Department of Pharmacy, Ernst Moritz Arndt University, Greifswald, Germany

Similar *in vitro* drug release as a surrogate of therapeutic equivalence of locally acting gastrointestinal products - what is the right *in vitro* method?

S. KLEIN

Received January 8, 2015, accepted February 22, 2015

Prof. Dr. Sandra Klein, Department of Pharmacy, Institute of Biopharmaceutics and Pharmaceutical Technology, Center of Drug Absorption and Transport, Felix Hausdorff Street 3, 17489 Greifswald, Germany
Sandra.Klein@uni-greifswald.de

Pharmazie 70: 535–542 (2015) *doi: 10.1691/ph.2015.5556*

There is - apart from clinical trials - an ongoing discussion on how to demonstrate therapeutic equivalence for locally applied and locally acting products in the gastrointestinal tract. Possibly, among other alternatives, *in vitro* drug release models could be considered surrogates of drug release and availability at the site of action. However, to date the conditions in which *in vitro* models provide valid surrogates of *in vivo* release and availability at the site of action would have to be defined. To demonstrate the potential applicability of *in vitro* test methods for screening therapeutic equivalence of locally applied and locally acting gastrointestinal products and also to get an idea of which would be the right dosage form for an individual patient a series of *in vitro* studies was performed comparing a variety of *in vitro* release methods ranging from pharmacopoeial methods to "patient-specific" release methods in examining drug release of four mesalazine tablet formulations intended for local drug delivery in the gastrointestinal tract. Results from this study indicated that pharmacopoeial quality control methods are hardly applicable to predict the therapeutic equivalence of such products. Moreover, comparison of the results obtained with the different *in vitro* methods reveal that a prediction of the therapeutic equivalence for locally acting products in the gastrointestinal tract is unlikely based on release profiles obtained in a single drug release experiment. However, results from the study also indicated that a set of individualized biorelevant *in vitro* test scenarios might be very useful for both demonstrating therapeutic equivalence and selecting the appropriate drug product for a particular patient.

1. Introduction

In September 2013 a concept paper on the development of a guideline on the demonstration of therapeutic equivalence for locally applied and locally acting products in the gastrointestinal tract was released for public consultation (EMA 2013). This concept paper discusses the need to expand the current "Note for Guidance on the Clinical Requirements for Locally Applied, Locally Acting Products Containing Known Constituents (CPMP/EWP/239/95)" (EMEA 1995) regarding the approaches for the demonstration of therapeutic equivalence of locally applied and locally acting gastrointestinal products. In contrast to the current guideline which defines the general requirements for all locally acting and locally applied products, the new guideline is aimed to address when and how to employ particular models to demonstrate therapeutic equivalence for gastrointestinal products (e.g. *in vitro* comparisons and pharmacokinetic comparisons) apart from clinical trials (EMA 2013).

In recent years, it has been agreed that drug release and availability at the site of action are the major factors determining the clinical response for locally applied and locally acting gastrointestinal products containing the same drug. Therefore, various alternatives, including *in vitro* models, are considered surrogates of drug release and availability at the site of action. However, according to the concept paper, the conditions under which such alternatives provide valid surrogates of *in vivo* release and availability at the site of action would have to be defined. These efforts give reason to present results from a case study which was performed to screen the applicability of different *in vitro* release methods for the purpose of demonstrating therapeutic equivalence in locally applied and locally acting gastrointestinal products and also for the purpose of selecting the right dosage form for individual patients.

Mesalazine (5-ASA) is an anti-inflammatory drug that is used to induce and maintain remission of inflammatory bowel diseases (IBDs) such as Crohn's Disease (CD) and Ulcerative Colitis (UC) (Sandborn and Hanauer 2003; Verzijl and Bodegraven 2003). The main principle of mesalazine action is a topical reduction of inflammation in the intestinal mucosa. Thus, an effective mesalazine therapy requires effective drug delivery to the sites of inflammation. However, in IBD both the pattern and the extent of inflammation underlie a high inter-individual variability (Ochsenkuhn et al. 2003). CD is a chronic transmural inflammation of the bowel which can affect the entire gastrointestinal tract, usually in a discontinuous pattern. The initial location of CD is most commonly in the lower ileum. From here the inflammation typically spreads towards proximal parts of the small intestine. However, the colon is also often involved. UC is a chronic inflammatory disease affecting only the colon and showing a continuous distribution in the gastrointestinal mucosa. Many patients suffer from a "proctitis" where

the focal point of the inflammation is found in the distal part of the colon and the rectum. However, from this origin, the inflammation often spreads proximally and in the most severe cases, the entire colon is affected.

In front of the background of the high inter-individual variability both in pattern and extent of inflammation in CD and UC, for discussing therapeutic equivalence and or to select the right dosage form for a particular patient based on *in vitro* release rates, it would be essential to simulate the properties of the intraluminal contents and the residence times in the different GI sections as close as possible. However, these aspects are typically not addressed in standard quality control (QC) methods. The first part of our study was therefore dedicated to investigate the *in vivo* predictivity of pharmacopoeial test methods (Ph.Eur. 2011; USP 2013) and of *in vitro* approaches addressing average intraluminal pH-conditions and tablet residence times in the human GI tract (Klein et al. 2005, 2008).

Ibekwe et al. (2008) presented results from a study examining the interplay of *in situ* gastrointestinal pH, transit time and feed status on the *in vivo* performance of Eudragit® S coated polymer systems in healthy individuals. In one arm of the cited study, a radiolabelled Bravo® pH capsule was swallowed by volunteers. With this radiolabelled Bravo® pH capsule it was possible to monitor intraluminal pH-conditions and transit through the gastrointestinal tract at the same time. Ibekwe et al. (2008) thus presented a valuable set of pH values and transit/residence times of monolithic dosage forms in individual subjects.

