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ORIGINAL ARTICLE

Investigation of dissolution behavior of diclofenac sodium extended release formulations under standard and biorelevant test conditions

Grzegorz Garbacz and Werner Weitschies

Department of Biopharmaceutics and Pharmaceutical Technology, Institute of Pharmacy, University of Greifswald, Greifswald, Germany

Abstract

Background: Dissolution characteristics of four extended release (ER) generic formulations of diclofenac sodium were examined. *Aim*: The aim of this study was to compare the drug dissolution behavior of diclofenac ER generics to clarify whether the products are characterized by comparable dissolution characteristics under the applied test conditions. *Methods*: The investigations were performed in the USP apparatus 2 and in the new biorelevant dissolution stress test device. *Results*: The experiments yielded striking differences between the generic formulations. Applying USP apparatus 2 it was noticed that the dissolution profiles of the products were distinctly affected by the stirring rate. Using the biorelevant dissolution stress test device susceptibility of the products to biorelevant stresses was observed. Change of pH within the experiments reduced the dissolution rates of all formulations and distinctly influenced their dissolution characteristics. *Conclusion*: The study demonstrates clearly the divergences in the dissolution behavior among the generic ER formulations of diclofenac sodium. The observed susceptibility of the tested dosage forms toward biorelevant stress bears in our interpretation the risk to cause unwanted fluctuations in drug plasma concentration profiles.

Key words: *Biorelevant dissolution testing; diclofenac extended release tablets; dissolution stress test; HPMC matrix tablet; pH change*

Introduction

Diclofenac is a non-steroidal anti-inflammatory drug with very well-characterized pharmacokinetic and pharmacodynamic properties[1,2]. It is widely used in the treatment of inflammatory diseases and is also administered as an analgesic in osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and acute muscle pain[3]. The favorable properties of diclofenac, such as high anti-inflammatory activity, good absorbability along the gastrointestinal (GI) tract, and lack of accumulation, result in a wide application of the substance in the routine pain treatment. Diclofenac is a phenylactetic acid derivate with a pK_a value of 4.0. Its solubility strongly depends on the pH value of the medium. In acidic solutions such as gastric juice the substance is practically insoluble (~3.6 μg/mL), but well soluble in media with pH values above the pK_a as for example in intestinal fluids (26 mg/mL)[4,5]. Numerous oral formulations with differing dissolution characteristics such as immediate release, delayed release, and extended release (ER) products have been developed. Furthermore, different salts of diclofenac with somewhat differing water solubility and crystal structure are in use[6–9].

In case of ER products based on hydrophilic matrices multiple peaks in the plasma concentration profiles have been reported[10–12]. This phenomenon was observed under fasting and fed conditions and has been intensively investigated and discussed elsewhere[13,14]. The studies demonstrated that when ER formulations were taken under fasting conditions, irregular patterns and multiple peaks with a rapid onset and no lag time in the plasma profiles were observed[5]. When the dosage forms were administered with food a single peak after a

Address for correspondence: Grzegorz Garbacz, Department of Biopharmaceutics and Pharmaceutical Technology, Institute of Pharmacy, University of Greifswald, Friedrich-Ludwig-Jahn-Strasse 17, 17487 Greifswald, Germany. Tel: +49 3834 864899, Fax: +49 3834 864886. E-mail: grzegorz.garbacz@ uni-greifswald.de

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lag time of 2–4 hours was noticed. As an explanation the significant difference in the solubility of the drug in acidic gastric milieu and in intestinal fluids was proposed. The irregular plasma concentration time profiles observed under fasting conditions were suggested to depend on the ability of the gastric acid to decrease the pH value of the drinking water taken with the tablets[5]. Another suggested explanation for this observation was the variability of gastric emptying during which the tablet passed into the small intestine with a higher pH value[15].

Furthermore, spontaneous changes in the dissolution characteristics of the dosage forms because of exposition to the acidic milieu were also discussed as a possible reason for the high variability of the drug plasma profiles[16]. It was demonstrated that the matrix formation process can be continued in gastric juice and the drug release process may in consequence depend on gastric residence time of the tablets and pH value and composition of the stomach milieu.

With respect to recent results it seems likely that the dominant peaks observed in the individual plasma profiles after administration of diclofenac ER tablets are caused by the susceptibility of the ER tablet matrix toward physical stress as it is present in the human GI tract[10]. In this study we were able to demonstrate that the irregular in vivo release behavior of the ER tablets observed after dosing under fasted conditions can be predicted by means of a novel biorelevant dissolution stress test device. This device is capable of simulating physical stress conditions that are known to be present under in vivo conditions. Accordingly, the device can be applied for the examination of the robustness of dosage forms toward biorelevant stress to identify the potential risk of undesired irregular release behavior in vivo, such as dose dumping.

The novel test device simulates three parameters that may affect drug dissolution from solid dosage forms: First, the physiological movement behavior of dosage forms is mimicked in a realistic way. GI transport is known to be inhomogeneous. Transport events are characterized by the presence of relative long phases of rest spiked with short periods of propulsion with velocities up to 50 cm/s[17,18]. Second, the influence of the impact of GI-specific pressure waves on the dosage form can be simulated in physiological scale[19–21]. Third, contact of the dosage form to the liquid phase can be interrupted as it may occur in vivo[22].

In this study four different diclofenac sodium ER tablets of a dose strength of 100 mg that are marketed in Europe were examined using a recently introduced biorelevant dissolution stress test device and apparatus 2 of the USP. The tablets are regarded as bioequivalent and can be freely substituted by each other as well as the original product during routine pain therapy. The dissolution experiments were performed under experimental settings identical to those introduced for the originator product Voltaren 100 mg retard[10]. In addition to the previous test conditions the impact of pH changes on the dissolution characteristic of all tested products was investigated under standard as well as stress test conditions. It was the aim of the study to compare the drug dissolution behavior of the generic formulations to clarify whether the products are characterized by comparable dissolution characteristics under applied test conditions.

Materials and methods

Dosage forms

In this study four ER tablet formulations of dose strengths of 100 mg of diclofenac sodium were examined. All tested ER formulations are based on hydrophilic matrices. Their composition is given in Table 1. The products are marketed in Europe.

Dissolution test experiments

The dissolution experiments were performed with phosphate buffer, pH 6.8 (USP), as dissolution medium. This medium assures sink conditions in the applied volumes over the duration of the experiment. As a model medium for the simulation of the gastric milieu, 0.1 mol/L HCl solution (pH 1.0) was applied.

Table 1. Composition and dimensions of the test formulations.

