac release is as expected fast and complete for the three forms. However, *in vivo* such favorable pH-conditions are only available in the small intestine. To reach this site, the drug has to pass the stomach. *In vitro*, the passage through the fasted stomach was simulated, first, by immersion of the drug in a standard acidic medium in which the drug thus hardly dissolves but is dispersed. This acidic pretreatment has a significant impact on the dissolution of the sodium and potassium salts in a standard simulated intestinal media. *In vivo*, this would correlate with a slower intestinal drug dissolution and would thus most likely also affect the absorption in a negative way. Particle size analyses confirm that immersion in the so simulated gastric fluid has a huge impact on the diclofenac salt particles. Dispersed in acidic media, the salt particles show the tendency to markedly increase in size. In contrast, particles of the free acid remain dispersed in the surrounding acidic environment without any significant particle growth. First results indicate distinct *in vitro* behaviors of the free acid and the salt forms of diclofenac regarding dissolution performances and particle size patterns under pH-change conditions.

After oral administration of solutions, suspensions, effervescent or dispersible preparations where the disintegration of the dosage form has already occurred, the drug may be released as early as in the oral cavity, i.e. be exposed to saliva. For diclofenac, the exposure to the mouth may become even more critical as drug dissolution may start in the neutral media of the saliva before reaching the stomach. As gastric residence, this may also potentially affects the further *in vivo* behavior. Therefore, it is of utmost importance not only to evaluate drug performance simulating conditions of the upper GI tract, but also to consider the mouth as a potential dissolution compartment. Based on literature data, this work has therefore been aimed at designing more predictive *in vitro* test setups. The biorelevant models mimic the conditions experienced by drugs after oral administration considering mouth exposure before storage in the stomach and further absorption in the small intestine. The design has been focused on media composition, pH value and volume together with suitable residence times (39–41). These biorelevant setups have been applied for further *in vitro* evaluations of the behavior of the three forms of diclofenac.

Dissolution testing of diclofenac has been thus performed in simulated saliva prior to simulated GI fluids and compared to compendial dissolution methods. Various factors that do not apply to the GI dissolution are specific in evaluating *in vitro* drug dissolution in the oral cavity conditions. Amongst others, these include the small volume available for disintegration and/or dissolution and the short residence time. The unique media composition and the potential for incomplete dissolution are of utmost importance (25). As a result of the small fluid volume, tremendous drug concentration can be obtained in the saliva resulting in dissolution conditions that may be far from *sink* conditions. In simulated saliva of high pH value (pH 7.4), diclofenac salts are rapidly and almost completely dissolved suggesting that *in vivo* both substances would be swallowed almost as solutions. In contrast, with only very slight dissolution in these conditions, diclofenac free acid would enter the stomach mostly as dispersed particles.

Simulated prior mouth contact proves to result in drug solubilization in saliva and to affect the further *in vitro* performance of the drug solution or suspension. The dissolution results indicate that *in vivo* an immediate drug precipitation is likely upon contact with fasted gastric fluid. In agreement with the solubility data, this is the result of the strong decrease in pH when switching from oral to gastric conditions. Interestingly, it became obvious that diclofenac dissolution patterns in acidic contents highly depend on its form. Whereas, most probably due to low prior dissolution in simulated saliva (SSF), the free acid almost immediately shows complete precipitation at gastric pH conditions (SGF/FaSSGF); both salts show a transitional state before complete precipitation occurs. This particular pattern, characterized by an increase and subsequent decrease of the amount of drug dissolved, could not have been seen applying compendial dissolution method.

Subsequent drug dissolution in intestinal conditions is rapid and almost complete for all drug forms.

To investigate the peculiar diclofenac precipitation patterns in simulated gastric conditions, drug particles were further characterized *in vitro* applying the newly designed biorelevant models. These studies were performed mimicking the less favorable physiological conditions of the oral cavity and the fasted stomach that diclofenac can experience *in vivo*. *In vitro*, the saliva was therefore simulated with a neutral pH value in which diclofenac shows better solubility properties (SSF pH 7.4) and combined with a volume of the fasted stomach fluid in which a pronounced precipitation tendency can be expected (50 mL of SGF or FaSSGF pH 1.6). With chemical and physical state analyses, it was possible to clearly distinguish the behavior of the free acid from that of the salts of diclofenac. Although the free acid is almost not affected by the pH-change between simulated conditions in the oral cavity and the fasted stomach, both salts prove to undergo modifications of their chemical and physical states. Interestingly, these modifications follow transitional patterns and seem to be correlated to the transitional states observed in dissolution testing.

