HEINRICH HEINE
UNIVERSITÄT DÜSSELDORF

Paediatric drug development for praziquantel

Inaugural-Dissertation

zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Magdalena Münster
aus Rostock

Düsseldorf,
Februar 2018

aus dem Institut für Pharmazeutische Technologie und Biopharmazie
der Heinrich-Heine-Universität Düsseldorf

Gedruckt mit der Genehmigung der
Mathematisch-Naturwissenschaftlichen Fakultät der
Heinrich-Heine-Universität Düsseldorf

Referent: Prof. Dr. Jörg Breitkreutz

Korreferent: Prof. Dr. Dr. h.c. Peter Kleinebudde

Tag der mündlichen Prüfung: 01.02.2018

“The distance is nothing when one has a motive.”

Jane Austen

For my loved ones

Contents

Abbreviations

API	Active pharmaceutical ingredient
BATA	Brief-access taste aversion
BCS	Biopharmaceutical classification system
CD	Cyclodextrins
CE	Complexation efficiency
DSC	Differential scanning calorimetry
EMA	European Medicines Agency
EU	European Union
EuPFI	European Paediatric Formulation Initiative
EX	Extrudate
FaSSIF	Fasted state simulated intestinal fluid
FDA	Food and Drug Administration
ffc	Flow function coefficient
GMS	Glyceryl monostearate
HME	Hot-melt extrusion
HP-β-CD	Hydroxypropyl-beta-cyclodextrin
MD	Maltodextrin
MDT	Mean dissolution time
MT	Mini-tablet
ODF	Orally disintegrating / orodispersible dosage form
ODMT	Orally disintegrating/ orodispersible mini-tablet
ODT	Orally disintegrating/ orodispersible tablet
PDCO	Paediatric Committee
Ph. Eur.	European Pharmacopoeia
PIP	Paediatric Investigation Plan
PM	Physical mixture
PZQ	Praziquantel
r. h.	Relative humidity
RSD	Relative standard deviation
SBE-β-CD	Sulfobutylether-beta-cyclodextrin
SD	Spray-dried powders
SEM	Scanning electron microscopy
S.D.	Standard deviation
SLE	Solid lipid extrusion
spSGF	Simulated gastric fluid sine pepsin
SSF	Simulated salivary fluid
STEP	Safety and Toxicity of Excipients for Paediatrics
T_g	Glass transition temperature
US	United States
WHO	World Health Organization
XRPD	X-ray powder diffraction

1. Introduction

1.1. The need for improving paediatric medicines

Recent decades demonstrated a sustainable improvement of pharmaceutical formulation development, such as strategies for processing and formulating poorly water soluble drugs, drug release control, carrier systems and medical devices. Nevertheless, the availability of appropriate, effective and safe oral drug dosage forms for challenging patient populations like children remains a major gap in medical care. Inappropriate medications, mainly due to the unacceptable taste of liquid formulations or swallowing issues when administering solids, have a strong negative impact on the compliance of children and cause difficulties for parents in feeding medications to their children [1, 2]. Thus, the compliance rate varies greatly from 11 to 93% [3].

The unmet treatment needs of the paediatric population are reflected by the alarming extent of unauthorized and off-label use of medicines in children. The unauthorized application includes e.g. manipulation of the dosage form for an easier administration (e.g. segmenting tablets, cutting transdermal patches). Off-label is defined as the use of a medicine outside the specifications of the marketing authorization and the summary of the product characteristics, such as the use for another indication or a deviating dose and age group [4, 5]. In 2010 the European Medicines Agency (EMA) published the “report on the survey of all paediatric uses of medicinal products in Europe” collecting data on all applied medicines in paediatrics [6]. The report revealed a widespread off-label and unauthorized use of 45 to 60% off all prescriptions in children in the European Union (EU). Even higher rates up to 90% were obtained in preterm and term neonates. The main affected medicine areas were the intensive care unit, gastroenterology (reflux), cardiovascular (hypertension) and respiratory (asthma) medications.

To improve the unacceptable situation of the medicinal treatment of children, the Paediatric Regulation (EU 1901/2006 of the European Parliament and of the Council on medicinal products for paediatric use) entered into force in 2007. Since then, the EU enforces a Paediatric Investigation Plan (PIP) from companies for a marketing authorization of any new medical product and line extensions for products authorized in an EU member state. This PIP includes development of a formulation suitable for paediatric application of the medicinal product such as the clear target indication, targeted age groups, age-appropriate formulations (dosage form, route of administration, excipients, preservatives, coloring, flavoring agents) and clinical studies (e.g. in juvenile animals). The PIP has to be sent to the EMA for approval as soon as the adult pharmacokinetic studies are finalized and is assessed by the Paediatric Committee (PDCO). Waivers are possible for products that are not applicable for children based on the safety of the product, the indication or the therapeutic benefit over existing therapies. Deferrals are approved if additional information of clinical studies is awaited. Moreover, as the PIP is required at an early stage of development, modifications of the proposal can be submitted during further development. A completed PIP approval is rewarded with a 6 months extension of the patent protection, whereas a non-agreed PIP leads to a blocked marketing authorization of the medicinal product [7]. This was adapted on

the formerly introduced US Food and Drug Administration Modernization Act of 1997 concerning regulations of paediatric medicines and paediatric labeling in the US [8]. The Paediatric Regulation aims to increase the quality of medicinal products for the paediatric population and to ensure high quality research, safety, efficacy and authorization of such medicines [7].
In view of improving paediatric medications, the European Paediatric Formulation Initiative (EuPFI) was founded in 2007. This collaboration of academia, industry and clinical pharmacy focusses on age appropriate formulations and delivery devices for children, excipients, taste masking strategies and assessment and biopharmaceutics of children [9]. As one objective, the Database of Safety and Toxicity of Excipients for Paediatrics (STEP database) was created. It addresses the need for shared information on pharmacology, toxicology and safety of excipients in children [10]. The annually conferences of the EuPFI offer the opportunity to discuss and present improvements of better medicines for children as well as regulatory aspects for PIPs [9].

Whereas tablets and capsules are the commonly preferred oral dosage form for adults, oral liquids are traditionally the first choice for children as the child's ability to swallow tablets and capsules has been insufficiently reported [11]. Caregivers or parents tend to crush tablets or to open capsules and sprinkle the content onto food before administration to children [4]. Oral liquid dosage forms are well accepted for children, parents and health professionals. Advantages of liquid formulations are the ease of ingestion or administration as well as a high dose flexibility. However, challenging are stability issues (chemical, physical and microbiological), especially in developing countries with climate zones III to IVb (hot zones) and the subsequent use of preservatives. Further disadvantages are the limited taste masking opportunities accompanied with low dose accuracy, if the child refuses the medicine, and the lack of controlled release properties. Thus, in the recent years, there is an increasing focus on solid dosage forms providing a higher flexibility and easier swallowing than usual tablets [11]. Such flexible oral dosage forms are multiparticulates like pellets and mini-tablets and orally disintegrating drug formulations including oral lyophilisates, orodispersible tablets, orodispersible granules and orodispersible films [12].
In 2006 the EMA released the reflection paper "Formulations of Choice for the Paediatric Population" collecting the available data on paediatric drug delivery. The document promotes the development of paediatric formulations meaning authorized, safe and effective dosage forms for all age groups. The term children was thereby separated into five age groups: preterm newborn infants, term newborn infants (0 – 27 days), infants and toddlers (1 – 23 months), children (2 – 11 years) and adolescents (12 – 16 or 18 years). In terms of the child's ability to swallow solid oral dosage forms, it was useful to divide children in preschool (2 – 5 years) and school age (6 – 11 years) children. Within the paper, the appropriateness of various available dosage forms was classified for each age group. It claimed that for children up to 2 years liquids are preferred over solid dosage forms accompanied with the difficulties regarding swallowing tablets and capsules. Powders, multiparticulates and orodispersibles were ranked in between. Orodispersible dosage forms were included as a promising dosage form for use in younger children as they can be administered without water. Powders or multiparticulate formulations (e.g. granules, mini-tablets)

were proposed in terms of taste masking. Due to the fast changing pharmacology of neonates, infants and children, it is most likely that no single formulation would be applicable for all age groups [13].

Further documents such as the “Guideline on Pharmaceutical Development of Medicines for Paediatric Use” released by the EMA in 2014 [14] and the World Health Organization (WHO) “Report of the informal expert meeting on dosage forms of medicines for children” of 2008 [15] and more related releases and campaigns of the WHO such as the “Make Medicines Child Size” campaign [16] encouraged and guided paediatric drug development [17]. For the consideration and choice of safe and appropriate excipients, the EMA Guideline of 2014 [14] listed available information sources in hierarchy to support the pharmaceutical development. All released documents of the EMA and the WHO proposed a rethinking of liquids over solids as the first choice of medicines for paediatrics. Powders, multiparticulates and orodispersible dosage forms were discussed to be appropriate from an age of 2 or 6 years. The main concern for the use of solids in children below 2 years is the risk of inhalation of solid particles and aspiration during administration [18]. The lack of valid scientific data on the acceptability and suitability of solid dosage forms in children from birth to adolescence led to an enhanced focus on this research area.

1.2. Mini-tablets and orodispersible dosage forms for paediatric use

As mentioned before, liquid dosage forms offer an ease of ingestion, but involve several risks such as stability, palatability in terms of taste masking with subsequent risky dose accuracy and dispensing errors [19]. A ready-to-use solid oral dosage form offers a high dosing accuracy and is most convenient for packaging, transport and administration. However, in case of swallowing difficulties, especially for paediatrics and geriatrics, traditional solid dosage forms need to be improved. Orally disintegrating / orodispersible dosage forms (ODFs) and mini-tablets (MTs) address that need and are suitable for a broad range of patients including adults and children.

ODFs (tablets, granules, films or oral lyophilisates) are stable in the solid state in the packaging material, but disintegrate in the mouth within a few seconds forming drug solutions or suspensions. The European Pharmacopoeia (Ph. Eur.) defines orodispersible tablets (ODTs) as uncoated tablets with a maximum *in vitro* disintegration time in water of 3 min. The Food and Drug Administration (FDA) suggests a faster disintegration within 30 s [20]. Manufacturing techniques for the production of ODTs include direct compression or granulation with the incorporation of superdisintegrants or effervescent powders and others [12]. The rapid disintegration is accompanied with an easier administration and use without additional water. This results in an improved adherence especially for patients with dysphagia (swallowing dysfunction) [1]. The children’s ability and willingness to take orodispersible formulations among other solid oral dosage forms was recently demonstrated and surveyed by Ranmal *et al.* [21]. The effect of particle sizes and granule characteristics on the palatability and mouthfeel of ODTs was evaluated by Kimura *et al.* in adult healthy volunteers [22]. They determined a negligible mouthfeel of granules out of ODTs for particle sizes smaller than 200 µm. In addition, they demonstrated an improvement of the overall palatability of the ODTs using mannitol in the formulation. The advantageous characteristics of mannitol for a rapid disintegration,

a sweet taste and pleasant mouthfeel of orodispersible formulations was confirmed by various studies [12]. Accompanied with the fast disintegration of ODFs is the high importance of taste masking of unpleasant active pharmaceutical ingredients (APIs) due to the subsequent rapid contact with the taste buds [23]. A successful embedding of the API for taste masking in such dosage dorms can be achieved using e.g. hot melt extrusion, spray-drying or fluid bed coating and is explained later in detail.

MTs are defined as small tablets with a diameter of 1 to 3 mm [24]. They can be produced using conventional tablet presses and offer all advantages of solid dosage forms. In addition, they provide a very high flexibility and accuracy regarding dosing [25], especially for low dose drugs [26]. In terms of safety issues and therapeutic failure, dose accuracy still remains a problem, especially for children and elderly people. MTs provide a low risk of dose dumping, an easy administration due to an improved swallowability and a low risk of gastrointestinal tract irritation compared to large and slowly dissolving tablets [25-27]. Different applications for MTs have been demonstrated including sustained [28, 29] or biphasic drug release [30, 31]. The feasibility of formulating 1 mm and 2 mm MTs via direct compression and roller compaction was proven by Tissen *et al.* [32]. Mitra *et al.* [19] currently demonstrated the manufacturing quality of 3 mm MTs and applicability as single or multiple unit dosage forms. Commercially available products with MTs are e.g. Zalviso®, Orfiril Long®, Levetiracetam Desitin® or Lamisil®.

Orally disintegrating mini-tablets (ODMTs) are even more suitable for children and adults with swallowing issues [33]. They combine the advantages of solid dosage forms over liquids and further increase the compliance of patients as the administration is possible without additional water. Moreover, they improve the safety of MTs for small children as the risk of particle inhalation is decreased [12]. The combination of MTs and ODTs was firstly evaluated by Stoltenberg and Breitkreutz [33]. They successfully prepared drug-free and hydrochlorothiazide ODMTs of 2 mm with different ready-to-use tableting excipients via direct compression.

In view of regulatory aspects, MTs and ODMTs are considered as usual tablets or granules and do not require additional quality attributes. However, there is a lack of appropriate analytical methods adapted to the small tablet size from 1 to 3 mm and subsequent decreased drug content. As an example, alternative or adapted disintegration tests were described in literature [19, 33].

The acceptance of MTs was proven in adults, children from 6 months to 6 years and in infants in comparison to other liquid and solid dosage forms [18, 34-41], as well as in cats [42]. Klingmann *et al.* [18, 35] and Spomer *et al.* [34] explored the applicability of uncoated drug-free 2 mm tablets in children from 6 months to 6 years and newborns from 2 to 28 days in comparison to a sweet tasting syrup. MTs were a valuable alternative to syrup and were more or equally accepted than the liquid formulation. This is an emerging success as it was reported so far that children until 4 or 5 months of age possess an extrusion reflex enabling them to swallow only liquids as a preventive mechanism against solid objects entering the oral cavity [43]. The suitability of 3 mm tablets was shown for age groups from 2 to 6 years for the administration of one single tablet [36] and in units from 5 or 10 for children of 2 and 3 years [37]. Tablets of 4 mm confirmed the equivalent acceptability of small tablets compared to powder, suspension or syrup for children from 1 to 4 years [38, 39]. In

conclusion, these studies demonstrated an overall positive response of children to MTs and demonstrated the usability of MTs as an appropriate solid oral dosage form. The use of MTs instead of liquid dosage forms for children of all groups would be an improvement in drug delivery in terms of simplicity and flexibility of administration, stability, controlled or sustained release and especially for taste masking.

1.3. Praziquantel as model compound

An example for a pharmaceutical compound administered in adults and children is praziquantel (PZQ, 2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one). According to the WHO it belongs to the "Model List of Essential medicines" (20th edition, March 2017). As a safe and effective anthelminthic compound it is the recommended API in the control and treatment of schistosomiasis in humans and widely applied in the veterinary area against worm infections [44]. A drawback is its intensive bitter and metallic taste, which is often accompanied by poor compliance with oral dosage forms, especially for children and picky animals like cats [45].

Schistosomiasis is one of the most neglected tropical diseases in Africa. It affects almost 240 million people in 78 countries in Africa. 700 million people are at risk [46]. Schistosomiasis is caused by the infection of parasitic worms that develop in freshwater snails. The trematodes can penetrate the skin and travel through the body via the lymph and blood system. They feed on red blood cells resulting in an anemia. Moreover, they reside in mesenteric veins, the bladder, colon, liver and cause abdominal pain, diarrhea and blood in the urine and stool as well as multiple infections. The chronic disease can end up in serious liver and kidney damage and infertility. For children, there is a risk of impaired growth and cognitive development [47]. PZQ disrupts the calcium homeostasis of the trematodes and causes a spasmodic muscular contraction and immobilization of the worm's body ending up in the excretion of the worms [48].