Formulation no.	Dimensions Ø × H (mm)	Mass (mg) (mean 10 tablets ± SD)	Excipient
1	9.1 × 3.8	0.243 ± 0.003	Lactose monohydrate, hypromellose, magnesium stearate
2	9.1 × 3.5	0.278 ± 0.003	Cetyl alcohol, hypromellose, macrogol 6000, magnesium stearate, povidone K 30, polysorbate 80, sucrose, talcum, titandioxide, iron oxide
3	9.1 × 4.3	0.343 ± 0.003	Diethylphthalate, ethylcellulose, hypromellose, macrogol 4000, magnesium stearate, povidone, stearic acid, talcum, iron oxide, titandioxide
4	9.05 × 4.3	0.344 ± 0.004	Diethylphthalate, ethylcellulose, hypromellose, macrogol 4000, magnesium stearate, povidone K 25, stearic acid, talcum, titandioxide, iron oxide

Standard dissolution test

The dissolution behavior of diclofenac 100 mg ER tablets was examined using USP apparatus 2 (paddle apparatus, PT-DT 7; PharmaTest, Hainburg, Germany). The device was operated at stirring rates of 50 and 100 rpm at 37°C. The volume of the dissolution medium was 1000 mL.

The determination of the dissolved drug was carried out using a UV spectrophotometer (UV 1650; Shimadzu, Duisburg, Germany) equipped with a multicell positioner. The analysis proceeded in a closed flow-through system. The circulation of the dissolution medium in the system was induced by a peristaltic pump (IPC-N 16; Ismatec, Wertheim-Mondfeld, Germany), which was driven at a flow rate of 10 mL/min in 10-minute-long pump intervals (8 minutes of pumping and 2 minutes of rest). Then the medium was fed into a quartz flow-through cell (light path 2 mm; Hellma, Müllheim, Germany). The absorption was measured in intervals of 5 minutes in a differential mode at 276 (signal) and 450 nm (background). Data were recorded and processed using commercial software (UV-Probe; Shimadzu). The loss of the dissolution medium was determined immediately after completion of the experiment for each vessel. The decrease in the volume of the dissolution media was caused by evaporation, which assumedly had a linear character. Therefore, the proportional decrease in the volume of dissolution media was taken into consideration for each of the measurement points.

In a further experimental setting a pH change was performed. Here, the tablets were preincubated in the dissolution vessels in 20 mL of pH 1.0 HCl solution for 1 hour. The pH value of the medium was checked prior to and after the incubation. Thereafter, 980 mL of phosphate buffer (pH 6.8) at 37°C was added. The determination of the amount of dissolved drug was performed as described above and the fraction of drug dissolved in the acidic medium was considered in the calculations as the first data point, which was determined immediately after the addition of phosphate buffer with pH 6.8.

Dissolution stress test

To simulate the impact of physiological mechanical stress that may occur during the GI transit of the dosage form, dissolution experiments were performed using the biorelevant dissolution stress test device.

The device, presented in Figure 1, was introduced recently and its detailed description is given elsewhere[10]. Briefly, in the dissolution stress test apparatus the tablets are exposed to sequences of agitation including movement and pressure waves as well as phases of rest as they occur under in vivo conditions. The device consists of a central apparatus axis, with six

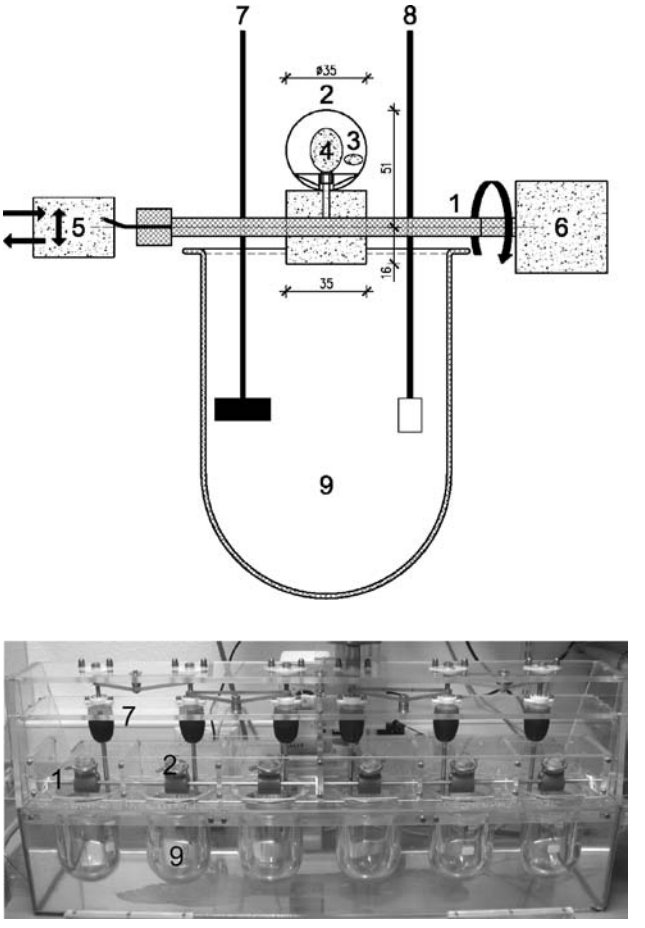

Figure 1. Schematic (a) and photographic (b) representation of the biorelevant dissolution stress-test device: 1, central axle (Ø8 mm); 2, steel netting wire chamber (Ø35 mm, mesh size 0.5 mm, wire 0.1 mm); 3, dosage form; 4, inflatable balloon; 5, solenoid valve system; 6, stepping motor; 7, stirrer (blade 35 × 15 mm); 8, sampling; 9, standard dissolution vessel.

steel wire netting spheres in which the dosage forms are hosted. Each chamber is divided into two parts. The bottom part is screwed onto the central pipe by a PVC bush and by a profiled nozzle. The central pipe is attached by teflon handles, placed on the deck plate of the device close above the top edges of linearly placed standard dissolution vessels in their symmetry plane in such a way that each sphere operates in a separate vessel. The central axis is coupled on one end with a pressure regulation unit by a rotating joint and on the other end with a stepping motor. Pressure waves are generated by pulsatile inflation and deflation of balloons inside the chambers. The inflation and deflation is controlled by synchronized switching of solenoid valves. The pressure value is regulated by a computer-controlled pressure-reducing device. The central axis is driven by a computer-driven stepping motor. The test parameters are controlled by self-made software based on LabView 7.1 (National Instruments, Austin, TX, USA).

Table 2. Arrangement of the stress sequences and the test programs applied in the dissolution stress test experiments.

Activity pattern	3 pressure waves (300 mbar per 6 seconds each)	Rotation 100 rpm (duration 1 minute)	Rotation 10 rpm (duration 0.5 minutes)
Sequence 1	Not applied	Every 20 minutes	Not applied
Sequence 2	Every 20 minutes	Not applied	Not applied
Program 1	At 5 minutes at 5 hours	At 5 minutes at 5 hours	Every 10 minutes (starting after15 minutes)
Program 2	At 30 minutes at 5 hours	At 30 minutes at 5 hours	Every 10 minutes (starting after 40 minutes)
Program 3	At 60 minutes at 5 hours	At 60 minutes at 5 hours	Every 10 minutes (starting after 70 minutes)

All stress test experiments were carried out in 1200 mL of phosphate buffer, pH 6.8 (USP). This volume assures the complete immersion of the steel wire netting spheres in the dissolution medium during the rotational movement and the phases of the rest when the spheres are placed vertically down. The medium was homogenized by a steel paddle stirrer operated at a rotational speed of 100 rpm.