The objective of the second part of our study was to screen the applicability of so called patient-specific *in vitro* methods for predicting site and extent of *in vivo* drug release. For this purpose we designed new release methods simulating intraluminal pH-conditions and residence times in individual subjects by utilizing several individual data sets reported from the Ibekwe study. Moreover, in a final set of *in vitro* experiments we combined patient-specific pH-conditions and residence times with typical gastrointestinal shear forces and pressures in the GI tract that might affect drug release in the corresponding individuals (Shameem et al. 1995; Kamba et al. 2000; Cassilly et al. 2008; Garbacz et al. 2008; Garbacz et al. 2010; Goodman et al. 2010).

2. Investigations and results

To screen the *in vivo* predictivity of different *in vitro* methods a series of release experiments was performed with four enteric coated tablet formulations that are currently used in oral regimens of CD and/or UC. These were Claversal® and Salofalk® 500 mg tablets, Asacol® 400 mg tablets and mezavant® 1200 mg gastro-resistant prolonged release tablets.

2.1. Release profiles obtained with pharmacopoeial test methods

The first set of experiments was performed with the USP method for "Mesalamine delayed-release tablets" (USP 2013). The second set of experiments was performed with the Ph.Eur./USP drug release standard for delayed-release articles, method A (Ph.Eur. 2011; USP 2013).

Figure 1 shows the release profiles that were obtained with the USP method for mesalamine delayed-release tablets (USP 2013).

All tablets showed enteric properties and started to release the active after about the same lag time. Drug release from Asacol® 400 mg tablets was similar to that of Claversal® and Salofalk® 500 mg, despite the Asacol tablet being essentially different from the other two formulations (Eudragit® S *versus* Eudragit® L coating).

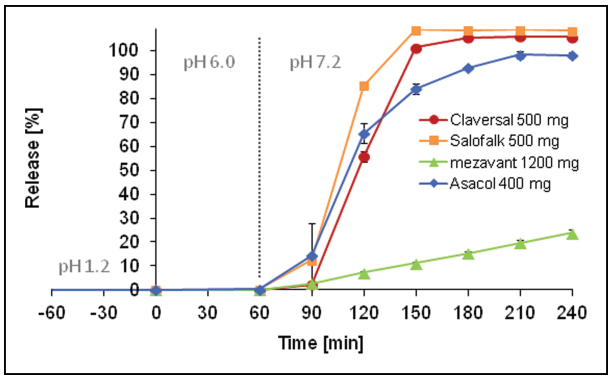

Fig. 1: Mesalazine release in the Paddle apparatus applying the USP QC method for mesalamine delayed-release tablets, gastric residence time is plotted left from x-axis, mean of n = 3, ± S.D.

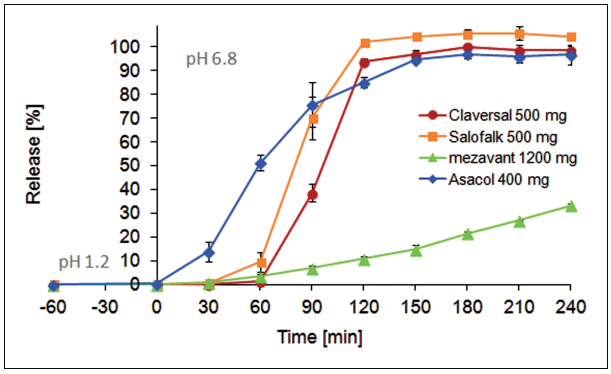

Fig. 2: Mesalazine release in the paddle apparatus applying pharmacopoeial QC conditions for delayed release articles, gastric residence time is plotted left from x-axis, mean of n = 3, ± S.D.

Figure 2 shows the results obtained with the Ph.Eur./USP drug release standard for delayed-release articles (Ph.Eur. 2011; USP 2013).

The rank order of the profiles obtained in this setup was different from that obtained with the USP method for mesalamine delayed-release tablets. Unexpectedly, despite of its Eudragit® S coating which compared to the Eudragit® L coating of the Claversal® and Salofalk® tablets, should dissolve at higher pH, Asacol® showed the fastest onset of drug release. For the mezavant® 1200 mg tablets similar release profiles were obtained in both pharmacopoeial setups. Drug release of this formulation was very slow and incomplete within the test duration.

2.2. Release profiles obtained when simulating average fasted gastrointestinal pH profiles

The next set of experiments was performed applying a compendial pH-gradient method in USP apparatus 3, the Reciprocating Cylinder apparatus (RRT 10 Erweka GmbH, Heusenstamm, Germany) to simulate average fasted gastrointestinal pH-conditions and residence times in healthy subjects (Klein et al. 2005; Klein et al. 2008). These dissolution test methods reflect the changing environment as a dosage form moves through the gastrointestinal tract and originally had been developed to predict the gastrointestinal release profile of different mesalazine formulations. Since most of the tested dosage forms are recommended for administration in the fasted state, the test set-up used in this study was designed to simulate fasted intraluminal conditions. Fasted state residence times in the different regions of the gastrointestinal tract were matched to mean transit times reported in several gamma-scintigraphy and magnetic-

Table 1: Test setup for simulating an average continuous and discontinuous passage through the fasted gastrointestinal tract of healthy subjects