PZQ is currently used in mass control programs coordinated by the WHO as the recommended therapy against schistosomiasis as it has only minor and transient side effects. It is applied as mass drug administration for morbidity control to school-age children and adults at risk [44]. In 2015, more than 66.5 million people were treated with PZQ usually with 600 mg tablets (Cesol®) as a single oral dose (40 mg/kg) donated by Merck KGaA [49]. The treatment is not suitable for pre-school age children and infants which account for 10% of the infected population. They are seen as a high risk group and play a key role in local disease transmission. These very young children cannot swallow the donated tablets due to their large size and bitter taste [50]. Thus, this treatment need has to be addressed by developing an appropriate taste masked paediatric formulation. The Pediatric Praziquantel Consortium under the leadership of Merck KGaA focusses on the development of an orodispersible formulation for children younger than 6 years.

Despite the bad taste, PZQ has a low solubility in water with a subsequent classification to the biopharmaceutical classification system (BCS) class 2, thereby enhancing challenges for drug product formulations [51]. The aqueous solubility is pH-independent as PZQ is a non-ionic compound. As a hydrophobic molecule it has a log P of 2.5 (logarithmic octanol/water partition

coefficient) [52]. PZQ is a small molecule with a molecular weight of 312.41 g/mol. The chemical structure is depicted in **Figure 1**.

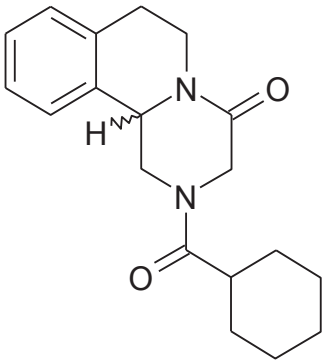


Figure 1. Chemical structure of praziquantel.

1.4. Taste

The taste of a medication can be the key barrier for the compliance and the success of the therapy independent on the age of the patient or the illness. In general, there are five basic modalities of taste: sweetness, sourness, saltiness, bitterness and umami. Taste perception is mediated by taste receptor cells on the tongue, soft palate and upper throat. They consist of ion channels for saltiness and sourness or G protein-coupled receptors for bitter, sweet and umami tastes. Approximately 100 taste receptor cells are clustered onion-like forming the taste buds [53]. The taste results from the downstream transduction after activation of the taste receptor including depolarization of the cells or the release of messengers and subsequent neurotransmitters. The signal is carried by neurons via the cranial nerves to the brain [54].

The ability to perceive the taste of certain substances and individual variations regarding taste preferences is due to genetic variants, expression and individual brain activity and cortical responses to different tastes [53, 54]. Genetic variants in the receptors affect taste perception within and across species. As an example, the T2R receptor family (approximately 25 G protein-coupled receptors) mediate bitter taste [55]. Due to polymorphisms in the proteins, bitter substances such as quinine, propylthiouracil or phenylthiocarbamide are perceived strongly different in humans [56]. The varying cortical responses on taste transduction is based on learning, experiences and genetics [53]. Moreover, evolutional aspects affect taste perception. In this way, toxic plants exhibit a bitter taste thereby preventing the intake with subsequent intoxications [56].

Most of pharmaceutical compounds provide a bitter, metallic or spicy taste, thereby decreasing the acceptability and compliance [54]. Formulation development for those substances gets more complicated as the taste sensitivity between children and adults varies greatly. Children show a higher preference for sweetness, umami, saltiness and sourness relative to adults and a strong dislike for bitter tasting liquids [54].

1.5. Taste assessment tools

In view of saving costs in product development of a compound it is reasonable to screen APIs and clinical formulations regarding their taste as early as possible. As a consequence of the high individual variability of taste perception, human taste panels remain the gold standard for taste assessment in drug product development [57]. Healthy and ideally trained volunteers evaluate the taste of the API and the taste masking effect of different formulations. In the early stage of development, human studies are questionable due to ethical and toxicological reasons, especially when testing new drugs with unknown safety. Moreover, there are no standardized protocols regarding panelists, test design and objective determination for taste assessment using scores or scales. The subjectivity of the panel members based on their nationality, eating habits and age remains a serious problem [58]. In addition, the generated data evaluated in adults cannot be easily transferred to medications for children who demonstrate even more difficulties in terms of a differentiated taste perception [59]. Younger children are most likely not adequately capable to express their taste sensation and mouth feeling [60].

Other *in vivo* methods representing alternatives to human taste assessment tools include animal preference tests using dogs, cats, rats or mice, fish or drosophila. Furthermore, electrophysiological methods in primates were reported. These *in vivo* methods were recently reviewed by Mohamed-Ahmed *et al.* [61] and Maniruzzaman *et al.* [62] and compared to *in vitro* methods regarding various parameters such as the necessary time for data collection, ability to screen pure drugs and formulations, correlation to human *in vivo* data, validation potential and costs. *In vitro* methods for the evaluation of taste masking effects involve electronic taste-sensing systems (electronic tongues) or cell based systems using calcium imaging as well as drug release studies [61, 63]. They are mostly based on determined taste thresholds in humans.

The rodent brief-access taste aversion (BATA) model is an efficient non-human taste assessment tool. In the BATA model, mildly water-deprived rats or mice assess several taste samples. Different concentrations of one compound and solubilized mixtures are presented to the rodents in several sipper tubes in a randomized order during one experiment. Within a time period of a few seconds the rodents can lick each solution separately. The number of licks is recorded electronically by a lickometer. A compound with an aversive taste decreases the number of licks in comparison to the reference defined as deionized water or pure excipient solution [64]. Thus, the number of licks is inversely proportional to the aversiveness of the samples. The concentration-dependent results are analyzed by an E_{max} model for lick numbers, lick ratios and to reliably identify the concentration of the compound that suppresses 50% of the licks in comparison to water (IC_{50}) [65]. Several studies showed the great potential of rat models designed for the measurement of the palatability of different compounds [66-69] and the correlation to human taste panels [70-74] revealing the same ranking order of the aversive compounds as in the human taste panels. Devantier *et al.* [70] demonstrated the ability of rats to determine the taste intensities of quinine, ciprofloxacin, clarithromycin and nystatin in comparison to a human taste panel. Clapham *et al.* [73] evaluated the BATA model for further various bitter compounds. The rats exhibited the same rank order of bitterness for the tested compounds as in humans. This was confirmed by Rudnitskaya *et al.* [71]

and Soto *et al.* [74]. Including usability (e.g. necessary time for data collection), ability to screen pure drugs and formulations, correlation to human *in vivo* data, validation potential (e.g. specificity, accuracy) and costs, the BATA model is superior to other *in vivo* and *in vitro* taste assessment tools [61]. Due to the short period of time of exposure to samples, the intake of each compound is limited, to avoid toxic side effects. New chemical entities with unknown toxicity and well-known APIs can be screened in early development as pure drugs or in preclinical or clinical formulations [61]. Nevertheless, limited assumptions can be made for non-stabilized suspensions, which can block the sipper tubes by sedimentation.

Among *in vitro* methods, the electronic tongue as an artificial taste assessment technique is the most useful non-human taste assessment tool [61, 75]. Electronic tongues are analytical sensor array systems [76]. The equipped sensors vary in their composition, properties and selectivity for different substances [77]. The applied measurement principle can be based on potentiometry, voltammetry, amperometry or others [78]. Electronic tongues imitate the interaction of dissolved substances in the saliva with the taste buds [79]. In this way, they can be applied for the characterization of pure substances and formulations in aqueous solutions. The activity of a compound logarithmically affects the measured signals of the electronic tongue in accordance to the human taste [77, 79]. A concentration dependent signal for the pure compounds as a calibration is the prerequisite for the analytics of various formulations. Subsequently mixtures can be compared regarding their taste masking efficiency using multivariate data analysis [80]. The applications of the electronic tongue are various: medical diagnostics, quality control, toxicity studies, stability, taste assessment and comparison of products in the field of food and pharmaceutical industries [79, 81, 82]. The Insent taste sensing system with a potentiometric measurement principle was the first marketed electronic tongue [83] based on the research of a Japanese group [84]. The αAstree electronic tongue is the second commercially available system based on chemically modified field effect transistor technology [80]. The application of both systems for pharmaceutics was summarized by Woertz *et al.* [76, 79]. The implementation of these systems for taste assessment in pharmaceutical formulation development for liquid and solid dosage forms was reviewed by various studies [71, 80, 85-95]. Furthermore, correlation to human taste panels was shown in the literature [71, 80, 87, 92-94]. With regard to safety, toxicity and objectivity electronic tongues are preferable over human taste panels. They can provide an early screening of especially new drugs of unknown toxicity and the relative optimization of preclinical formulations [61]. Nevertheless, as they only provide relative data and no overall taste impressions, the assumptions of the results are limited without correlation to human data.

In vitro dissolution using release studies is also a common approach to determine taste masking effects. It is assumed that only the dissolved amount of API causes the aversive taste of the formulation through the interaction with the taste buds [96]. In this connection, the drug release of the formulations can be used as an indicative tool for evaluating taste masking efficacy especially for poorly soluble drugs and coated formulations [97]. This approach provides limited predictions for the *in vivo* taste perception of the formulations without knowing human taste thresholds [61]. Moreover, dissolution setups in the literature vary greatly regarding dissolution media in terms of

buffer composition, pH and volume, sampling time points, in-line or off-line determination of the API concentration and temperature settings [58]. However, the data supports pharmaceutical development in the early screening of pure APIs and preclinical formulations.

1.6. Taste masking strategies

The importance of an acceptable palatability of the dosage form has been recognized by regulatory authorities in the same way as the use of suitable dosage forms for children [2]. Taste masking as a tool for palatability is included in the "Guideline on Pharmaceutical Development of Medicines for Paediatric Use" of the EMA of 2014 and the Paediatric Regulation and is a mandatory part of the Paediatric Investigation Plan.

Various taste masking strategies can be applied to achieve a successful palatability of an unpleasant API (**Figure 2**). They have been reviewed by various authors [2, 96, 98, 99]. A pragmatic approach of caregivers and parents administering dosage forms to their children is to manipulate the medication to improve or obscure the taste. Tablets are crushed to sprinkle them onto food such as fruit jelly or yoghurt or to mix them with food or beverages [4]. According to the reflection paper of the EMA in 2006 this should be the last resort [13]. Associated risks with manipulating medicines are the subsequent unpredictable stability of the API, compatibility with the used food or beverages and destruction of modified release systems and coatings such as an enteric coating [100].

Scientific approaches directly address the taste buds antagonizing the bitter receptors or blocking the taste transduction. The use of bitter receptor antagonists is limited and mostly needs to be adapted on a case by case basis as the interaction of the API and the bitter receptors in the mouth is not fully predictable [2]. The bitter blockers such as lipoproteins, compete with the API to bind to the receptor site of the G-protein [98]. The specific inhibition of the T2R receptor family mediating bitter taste is still a scientific approach and not commercially marketed. Moreover, the safety and toxicology of such substances is not determined, especially not for children [2].

The most robust and effective taste masking methods applicable as platform strategies are implemented in the pharmaceutical development of the dosage form. These methods can be focussed on the modification of the API itself or the final liquid or solid dosage form. Modification of the API in terms of lowering the solubility is based on the assumption that only the dissolved amount of the unpleasant API interacts with the taste buds and subsequently transmits the aversive taste [96]. The modification can be applied using a prodrug, another salt or fixing the pH of the formulation for APIs with a pH-dependent solubility. The efficacy of these techniques is limited for APIs with a very low taste threshold, if a small dissolved amount is unavoidable independent on the modification of the API. Moreover, the modification of the API can be accompanied with changes in the pharmacokinetics and safety or toxicology of the formulation [2]. A promising alternative to prevent the interaction of dissolved API in the saliva with the taste buds is the complexation with ion-exchange resins or cyclodextrins (CDs). Ion-exchange resins are only applicable for ionic compounds. Thus, they are not appropriate for the chosen model compound PZQ in this study. CDs are capable of forming inclusion complexes with a variety of molecules thereby enhancing the

solubility of the molecule and creating a molecular barrier around it [2]. They are cyclic oligosaccharides obtained from starch and shaped as truncated cones [101].

A flexible concept for the development of liquid and sold dosage forms is the addition of sweeteners or flavors. Bulk and intense sweeteners, e.g. sucrose, saccharin sodium, acesulfame potassium or aspartame, as well as flavors like cherry, raspberry, peach or chocolate can be very effective in changing the overall taste of the formulation. However, they only obscure the bitter taste of the API. Other drawbacks are the possible toxicity, and risk of allergies and sensation. Flavors can be challenging from a regulatory perspective as they are mostly complex compositions and preferences vary from country to country [2].

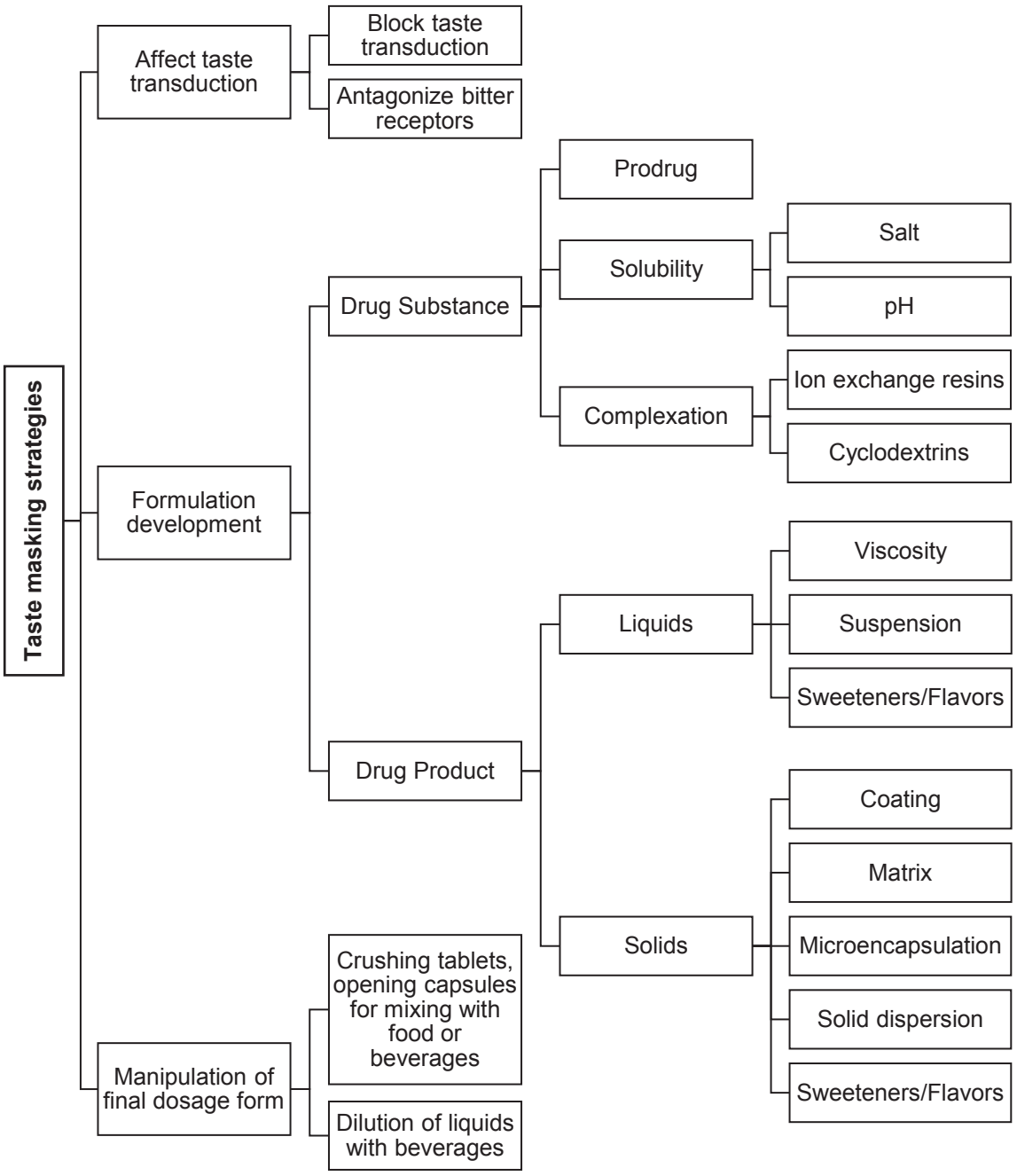


Figure 2. Taste masking strategies, modified according to Walsh *et al.* [2].