During *in vitro* biorelevant dissolution testing, diclofenac RC A is formed from diclofenac salts to a small extent in SGF/FaSSGF. Its dissolution pattern is similar to those of the salts, i.e. also exhibiting a transitional state. Diclofenac RC A is not formed from the free acid. However, diclofenac RC A almost disappears in the following stage in FaSSIF. The appearance of diclofenac RC A during the *in vitro* dissolution testing of the salts might be explained by a reversible chemical transformation, i.e. intramolecular cyclization. In that case, a part of the dose would undergo the intramolecular cyclization when precipitating in SGF/FaSSGF, which reverses when switching to FaSSIF. The intramolecular cyclization of diclofenac has been previously observed in a surrounding solution of acidic pH and here it was also reported to be reversible when the solution changes to neutral pH (42). Due to its reversibility, this particular event is considered to very unlikely impact *in vivo* performances in the upper small intestine, i.e. by decreasing the drug amount available for dissolution (29). However, the influence of such instable chemical transformation on the parent drug crystals themselves (e.g. surface irregularity, roughness and area) should not be avoided. As a precipitation-related event, it could all the more affect drug crystals in a peculiar or unpredictable way.

When first exposed to SSF, drug crystallization is observed from the three chemicals when switching to SGF/FaSSGF. The high precipitation and crystallization rates observed in the dissolution profiles are most likely a result of the strong drug supersaturation in the simulated gastric media. However, this effect is only noticed for the salts and not for the free acid. From the salts, dissolution curves are in good agreement with data obtained by image analysis and optical morphogranulometry where, immediately after media change from SSF to FaSSGF, numerous dispersed small particles are detected which rapidly increase in size over time.

The observed successive steps are characteristic of the crystallization process: the first nucleation with appearance of small particles followed by the crystal growth leading to particles of larger sizes. With the exposition time, the number of salt particles dispersed in SGF/FaSSGF remains stable but even larger particles are detected. This phenomenon is likely the result of an agglomeration process that succeeds the crystallization. This explanation is clearly confirmed by microscopic pictures where clusters of particles of different sizes can be observed. Thus, salt particles dispersed under stomach-relevant conditions are rather present as agglomerated particles than single particles. Behaving totally differently, only very slight crystallization is observed from diclofenac free acid the particles of which do almost not change in shape, size and number; and also no formation of agglomerates is evident.

Thermal and structural analyses reveal that SSF-pretreated crystals from diclofenac free acid remain identical in SGF/FaSSGF, whereas crystals from the salts undergo transformations in identical conditions. In SGF/FaSSGF, particles obtained from diclofenac sodium and potassium are in the form of free acid. This is in good agreement with the well-known protonation of salts from acidic compounds when exposed to acidic media. However, crystals formed in SGF/FaSSGF are in some extent structurally different. Surprisingly, these differences in structure are dependent on the salt form but also on the composition of the acidic media. SSF-pretreated crystals from the salts show very similar internal structures to free acid crystals in FaSSGF, but not in SGF. These contrasting results might be attributed to the bile effect on diclofenac crystals that could only be observed in FaSSGF.

Data generated in this study clearly demonstrate differences in *in vitro* behavior of the unmodified form of diclofenac and its salt counterparts under biorelevant conditions of the mouth and the GI tract. Most of these behaviors appear to be determined by the contact of the chemicals with the simulated environment of the stomach where the surrounding conditions extremely lower drug solubility. This becomes even more critical when taking account a previous contact with simulated saliva (SSF). However, although the salt forms prove to undergo chemical and physical state modifications under extreme pH-changes, the free acid is almost not affected. Due to low dissolution in SSF, subsequent *in vitro* precipitation in SGF/FaSSGF is limited. In contrast, diclofenac salts are partially or almost totally released in SSF. Entering into SGF or FaSSGF, the resulting solution/suspension of the drugs meets extreme stress conditions that alter their stability. First of all, the drugs precipitate and crystallize in the free acid form as a result of the salt protonation. Besides this, both chemical instability coming along with the formation of a cyclic compound (e.g. through intramolecular cyclization) and physical instability with the build of agglomerates are reported. These events show transitional patterns and even seem to occur in an unpredictable way.