GI segment	Medium	Residence times	
		Discontinuous SI passage	Continuous SI passage
Stomach	SGFsp pH 1.8	60 min	60 min
Proximal jejunum	Blank FaSSIF pH 6.5	15 min	45 min
Distal jejunum	Blank FaSSIF pH 6.8	15 min	45 min
Proximal ileum	Blank FaSSIF pH 7.2	30 min	45 min
Distal ileum	Blank FaSSIF pH 7.5	120 min	45 min
Proximal colon	SCoF pH 5.8	360 min	360 min

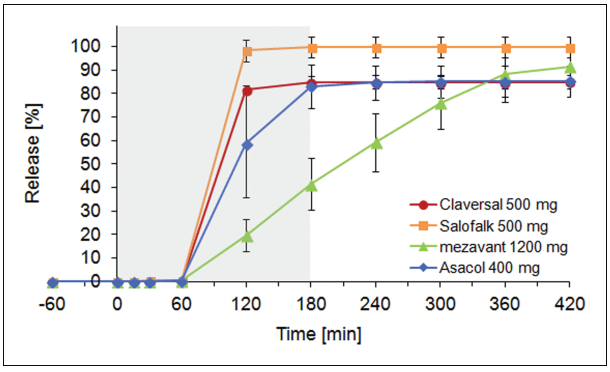

Fig. 3: Mesalazine release in the reciprocating cylinder apparatus simulating a fasted discontinuous SI passage (shaded area), gastric residence time is plotted left from x-axis, residence in jejunum and ileum is separated by a dotted line, mean of n = 3, ± S.D.

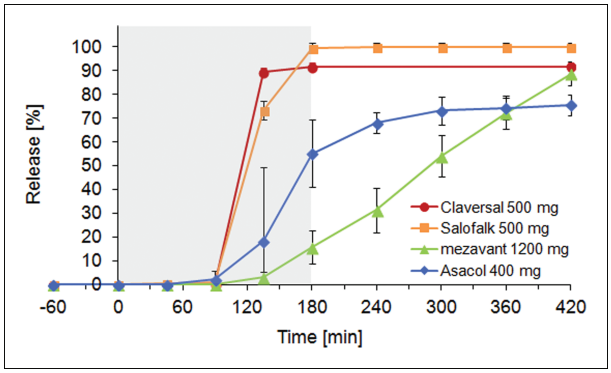

Fig. 4: Mesalazine release in the reciprocating cylinder apparatus simulating a fasted continuous SI passage (shaded area), gastric residence time is plotted left from x-axis, residence in jejunum and ileum is separated by a dotted line, mean of n = 3, ± S.D.

marker-monitoring studies (Davis et al. 1986; Hardy et al. 1993; Weitschies 2001). The pH values used to simulate the different sections in the gastrointestinal tract resulted from various clinical trials examining the pH-profile in healthy subjects and patients with CD or UC, respectively (Fallingborg et al. 1998; Press et al. 1998; Ewe et al. 1999; Nugent et al. 2001). Since passage through the gastrointestinal tract can follow different patterns, both a discontinuous and a continuous passage through the small intestine (SI) were addressed in the experiments (Table 1).

Figures 3–4 show the results obtained in fasted pH-gradients during a discontinuous or continuous simulated gastrointestinal passage, respectively. Compared to the official test methods, these methods should give a better idea of when and to which extent mesalazine might be released during a GI passage in an "average" fasted patient.

Results from both setups indicate that *in vivo* drug release of all formulations is likely to initiate in the ileum. After the initial lag time *in vitro* drug release of the Eudragit® L coated Salofalk® and Claversal® 500 mg tablets was complete before simulating ileocecal passage. In contrast, the release behavior of Asacol® 400 mg and mezavant® 1200 mg was affected by the test design. When comparing results from setups simulating a discontinuous or continuous passage, before simulating entry into the colon both tablet formulations had released a higher amount of drug in the discontinuous setup, i.e. ~ 83 *vs.* 68 % for Asacol® 400 mg and 42 *vs.* 16 % for mezavant® 1200 mg.

2.2.1. Simulation of patient-specific gastrointestinal conditions

In the following set of experiments fasted gastrointestinal pH-conditions and residence times in three different subjects were simulated with the reciprocating cylinder apparatus (Tables 2–4).

The *in vivo* data used for designing the three *in vitro* test scenarios were taken from Ibekwe et al. (2008). The particular data sets were chosen to reflect i) pH-conditions and residence times similar to those used in the experiments simulating pH-profiles in an "average" patient (Klein et al. 2005, 2008) (patient A), ii) an unexpectedly high pH in the proximal small intestine and long residence in the distal small intestine and ileocecal junction (ICJ) (patient B), and iii) no significant pH increase along the small intestine (patient C). The data set utilized to design the *in vitro* scenarios was taken from the study arm where the radiolabelled Bravo® pH capsule was administered after an overnight fast.

The final set of experiments was performed in the biorelevant stress test device (Garbacz et al. 2010), where in addition to the pH-conditions and residence times given in Tables 1-3, pressure that may act on the dosage forms during gastric emptying and ileocecal passage and mechanical stress that may be caused by small intestinal transport events were simulated (Garbacz et al. 2008).

Figures 5–7 show the release profiles obtained with the two different setups addressing the pH conditions, residence times (and mechanical impacts) during the gastrointestinal passage in individual subjects.