The most useful platform technology is to create a physical barrier around the API for taste masking in solid oral dosage forms. This approach includes the encapsulation of the API via spray-drying, fluidized bed coating, phase separation, hot melt coating or solvent evaporation with various excipients in aqueous or organic systems. In terms of orodispersible formulations the creation of a physical barrier around the API is the most suitable. Moreover, as recent studies revealed the natural tendency to chew MTs it is more effective than coating the final dosage form [34, 36]. Nevertheless, the coating should not diminish the release of the API in the gastro-intestinal tract and affect the absorption and consequently the bioavailability of the API. Other applicable techniques are extrusion, freeze-drying or granulation for embedding the unpleasant API in a matrix and thereby delaying the interaction with the taste buds. A chemical or physical interaction between the API and the carrier can enable the adequate release profile for the taste masking approach [98]. This can be realized as well in solid dispersions via hot melt extrusion, spray-drying, spray congealing or coprecipitation [99]. Extrusion and spray-drying are well-known and broadly applied techniques that will be explained in detail in the next sections.

Finally, the dosage form such as a tablet, can be coated with a saliva-resistant or modified release layer using a conventional drum coater or fluidized bed system for MTs or granules. In terms of children this can be a risk, as they are likely to chew solid dosage forms cracking the taste masked coating before swallowing them [35].

1.7. Extrusion

Extrusion is a processing technology in which materials are exposed to mechanical or thermal stress until they flow through a die [102]. It involves feeding, mixing and compacting, as well as softening or melting depending on the applied temperature of a powder blend to a strand-like product of a uniform shape [103]. The shape and size of the extrudate strands are defined by the used die. The conveyer barrel is divided in sections providing different heating zones [104]. Extruders with two co-rotating screws in the barrel (twin-screw) are preferred based on the best mixing capability [105].

Hot-melt extrusion (HME) under elevated temperatures has become a well-known processing technology for the preparation of solid dispersions of APIs into polymer or lipid matrices [102]. This offered various applications such as improving the solubility and bioavailability of APIs, modified, extended and controlled drug delivery as well as taste masking [102, 106]. HME has covered a platform for oral, parenteral and topical dosage forms, as well as medical devices with various marketed formulations [105]. Advantages of HME include continuous design reducing production times, solvent-free set up, robust process, easy to scale-up and applicability to moisture-sensitive APIs. Limitations are the application to heat-sensitive APIs and carriers and the necessary good flowability needed for a constant and precise feeding of the extruder [106].

An effective taste masking using HME is usually facilitated with the selection of appropriate excipients. Different polymeric systems can be applied to create a physical barrier around the API thereby inhibiting the interaction of the unpleasant compound with the taste buds in the saliva. In this way, the effective taste masking using HME was evaluated for various APIs using polymers

with a pH-dependent solubility (e.g. insolubility in the saliva at pH 6 – 7) or insolubility but slow permeability in water. Gryczke *et al.* [23, 106] demonstrated the successful taste suppression of verapamil and ibuprofen using Eudragit® L100, L100-55 and E PO in HME. Strong drug-polymer interactions and a molecular dispersion of the API in the polymer matrix during the extrusion process led to an efficient taste masking in the *in vivo* panelist's score without diminishing dissolution properties. Maniruzzaman *et al.* [62, 107] confirmed HME as an effective tool for taste masking. The taste of the cationic APIs propranolol, verapamil, diphenhydramine and cetirizine could be improved by extrusion with anionic Eudragit® L100, L100-55 and S100, verified in healthy volunteers and using the electronic tongue. Paracetamol was extruded with Eudragit® E PO and Kollidon® VA64 showing a significant taste suppression analyzed with the electronic tongue.

Besides HME, solid lipid extrusion (SLE) has been demonstrated as advantageous for the solubility and permeability of heat- or water-sensitive and volatile APIs [108-111] and provided applications for controlled release [108, 112, 113] and taste masking [108, 114, 115]. SLE is described as a robust and uncomplicated process to disperse an API in a lipid matrix [115]. The extrusion temperature is set below the melting point or range of the lipid to avoid complete melting but soften it [112]. Solid lipids are advantageous pharmaceutical excipients being low cost, natural and biodegradable products with physiological, non-toxic properties [112]. However, they are complex chemical and physical compositions observing wide melting ranges [116]. They consist of crystal structures including alpha and beta modifications which show different polymorphic transition temperatures and melting points and can lead to stability issues [117]. The unstable alpha form with a lower dense of packaging mode is usually generated by rapid cooling of molten lipid and subsequently transforms toward the stable densest packaged beta form [112]. Differential scanning calorimetry is a fundamental technique for the characterization of the thermal behavior of these lipid excipients [116]. The transformation of lipids affects the morphology, surface area, surface shape and wettability of the particles, so called blooming effect [118, 119]. It ends up in changes in the drug release as the dissolution through a lipid matrix is diffusion controlled [112]. The result is a change of the quality of the product, reported in various studies [109, 112, 118]. Nevertheless, Windbergs *et al*. [119] demonstrated SLE without development of blooming on the surface keeping the extrusion temperature between the melting points of the alpha and beta form. In this way, the partial molten lipid directly solidified in its stable beta form.

The use of lipid matrices for extrusion in view of taste masking was reported the first by Breitkreutz *et al.* [108] for sodium benzoate. Further studies confirmed the usability of SLE for the production of controlled [113], sustained [112, 120] and immediate [109, 114, 121] release lipid matrix systems. Both techniques, HME and SLE, can be combined with a spheronization or milling step obtaining pellets or a powder for further processing to a tablet or capsule [2].

1.8. Spray-drying

Spray-drying is a process to produce dry powders from a fluid by atomization in a hot drying gas stream. This is generally air and nitrogen. The drying is applicable for solutions, suspensions, emulsions or melts [122]. Spray-drying involves atomization of the pumped fluid through a nozzle, contact of the liquid droplets with the drying gas, formation of dry particles in the drying chamber through evaporation and separation of particles from the air using a cyclone followed by collection in a device [123]. The method is single-step, continuous, rapid and scalable for laboratory and industrial setups. The high surface to volume ratio of the atomized fluid is accompanied with a fast drying and cost-effective process [124]. Spray-drying generates particles from nano to micron size [123]. The properties of the particles are affected by the process parameters such as the concentration, viscosity and boiling point of the liquid feed, the dosing of the liquid, flow rate of atomizing and drying air, nozzle size, gas inlet temperature and dimensions of the drying chamber limiting the residence time of the particles in the drying system [122, 123]. As the evaporation is very fast and the residence time of dry particles is typically limited to a few ms, the heat exposure is extremely low (ms to s). Moreover, during solvent evaporation the droplet cools to the wet bulb temperature delaying the heat exposure of the particles. Thus, spray-drying is favorable for heat-sensitive APIs and prevents drug and carrier degradation.

Besides residual solvent, a main challenge of the spray-drying process is the yield which strongly depends on the work scale [122]. In laboratory scale the yield in conventional spray-dryers is not optimal at 20 to 70% due to the loss of particles on the wall of the drying chamber. Moreover, very fine particles below 2 µm can directly pass into the exhaust air due to an insufficient separation in the cyclone. The separation of dispersed particles from the gas phase in the cyclone is achieved based on the density differences between solid particles and the gas phase. The rotating vortex in the cyclone forces the dense particles to the wall of the cyclone and down to the collecting vial due to their lag in velocity in the accelerated flow field compared to the gas phase. Smaller particles can be entrained with the gas phase [124]. This strongly affects small batch sizes. The fraction lost decreases in larger scale setups [122]. Moreover, the small particle size and low bulk density of spray-dried powders generally results in a poor flowability [123]. Further compaction or granulation can be applied to improve the flowability and handling of the powders for processing to tablets or other solid dosage forms [123].

Spray-drying is a widely used technique in pharmaceutical, chemical, cosmetic and food industry [122]. It is applicable for drying pure drugs or dehydrating multicomponent liquids to matrix powder systems or to encapsulate or coat drugs. The particles can be administered via the oral, pulmonary, parenteral, nasal, ophthalmic or vaginal routes. Various specialized applications exist such as controlled release, targeted drug delivery, bioavailability and solubility enhancement, degradation protection or liposomes [122]. In view of pharmaceutics the process is of high interest for manufacturing amorphous solid dispersions using polymer matrices [125]. Paudel *et al.* [124] recently summarized frequently used carriers in formulating spray dried dispersions including polymers, polyethylene oxides, lipids and carbohydrates. The choice of the adequate carrier system is based on various considerations such as miscibility with the drug, intermolecular interaction (e.g.

H-bonding, electrostatic, van der Waals, ionic or hydrophobic interactions) and physicochemical properties (e.g. solubility, melting point, thermal stability). Furthermore, the addition of a third component to the liquid feed facilitating a more convenient spray-drying process (e.g. surfactant) and downstream of the powder (e.g. disintegrant, binder, glidant) is common practice [124].

Applying a physical barrier around the aversive API is an effective taste masking strategy [2]. Spray-drying has been demonstrated as a suitable technique for this approach using polymer matrices as the carrier system [99, 126, 127]. Preparing spray-dried particles with polymers which are insoluble in the saliva, APIs were taste masked without diminishing the bioavailability in the gastro-intestinal tract [128].

2. Aim of the thesis

The objective of this thesis was to identify, conduct and compare various taste masking strategies in the pharmaceutical development of liquid and solid oral dosage forms for paediatrics.

Praziquantel was chosen as a suitable model compound with regard to its generally known aversive and bitter taste. Moreover, praziquantel is currently used against schistosomiasis in humans and various worm infections in animals including difficult patient populations such as children and picky animals like cats. There is a high need for a taste masked and acceptable formulation as especially very young children cannot swallow the currently used tablets due to their large size and bitter taste. Based on the chemical and physical characteristics of praziquantel, appropriate taste masking techniques should be applied among the broad range of possible taste masking strategies for liquid and solid oral dosage forms suitable for the administration to paediatrics.

With regard to liquid formulations, the focus was set on the complexation of praziquantel in solution to efficiently inhibit the interaction of the compound with the taste buds during oral uptake. For this purpose, different cyclodextrins were examined. In addition, various maltodextrins were evaluated as an alternative to cyclodextrins and as an efficient and safe taste masking technique in solution for paediatrics.

With regard to solid formulations, mini-tablets and orodispersible mini-tablets were determined as the most applicable and flexible dosage forms. As a prerequisite for the processing of taste masked formulations, various excipients for the direct compression to mini-tablets and orodispersible mini-tablets with a diameter of 3 mm should be evaluated. Furthermore, different analytical methods were assessed comparatively as the small size of the tablets was accompanied with limitations of the usually used analytical equipment for tablets.

In terms of taste masking in mini-tablets, different manufacturing methods including solvent casting, spray-drying and extrusion had to be examined to encapsulate and embed praziquantel. For this purpose, suitable excipients were identified. Subsequently, the formulations were further processed to mini-tablets and systematically analyzed.

Furthermore, this work should evaluate in vitro and in vivo taste assessment tools regarding the applicability for praziquantel and taste masked formulations.

3. Results and discussion

This work focused on the development, manufacturing and characterization of taste masked liquid and solid oral dosage forms for praziquantel for paediatrics. In the following, the thesis is divided in two main chapters separating liquid and solid dosage forms.

3.1. Liquid dosage forms

As described in the introduction (1.6, **Figure 2**) there are various taste masking strategies in the pharmaceutical development of liquid dosage forms such as the modification of the API (prodrug, salt), the use of ion-exchange resins, cyclodextrins, sweeteners or flavors. The modification of praziquantel was excluded as this can be related to changes in the pharmacokinetics and toxicology of the compound. Moreover, ion exchange-resins are not suitable due to the non-ionic characteristics of praziquantel. Flavors are complex compositions with varying acceptance from country to country [2]. Thus, the focus was set on the complexation of praziquantel with cyclodextrins. Furthermore, maltodextrins were evaluated as a promising alternative to cyclodextrins regarding an efficient taste masking in solution for paediatrics. As another important objective, praziquantel and taste masked formulations were assessed using *in vitro* and *in vivo* taste assessment tools.

Parts of this chapter have been published as:
Münster, M., Mohamed-Ahmed, A.H.A., Immohr, L.I., Schoch, C., Schmidt, C., Tuleu, C., Breitkreutz, J., 2017. Comparative in vitro and in vivo taste assessment of liquid praziquantel formulations. Int. J. Pharm. 529, 310-318.
DOI: 10.1016/j.ijpharm.2017.06.084.

3.1.1. Complexation with maltodextrin or cyclodextrin

Cyclodextrins (CDs) are cyclic oligosaccharides obtained from starch [129]. They are composed of α-D-glucopyranose units linked with α-1,4-glycosidic bonds [101] (**Figure 3**). The nomenclature is depending on the number of units: α-CDs (six), β-CDs (seven) and γ-CDs (eight) [130]. The β-CD and its derivatives are the most commonly used CDs in the pharmaceutical industry [131]. Shaped as truncated cones CDs provide a hydrophilic outer surface due to hydroxyl groups and a lipophilic inner cavity. The hydrophilic outer surface enables a good water solubility, which can be increased by chemical modification with hydroxyl and other functional groups [2]. The cavity provides a favorable environment for lipophilic molecules. Water molecules located in the cavity can be easily substituted with less polar substances. Moreover several interactions such as hydrogen bonds, van der Waals', electrostatic, charge-transfer and hydrophobic binding lead to host-guest type inclusion complexes and partly or complete encapsulation of the drug [129, 132, 133]. This is applied for increasing drug solubility, bioavailability or stability and decreasing volatility, sublimation or

unpleasant taste and smell [133-136]. The efficacy of CDs for these applications needs to be optimized on a case-by-case basis as it highly depends on the properties of the API and the selected CD. Drawbacks of the use of CDs are the possible effect of the complex on the bioavailability and pharmacokinetic or pharmacodynamics of the encapsulated API [137].

Orally administered CDs are practically non-toxic due to the lack of absorption in the gastrointestinal tract [2]. The derivatives hydroxypropyl-beta-cyclodextrin (HP-β-CD) and sulfobutyl ether-beta-cyclodextrin (SBE-β-CD) appear even safer than the native β-CD [137, 138]. HP-β-CD was well tolerated in humans for one to two weeks with daily doses of 4 – 8 g [139]. However, higher amounts of 16 – 24 g showed an increase in the incidence in soft stools and diarrhea. Further findings upon oral administration where cecal enlargement and renal effects due to systemic absorption [137]. The daily oral dose for HP-β-CD as oral pharmaceutical may reach 8 g/day and the NOAEL (No Observed Adverse Effect Level) for SBE-β-CD for rats and dogs after three months is 3.6 mg/kg/day [140]. There is no data available for children below two years. Juvenile rats observed the same findings as reported for adult rats [141].