Through the results of this work, it is also demonstrated that *the in vitro* features of diclofenac are strongly determined by the conditions of characterization applied, as summarized in Fig. 9. When investigated with various combinations of media, diclofenac precipitation always results in similar equilibration concentrations after 30 min in SGF/FaSSGF. However, different precipitation patterns are correlated to different drug particle characteristics and behaviors affecting further *in vitro* drug performance. For diclofenac salts, the precipitation is less pronounced in FaSSGF than in SGF and is characterized by a gradual agglomeration process which occurs almost immediately in SGF. Moreover, the FaSSGF stage leads to salt crystals similar to free acid crystals; this is not the case in SGF. It is thus suggested that bile compounds have a strong influence on diclofenac precipitation. This influence could further explain the faster dissolution performance of diclofenac salts observed in FaSSIF when FaSSGF simulates gastric conditions compared to SGF. As bile compounds could affect the drug crystals formed during precipitation (e.g. improved wettability, reduced agglomeration, smaller agglomerate sizes), the dissolution of the drugs pretreated with FaSSGF could be promoted in FaSSIF. It becomes thus obvious that studying diclofenac dissolution under compendial conditions could lead to poor predictive value of the *in vitro* dissolution profiles. This was confirmed in this study as in the compendial acidic stage, no peculiar salt dissolution pattern can be seen. In addition, the salts built much larger agglomerates in this acidic medium, i.e. particles with smaller surface area, which dissolve slower in the subsequent basic stage. The study of diclofenac under biorelevant conditions may thus be a prerequisite for forecasting its *in vivo* features. Here, the most predictive *in vitro* conditions would have most probably taken place in the following successive media: SSF-FaSSGF-FaSSIF. Under that scheme, the *in vitro* agglomeration of diclofenac salts in FaSSGF proves to be closely linked to their precipitation, and thus, might happen in an irregular and even unpredictable way. It might be so very hard to forecast the size or the number of agglomerates that can be built *in vivo*. Occurring *in vitro* in this work, this peculiar event would be also expected *in vivo*. Based on *in vitro* dissolution data, the dissolution of the drug in FaSSIF is not affected by drug agglomerates formed in FaSSGF. This is consistent with very high solubility data and D/S ratios for diclofenac salts in FaSSIF which suggest that their solubilization in this medium would not be a limiting factor. In a like manner, it might be expected that, the rate and extent of *in vivo* drug dissolution in this site would not be critical determining factors in the absorption rate of diclofenac (3).

However, the designed *in vitro* models do not take physiological factors of the human body, e.g. the emptying of gastric contents, into consideration. *In vivo*, after oral administration, the drug is retained in the stomach until it is delivered to the small intestine and subsequently absorbed. Under these circumstances, the absorption rate of diclofenac would be controlled by gastric-emptying (3). The very poor solubility of diclofenac in the stomach, and so its subsequent precipitation, have already been supposed to possibly result in fractionated emptying of the drug into the small intestine; undissolved drug particles being likely longer retained in the stomach (5,11,13,14). Moreover, no agglomeration phenomenon of the drug was taken into consideration at that time. It might be doubted that, differently sized agglomerated particles formed as the drug precipitates, would be emptied from the stomach at once. Indeed, a longer retention capacity of agglomerates in the stomach could easily be expected, and a discontinuous gastric emptying of these differently sized agglomerates might be more probable. Moreover, only a slight resting volume is available in the fasted stomach, providing a small volume of liquids with which undissolved drug particles can be emptied. This hypothesis can be further supported by *in vitro* data of particle size and image analyses which highlight large sizes and also

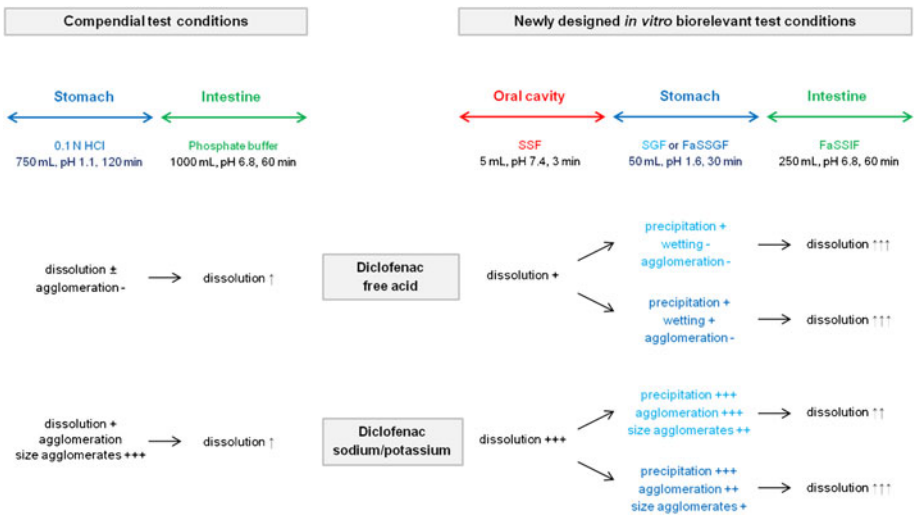

Fig. 9 Comparative *in vitro* features of diclofenac free acid, sodium and potassium depending on the characterization test conditions: compendial test conditions (left) or newly designed biorelevant test conditions (right).

marked irregular shapes of the agglomerates built. In such a case, fractionated gastric emptying times would induce multiple dissolution rates and consequently, multiple absorption rates of the drug in the duodenum. This explanation is in good agreement with previous work of Oberle *et al.* on cimetidine, stipulating that variable gastric emptying rates can lead to variable absorption rates from the GI tract (43). In their study, this was correlated to the appearance of double peaks in plasma concentration-time profiles.