Even though in one of the simulated patients pH in the upper small intestine was rather high (pH 7.0, patient B), no drug release could be observed of any of the tested formulation in simulated pH-conditions of the upper small intestine. However, the overall release profiles obtained in the three different patient-specific test scenarios clearly indicate that the site and extent of drug release from the different tablet formulations is strongly dependent on both pH and residence time in the respective sections the gastrointestinal tract. Results obtained with the stress

Table 2: Simulation of a fasted gastrointestinal passage in Patient A

GI segment	In situ pH	Medium	Residence time
Stomach	1.8 ± 0.3	SGFsp pH 1.8	36 min
Proximal SI	6.0 ± 0.4	Blank FaSSIF pH 6.0	70 min
Mid SI	7.0 ± 0.8	Blank FaSSIF pH 7.0	73 min
Distal SI, ICJ	7.2 ± 0.1	Blank FaSSIF pH 7.2	10 min
Proximal colon	7.1 ± 0.3	Blank FaSSIF pH 7.1	240 min

Table 3: Simulation of a fasted gastrointestinal passage in Patient B

GI segment	In situ pH	Medium	Residence time
Stomach	1.3 ± 1.2	SGFsp pH 1.3	38 min
Proximal SI	7.0 ± 0.4	Blank FaSSIF pH 7.0	32 min
Mid SI	6.3 ± 0.5	Blank FaSSIF pH 6.3	32 min
Distal SI, ICJ	7.5 ± 0.2	Blank FaSSIF pH 7.5	199 min
Proximal colon	6.5 ± 0.2	Blank FaSSIF pH 6.5	240 min

Table 4: Simulation of a fasted gastrointestinal passage in Patient C

GI segment	In situ pH	Medium	Residence time
Stomach	1.1 ± 0.2	SGFsp pH 1.1	43 min
Proximal SI	6.2 ± 1.2	Blank FaSSIF pH 6.2	126 min
Mid SI	6.7 ± 1.2	Blank FaSSIF pH 6.7	125 min
Distal SI, ICJ	6.7 ± 0.9	Blank FaSSIF pH 6.7	76 min
Proximal colon	5.8 ± 0.2	SCoF pH 5.8	240 min

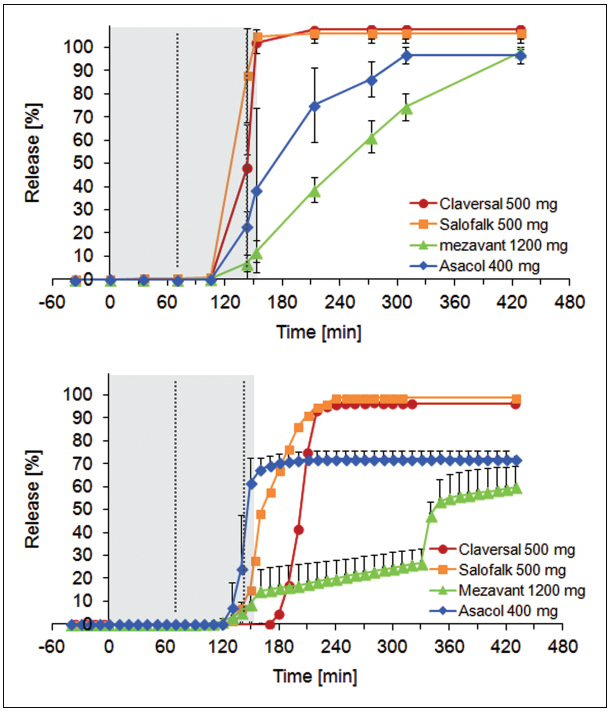

Fig. 5: Mesalazine release (mean of n = 3, ± S.D.) during a simulated GI passage in patient A: upper panel: reciprocating cylinder apparatus, lower panel: stress test apparatus, gastric residence: left from x-axis; SI residence: shaded area, residence in the different SI sections (proximal SI, mid SI, distal SI) is separated by dotted lines.

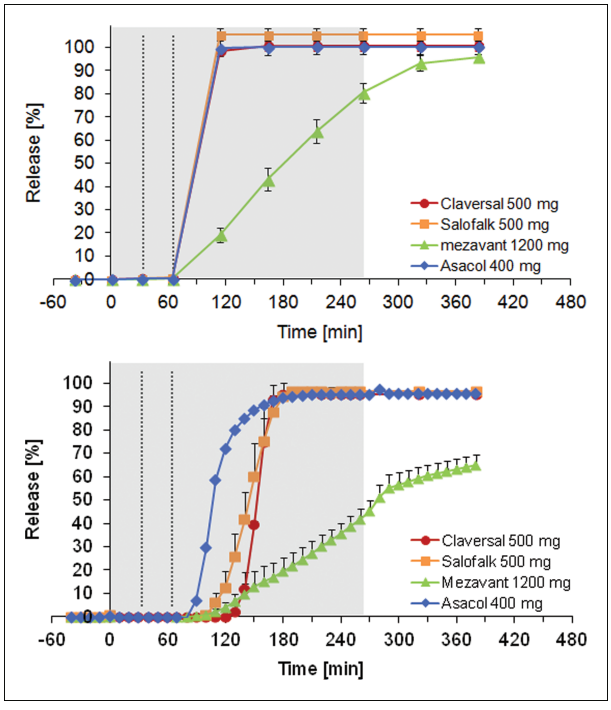

Fig. 6: Mesalazine release (mean of n = 3, ± S.D.) during a simulated GI passage in patient B: upper panel: Reciprocating Cylinder apparatus, lower panel: Stress test apparatus, gastric residence: left from x-axis; SI residence: shaded area, residence in the different SI (proximal SI, mid SI, distal SI) sections is separated by dotted lines.

test apparatus underline the observations made in the experiments with the reciprocating cylinder apparatus and in addition illustrate that drug release of the tested formulations can also be affected by mechanical stress.