The dissolution stress test apparatus was driven in two stress sequences as well as three test programs. The arrangements are summarized in Table 2. Sequence 1 is composed of phases of dynamic stress generated by the rotation of the central axis at a rate of 100 rpm over a period of 1 minute, which was repeated every 19 minutes. The sequence 2 is composed of three symmetrical pressure waves of 6-second duration and 300 mbar, which were repeated every 20 minutes. To mimic the mechanistic aspect of the GI transit under fasting conditions three test programs were applied. The programs are composed of phases of agitation initiated by rotational movement of the central apparatus axis, which simulates the events of transport followed by phases of pressure waves imitating GI tract motility events. The stress events were identical in all programs and were composed of three symmetrical pressure waves of 300 mbar of 6 seconds duration each (an element of the sequence 2). The pressure waves were followed by 1 minute of rotation at 100 rpm (an element of the sequence 1). It was the intent of this stress phase to simulate the harsh conditions that may occur during gastric emptying and duodenal passage of tablets. The time point of the occurrence of the first agitation phase was varied among the test programs to simulate the variability of gastric emptying under fasting conditions. In program 1 the agitation phase was applied at 3 minutes mimicking very rapid gastric emptying. In program 2 the first stress phase was applied at 30 minutes simulating typical gastric emptying under fasted conditions. In program 3 the lag time prior to simulation of the stress phase was elongated to 60 minutes to mimic late gastric emptying.

The small intestinal transport in the programs 1–3 was simulated as five rotations of the apparatus axle at 10 rpm, which were repeated every 10 minutes. Between the rotations the probe chambers remained in a fixed position, with the dosage form being submersed in the dissolution medium. At 5 hours in all test programs colon arrival was simulated, using the identical conditions as for gastric emptying (three pressure waves of 300 mbar lasting 6 seconds each followed by 1-minute rotation at 100 rpm). These agitation phases were intended to mimic the passage of the dosage forms through the ileocecal junction as a region with known strong motor activity and high pressure gradients[20].

The pH change was investigated in the experiments carried out using program 3. The dosage forms were preincubated in 20 mL HCl solution (pH 1.0) at 37°C for 1 hour. Thereafter they were transferred into the probe chambers and placed into the phosphate buffer solution (pH 6.8) of the USP (1180 mL) for 1 minute prior to simulation of the first high stress phase. The acidic media together with fragments of the tablets were transferred into the buffer solutions. The amount of dissolved drug was determined and considered in the calculations as the first data point, which was determined immediately after the transfer of the acidic media into pH 6.8 phosphate buffer.

The determination of the amount of the drug dissolved in the experiments carried out with the biorelevant dissolution stress test device was conducted by means of UV–Vis spectroscopy (Cary 50, Varian, Palo Alto, CA, USA) equipped with a multichannel fiber-optic system. The measurements were carried out using optical probes of 2 mm light path at an averaging time of 1 second per wave length. The absorption was measured in intervals of 5 minutes at 276 (signal) and 450 nm (background) and calculated as the difference. Data acquisition and processing were performed using commercial software.

The loss of the dissolution media because of evaporation was determined and considered in the calculations identically as described for standard dissolution test.

Statistical analysis

The dissolution profiles obtained under the various test conditions were compared using the statistical method developed by Pillay and Fassihi[23]. Determined were

times for dissolution of 30, 50, and 90 mg of the drug load as well as the mean dissolution times ($MDT_{30\ mg}$, $MDT_{50\ mg}$, $MDT_{90\ mg}$) were calculated. The equality of variances within the data groups was investigated using the Brown–Forsythe test for $P > 0.05$. The statistical significance of the observed differences in the dissolution profiles was determined by multivariate analysis of variance (ANOVA/MANOVA) with the post hoc test NIR. The data were processed by commercial software.

Results

Standard dissolution test

The results of the tests carried out without pH-change procedure yielded that the tested formulations are characterized by various dissolution characteristics. In the case of formulation 1 rapid dissolution of the drug was observed with approximately 90 mg diclofenac being dissolved within 0.17 hours at 100 rpm and within 0.83 hours at 50 rpm, respectively (Figure 2a). The differences in the $MDT_{90\ mg}$ are of statistical significance. The tablets showed intense swelling and disintegrated within 10 minutes at 100 rpm (Figure 3). At 50 rpm swelling proceeded slower and the disintegration process was completed within approximately 2 hours. However, the divergences calculated for $MDT_{30\ mg}$ and $MDT_{50\ mg}$ at 50 and 100 rpm were not significant.

In case of formulation 2 strong divergences were observed between the dissolution profiles obtained at 50 and 100 rpm (Figure 2b). At 50 rpm the tablets remained on the bottom of the dissolution vessel during the analysis and drug release was linear with a rate of approximately 6 mg/h. The $MDT_{30\ mg}$ at 50 and 100 rpm amounted to 2.19 and 1.77 hours and the differences were not significant. At 100 rpm the tablets detached from the bottom of the vessels after approximately 3.5 hours and, thereafter, moved freely in the dissolution medium. Many impactions of the tablets against the paddle and against the walls of the dissolution vessels were observed. As a consequence in the time interval between 4 and 7 hours complete disintegration of the matrices occurred. The calculated $MDT_{50\ mg}$ and $MDT_{90\ mg}$ at 50 rpm amounted to 4.86 and 15.01 hours and differed significantly from corresponding $MDT_{50\ mg}$ and $MDT_{90\ mg}$ calculated for 100 rpm, which amounted to 3.57 and 4.37 hours, respectively.

Drug release from formulation 3 was continuous at both stirring rates with a higher dissolution rate of approximately 16 mg/h at 100 rpm (Figure 2c). However, at time points later than 5 hours the individual