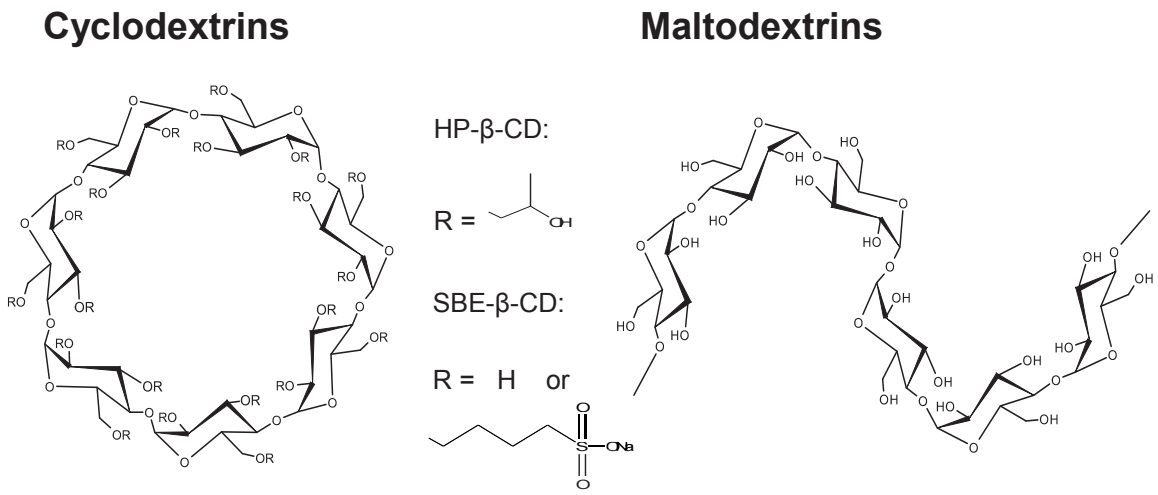


Figure 3. Chemical structures of hydroxypropyl-beta-cyclodextrin (HP-β-CD), sulfobutylether-beta-cyclodextrin (SBE-β-CD) (left) and maltodextrins (right).

Maltodextrins (MDs) are excipients of lower safety concerns than CDs and can evenly provide taste masking and solubility enhancement [88, 142]. They consist of d-glucose units (amylose and amylopectin) connected in chains of variable length with α-1,4-glycosidic and few α-1,6-glycosidic bonds derived from starch. Thus, they depict an open chain structure of CDs [143] (**Figure 3**). Different types can have different dextrose equivalents characterizing the percentage of reducing sugars [144]. The incorporated amylose builds up a helical structure in aqueous media [145]. In this way MDs can provide inclusion complexes by hydrophobic and van der Waals' interactions and can encapsulate hydrophobic drugs just as CDs [146-148]. Moreover, they are accepted as safe excipients with no limited daily intake [149]. Due to their widely use in infant formula and nutritional supply they represent a promising alternative to CDs. Some suppliers provide MDs with especially high amylose content such as Roquette with Kleptose® linecaps 17.

The most common type for inclusion complexes between an API and a CD is 1:1 [150]. A phase solubility study according to Higuchi & Connors [151] classifies the stoichiometry of the formed interaction and determines the apparent stability constant ($K_{1:1}$) with the following equation:

$$K_{1:1} = \text{slope}/(S_0 * (1 - \text{slope}))$$ Eq. 1.

An excess amount of API is added to aqueous solutions of CDs with increasing concentration. The resulting dissolved API concentration is depicted against the concentration of the CD. The $K_{1:1}$ value is calculated from the slope of the phase solubility profile and the intrinsic solubility of the API (S_0). A linear profile indicates a stoichiometry of 1:1 API:CD, a positive or negative deviation from linearity can be associated with a higher order interaction such as 2:1 or 3:1 API:CD or a self-association of CDs such as 1:2 or 1:3 API:CD [152].
The complexation efficiency (CE) can be determined with the following equation:

$$CE = [API/CD]/[CD] = \text{slope}/(1 - \text{slope})$$ Eq. 2.

The CE is independent on S_0 and a more convenient method to compare different APIs and CDs during formulation development. It determines the ratio of dissolved complex (API/CD) and free CD and can be used to calculate the increase in formulation bulk involving the molecular weights (MW) [150]:

$$API : CD = 1 : (1 + 1/CE)$$ Eq. 3.

$$\text{Increase in formulation bulk} = MW_{CD}/MW_{API} * (1 + 1/CE)$$ Eq. 4.

Previous studies investigated the successful complex formation of PZQ with CDs characterizing the interaction and the solubility and dissolution improvement of PZQ [132, 153-158].
The focus of this study was to evaluate the taste masking effects of CDs for PZQ in comparison to MDs. Phase solubility studies were performed with HP-β-CD, SBE-β-CD and different MDs: Maltodextrin DE 13.0 – 17.0, Kleptose® linecaps 17 (DE 17) and Glucidex® 17 (DE 17). Formulations with CDs were additionally analyzed in solid state after spray-drying.
The efficiency of taste masking *in vivo* was investigated and discussed in 3.1.2.2.

3.1.1.1. Phase solubility study

The ability of MDs and CDs to improve the solubility of PZQ and hence provide taste masking was conducted with phase solubility studies according to Higuchi and Connors [151] as described in 6.2.1.1 and analyzed as reported in 6.2.2.1 and 6.2.2.2.

Excess amounts of PZQ (10-fold based on the thermodynamic solubility) were added to aqueous solutions of 2, 5, 10, 15 and 20 mM of the MDs or the CDs (n = 3). The subsequent concentration of PZQ was plotted against the concentration of the MDs and CDs (**Figure 4**). The intrinsic solubility of PZQ in water was determined as 0.71 mM (**Figure 4**).

Both CDs (HP-β-CD and SBE-β-CD) significantly increased the intrinsic solubility of PZQ in water (0.71 mM) up to 5 to 7-fold at the highest concentration of 20 mM CD ($p < 0.001$) (**Figure 4**). These findings confirmed previous results of Arrua *et al.* [132] and Maragos *et al.* [156]. A linear profile of the solubility increase of CDs indicated a stoichiometry of 1:1, a positive or negative deviation from linearity would be associated with a higher order interaction or a self-association of CDs [152]. Whereas this conclusion is in line with Maragos *et al.* [156], de Jesus *et al.* [154] and El-Arini *et al.* [157], other studies reported a stoichiometry of 1:2 [132].

The apparent solubility constant $K_{1:1}$ and the complexation efficiency (CE) were calculated from the slope of the plot (**Table 1**). SBE-β-CD showed the highest solubility enhancement and a CE of 0.26 meaning that one out of five SBE-β-CD molecules complexed the drug. For HP-β-CD a lower CE of 0.16 was observed. Less than one out of seven HP-β-CD molecules interacted with the drug. Loftsson *et al.* summarized the reported complexation studies appointing an average CE for HP-β-CD of 0.39 ± 0.47 for 13 different drugs in water [150]. High values for $K_{1:1}$ above $1 \times 10^5\ M^{-1}$ can lead to negative effects on the bioavailability of a compound [137]. A strong affinity of a CD to a specific compound can result in issues regarding the complete dissociation of the drug from the CD in the gut. As the $K_{1:1}$ values for HP-β-CD and SBE-β-CD in this study are below this critical value, no issues were anticipated *in vivo*. Nevertheless, the necessary high amount of CDs led to a high increase in formulation bulk. This is not favorable due to the relatively high dose of praziquantel of 40 – 60 mg/kg bodyweight [48].

The calculations for the CE were not applicable for all MDs as there was no remarkable slope of the phase solubility profiles. A solubility enhancement as it was shown for loperamide, dextromethorphan or dimenhydrinate could not be confirmed for PZQ [88, 142]. The incorporated amylose builds up a helical structure in aqueous media [145]. In this way maltodextrins can provide hydrophobic and van der Waals' interactions to hydrophobic drugs [146-148]. Nevertheless, these findings could not be confirmed for PZQ in this phase solubility study (**Figure 4**). There was no difference between Kleptose® linecaps providing a high content of amylose and the MD of Sigma Aldrich or Glucidex®.

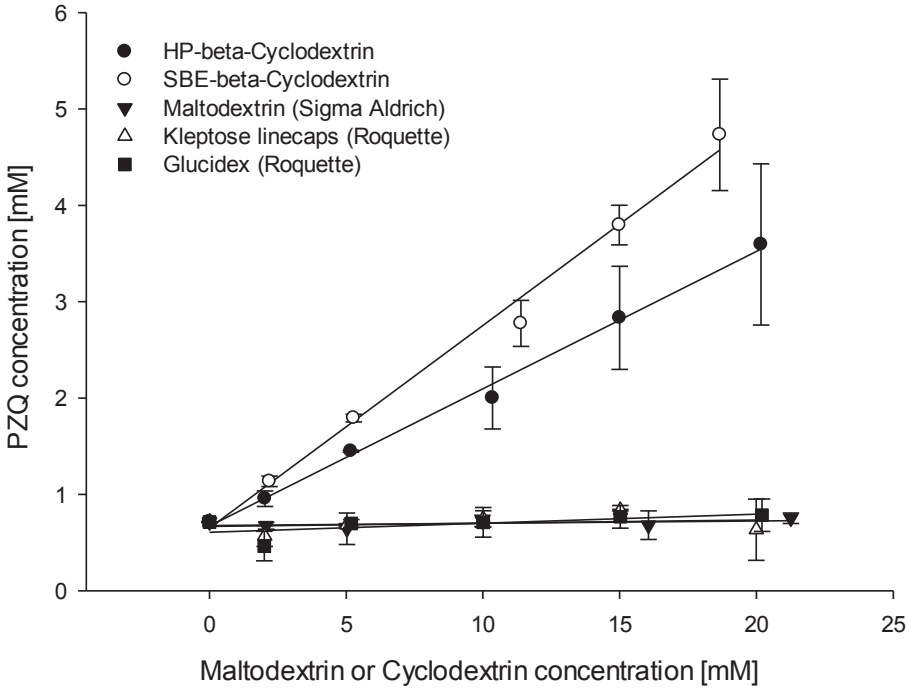


Figure 4. Phase solubility diagram. Solubilized concentration of praziquantel (PZQ) plotted against the concentration of hydroxypropyl-beta-cyclodextrin (HP-beta-Cyclodextrin), sulfobutylether-beta-cyclodextrin (SBE-beta-Cyclodextrin) and all maltodextrins. Arithmetic mean ± S.D. (n = 3).

Table 1
Key figures calculated from the slope of the phase solubility study.

Excipient	Slope	$K_{1:1}$ [M^{-1}]	CE	PZQ: CD	Increase in formulation bulk
HP-β-CD	0.14	228.71	0.16	1: 7.25	32.19
SBE-β-CD	0.21	365.11	0.26	1: 4.85	33.58
Maltodextrin DE 13.0 – 17.0	N/A	N/A	N/A	N/A	N/A
Kleptose linecaps® 17	N/A	N/A	N/A	N/A	N/A
Glucidex® 17	N/A	N/A	N/A	N/A	N/A

The taste masking effectiveness of maltodextrins in comparison to cyclodextrins were evaluated in 3.1.2. Based on the results of this phase solubility study a higher taste masking ability of both CDs in comparison to the MDs was expected.

3.1.1.2. Solid state characterization

Solid oral dosage forms are most convenient for packaging, transport and use. Thus, based on the promising results of the phase solubility study (0) solutions with PZQ praziquantel and HP-β-CD or SBE-β-CD were spray-dried for the solid state characterization. The purpose was to identify the need of a complex formation in solution and subsequent spray-drying in comparison to the preparation as a physical mixture (PM) in a mortar in the same ratio of API: CD (w/w). A complexation in dry powder is possible when the CD exchanges the included water (crystal water) with the guest molecule. This formation can proceed during mixing and storage [135].
Spray-drying was conducted as described in 6.2.1.3. All parameters were adapted in order to maximize the particle size of the resulting powder resulting in ranges from 4 to 35 µm (**Figure 5**).

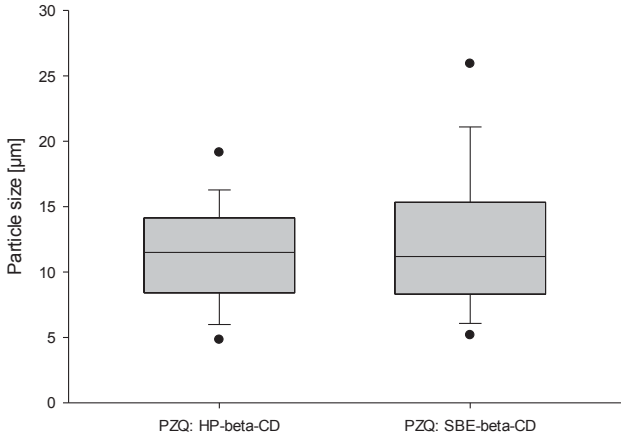


Figure 5. Particle size distribution of spray-dried powders with praziquantel (PZQ), hydroxypropyl-beta-cyclodextrin (HP-beta-CD) and sulfobutylether-beta-cyclodextrin (SBE-beta-CD) representing the median, 1st and 3rd quartile and 5th and 95th percentile (n = 100).

A process yield of 69.2% was achieved for PZQ: HP-β-CD and 72.9% for PZQ: SBE-β-CD. Only a small amount was lost in the drying chamber. The powders were not electrostatic with a visually good flowability. Scanning electron microscopy (SEM) was performed to examine the morphology of the spray-dried particles (**Figure 6**) in comparison to the physical mixtures. Both spray-dried formulations exhibited spherical particles with wrinkled surfaces in contrast to the needle shaped bulk PZQ and round CDs. This has already been reported for spray-dried CDs [159-161]. The particle shape depends on the evaporation and diffusion rate of the droplets in the spraying chamber expressed as the Peclet number [162]. The Peclet number is influenced by physicochemical characteristics of the spray-dried materials (saturation, crystallization kinetics), the used solvents and the process parameters (solid content, inlet temperature, atomizing air and nozzle size) of the process. CDs are associated with a high Peclet number [163] leading to deflated-ball like shapes [164]. The evaporation at the surface of the droplets is fast compared to the diffusion process of the incorporated substances. A shell is formed at the surface due to local high concentration and viscosity. During process the remained water in the core further diffuses or evaporates resulting in a collapsed or wrinkled skin of the particles [162].