3. Discussion

According to the "Concept paper on the development of a guideline on the demonstration of therapeutic equivalence for locally applied and locally acting products in the gastrointestinal tract"

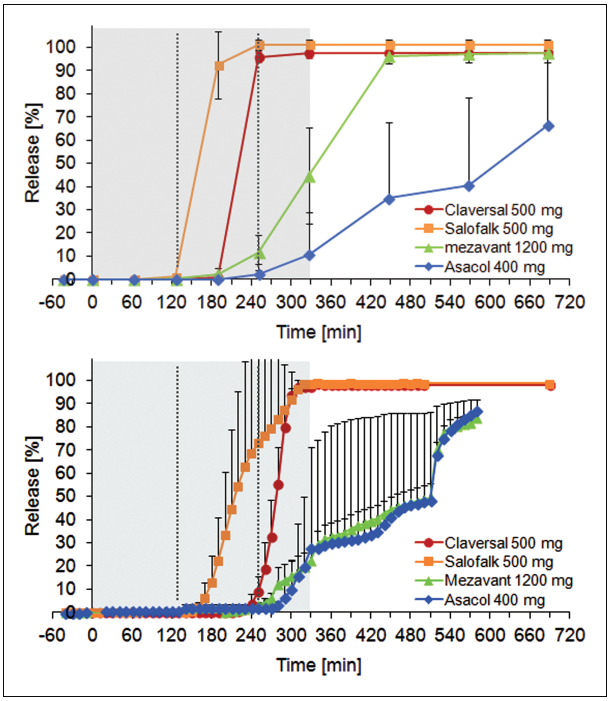

Fig. 7: Mesalazine release (mean of n = 3, ± S.D.) during a simulated GI passage in patient C: upper panel: reciprocating cylinder apparatus, lower panel: Stress test apparatus, gastric residence: left from x-axis; SI residence: shaded area, residence in the different SI sections (proximal SI, mid SI, distal SI) is separated by dotted lines.

in vitro models could be considered surrogates of drug release and availability at the site of action. This raised the question, if it would be possible to define an adequate *in vitro* setup that enables an exact prediction of both the site and extent of drug release in the lumen of the gastrointestinal tract. For the pharmaceutical industry, particularly for companies developing generic formulations such alternative approaches would indeed be of great benefit since the acceptance of *in vitro* models for indicating therapeutic equivalence of locally acting drug products would help to save time and costs. However, the question is now how to define appropriate test conditions for this purpose. Oral mesalazine formulations represent a relevant case example to discuss this question since i) there is a variety of mesalazine formulations available on the market, ii) most of these formulations are enteric coated, i.e. are characterized by pH-dependent drug release, iii) mesalazine itself has a pH-dependent solubility and iv) the drug is used in the treatment of both CD and UC, two IBDs that are characterized by different patterns of inflammation and thus require the administration of dosage forms with particular release patterns. Since an effective anti-inflammatory therapy of the two diseases relies on effective luminal drug concentrations at the site(s) of inflammation, it is crucial to select the right dosage form for each individual patient. To be reliable and predictive of both the efficacy and the safety of the locally acting drug product, an *in vitro* method must thus address all parameters that can affect the *in vivo* drug release of the product. The present set of experiments was performed to screen the applicability of different *in vitro* drug release methods, including pharmacopoeial methods (Ph.Eur. 2011; USP 2013), pH-gradient methods that simulate gastrointestinal pH-conditions in "average" fasted subjects (Klein et al. 2005, 2008) and, patient-specific models simulating intraluminal fasted pH-conditions and passage times (and gastrointestinal stress events) in three fasted individuals.

Results from the experiments performed with the pharmacopoeial test methods (Figs. 1–2) indicated that these methods might hardly be applicable for predicting therapeutic effectiveness of a particular product. These methods represent two/three stage models representing a simplistic snapshot of a dosage forms gastric and small intestinal residence. The release profiles obtained when studying drug release of the four enteric-coated mesalazine tablets with the two pharmacopoeial setups were characterized by different lag times before initial drug release and also the order of the profiles changed with the test design. Since the cited test methods do neither address typical residence times in the different gastrointestinal segments nor the detailed pH changes during small intestinal passage, results obtained with these methods might be useful for QC purposes but do not allow for estimating site and extent of *in vivo* drug release of the formulations tested.

Since the *in vitro* methodology was already proven to be predictive for *in vivo* drug release of a new site-specific delivery system intended to be applied in UC therapy (Klein et al. 2008), results obtained from the simulation of average fasted gastrointestinal pH-conditions and transit times in the reciprocating cylinder apparatus were supposed to give a much better estimate of timing, site and extent of *in vivo* drug release. Independent of simulating a continuous or a discontinuous passage through the small intestine, the onset of drug release from all tested formulations could be observed under simulated ileal conditions (Figs. 3–4). However, whereas drug release of the two Eudragit® L coated formulations Salofalk® and Claversal® 500 mg was similar and complete within the simulated small intestinal passage, significant differences could be observed in the amount of mesalazine released from Asacol® 400 mg and mezavant® 1200 mg when comparing profiles obtained in the continuous and the discontinuous setup. Since the gastrointestinal passage of an orally administered dosage form is a process that is subject to high intra- and interindividual variability, these observations already reveal that there will be no one-fits-all *in vitro* test method being predictive for the *in vivo* efficacy or the therapeutic equivalence of (a) locally acting gastrointestinal drug product(s).

The development of test setups simulating gastrointestinal pH-conditions in an "average" patient had been an important step towards the prediction of *in vivo* drug release of oral modified-release dosage forms. However, in recent years, it became obvious that site and extent of *in vivo* drug release from oral modified-release dosage forms is not only a result of pH and residence times but can also be affected by other physiological parameters, like for instance the composition and ionic strength of the gastrointestinal fluids (Fadda et al. 2009) or the physical stress acting on the dosage form during gastrointestinal passage (Garbacz et al. 2010). The latter aspect is particularly true for non-disintegrating monolithic dosage forms (Garbacz and Klein 2012), such as enteric-coated mesalazine tablets. Another point to consider is that an *in vitro* setup simulating average gastrointestinal pH-conditions and transit times might not adequately address intra- and inter-subject variability of the parameters relevant to drug release in the gastrointestinal tract.