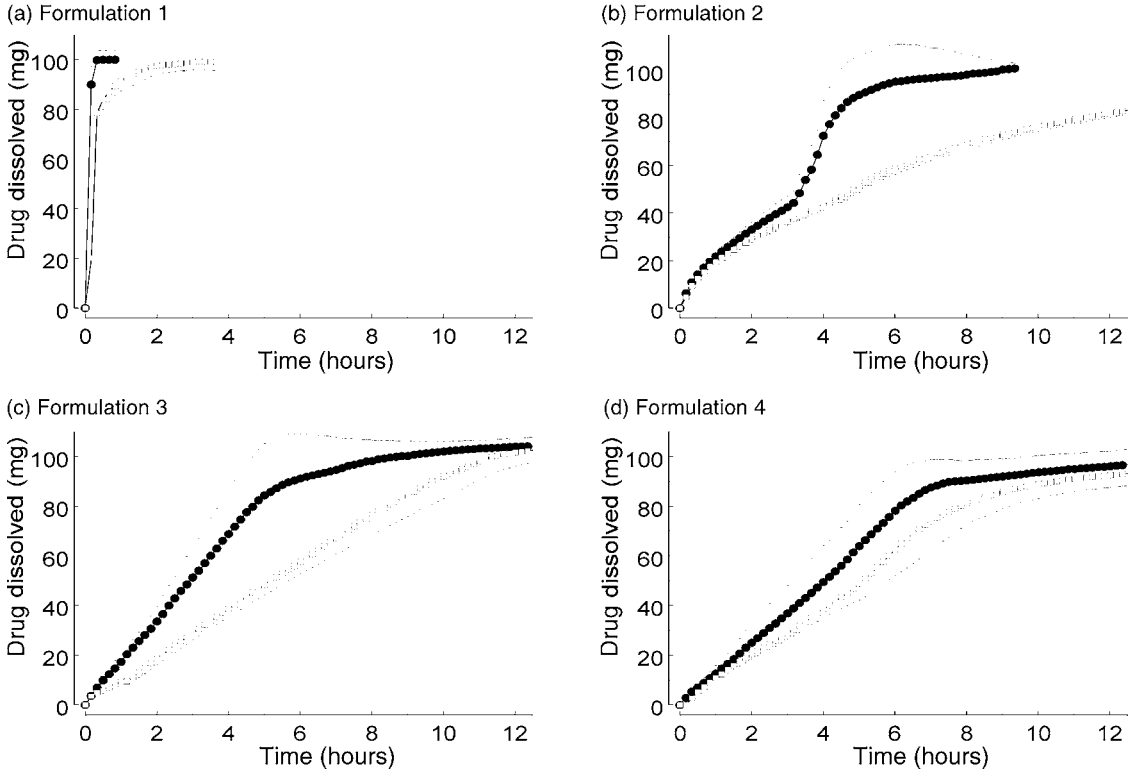

Figure 2. Mean drug release profiles ($n = 6$) of diclofenac retard tablets in the paddle apparatus at 37°C, stirring speed of 50 (□) and 100 rpm (●) and fill volume of 1000 mL phosphate buffer (pH 6.8) USP. SDs are shown by the error bars.

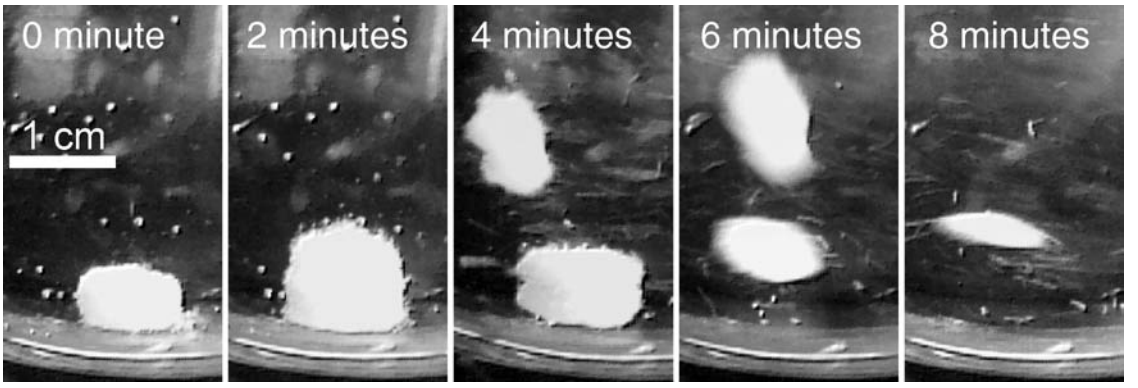

Figure 3. Disintegration behavior of formulation 1 during dissolution test in apparatus 2 of the USP at 100 rpm 37°C and 1000 mL fill volume.

dissolution profiles were highly variable. Two of the six tested tablets were located directly under the stirrer blade in the center of the dissolution vessel, resulting in coning behavior. The $MDT_{90\ mg}$ was achieved after 5.92 hours at 100 rpm and after about 9.95 hours in the experiments at 50 rpm. The observed differences in the dissolution profiles obtained at 50 and 100 rpm for MDT of 30, 50, and 90 mg were of statistical significance.

Drug release from formulation 4 was only slightly affected by the stirring rate (Figure 2d). Diclofenac dissolution at 50 and 100 rpm was linear with dissolution rates of approximately 10 and 12 mg/h, respectively. During the dissolution tests it was observed that the tablets' matrices formed sediments on the bottom of the vessels. The divergences of the dissolution profiles for $MDT_{30\ mg}$, $MDT_{50\ mg}$ as well as $MDT_{90\ mg}$ were not significant. The $MDT_{90\ mg}$ amounted to 10.88 at 50 rpm and 8.61 hours at 100 rpm.

Change of pH resulted in USP apparatus 2 at a stirring rate of 100 rpm in the prolongation of the dissolution process for all tested formulations (Figure 4). The most distinct decrease in the dissolution rate was observed for formulation 1. Here, 90 mg of the drug was dissolved within 9.18 hours with a rate of about 12 mg/h (Figure 4a). In this case, the tablets formed swollen matrices that moved freely in the dissolution medium and showed no sign of disintegration until complete drug release. The changes in the dissolution characteristic for calculated mean time points for dissolution of 30, 50, and 90 mg were significant.

The dissolution process of formulation 2 was also linear with a rate of about 8 mg/h (Figure 4b). All tablets remained for about 8 hours at the same position within the vessel. This is 5 hours longer than in the dissolution experiments without pH-change procedure. Consequently, a decrease of the release rates was observed, with $MDT_{90\ mg}$ of approximately 12.11 hours. The change of the dissolution characteristic of the formulation 2 because of preincubation in the acidic medium was also of statistical significance for $MDT_{30\ mg}$ and $MDT_{50\ mg}$ if compared to the tests without prior incubation (Figure 4b).

In case of formulation 3 the incubation of the tablets in the acidic medium resulted in a decrease of the dissolution rate from about 16 mg/h observed in the experiments without pH change to about 13 mg/h (Figure 4c). The calculated MDT amounted to $MDT_{30\ mg}$ 4.56 hours, $MDT_{50\ mg}$ 5.96 hours, and 8.41 hours for $MDT_{90\ mg}$ and differed significantly from the corresponding MDT at 100 rpm without preincubation in the acidic media.

Change of pH caused, in case of formulation 4, a lag phase of about 2 hours (Figure 4d). Visual inspection after 1, 2, and 3 hours yielded that the outer layers of the tablets were intact. The dissolution rate observed after the lag time was distinctly lower than in the experiments without pH change with approximately 5 mg/h resulting in the release of 30 mg diclofenac within 10.02 hours and 50 mg within 18.74 hours, respectively. The increase in the dissolution rate observed after approximately 4 hours was initiated by rupture of the tablets' surfaces. The individual time point of the rupture varied. This resulted in divergences in the individual dissolution profiles of the tablets. After 24 hours the matrices were strongly deformed but did not disintegrate. The recovered drug amounted to 103 mg and was calculated as the sum of the fraction of diclofenac sodium dissolved and the amount of the drug that was still present in the residues of the tablets. The divergences of the dissolution profiles observed under pH change conditions were significant in comparison to dissolution profiles obtained at 100 rpm in USP phosphate buffer as sole dissolution medium.