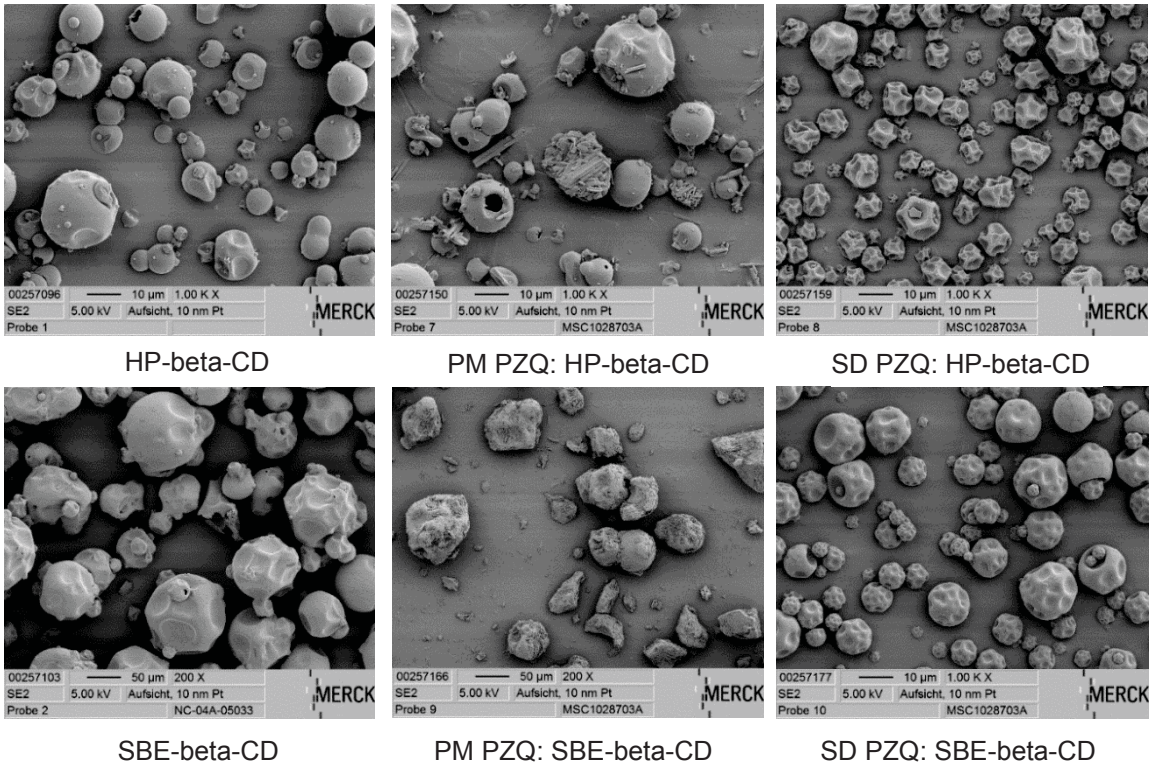


Figure 6. Scanning electron microscopic pictures of bulk hydroxypropyl-beta-cyclodextrin (HP-beta-CD) and sulfobutylether-beta-cyclodextrin (SBE-beta-CD), physical mixtures (PM) of praziquantel (PZQ) with both CDs in the ratio 1:1 (M/M) and spray-dried (SD) powders.

After spray-drying PZQ is completely in amorphous state in both formulations as shown in the data of the differential scanning calorimetry (DSC) (**Figure 7** and **Figure 73** in appendix) and X-ray powder diffraction (XRPD) (**Figure 8** and **Figure 74** in appendix). Crystalline PZQ melted at 140°C (Onset). This peak appeared in both PMs, but not in the spray-dried powders. The broad endothermic peak before indicated the dehydration process. The XRPD patterns of the crystalline PZQ observed characteristic peaks at 3.95, 6.30, 6.65, 7.93, 8.09, 15.24, 16.40, 16.93, 20.10 and 25.62° among many others [52, 165, 166]. The peak at 25.54° in the spray-dried mixture of PZQ and SBE-β-CD was assigned to the pure CD. Minor recrystallization of the CD during spray-drying might occurred due to the supersaturation at the surface of the particles during fast evaporation [162].

It can be concluded that the API is more efficiently encapsulated or bonded to the CDs out of a spray-dried solution than from a physical mixture in accordance with other studies [167-172]. NMR spectra of Arrua *et al.*[132] proofed no complex formation of PZQ with CDs in physical mixtures, whereas PZQ was in amorphous state in the complexes with HP-β-CD out of solvent evaporation. This is of high importance for a promising taste masking effect. Nevertheless an amorphous API provides a faster and higher solubility due to a decreased lattice energy and an improved wettability of the drug molecules [173-175] counteracting this approach for taste masking.

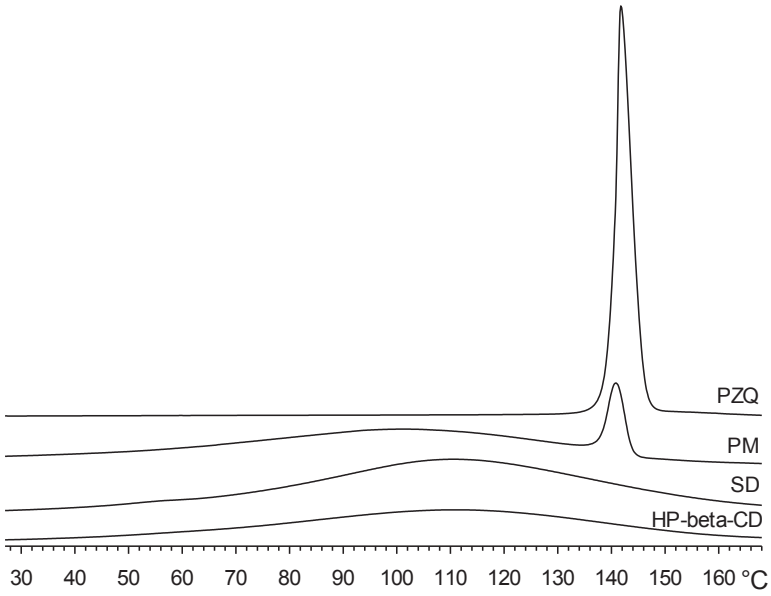


Figure 7. Endothermic thermograms of differential scanning calorimetry of crystalline praziquantel (PZQ), physical mixtures (PM) with hydroxypropyl-beta-cyclodextrin (HP-β-CD) and spray-dried (SD) powders.

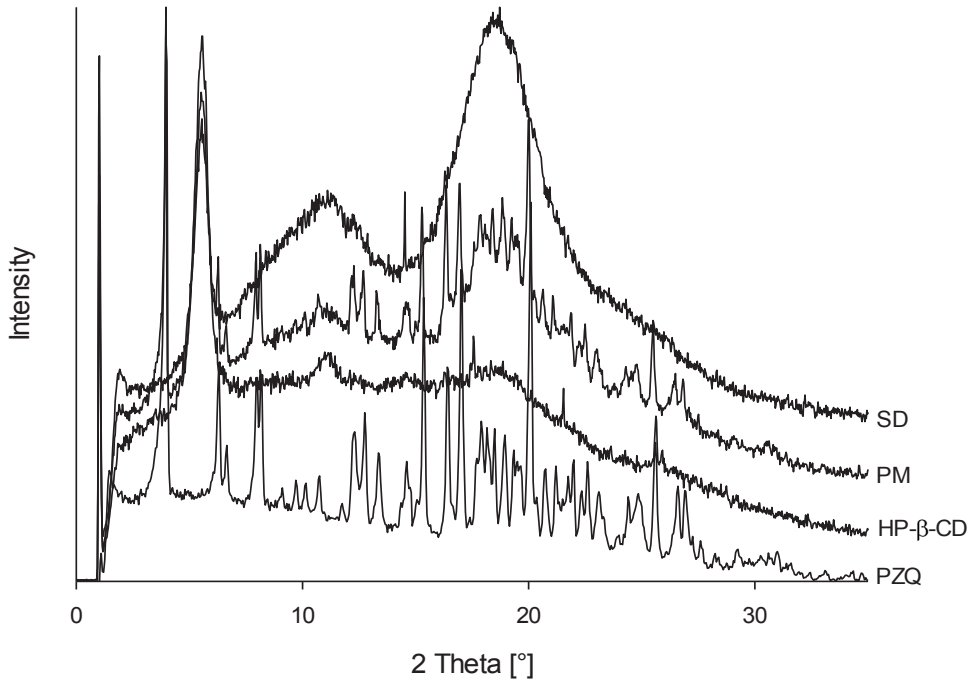


Figure 8. X-ray powder diffraction patterns of praziquantel (PZQ) with hydroxypropyl-beta-cyclodextrin (HP-β-CD), physical mixture (PM) in the ratio 1:1 (Mol/Mol) and spray-dried (SD) powder.

Therefore non-sink dissolution experiments as described in 6.2.2.9 were conducted in three different media: simulated salivary fluid pH 6.8 (SSF) (**Figure 9**), simulated gastric fluid sine pepsin pH 1.2 (spSGF) and fasted state simulated intestinal fluid pH 6.5 (FaSSIF) (**Figure 10**). PZQ exhibited a pH-independent solubility of 0.18 – 0.20 mg/ml in all media (release of 14 – 16%). The

spray-dried formulations immediately solubilized PZQ at the first sampling time point: after 1 min in SSF and 5 min in spSGF and FaSSIF independent on the pH and the ionic composition of the buffers (release of 100%). The complex enabled a 5-fold higher solubility than the pure crystalline PZQ. The PMs showed a slower solubilization of PZQ (maximum release of 80%) and higher deviations. This can be explained by the amorphous and complexed state of PZQ in spray-dried powders in contrast to free crystalline amounts in the physical mixtures.

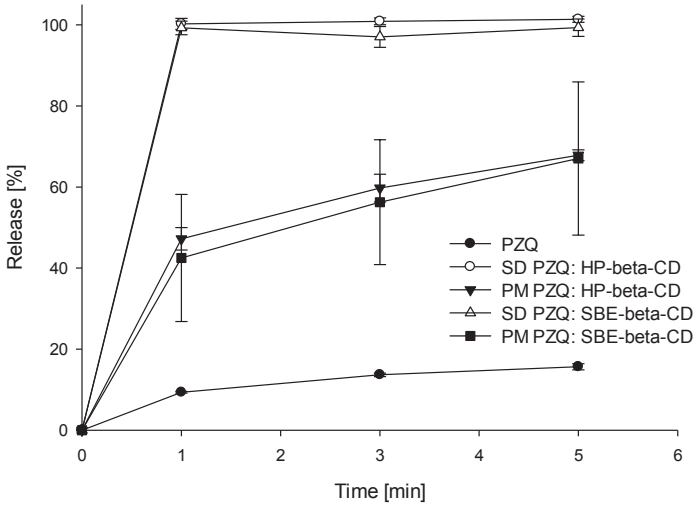


Figure 9. Praziquantel (PZQ) release of spray-dried (SD) powders with hydroxypropyl-beta-cyclodextrin (HP-β-CD) and sulfobutylether-beta-cyclodextrin (SBE-β-CD) in SSF in comparison to crystalline PZQ and the physical mixtures (PM). Arithmetic mean ± S.D. (n = 6).

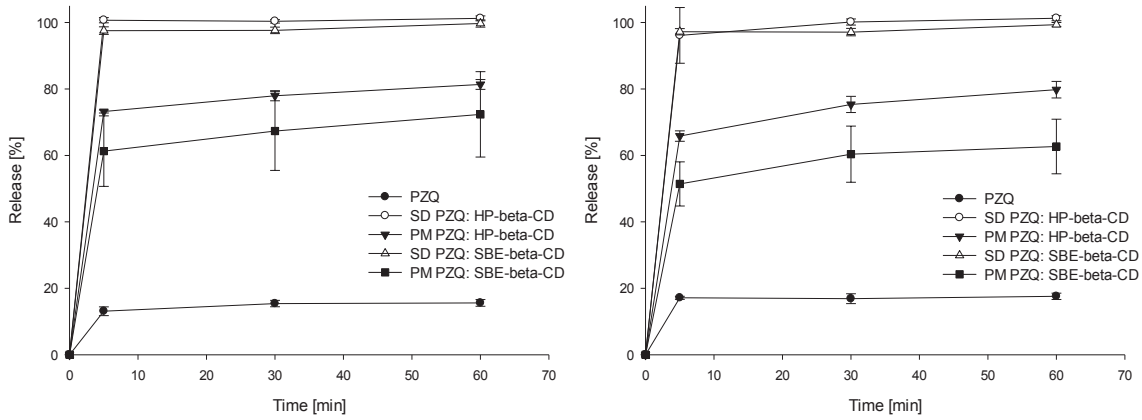


Figure 10. Praziquantel (PZQ) release of spray-dried (SD) powders with hydroxypropyl-beta-cyclodextrin (HP-β-CD) and sulfobutylether-beta-cyclodextrin (SBE-β-CD) in spSGF and FaSSIF in comparison to crystalline PZQ and the physical mixtures (PM). Arithmetic mean ± S.D. (n = 6).

The stability was investigated during storage at 40°C and 75% relative humidity (r. h.) for up to 8 weeks. Non-sink dissolution and DSC measurements were performed directly after production (t0) and after 1 (t1), 2 (t2), 4 (t4) and 8 (t8) weeks (**Figure 11**, **Figure 12** and **Figure 75** in appendix). No recrystallization of PZQ was observed within spray-dried powders during storage leading to stable and reproducible dissolution profiles. There was no statistical significance between values for all storage time points (p = 0.05) for both CDs. DSC results of the PM still revealed crystalline

PZQ. The solubility of the physical mixture of PZQ with SBE-β-CD significantly increased ($p < 0.001$) after 8 weeks but is still significantly lower than the values of the spray-dried powders ($p < 0.001$).

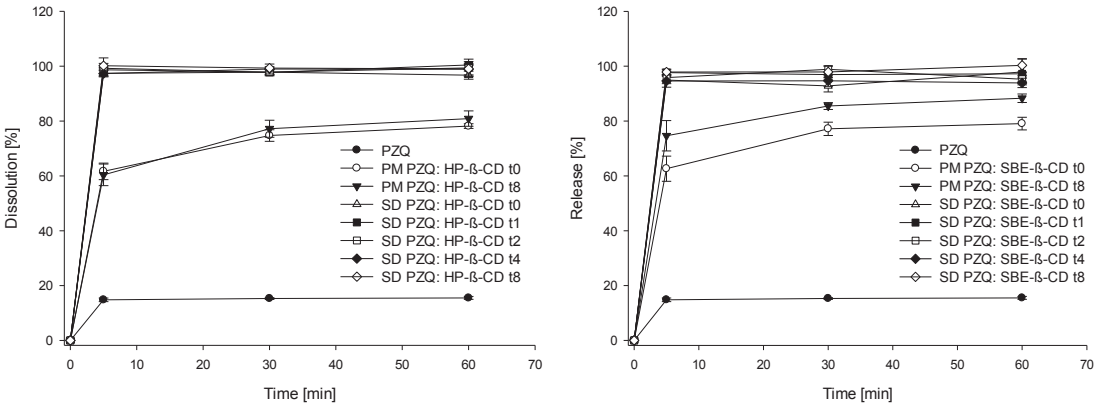


Figure 11. Praziquantel (PZQ) release of spray-dried (SD) powders with hydroxypropyl-beta-cyclodextrin (HP-β-CD) and sulfobutylether-beta-cyclodextrin (SBE-β-CD) in FaSSIF pH 6.5 in comparison to crystalline PZQ and the physical mixtures (PM) directly after production (t0) and after storage of 1 (t1), 2 (t2), 4 (t4), and 8 (t8) weeks. Arithmetic mean ± S.D. (n = 6).