For these reasons, a final series of experiments was performed applying a novel test design, in which *in vitro* test settings were adapted to individual gastrointestinal pH-values and residence times reported in the literature (Ibekwe et al. 2008). In the first set of experiments so-called patient-specific *in vitro* models reflecting intraluminal fasted pH-conditions and passage times in three fasted individuals were established to study the robustness of drug release of the four enteric-coated mesalazine tablet formulations towards variations in gastrointestinal pH and residence times. In the second set of experiments individual pH-conditions and residence times were combined with simulated gastrointestinal stress events as they can occur during gastric emptying, passage through the small intestine and passage through the ileocecal junction.

However, at this point, it should be noted that even though focussing on the establishment of advanced individualized methods, in the present study, it was necessary to make some compromises in the test design. As we had no access to detailed data sets of CD and UC patients, the individual pH-profiles and residence times simulated in the "patient-specific experiments" were from healthy subjects. The media used to simulate intraluminal conditions were aqueous buffer systems with no bile salts and phospholipids added (blank biorelevant media). Moreover, for each individual subject and each gastrointestinal segment we had to work with a fixed combination of pH and residence time. This scenario does not reflect every detail of the fluctuating intraluminal conditions when a dosage form passes along the gastrointestinal tract. Nevertheless, results obtained in this last part of our study clearly indicate the importance of adequately addressing the individual patient when selecting the optimal dosage form and also when screening therapeutic equivalence of locally applied and locally acting gastrointestinal products.

As expected, in all simulated individuals, the four tablet formulations showed enteric properties. The release profiles obtained when simulating gastrointestinal pH conditions and residence times in patient A in the reciprocating cylinder apparatus were similar to those obtained with the "average fasted patient" setup. When using the same apparatus to simulate the gastrointestinal passage in patient B, even though the formulations bear different polymer coatings, a burst-like drug release with superimposable release profiles could be observed for Salofalk® 500 mg, Claversal® 500 mg and Asacol® 400 mg tablets when the pH was changed to ileal conditions in that patient (pH 7.5). This observation was clearly a result of the media pH which for the polymeric coating materials of all three formulations was quite higher than the minimum pH required for polymer dissolution. The time before the onset of drug release from the mezavant® 1200 mg tablets which are also coated with a gastro-resistant pH-dependent polymer film that is intended to break down at or above pH 7 (Eudragit® L/S mixture) was also a result of the intraluminal pH-conditions. However, since the tablet core represents a prolonged-release tablet where mesalazine is embedded into a matrix of hydrophilic and lipophilic excipients, a burst release was not seen. When simulating a gastrointestinal passage in patient C in which intraluminal pH did never increase pH 7, essentially different release profiles were obtained for the different tablet formulations. According to the *in vivo* results, in this individual subject Salofalk® and Claversal® 500 mg tablets would completely dissolve in the mid small intestine, whereas Asacol® 400 mg would hardly release any drug in the small intestine and subsequent drug release in the proximal colon would also be poor and incomplete. Even though at a first sight, the release profiles of the mezavant® tablets looked very similar in the three simulated patients, with a closer look it became obvious that the amount of drug released before the simulated ileocecal passage ranged from ~15 % in patient A through ~ 45 % in patient C to ~80 % in patient B.

It can be summarized that the results obtained in these first experiments with individualized setups, tell their own tale and point out the importance of addressing different patient scenarios. Since neither the pH-conditions in the gastrointestinal tract nor the residence time of a dosage form in the different gastrointestinal sections follow a fixed scheme, the results again confirm that release data obtained with a simple test setup can be totally misleading when the aim is to predict the ability of a dosage form to release the active at a particular site and to a particular extent. The results obtained in the stress test experiments, where in addition to pH and residence times, also typical gastrointestinal stress was addressed, also confirm this statement since in this setup, even though the same media set and residence times were applied, the release behavior of the test formulations was again different. The longer lag times before initial drug release that were observed in this setup were most likely a result of the hydrodynamic conditions. Whereas in the reciprocating cylinder setup the cylinder holding the dosage form is continuously agitated with 10 dpm, in the stress test device there are long phases where the mesh screen holding the dosage form is simply immersed in the release medium which itself is agitated by a small stirring device. In these phases the shear forces acting on the dosage form are likely to be much lower than during continuous reciprocation in the reciprocating cylinder apparatus resulting in slower dissolution. After the initial lag time, still a relatively fast drug release could be observed for Salofalk® and Claversal® 500 mg tablets. However, according to the test results, in patient A, this would no longer take place in the ileum, but in the proximal colon. In the same simulated patient, drug release from Asacol® 400 mg tablets was incomplete and it became also obvious that pressure acting on the dosage form during simulated pyloric and ileocecal passage had a significant impact on the release behavior of the mezavant® 1200 mg tablet formulation. The same observation was made in the stress test scenario simulating patient B. In contrast, stress test experiments simulating conditions in patient C resulted in highly variable release profiles for the mezavant® formulation. Overall mechanical pressure seemed to result in compaction of the tablet core matrix which came along with lower drug release rates.