Dissolution stress test

The drug dissolution profiles that were obtained applying the simplified agitation sequences that are characterized by continuous repetition (every 20 minutes) of

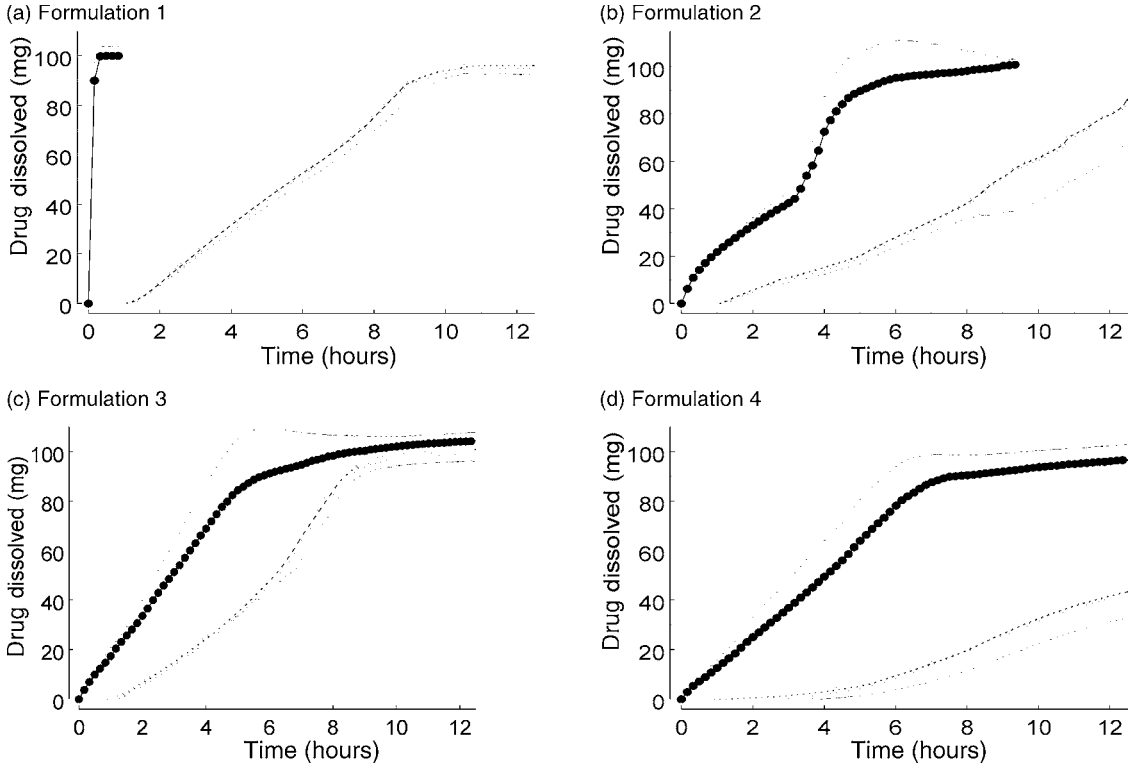

Figure 4. Mean drug release profiles (n = 6) of diclofenac retard tablets in apparatus 2 of the USP at 37°C, obtained in the USP (pH 6.8) phosphate buffer solution at 100 rpm after incubation of the tablets in HCl (pH 1.0) for 1 hour (◇) and without prior incubation (●). SDs are shown by the error bars.

rotation events (sequence 1) and pressure waves (sequence 2) are presented in Figure 5. Both types of stress applications resulted in an immediate increase in the amount of the drug dissolved in case of the formulations 1 and 2 and a differential behavior in case of the formulations 3 and 4. In general, the pressure events produced more distinct changes in the dissolution profiles.

In the case of formulation 1 dissolution was rapid like it was observed using USP apparatus 2 at 100 rpm (Figure 5a). Complete dissolution was reached after complete disintegration of the tablets. In case of formulation 2 $MDT_{90\,mg}$ amounted 1.53 hours for pressure sequence and 1.76 hours for rotation sequence which is significantly faster than in USP apparatus 2 (Figure 5b). Drug release from formulation 3 was linear under appliance of stress events in the form of rotational movements (Figure 5c). The dissolution rate was approximately 20 mg/h, which is significantly higher than under standard conditions at 100 rpm. Agitation caused by the pressure waves resulted in an increase for about 3–5 mg in the amount of the drug dissolved immediately after the stress phases. In case of formulation 4 drug release was almost unaffected by the applied stress and the observed dissolution rates differed significantly from the values obtained under standard conditions at 50 and 100 rpm. Differences in the dissolution profiles observed under both stress sequences in $MDT_{50\,mg}$ and $MDT_{90\,mg}$ were also significant.

The dissolution profiles obtained using the dissolution programs are shown in Figure 6a–c. Applying program 1 it was observed that the first stress phase simulated after 3 minutes did not affect the dissolution profiles of all tested formulations. The drug dissolution proceeded with the highest rate for formulation 1 with dissolution of 50 mg within 1.68 hours and 90 mg within 5.09 hours, which is significantly slower than in the apparatus 2 of the USP. For the other formulations the amount of the drug dissolved within 5 hours ranged from about 60 mg in the case of the formulation 3 to about 80 mg in the case of formulation 2. During the stress phase simulated at 5 hours the formulations 1, 2, and 3 disintegrated and the remaining drug load rapidly dissolved. In the case of formulation 4 partial disintegration and formation of a sediment were observed, whereas an increase in the amount of drug dissolved was not registered. Drug dissolution proceeded in a regular manner with a $MDT_{90\,mg}$ of 6.34 hours, which resembled the dissolution rate observed under standard conditions.