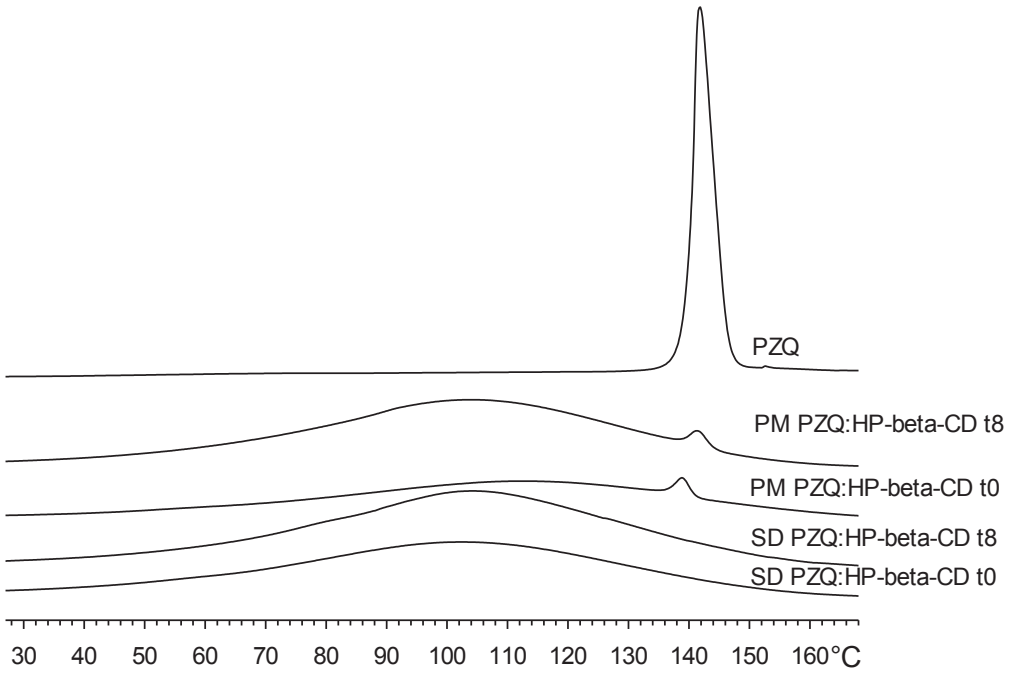


Figure 12. Endothermic thermograms of differential scanning calorimetry of crystalline praziquantel (PZQ), physical mixture (PM) with hydroxypropyl-beta-cyclodextrin (HP-β-CD) and spray-dried (SD) powder directly after production (t0) and after storage of 8 weeks (t8) at 40°C and 75% r. H..

As a summary, spray-drying of complexes of PZQ with both CDs prepared in solution provided a more efficient bonding in comparison to a physical mixture resulting in a higher and faster release and solubility with no detectable crystalline PZQ. Stability data proved that CDs are a stable system to complex PZQ enabling a fast and stable drug solubilisation.

3.1.2. Taste assessment of liquid dosage forms

As described in the introduction (1.5), there are various *in vitro* and *in vivo* taste assessment tools representing alternatives to human taste panels. The electronic tongue and the BATA model were chosen as the most promising taste screening tools. Firstly, the applicability of both methods was evaluated for the pure compound PZQ. Secondly the efficacy of the BATA model was evaluated by comparing PZQ taste masking capabilities of the aforementioned MD and CDs.

3.1.2.1. Electronic tongue

Within this study the applicability of the electronic tongue as a taste assessment tool was investigated for PZQ with the commercially available system TS-5000Z (Insent, Atsugi-chi, Japan). This electronic tongue was chosen due to the previous shown successful performance qualification according to ICH guideline Q2 in Woertz *et al.* [77]. It is composed of a sensor unit with a sample table with two circles of sample positions, two sensor heads with up to eight sensors at a robot arm and a data recording system. The measurement principle of this electronic tongue is potentiometric with an Ag/AgCl reference electrode attached to each sensor head [79]. Each sensor is associated with a specific taste: sourness, bitterness, umami, sweetness and saltiness. This is based on different compositions of the lipid membranes of the sensors and their resulting sensitivity for the detection of groups of molecules [83]. As an example, the sensors SB2AC0 and SB2AN0 consist of anionic lipids (phosphoric acid di-n-decyl ester), thus more applicable for cationic substances. The results are obtained as voltage changes in the membrane potentials in relation to a reference solution, recorded as mV values. In the measurement procedure the sensors are firstly immersed in the reference solution (30 mM potassium chloride and 0.3 mM tartaric acid in distilled water) and afterwards in the sample solution. The difference of both potentials determines the intensity of the tested sample based on the interaction of the dissolved molecules in water with the lipid membranes of the sensors [176]. In accordance to the human taste the activity of the compound logarithmically affects the measured signals based on the Nernst equation (U = electrode potential, U^0 = standard electrode potential, R = universal gas constant, T = temperature, z = ionic valence of the substance, F = Faraday constant, a_i = activity of the substance) [79].

$$U = U^0 + RT/zF \ln a_i \qquad \text{Eq. 5.}$$

Previous studies confirmed a logarithmic concentration sensor response as the characteristic relationship for the Insent electronic tongue. This involves that above a specific threshold there is no clear differentiation of the intensity anymore [77].
After a short rinsing of the sensors the potential is measured again to evaluate the adsorption of substances on the sensors imitating the adsorption of bitter or astringent molecules on the human tongue. This is defined as aftertaste or change of membrane potential caused by adsorption (CPA) [83].

The electronic tongue can be used to evaluate various substances and complex formulations. As a prerequisite a calibration for the pure compounds needs to be performed proving a concentration dependent signal. Subsequently mixtures can be compared regarding their taste masking using multivariate data analysis [80]. An external standard is recommended to obtain reliable data, as the sensor response is affected by the environment, e.g. the temperature, and the age of the sensor [76]. In this study quinine hydrochloride (0.5 mM) was used as the external standard.
It has been reported that the detection of non-ionic substances by the commercially available sensors is limited and that the labelling for a specific taste is not necessary appropriate for all molecules [76]. Therefore new laboratory developed electronic tongue sensors were applied in addition to commercially available sensors.

3.1.2.1.1. Taste assessment of praziquantel using the electronic tongue

As mentioned before, a preliminary calibration with the pure compounds is mandatory for a reliable analysis and comparison of multicomponent systems. Pure PZQ was tested with four different concentrations ranging from 0.01 mM to 0.5 mM in distilled water to generate a calibration curve by means of the electronic tongue. The concentration range was comparable to other studies with other APIs [75]. A solution of 0.5 mM quinine hydrochloride was used as an external standard and as the first samples in the experiment set. The detection was evaluated with commercially available sensors (SB2AC0, SB2AN0, SB2AAE, SB2CT0, SB2CA0 and SB2BT0) and self-developed sensors named as sensor A to G (composition described in **Table 17**). The experimental procedure is described in detail in 6.2.3.1.
The sensor responses were determined as the change of the membrane potential (**Figure 13**). Depending on the composition of the sensor membrane negative or positive responses were obtained [83]. None of the sensors supplied a concentration-dependent signal for PZQ in the full concentration range. The commercially available sensors SB2AC0, SB2AN0 and SB2CA0 indicated a log-linear response from 0.01 to 0.1 mM, but not for 0.5 mM limiting the usability. This can be explained by the non-ionic character and the low solubility of PZQ in water resulting in only minor effects on the membrane potential of all tested sensors. The change of membrane potential as the response of the sensor to a sample solution can be generated by different mechanisms. The initial membrane potential of each sensor is formed when the lipid membrane is immersed in the reference solution. An electrical double layer is created at the surface depending on the composition of the incorporated lipids in the membrane. On the one hand, compounds in the sample solution can affect the membrane potential by ions directly altering the double layer. On the other hand, molecules can prevent lipid molecule dissociation which is responsible for the initial membrane potential. Furthermore, bitter compounds adsorb to the hydrophobic parts of the lipid membrane modifying the potential due to a varied charge density [83, 176]. Thus, a limited detection of the sensors has been expected for the non-ionic PZQ providing too little conductivity. Furthermore, it could be assumed that the adsorption of PZQ on the lipid membranes of all sensors is too low for reliable interaction and resulting measurement signals. Difficulties regarding the detection of other

neutral compounds have been reported before for ibuprofen, caffeine [76] and acetaminophen [77]. A higher concentration of PZQ could have improved the results but could not be provided due to the limited and pH independent solubility of PZQ in water. Modifying the media of the sample solution was not considered as previous studies demonstrated a shift to higher detection limits when evaluating different pH values and ionic concentrations [177]. As a consequence the detection of PZQ needs to be improved by developing sensors providing a higher sensitivity for the compound [178].

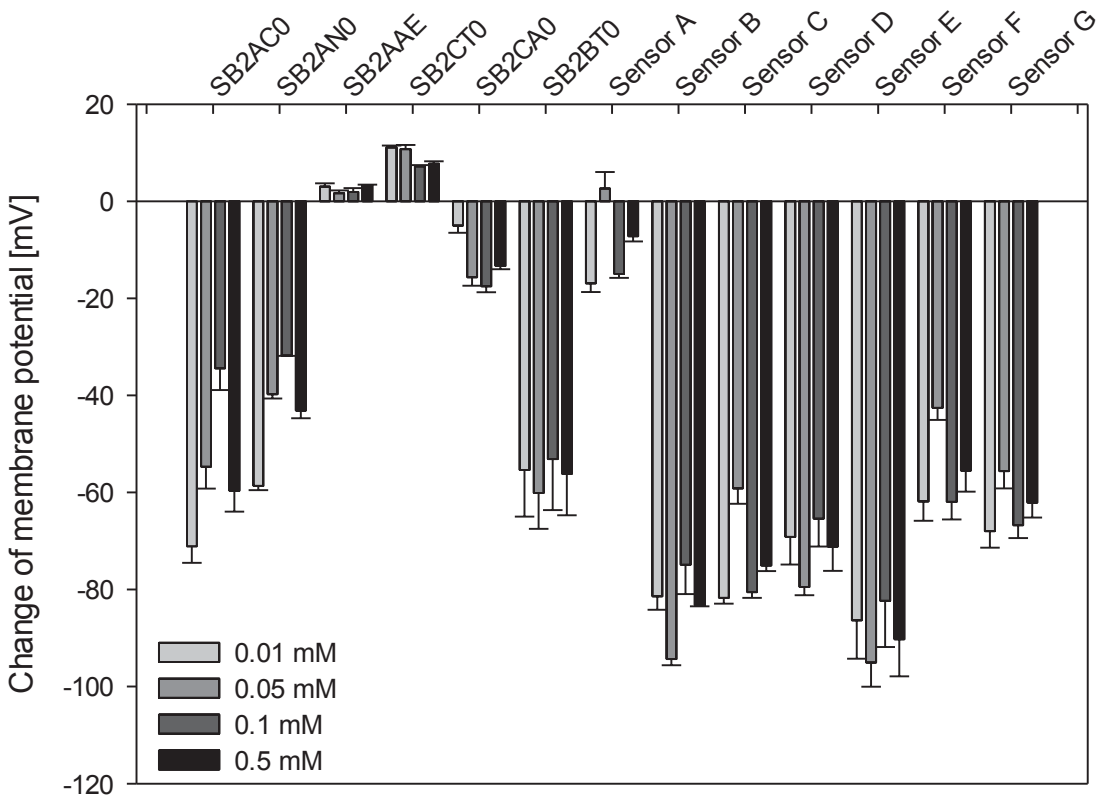


Figure 13. Calibration for electronic tongue. Sensor signals of four different concentrations of praziquantel in distilled water (0.01 – 0.5 mM). Arithmetic mean ± S.D. (n = 3).

The change of membrane potential caused by adsorption or so called aftertaste sensor responses (**Figure 14**) were smaller for the majority of the sensors due to the washing step in between. Sensor signals before and after washing were significantly ($p < 0.05$) different except for the sourness sensor SB2CA0 ($p > 0.05$). The highest difference between measurements before and after washing were observed for the cationic bitterness sensor SB2AC0. At 0.5 mM CPA values were more than 8 times lower compared to the change of membrane potential before washing ($p < 0.001$). This could indicate an interaction of PZQ with SB2AC0. Nevertheless none of the sensors provided a concentration-dependent response. As similar to the previous experiment (**Figure 13**) deviations between concentrations for each sensor are below the measured standard

deviations and do not provide additional insights regarding the taste intensity of PZQ or the reasonable applicability of one of the sensors in this study.

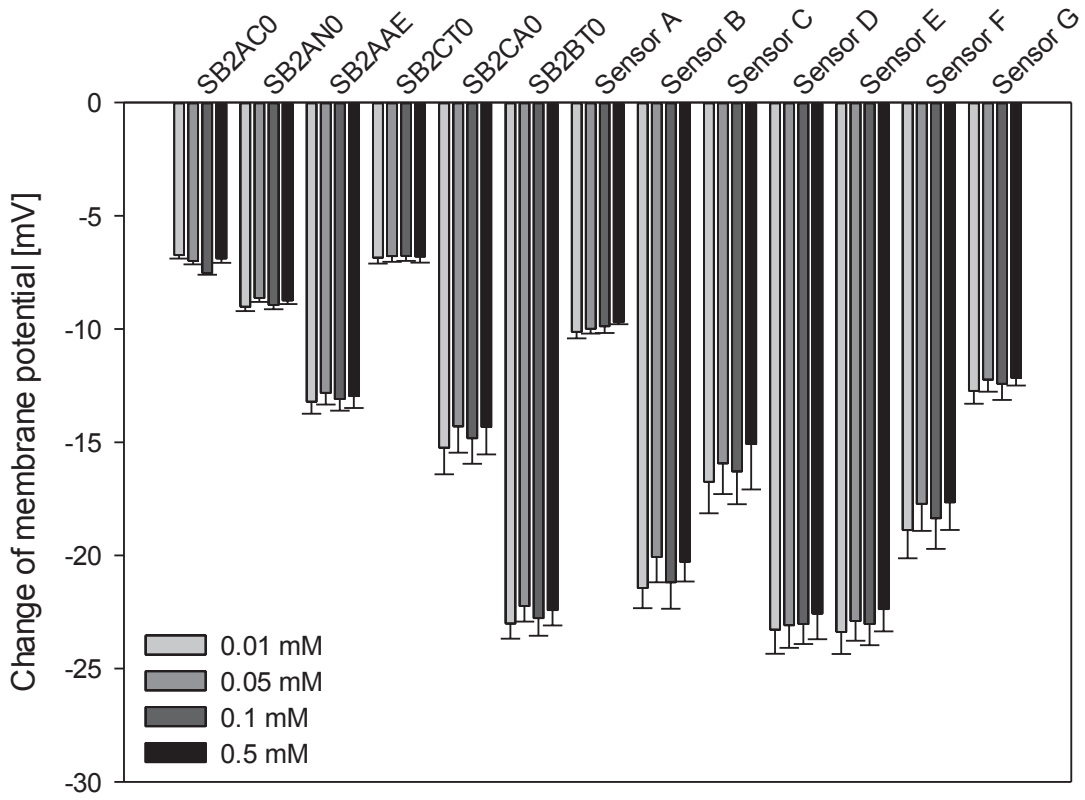


Figure 14. Measurement of aftertaste of electronic tongue. Change of membrane potential caused by adsorption (aftertaste) of four different concentrations of praziquantel in distilled water (0.01 – 0.5 mM). Arithmetic mean ± S.D. (n = 3).

As a conclusion the electronic tongue as an alternative taste assessment tool to *in vivo* taste studies is not applicable for PZQ so far. None of the sensors provided reliable results for the further evaluation of multicomponent systems. Thus, another described taste assessment tool was tested, the BATA model.

3.1.2.2. Rodent brief-access taste aversion model

Animal models such as the BATA model have demonstrated their suitability among several *in vivo* and *in vitro* taste assessment tools. Besides the electronic tongue and in terms of readiness, the rodent brief-access taste aversion (BATA) model is the most useful non-human taste assessment tool. Indeed various parameters (necessary time for data collection, ability to screen pure drugs and formulations, correlation to human *in vivo* data, validation potential and costs) were graded higher comparatively to other tools by Mohamed-Ahmed *et al.* [61]. Several studies showed the great potential of rat models designed for the measurement of the palatability of different compounds [66-69] and the correlation to human taste panels [70-73] revealing the same ranking order of aversive compounds like the human taste panels.