In summary, results from our first in *vitro* approaches in simulating gastrointestinal passage in individual subjects were very promising in terms of getting an idea of where and to which amount the drug mesalazine might be released *in vivo*. The results obtained with our individualized test methods indicate that drug release of mesalazine dosage forms will be highly affected by both, formulation design and a whole range of physiological parameters. Therefore, in future experiments, the "patient-specific" *in vitro* methods will be further fine-tuned to even better simulate media composition and residence times in the lumen of healthy subjects, CD and UC patients. Based on the observations made in the present study, mesalazine formulations that might show similar dissolution profiles in a certain *in vitro* setup will not necessarily do the same after oral administration to a certain patient. The same is likely to be true for the entire range of enteric coated products intended to show a local action in the gastrointestinal tract. Thus, when the aim is to use results from *in vitro* models as a surrogate for the *in vivo* performance of such dosage forms, it is important to address gastrointestinal conditions in a whole range of patients in the *in vitro* model.

Results obtained in the present study clearly indicate that simple QC methods are not applicable to predict the therapeutic equivalence of locally applied and locally acting products in the GI tract. It is overall unlikely to determine a "one-fits-all" *in vitro* method for this purpose. However, this does not necessarily mean that *in vitro* models cannot provide valid surrogates of *in vivo* drug release and availability at the site of action. With a set of physiologically-based dissolution models taking into account the particular features in gastrointestinal physiology and typical dosing scenarios in the target patient group, one should be able to estimate the *in vivo* performance of different formulations, to discriminate between formulations and finally to contribute to a safe and effective drug therapy for the individual patient.

4. Experimental

4.1. Materials and products studied

Mesalazine standard substance, batch # 107K1265 was purchased from Sigma Aldrich, Steinheim, Germany. The products studied were: Claversal® 500 mg tablets (batch # 184990, Recordati GmbH, Ulm, Germany),

Salofalk® 500 mg tablets (batch # 11K18919L, Dr. Falk Pharma GmbH, Freiburg, Germany), Asacol® 400 mg tablets (batch # 08B75A01, Meduna Arzneimittel GmbH, Aschaffenburg, Germany) and mezavant® 1200 mg gastro-resistant prolonged release tablets (batch # NX133, Shire, Berlin, Germany). Glacial acetic acid, potassium dihydrogen phosphate, potassium hydrogen phosphate, sodium chloride, sodium dihydrogen phosphate, sodium hydrogen phosphate, sodium phosphate, sodium hydroxide and hydrochloric acid were all of analytical grade and purchased commercially.

4.2. Drug release experiments and sample analysis

All *in vitro* drug release experiments were run in triplicate and results expressed as mean % (± SD) dissolved at the given sampling time. The following setups were applied:

4.2.1. Pharmacopoeial test methods

The initial set of experiments was performed using the USP method for mesalamine (mesalazine) delayed-release tablets (USP 2013), i.e. the paddle apparatus, starting with 500 mL 0.1 N HCl and a paddle agitation of 100 rotations per minute (rpm). After 2 h, the acid was replaced by 900 mL phosphate buffer pH 6.0. After another hour, the pH of the medium was adjusted to pH 7.2 by adding 50 mL of a 1.67 N NaOH solution and the experiment was continued for 3 h at a paddle speed of 50 rpm. Another set of experiments was performed using the Ph.Eur./USP drug release standard for delayed-release articles, method A (Ph.Eur. 2011; USP 2013), i.e. the Paddle apparatus at 50 rpm. The experiment was started in 750 mL 0.1 N HCl. After 1 h, 250 mL of a 0.2 N Na_3PO_4 solution was added to obtain a pH of 6.8. The test was then continued for 5 h In all experiments samples (5 mL) were periodically removed using a glass syringe and immediately filtered through a 0.45 μm cellulose acetate filter (Puradisc® FP 030/0.45 CA, Whatman GmbH, GE Healthcare & Sciences, Dassel, Germany).

4.2.2. Simulation of average fasted gastrointestinal pH profiles

Experiments were performed using a compendial pH gradient in USP apparatus 3 (Reciprocating Cylinder apparatus, RRT 10, Erweka GmbH, Heusenstamm, Germany) (Klein et al. 2005, 2008). Each vessel was filled with 200 mL of media at 37 ± 0.5 °C. Mesh sizes of 420 μm were used for both the top and bottom mesh of the glass cylinders holding the dosage forms throughout the test. For all experiments, a dip rate of 10 dpm was used. Samples (5 mL) were periodically removed with a glass syringe and immediately filtered through a 0.45 μm cellulose acetate filter (Puradisc® FP 030/0.45 CA, Whatman GmbH, GE Healthcare & Sciences, Dassel, Germany).

4.2.3. Simulation of patient-specific gastrointestinal conditions

Experiments with the reciprocating cylinder apparatus were performed with the same apperative settings as in the previous experiments. Stress test experiments were performed with the biorelevant stress test device developed by Garbacz (Garbacz et al. 2010) (Physiolution GmbH, Greifswald, Germany & Erweka GmbH, Heusenstamm, Germany). Maximum pressures as they can occur during gastric emptying and ileocecal passage were simulated by 3 consecutive pressure cycles of 300 mbar, each of them acting on the dosage form for 6 s. To simulate transport from the stomach into the small intestine or from the small intestine into the colon, respectively, both pressure phases were followed by 1 min of intensive dosage form movement in the test medium, i.e. by a fast rotation (100 rpm) of the mesh sphere holding the dosage form. Transport events in the small intestine were simulated by mesh sphere rotations at 10 rpm for 1 min taking place in 10 minute intervals.

4.2.4. Sample analysis

Following appropriate dilution all samples were analyzed at 231 nm (pH ≤ 1.8) or 331 nm (pH ≥ 5.8) with a UV spectrophotometer equipped with a 10 mm quartz glass cuvette (U 2000, Hitachi, Tokyo, Japan).