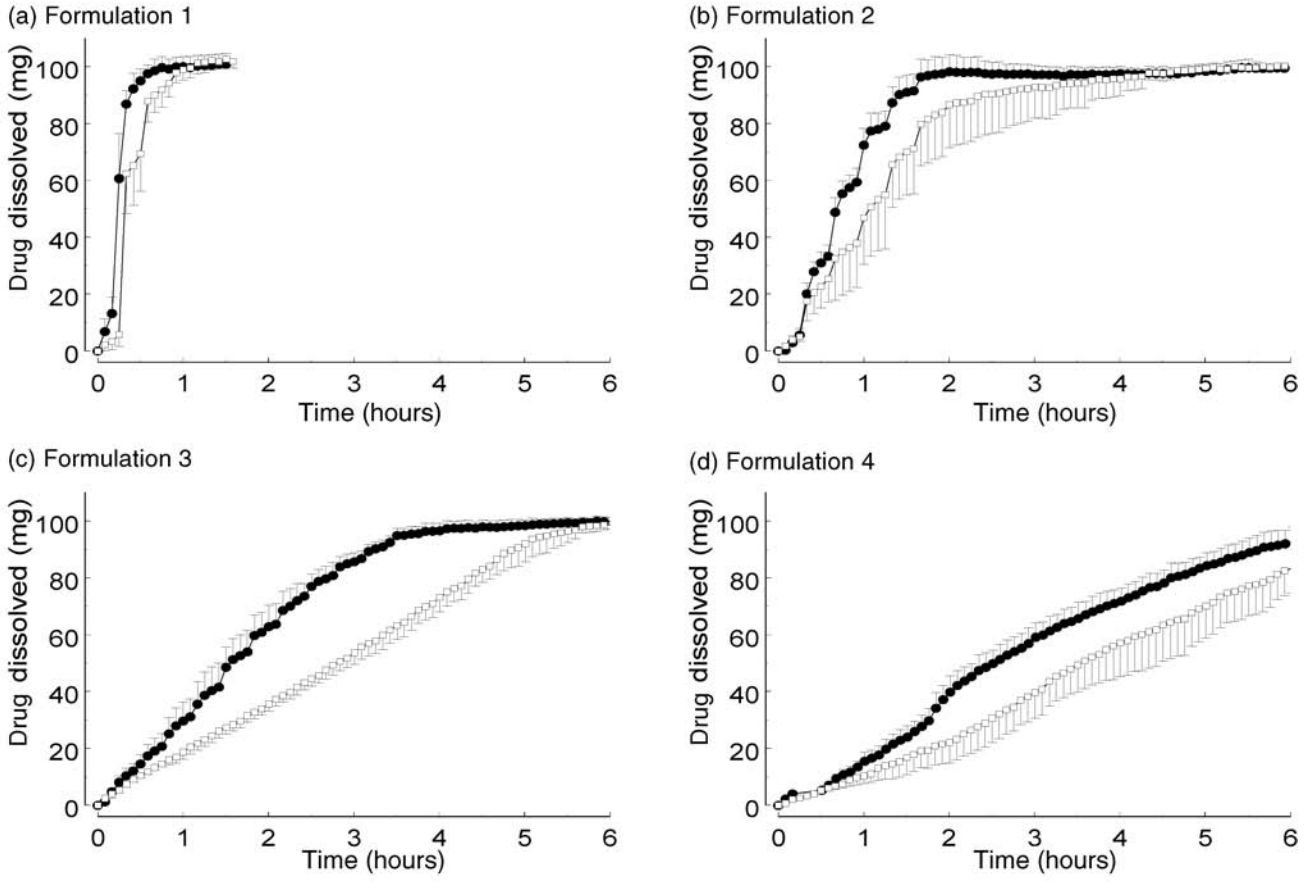

Figure 5. Mean drug release profiles (n = 6) from diclofenac retard tablets in phosphate buffer (pH 6.8) USP, using the dissolution stress test apparatus under simplified stress sequences: □, sequence 1 (rotation) and ●, sequence 2 (pressure). SDs are shown by the error bars.

The prolongation of the lag time of the dosage forms within the dissolution medium to 30 minutes in program 2 and 60 minutes in program 3 prior to simulated stress resulted in an increase in the susceptibility of the formulations 1 and 2 to the applied stress conditions. Drug dissolution and the disintegration process of the matrices of formulation 1 was completed immediately after the first stress phase, which resulted in rapid dissolution of 65 and 50 mg of diclofenac applying the programs 2 and 3, respectively. In the case of formulation 2 the applied stress resulted in rapid dissolution of 40 mg diclofenac applying program 2 and about 50 mg in case of program 3. It was observed that the tablets eroded, but they retained their shape and integrity. The dissolution rate prior to the second stress phase at 5 hours was significantly slower than in program 1. In case of formulations 3 and 4 the first stress phase did not significantly increase the amount of the drug dissolved and the dissolution rate within 5 hours. The stress phase simulated at 5 hours resulted in the disintegration of the tablets in case of formulations 2 and 3 followed by rapid dissolution of the remaining drug load. The amount of diclofenac that was dissolving rapidly was higher in program 2 and ranged from 15 mg for formulation 2 to 40 mg for formulation 3. Drug dissolution from formulation 4 was not affected by the programs 2 and 3 and proceeded with a similar rate as under program 1 and did not differ significantly from the dissolution profiles obtained in the USP apparatus 2 at 100 rpm.

The stress test results obtained under pH change yielded that the dissolution characteristics of all formulations were altered after incubation in 0.1 N HCl (Figure 7a–d). The most dominant and statistically significant modification of the dissolution profiles was observed for formulation 1 (Figure 7a). Interestingly, the first stress phase applied at 1 hour directly after the pH change had no relevant impact on drug dissolution. Dissolution proceeded with rate of approximately 12 mg/h, which is comparable to the dissolution rate obtained using USP apparatus 2 under the pH-change procedure; however, the divergences of the dissolution profiles were significant. The stress phase applied at