In the BATA model, samples are presented randomly to rats or mice in several sipper tubes and the number of licks recorded electronically by a lickometer is inversely proportional to the aversiveness of the samples [64]. Within a short time period of a few seconds the rodents can lick each solution separately. A compound with an aversive taste decreases the number of licks in comparison to the reference defined as deionized water [64]. The rodents can lick each solution separately for a few seconds. An E_{max} model is used to analyze the results. As mentioned for the electronic tongue, a prerequisite for the analysis of taste masked formulations is the analysis of pure compounds and a successful concentration-dependent signal. Lick numbers, lick ratios and the concentration of the compound that suppresses 50% of the licks in comparison to water (IC_{50}) are identified [65].

In the product development of a compound it is valuable to screen molecules regarding their taste as early as possible, ideally at the pre-candidate stage. Due to toxicology reasons human studies are not possible at that stage, whereas the BATA model enables a reasonable alternative [73].

Within this study the applicability of the BATA model was evaluated for the aversive compound PZQ and solutions with CDs or maltodextrin as taste masking agents. 6 different concentrations of PZQ were evaluated up to its maximum solubility (0.2 mg/ml). Kleptose® linecaps 17 was chosen as the most promising MD due to its high amylose content according to the supplier [179] and previous promising results regarding taste masking [88, 142].

3.1.2.2.1. Taste assessment of praziquantel using the BATA model

A mean value of 44.7 licks ± 1.1 was observed for deionized water as the reference of all 10 rats during both testing days of the pure compound. The water-deprivation of 22 h was suitable to encourage the rats to drink from the various sipper tubes.

As a calibration, 6 different concentrations of PZQ were tested in deionized water. The number of licks decreased with increasing concentration of PZQ (**Figure 15** and **Figure 16**). PZQ was fully tolerated and did not show a significant decrease in number of licks at the concentration of 0.005 mg/ml (45.3 ± 0.9 licks, 0 ± 1.2% lick inhibition) and 0.01 mg/ml (43.6 ± 1.2 licks, 1.2 ± 2.4% lick inhibition) in comparison to deionized water ($p = 0.896$ and 0.206, Kruskal-Wallis test). For a higher concentration of 0.03 mg/ml a significant decrease in lick number (32.9 ± 1.7 licks) was observed ($p < 0.001$, Kruskal-Wallis test), but was still well tolerated (26.4 ± 3.9% lick inhibition). An increase to 0.05 mg/ml PZQ was tolerated (34.6 ± 4.1%) with 29.2 ± 1.8 licks. The taste of PZQ was perceived aversive at 0.1 and 0.2 mg/ml (19.1 ± 1.5 licks, 57.3 ± 0.2% lick inhibition and 12.5 ± 1.2 licks, 72.0 ± 2.6% lick inhibition).

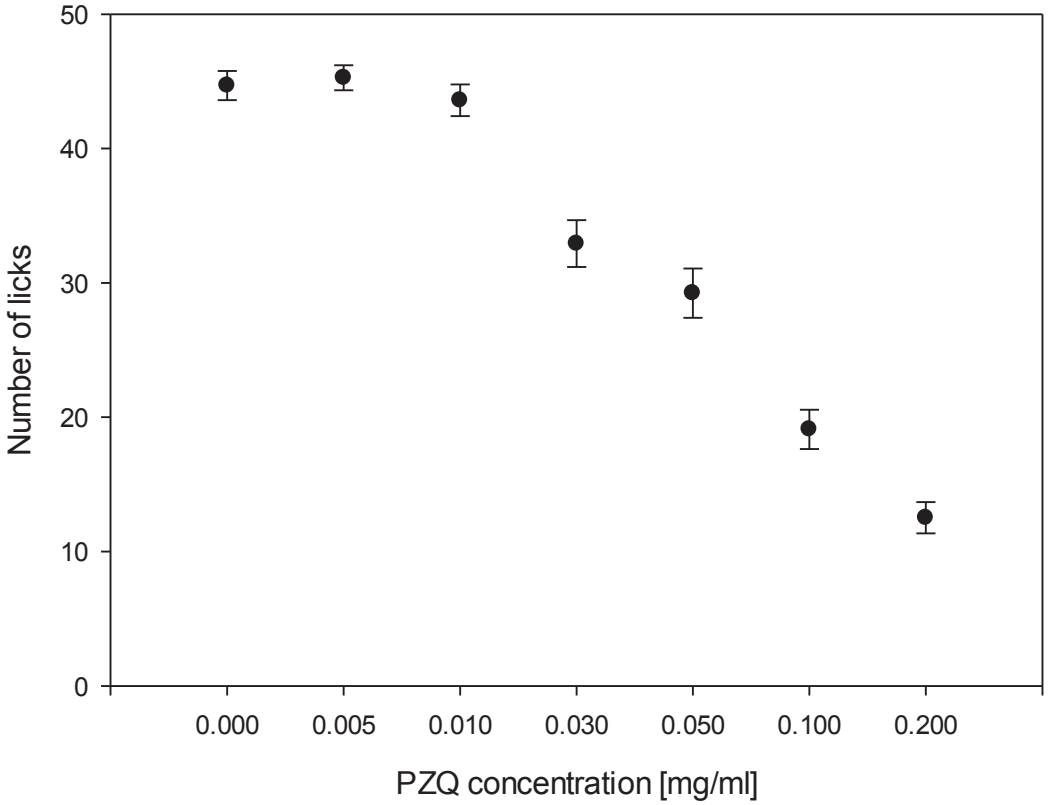


Figure 15. Calibration for BATA model. Recorded number of licks in BATA model as a function of praziquantel (PZQ) concentration in water. Arithmetic mean (n = 80) ± S.E.M..

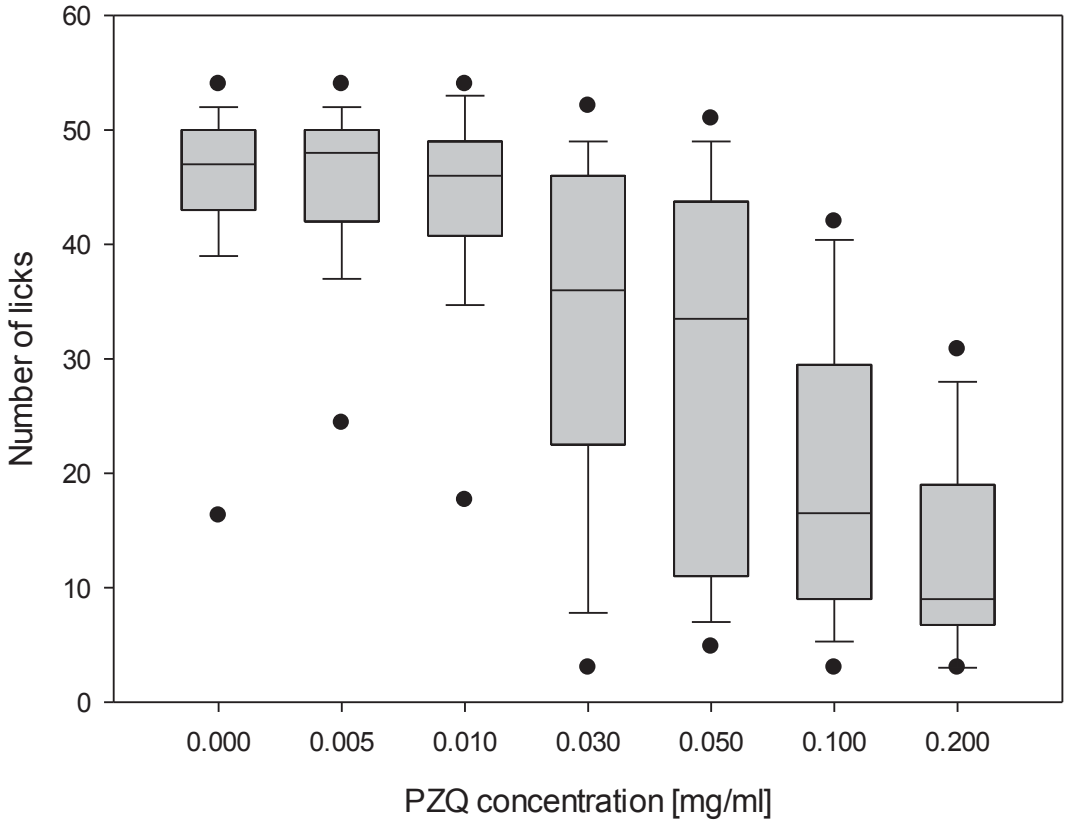


Figure 16. Calibration for BATA model. Box plot diagram of recorded number of licks in BATA model as a function of praziquantel (PZQ) concentration in water representing the median, 1st and 3rd quartile and 5th and 95th percentile (n = 80).

The IC_{50} value was determined as 0.06 mg/ml (95% CI 0.049-0.082). Thus PZQ showed an extreme aversive taste in the range of quinine hydrochloride [65], worse than other bitter compounds such as sildenafil citrate, caffeine citrate, diclofenac or paracetamol [64, 74]. These findings underline the importance of the taste masking for PZQ for the in use palatability of drug products.
The individual lick profiles for each rat demonstrate the sensitivity of all rats for PZQ (**Figure 76** in appendix). All rats responded with a decreasing number of licks with an increasing PZQ concentration. This is of high importance as it is well-known that mammalians vary greatly in their taste perception of bitter compounds due to a variability in receptor gene expression in the mouth [53, 180]. The variability of both testing sessions (day 1 and day 2) was not significant ($p > 0.1$, Kruskal-Wallis test) (**Figure 77** in appendix). Thus the rats did not get used to the aversiveness of PZQ. Furthermore this low variability strengthens the reliability of the results.

Three different concentrations of PZQ were tested with 20 mM MD or CDs. For comparison with pure PZQ in deionized water, the PZQ concentrations in the mixtures were set to the IC_{50} value of 0.06 mg/ml, the maximum solubility of the pure PZQ of 0.2 mg/ml and the maximum reachable PZQ concentration of 0.27 mg/ml in solution with MD and 1.3 mg/ml in solution with CDs.

The taste of pure 20 mM HP-β-CD (55.5 ± 1.2 licks) was similar to water (56.7 ± 1.4 licks) ($p = 0.149$, Mann-Whitney test) (**Figure 17** and **Figure 18**). However, pure 20 mM SBE-β-CD did significantly decrease the lick number to 43.2 ± 2.4 ($p < 0.001$, Mann-Whitney test). Yet, the profiles for formulations at 0.06 and 0.2 mg/ml indicate a taste masking effect for both CDs. The lick numbers measured at 0.06 mg/ml for PZQ with HP-β-CD (53.5 ± 1.8 licks) and SBE-β-CD (44.0 ± 2.2 licks) did not significantly differ from the pure excipient solutions ($p = 0.119$ and 0.616, Mann-Whitney test) indicating that these formulations were well tolerated by the rats (**Table 2**). In contrast, the pure PZQ was aversive/ untolerated at 0.06 mg/ml (IC_{50} value). The measured lick numbers significantly decreased for both CD formulations of 0.2 and 1.3 mg/ml ($p < 0.001$, Mann-Whitney test), but were better accepted at 0.2 mg/ml than the pure PZQ solution. The complexation of PZQ with CDs improved the solubility of PZQ to 1.3 mg/ml but did not achieve a taste masking. As the complexation of PZQ and CDs is an equilibrium, free drug molecules are in solution [152]. The amount of this free uncomplexed molecules increased with increasing drug concentration which might have lead in turn to a higher taste perception in the rats.

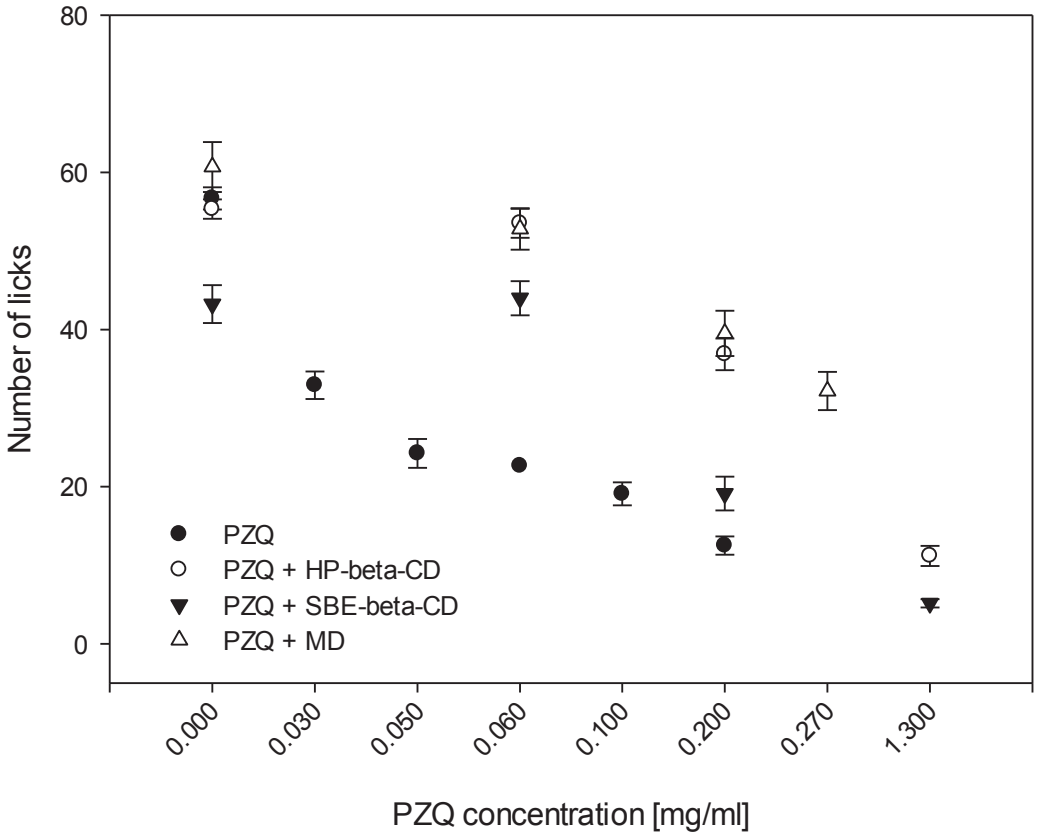


Figure 17. Comparison of taste masking efficacy in BATA model. Recorded number of licks in BATA model as a function of praziquantel (PZQ) concentration of pure PZQ solution and the solutions with hydroxypropyl-beta-cyclodextrin (HP-beta-CD) and sulfobutyl ether-beta-cyclodextrin (SBE-beta-CD) and maltodextrin (MD). The concentration of 0.000 mg/ml presents pure water in the case of PZQ and pure 20 mM CDs or MD in the case of the formulations. Arithmetic mean (n = 80) ± S.E.M..