Acknowledgements: This work was funded by the German Ministry of Economics and Technology (AZ V-630-F-157-2012/230). The author would like to thank Elisabeth Kersten for her support in performing the dissolution experiments and Physiolution GmbH, Walther-Rathenau-Strasse 49a, 17489 Greifswald, Germany for the provision of the stress test device for the duration of the experiments.

References

Cassilly D, Kantor S, Knight LC, Maurer AH, Fisher RS, Semler J, Parkman HP (2008) Gastric emptying of a non-digestible solid: assessment with simultaneous SmartPill pH and pressure capsule, antroduodenal manometry, gastric emptying scintigraphy. Neurogastroenterol Motil 20: 311–319.

Davis SS, Hardy JG, Fara JW (1986) Transit of pharmaceutical dosage forms through the small intestine. Gut 27: 886–892.

EMA (2013) Concept paper on the development of a guideline on the demonstration of therapeutic equivalence for locally applied and locally acting products in the gastrointestinal tract. C. f. M. P. f. H. u. (CHMP). London UK, European Medicines Agency. EMA/CHMP/558326/2013.

EMEA (1995) Note for Guidance on the Clinical Requirements for Locally Applied, Locally Acting Products Containing Known Constituents C. f. M. P. f. H. u. (CHMP). London UK, European Medicines Agency. CPMP/EWP/239/95.

Ewe K, Schwartz S, Petersen S, Press AG (1999) Inflammation does not decrease intraluminal pH in chronic inflammatory bowel disease. Dig Dis Sci 44: 1434–1439.

Fadda, HM, Merchant, HA, Arafat, BT, Basit, AW (2009) Physiological bicarbonate buffers: stabilisation and use as dissolution media for modified release systems. Int J Pharm 382: 56–60.

Fallingborg J, Pedersen P, Jacobsen BA (1998) Small intestinal transit time and intraluminal pH in ileocecal resected patients with Crohn's disease. Dig Dis Sci 43: 702–705.

Garbacz G, Klein S (2012) Dissolution testing of oral modified-release dosage forms. J Pharm Pharmacol 64: 944–968.

Garbacz G, Klein S, Weitschies W (2010) A biorelevant dissolution stress test device - background and experiences. Expert Opin Drug Deliv 7: 1251–1261.

Garbacz G, Wedemeyer RS, Nagel S, Giessmann T, Monnikes H, Wilson CG, Siegmund W, Weitschies W (2008) Irregular absorption profiles observed from diclofenac extended release tablets can be predicted using a dissolution test apparatus that mimics *in vivo* physical stresses. Eur J Pharm Biopharm 70: 421–428.

Goodman K, Hodges LA, Band J, Stevens HNE, Weitschies W, Wilson, CG (2010) Assessing gastrointestinal motility and disintegration profiles of magnetic tablets by a novel magnetic imaging device and gamma scintigraphy. Eur J Pharm Biopharm 74: 84–92.

Hardy JG, Harvey WJ, Sparrow RA, Marshall GB, Steed KP, Macarios M, Wilding IR (1993) Localization of drug release sites from an oral sustained-release formulation of 5-ASA (Pentasa) in the gastrointestinal tract using gamma scintigraphy. J Clin Pharmacol 33: 712–718.

Ibekwe VC, Fadda HM, McConnell EL, Khela MK, Evans DF, Basit AW (2008) Interplay between intestinal pH, transit time and feed status on the *in vivo* performance of pH responsive ileo-colonic release systems. Pharm Res 25: 1828–1835.

Kamba M, Seta Y, Kusai A, Ikeda M, Nishimura K (2000) A unique dosage form to evaluate the mechanical destructive force in the gastrointestinal tract. Int J Pharm 208: 61–70.

Klein S, Rudolph MW, Skalsky B, Petereit HU, Dressman JB (2008) Use of the BioDis to generate a physiologically relevant IVIVC. J Control Release 130: 216–219.

Klein S, Stein J, Dressman J (2005) Site-specific delivery of anti-inflammatory drugs in the gastrointestinal tract: an *in-vitro* release model. J Pharm Pharmacol 57: 709–719.

Nugent SG, Kumar D, Rampton DS, Evans DF (2001) Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs. Gut 48: 571–577.

Ochsenkuhn T, Sackmann M, Goke B (2003) [Inflammatory bowel diseases (IBD) – critical discussion of etiology, pathogenesis, diagnostics, and therapy]. Radiologe 43: 1–8.

Ph.Eur. (2011). European Pharmacopoeia 7th Edition, Council of Europe.

Press AG, Hauptmann IA, Hauptmann L, Fuchs B, Fuchs M, Ewe K, Ramadori G (1998) Gastrointestinal pH profiles in patients with inflammatory bowel disease. Aliment Pharmacol Ther 12: 673–678.

Sandborn WJ, Hanauer SB (2003) Systematic review: the pharmacokinetic profiles of oral mesalazine formulations and mesalazine pro-drugs used in the management of ulcerative colitis. Aliment Pharmacol Ther 17: 29–42.

Shameem M, Katori N, Aoyagi N, Kojima S (1995) Oral solid controlled release dosage forms: role of GI-mechanical destructive forces

and colonic release in drug absorption under fasted and fed conditions in humans. Pharm Res 12: 1049–1054.
USP (2013). USP 36 /NF 31. Rockville MD, United States Pharmacopoeia Convention, Inc.
Verzijl JM, Bodegraven AA (2003) Mesalazine - still a first-line therapy. Eur Pharmacother B. Briefing: 1–7.
Weitschies W (2001) Eine Reise durch den Verdauungstrakt. Pharm Ztg 146: 1108–1114.