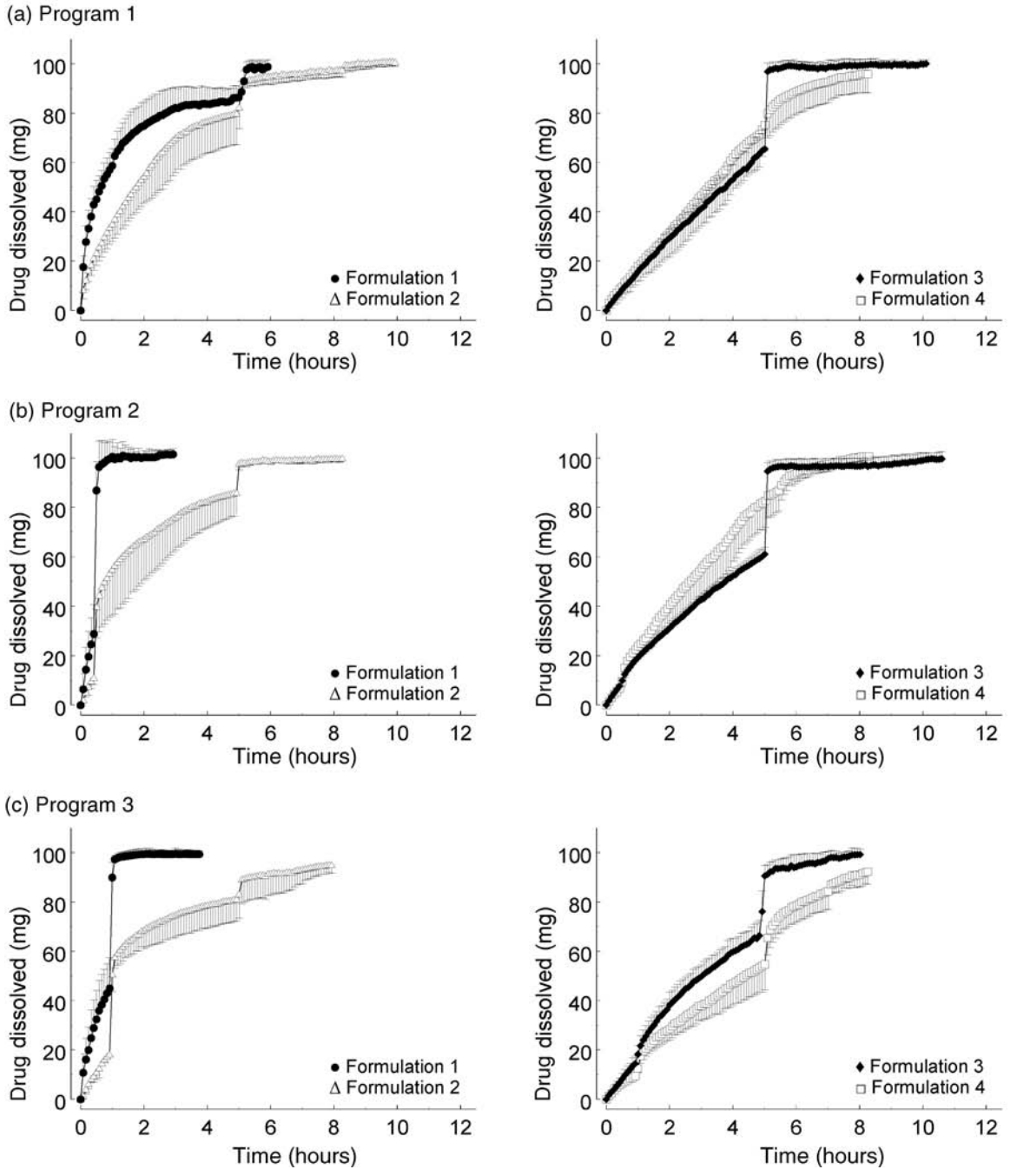

Figure 6. Mean drug release profiles (n = 6) of formulations 1–4 in phosphate buffer (pH 6.8) USP using the dissolution stress test apparatus under test programs 1–3: formulation 1 (●), formulation 2 (△), formulation 3 (◆), formulation 4 (□). The SDs are shown by the error bars.

5 hours resulted in a strong deformation of the tablets and a rapid dissolution of approximately 25 mg of the drug within 15 minutes. The $MDT_{90\ mg}$ was achieved within 4.01 hours, which is significantly faster than under standard conditions.

In case of formulation 2 the first stress phase yielded a rapid dissolution of 40 mg diclofenac. Thereafter a dissolution rate of 20 mg/h was obtained. $MDT_{90\ mg}$ was reached after 2.14 hours (Figure 7b). Significant divergences in the dissolution profiles in comparison to the tests obtained under program 3 without pH change were observed for $MDT_{30\ mg}$ as well as $MDT_{90\ mg}$, which amounted to 1.16 and 2.14 hours, respectively.

Drug release from formulation 3 was not significantly affected by the first stress phase (Figure 7c). The stress phase simulated at 5 hours provided an increase in the

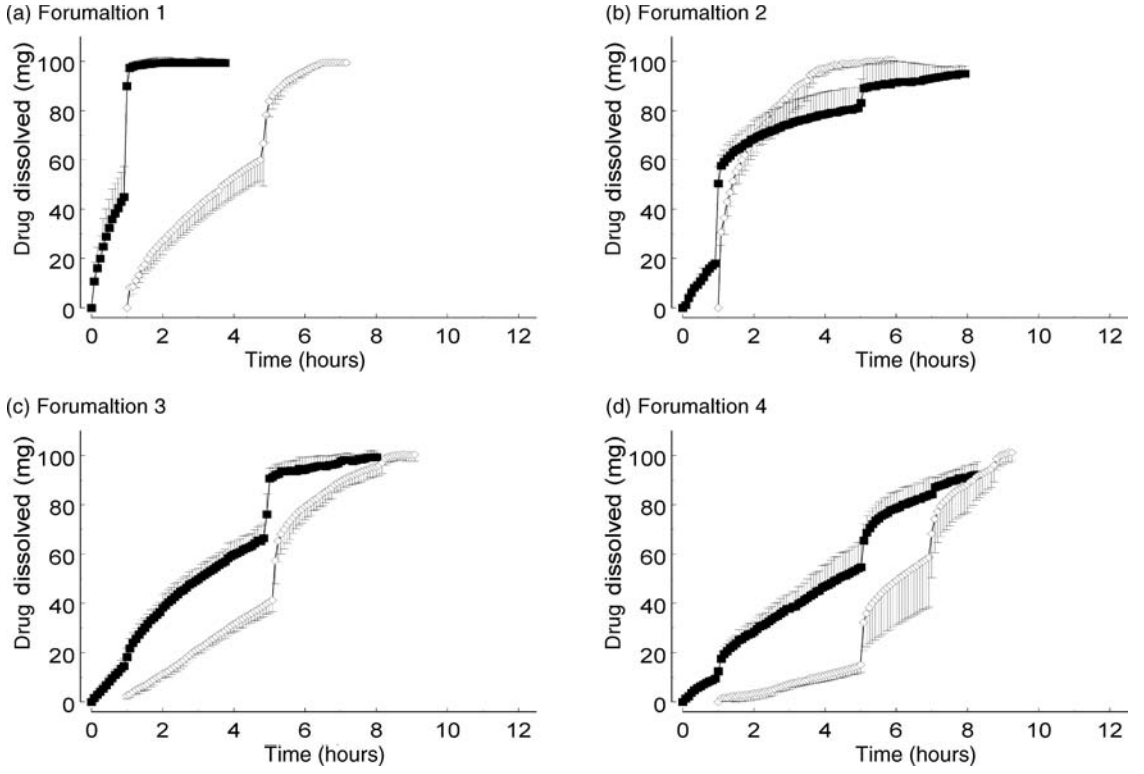

Figure 7. Mean drug release profiles ($n = 6$) of diclofenac retard tablets in the dissolution stress test apparatus under program 3 obtained in USP (pH 6.8) phosphate buffer after incubation of the tablets in HCl (pH 1.0) for 1 hour (◇) and without prior incubation (■). SDs are shown by the error bars.

amount of dissolved diclofenac in the range of approximately 20 mg. The $MDT_{50\ mg}$ and $MDT_{90\ mg}$ were reached at 3.56 and 4.69 hours, respectively.

The dissolution profiles of formulation 4 obtained under pH change conditions differed significantly from those observed in the previous experiments. The dissolution rate within the first 4 hours after pH change was slow and corresponded to the value observed in apparatus 2 of the USP. The applied stress events at 5 and 7 hours resulted in a significant increase in the amount of dissolved diclofenac (Figure 7d). After the stress events drug dissolution was significantly faster than in the dissolution stress test experiments that were performed without pH-change procedure. The mean times for dissolution of 30, 50, and 90 mg amounted to 4.68, 5.18, and 6.96 hours, respectively.

Discussion

According to the European guidelines as pharmaceutical equivalent defined are products that contain the same amount of the drug in the same dosage forms that meet the same comparable standards[24]. Pharmaceutical equivalence of a generic product with the originator product can be considered based on dissolution profiles that are composed of at least three time points, whereas any justified method to prove the similarity of the products is accepted[24]. Meanwhile the current guidelines request the extension of the in vitro tests for at least three charges of the reference and generic products to investigate the pharmaceutical equivalence[25]. However, it is still possible to select the dissolution methods and the sampling points in a way that will result in an achievement of small differences in dissolution behavior between the generic formulation and the originator.