In the present work, comparative *in vitro* evaluations were performed on the APIs. It seems however interesting to estimate the fate of diclofenac salts from formulations where the drug might highly precipitate in the stomach and a like agglomeration phenomenon might subsequently follow. Most sensitive formulations are certainly solutions, suspensions, but also effervescent, dispersible or orodispersible tablets. Indeed, these formulations are already in a disintegrated or disintegrated-like form before oral administration (solutions, suspensions, and effervescent or dispersible tablets) or the disintegration occurs in mouth saliva (orodispersible tablets). In addition, the drug may be exposed to the saliva and its dissolution may initiate. Then, after swallowing, the drug loaded content is retained in the stomach before it is emptied by the stomach and the drug absorbed in the small intestine. During the gastric storage time of this content, the drug might precipitate and irregularly form agglomerates of various sizes. Diclofenac single particles and/or agglomerated particles would be so suspended and dispersed in the small volume of gastric contents. Although insoluble diclofenac particles would most probably be emptied with liquid contents, part of the undissolved and agglomerated drug could be possibly left in the stomach and emptied later, causing subsequent increase(s) in drug concentration-time plasma curves. This hypothesis was also suggested in a literature work where *in vitro* irregular absorption profiles were studied using an *in vitro* device which simulates the physical stress events during GI transit (11). It is also in good agreement with pharmacokinetic data reported by Vidon *et al.* (11,29). Therefore, the results of this study could additionally help understanding the peculiar individual plasma profiles, i.e. multiple absorption peaks observed after dosing diclofenac salts from solutions, suspensions, effervescent or dispersible formulations.

The precipitation and the following agglomeration of diclofenac would not occur after oral administration of DR preparations. In that case, the presence of the enteric coating layer protects the drug from contacting the mouth and the acidic environment of the fasted stomach, preventing drug precipitation and instability. Because of a limited precipitation of diclofenac free acid and no formation of agglomerates in the stomach, these data could also explain why its administration by means of the clinical dispersible formulation results in a single peak in plasma profiles. Diclofenac free acid should therefore provide for a better *in vivo* stability after oral administration.

Drug absorption is a highly complex process based on physicochemical properties of the drug, physiological factors of the human body and features related to the drug formulation (1). Scientists have been striving for improving these aspects to achieve optimal drug absorption, thus for screening out and selecting drug candidates to consequently promote drug formulation development. In this area, testing conditions are of utmost importance. In this study, it has been shown that *in vitro* drug behaviors may strongly be determined by the conditions of characterization applied. Biorelevant test setups mimicking mouth and GI tract physiological conditions may represent a key tool for the understanding of *in vivo* drug features and thus the improvement of *IVIVC*. The use of these models may additionally provide valuable information in formulating particular drug delivery systems like effervescent or even orally dispersible formulations where drug exposure to the mouth may apply. For diclofenac, the free acid form which proves to withstand *in vitro* extreme changes along mouth and GI tract conditions should represent the most suitable candidate.

CONCLUSION

Biorelevant test setups intended to mimic the physiological conditions experienced by oral formulations along the passage through the mouth and the upper GI tract after their administration were designed and successfully applied to assessing and discriminating chemical and physical state behaviors of three different drug forms of diclofenac. Using the proposed *in vitro* models, the multiple-peak phenomenon in individual diclofenac salts plasma-concentration profiles observed after dosing a solution, suspension or dispersible formulation can be explained. Based on the results of the present investigation, it is likely that these multiple peaks are a result of irregular gastric-emptying of differently sized drug agglomerates formed during gastric residence. The use of the sodium or potassium form is suggested to be an issue in formulating diclofenac oral drug delivery systems where the drug may be exposed to the mouth and/or the stomach. The free acid form is supposed to withstand extreme pH-changes along the mouth and the upper GI tract and thus should represent the most suitable candidate for formulation development. This study indicates that, for diclofenac, biorelevant models may represent a key tool for the understanding of *in vivo* features of the drug and its dosage forms and may also be a prerequisite for establishing an *IVIVC*. This work may serve for the evaluation and the selection of drug candidates in the development of formulations, e.g. orodispersible dosage forms, where mouth exposure is an issue.

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