There is currently no obligation to investigate the pharmaceutical equivalence of generic formulations under uniform methods as, for example, the method used for the original product or the first generic formulation. Differing dissolution test methods or conditions might be one possible explanation for the striking differences in the dissolution behavior of the four tested generic ER formulations of diclofenac sodium when tested under uniform standard conditions (apparatus 2 of the USP). Furthermore, this may also serve as an explanation why the dissolution profiles of these four generic products are also differing from the dissolution characteristic reported previously for the original product[10].

The tested diclofenac sodium ER generic formulations are regarded as bioequivalent based on results of bioequivalence studies. However, considering the relative long product life time, scale up, and post-approval changes in the manufacturing as well as the test procedure are likely. In the European Community post-approval changes of pharmaceutical products are categorized into minor changes with of Type IA and Type IB as well as major changes of Type II[26]. In case of minor changes (like the replacement of an excipient with a comparable excipient or a change in tablet dimensions) it is required to demonstrate a comparable dissolution profile. However, it is not required to perform a new bioequivalence study. The equivalence test is to be performed according to the previously specified dissolution test method whose ability to predict the in vivo drug delivery behavior may be often poor. This may be critical as the applied changes can keep a potential risk of uncontrolled modifications of the in vivo drug delivery behavior.

Applying the stress factors in biorelevant fortitude it is possible to detect further dissimilarities in the dissolution characteristics among the formulations. We have recently demonstrated for the original product that the susceptibility of the drug dissolution behavior to the applied biorelevant stress is very likely to be the cause for strong fluctuations in the plasma concentration profiles as they are observed in vivo[10].

In case of diclofenac sodium such drug delivery characteristics of the formulations may result in an increase of the side effects and reduction of the dosage forms action time. However, in the case of substances characterized by narrow therapeutic index, the appearance of the steep rises in the drug–plasma concentration may bear potential risk for the therapy efficiency and safety and therefore should be prevented[27,28].

Based on the study results it is possible to carry out a draft evaluation of the biopharmaceutical quality of the tested formulations. The observed divergence of the dissolution profiles observed in the case of formulations 2 and 3 in the USP apparatus 2 at 50 and 100 rpm provided evidence of the susceptibility of the tablets to the intensified test conditions. With respect to the movement behavior of some of the tablets during the analysis the application of a sinker could be advantageous by preventing impaction of the tablets against the paddle and the wall of the dissolution vessel. However, considering the hydrodynamic conditions of the standard dissolution vessel directly below the paddle, where sinkers are predestined to remain, the impact of the increase in stirring rate on media velocities and drug dissolution in the central part of the vessel is only minor[29,30].

In the visual inspections within the analyses in the paddle apparatus we were able to detect fast and pH-dependent swelling and disintegration behavior of formulation 1. It is likely that applying dissolution media containing 1% (w/v) of Tween 20, as proposed previously for the screening analysis of ER products containing diclofenac sodium, further differences among the formulations may be observed[31].

Biorelevant test conditions can be employed applying the recently introduced dissolution stress test device that enables simulation of mechanistic aspects of the GI transit[10]. Applying this device it was shown that formulations 1–3 are prone to the mechanical agitation and disintegrate under stress events of biorelevant fortitude. Running the simplified stress sequences 1 and 2 (Figure 5a and c) it was observed that the drug delivery profiles of formulations 1 and 3 were affected more distinctly by the simulated pressure events that became the most critical stress factor that affected the drug delivery characteristic. The pressure waves influenced the retardation principles of the tablets causing their deformation and resulted in an immediate increase in the dissolution rates. The stress conditions did not influence the drug dissolution profiles of formulation 4 to a notable extent. Formulation 4 delivered diclofenac in a regular manner despite a strong deformation that was observed under applied stress.

Generally, it can be noticed that the prolongation of the incubation time of the dosage forms in the test medium resulted in an increase of the impact of the applied stress on the dissolution profiles. This was due to successive hydration of the tablets that became consequently more prone to the applied agitation. This bears, in our interpretation, a potential risk of dosage form related interactions in vivo. It is well recognized that especially the transport events like gastric emptying or ileocecal passage of dosage forms are associated with a strong peristaltic activity with pressure events of up to 300 mbar and causes a so-called jet-like propulsion of the chyme[17–19,32–34]. Such physical stress may result in the destruction of the control mechanism of the dosage form in vivo and provoke dose dumping. In all test programs the stress phase at 5 hours resulted in the disintegration of the tablets of formulations 2 and 3 and rapid dissolution of the remaining drug load. Similar response of the dosage forms to the applied stress conditions was observed for Voltaren 100 mg retard[10].

Applying the pH-change procedure a general prolongation of the drug dissolution process was observed under standard as well as under stress test conditions. Similar effects of the pH-change procedure on the dissolution rates were studied for Voltaren 100 mg retard and other ER formulations previously[4,16]). In our interpretation the effect is related to the poor solubility of diclofenac in the acidic milieu, which results in changes of matrix formation processes and in consequence in the reduction of the drug dissolution rates. Such effects were observed by other authors for hydrocolloid matrices

loaded with drugs of different solubility, which in detailed investigations found that drug solubility is an essential factor affecting matrix formation processes and release mechanisms[35,36]. It is noticeable that the applied pH-change procedure does not reflect the complete dissolution kinetics in the different compartments of the GI tract and was only adopted for screening analysis of the products.

In this study the visual inspections during the dissolution experiments in the USP apparatus 2 yielded changes in the disintegration behavior of all the formulations. These were most pronounced in the case of formulation 1. Interestingly, after incubating the tablets for 60 minutes in 50 mM phosphate buffer solutions of pH 3.0 and 4.5 a prolongation of the disintegration times and a reduction of the dissolution rates were not observed (data not shown). To our expectation, the observed dependency of the dissolution behavior of the product on to the pH of the environment may result in a high variability of the plasma concentration time profiles.

It should be kept in mind that the applied test procedures only sketchily simulate some of the complex aspects of the physiology of the GI tract and will have to be further developed to enable the testing of dosage forms under even more realistic conditions. Modifications of the novel test device aiming at reducing the volume of dissolution media to mimic physiological conditions are strongly recommended[22]. The application of novel biorelevant dissolution media for simulation of postprandial conditions is also advisable[37–39]. Finally, the parameters of the applied test conditions in the biorelevant dissolution stress test device should be modified to enable a more realistic simulation of postprandial conditions.

Conclusions

The study demonstrates clearly the divergences in the dissolution behavior among the generic ER formulations of diclofenac sodium. The results show that the dissolution characteristics of all tested formulations are strongly dependent on the test conditions and could be distinctly influenced by the mechanical stress as it may occur in vivo.

In our interpretation such susceptibility of dosage forms to stress events of biorelevant fortitude may be the main reason for irregularities in the drug plasma profiles that were investigated under identical experimental settings for Voltaren 100 mg retard in our previous study[10]. Appliance of the pH-change procedure yielded the susceptibility of the tablet matrices within the formation phase due to the pH value of the environment. However, the detailed explanation of the mechanisms thereof requires detailed investigations. The changes of the matrix formation processes and so in the drug dissolution characteristics may be an additional factor resulting in variability of the drug dissolution behavior in vivo. The clinical relevance of the observed dissimilarities in the drug dissolution behavior among the formulations remains to be intensively investigated.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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