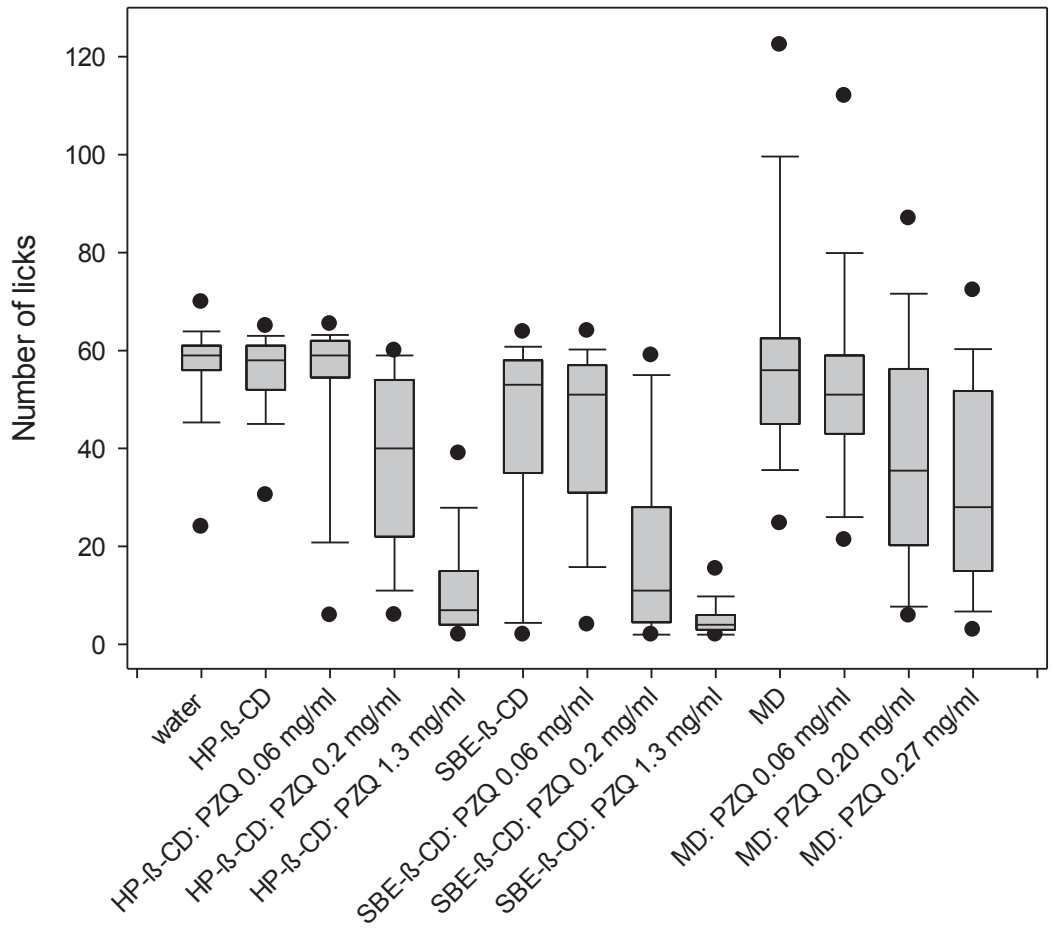


Figure 18. Comparison of taste masking efficacy in BATA model. Box plot diagram of recorded number of licks in BATA model as a function of praziquantel (PZQ) concentration in water. Compared are pure PZQ, complexes with hydroxypropyl-beta-cyclodextrin (HP-beta-CD) and sulfobutyl ether-beta-cyclodextrin (SBE-beta-CD) and the maltodextrin (MD) Kleptose® linecaps. Displayed are the median, 1st and 3rd quartile and 5th and 95th percentile (n = 80).

HP-β-CD showed a significant higher numbers of licks ($p < 0.001$, Mann-Whitney test) than SBE-β-CD at all concentrations. These findings are contrary to the results of the phase solubility study. SBE-β-CD led to a higher linear increase of the solubility of PZQ resulting in a higher $K_{1:1}$ and CE than HP-β-CD (**Figure 4**). Thus it was assumed that this higher affinity of PZQ to SBE-β-CD would lead to a higher taste masking efficiency. However, the interaction and complexation of PZQ with the CDs is a rapid equilibrium with free drug and CD molecules. Arrua *et al.* [132] described the presence of free PZQ in complexes with CDs. Moreover, CDs can form inclusion and non-inclusion complexes where the compounds interaction is located on the surface of the CD [150]. In this way the compound is not shielded completely for taste perception and the affinity of the drug to the CD might not be solely representative for the taste-masking effect. The analysis of the solid state revealed no free crystalline PZQ after spray-drying, but did not determine the location of the interaction. In addition, the results of the pure excipients outlined an aversiveness of the pure SBE-

β-CD in comparison to the pure HP-β-CD. In this study, the phase solubility results were not predictive for the *in vivo* taste assessment in the BATA model.

The MD did not affect the number of licks in comparison to water ($p = 0.810$, Mann-Whitney test) (**Figure 17** and **Figure 18**). The solution was fully tolerated according to the % lick inhibition (**Table 2**). In contrast to our expectations based on the phase solubility study, the BATA model revealed a taste masking effect of the MD up to 0.06 mg/ml PZQ. The result for this PZQ concentration did not differ significantly from the pure excipient ($p = 0.092$, Mann-Whitney test) and was well tolerated. Increasing the concentration of PZQ up to 0.2 and 0.27 mg/ml decreased the number of licks significantly to 39.5 ± 2.9 and 32.2 ± 2.4 licks ($p < 0.001$, Mann-Whitney test), but the solutions were still classifiable as tolerated. This can be explained by the helical structure of the incorporated amylose providing a barrier between PZQ and the taste buds in the mouth of the rats resulting in a decreased taste perception [145]. As previously mentioned MDs can provide inclusion complexes by hydrophobic and van der Waals' interactions and can encapsulate hydrophobic drugs just as CDs [146-148]. In the case of PZQ this did not affect the solubility of PZQ but could have resulted in the lower aversiveness of the solutions. Moreover the high amount of MD in the solution could also have led to an increased viscosity and consequently to a taste masking effect drug by decreasing the diffusion of PZQ to the taste buds [2]. It should be considered that the molecular weight of the MD is much higher in comparison to the CDs. Solutions with CDs contained 2 – 4% (w/w) in comparison to 25% (w/w) with MDs. Nevertheless, as there is no limited daily intake for MDs the concentration could even be increased to 30 to 40% (w/w) as it is common in syrups.
The taste masking of 0.06 mg/ml PZQ was achieved by using 20 mM of either MD or HP-β-CD. SBE-β-CD was significantly less effective ($p < 0.001$). At higher concentrations (0.2 mg/ml), there was significant reduction of licks for all the formulations regardless of the taste masking agent used. However, the formulation with SBE-β-CD was still significantly less useful compared to the other formulations containing MD or HP-β-CD ($p < 0.001$, Mann-Whitney test). Formulations with MD and HP-β-CD were tolerated by the rats with no significant difference between the number of licks ($p = 0.865$, Mann-Whitney test).

Table 2
Comparative percentage of lick inhibition of pure praziquantel (PZQ) in water and with the addition of 20 mM hydroxypropyl-beta-cyclodextrin (HP-β-CD), 20 mM sulfobutyl ether-beta-cyclodextrin (SBE-β-CD) and 20 mM maltodextrin (MD).

PZQ concentration [mg/ml]	Pure PZQ	PZQ + HP-β-CD	PZQ + SBE-β-CD	PZQ + MD
	Percentage of lick inhibition			
0		2.4 ± 2.2	23.9 ± 4.3	-4.3 ± 5.5
0.06	50 (IC_{50})	5.6 ± 3.3	22.4 ± 3.9	9.3 ± 4.6
0.2	72.0 ± 2.6	34.9 ± 3.6	66.3 ± 4.2	32.1 ± 5.0
0.27				44.7 ± 4.2
1.3		80.3 ± 2.3	90.9 ± 0.9	

3.1.3. Summary and outlook

Different taste assessment tools were applied to evaluate the taste masking efficiency of CDs and MD for PZQ. The electronic tongue and the BATA model were chosen as the most useful non-human taste assessment tools for liquid dosage forms. The evaluated sensors of the electronic tongue in this study were not applicable for the aversive compound PZQ due to the non-ionic characteristic and the low solubility of PZQ in water. None of the tested sensors provided conclusive responses for PZQ and hence for further evaluation of multicomponent systems. In contrast, the rats in the BATA model demonstrated a concentration-dependent sensitivity to PZQ aversiveness leading to an IC_{50} value of 0.06 mg/ml. During the experiment a maximum concentration of 0.01 mg/ml of PZQ in water was identified as well tolerated with no significant difference to pure water. The BATA model was shown to be a useful taste assessment tool for PZQ for the comparison of formulations at an early stage in development, avoiding challenging human taste panels.

Based on these findings the taste masking efficiency of liquid formulations for PZQ was evaluated and the use of MD was compared to two CDs. Despite the fact that the phase solubility study identified SBE-β-CD as superior to HP-β-CD and the MD, the BATA model revealed the MD as efficient as both CDs to mask the aversive taste of PZQ. Moreover, SBE-β-CD was significantly less useful compared to the other formulations containing MD or HP-β-CD in the BATA model accompanied with a less acceptable taste of the pure excipient solution. The increased drug solubility due to complex formation with both CDs did not offer an additional benefit as both solutions were assessed as highly aversive or highly untolerated.

With regard to the final dosage form MDs would be preferable over CDs due to various reasons such as excipient costs and safety. A liquid formulation development with CDs would not be favorable due to the necessary high volume (> 27 ml/kg bodyweight) of one dose of PZQ. Assuming a single dose of 40 mg/kg bodyweight of PZQ, already a child of 10.3 kg (2 years) would exceed the acceptable daily oral dose for HP-β-CD of 8 g/day. Maltodextrins with no limited daily intake offer a promising alternative in drug product development especially for liquid dosage forms, e.g. in terms of a syrup. As MDs are more harmless excipients than CDs, they could provide a viable alternative in terms of taste-masking of liquids.

3.2. Solid dosage forms

Considering suitable solid oral dosage forms for children, adults and elderly, MTs and ODMTs were chosen as the most flexible and acceptable dosage forms (as described in 1.2). For this purpose, a variety of excipients were examined for the direct compression to MTs and ODMTs. In addition, different analytical equipment and methods were tested regarding the applicability for MTs.
Various taste masking strategies can be applied for solid dosage forms as described in the introduction (1.6, **Figure 2**). The focus was set on the encapsulation and embedding of PZQ in a polymer and lipid matrix via extrusion and spray-drying to efficiently taste mask the API. The most suitable polymer was identified via a small scale solvent casting method. Finally, the taste masked formulations were processed to MTs. Sweeteners and flavors were excluded within this study as they only obscure the taste and need to be optimized on a case by case basis.

Parts of this chapter have been published as:
Münster. M., Schoch, C., Schmidt, C., Tuleu, C., Breitkreutz, J., 2017. Multiparticulate system combining taste masking and immediate release properties for the aversive compound praziquantel. Eur. J. Pharm. Sci. 109, 446-454.
DOI: 10.1016/j.ejps.2017.08.034

3.2.1. Tableting of orodispersible mini-tablets

MTs are defined as small tablets with a diameter of maximum 3 mm [24]. The target diameter of this work was set to 3 mm, as Praziquantel has a dose of 40 – 60 mg/kg [48] and the tablet mass increases exponentially with increasing diameter. The mass of a tablet is proportional to the volume, calculated for a flat tablet from the radius (r) and the height (h):

$$V = \pi * r^2 * h \qquad \text{Eq. 6.}$$

In this way, the use of 3 mm MTs highly decreases the number of tablets that are needed for a single dose of PZQ and simplifies the administration. Moreover, ODMTs were aimed, as they rapidly disintegrate into small particles in the saliva and improve the ease of ingestion of the medicines to children and adults with swallowing difficulties [33].
The production of the ODMTs was conducted via direct compression as the most time and cost saving method [181]. Therefore, powder mixtures needed a good flowability to achieve consistent filling of the tablet dies resulting in low variability regarding the tablet weight, drug content and crushing strength during tableting. A high compactibility was required for a good mechanical stability and low friability combined with a fast disintegration and drug release. The aim was to identify the most suitable excipients compromising all parameters for ODMTs. Parteck® M100 and Flowlac® 100 were chosen as water-soluble excipients enabling a fast disintegration. Prosolv® ODT and

Emdex® + Prosolv® SMCC are ready-to-use formulations for the development of ODTs. Prosolv® ODT was adjusted mixing Parteck® M100 and Vivapur® 102.
As mentioned before (1.2), MTs are not differentiated from larger sized tablets in terms of regulatory aspects and quality attributes. Nevertheless, the small size of MTs is accompanied with common analytical equipment for tablets. Thus, varying analytic methods were examined regarding the applicability for MTs.

3.2.1.1. Formulations and tableting

The composition of formulations is described in **Table 3**. Kollidon® CL-SF was chosen as it has been shown to be an effective disintegrant from 2 to 5% (w/w) for robust ODTs with adequate disintegration times, hardness and friability [23]. All powder mixtures were analyzed regarding their flowability and compressibility as described in 6.2.2.11.
According to Jenike [182] all formulations were free-flowing (ffc = 4 – 10) except Emdex® + Prosolv® SMCC with a flow function of 20.1 (easy-flowing). Flowlac® 100 and Emdex® are spray-dried excipients providing spherical particles with a narrow particle size distribution from 100 – 200 µm resulting in the best flow properties of the mixtures.
Parteck M®100 and the mixture Parteck® M100 + Vivapur® 102 showed the highest compressibility as those powders exhibited the lowest bulk densities according to the supplier's information: 0.5 – 0.6 mg/ml for Parteck M®100 and 0.28 – 0.33 mg/ml for Vivapur® 102. The results for compressibility for Flowlac® 100 and Prosolv® ODT are in the same range with bulk densities of approximately 0.6 mg/ml. The formulation consisting of Emdex® and Prosolv® SMCC exhibited the lowest value accompanied with the highest bulk density of 0.7 g/ml.

Table 3
Percentage composition and characteristics of formulations for tableting.

	Parteck® M100	**FlowLac® 100**	**Prosolv® ODT**	**Parteck® M100 + Vivapur® 102**	**Emdex® + Prosolv® SMCC**
Parteck® M100	94			65.5	
FlowLac® 100		94			
Prosolv® ODT			97		
Vivapur® 102				23.5	
Aerosil® 200				2.5	
Emdex®					83.5
Prosolv® SMCC					13.5
Kollidon® CL-SF	4	4		5.5	
PRUV®	2	2	3	3	3
Flow function coefficient (ffc)	6.5	11.4	9.6	5.2	20.1
Compressibility [%]	17.2	14.6	14.0	15.8	7.1

All formulations were successfully directly compressed into biconvex mini-tablets with a diameter of 3 mm and tablet heights of 2.0, 2.5 and 3.0 mm. Compaction forces from 0.4 to 2.3 ± 0.04 kN were applied.

Table 4 summarizes the tablet weights and relative standard deviations (RSD) (n = 10) for a representative selection of produced MTs with a tablet height of 2.5 mm and compaction forces from 0.8 to 1.5 kN. All values for RSD for the formulations were in the same range. There was a clear correlation to the flow function of each formulation. A high value for ff resulted in a uniform distribution of the powder in the dies of the tablet press during the complete process leading to low variability of the tablet weight. Thus, Emdex® + Prosolv® SMCC, showing the best flow properties, exhibited the smallest RSD followed by Flowlac® 100, Prosolv® ODT, Parteck® M100 and Parteck® M100 + Vivapur® 102 with the lowest ff and highest RSD.

Table 4
Tablet weights and relative standard deviations for tablets with 2.5 mm tablet height.

Fomulation	Compaction force [kN]	Tablet weight [mg]	RSD [%]
Parteck® M100	0.8	16.9	2.6
	1.2	17.5	1.7
	1.5	19.0	1.6
Flowlac® 100	0.8	18.9	2.1
	1.2	19.7	0.9
	1.5	20.5	1.1
Prosolv® ODT	0.8	18.4	1.7
	1.2	19.7	1.7
	1.5	20.8	2.2
Parteck® M100 + Vivapur® 102	0.8	17.5	1.9
	1.2	18.5	2.7
	1.5	19.1	1.4
Emdex® + Prosolv® SMCC	0.8	20.0	1.3
	1.2	21.2	0.8
	1.5	22.1	1.6

3.2.1.2. Crushing force and friability

All tablets (of the formulations and varied compaction forces and tablet heights) were analyzed regarding hardness and friability as described in 6.2.2.12 and 6.2.2.14. The hardness was evaluated with the compact tablet tester MultiCheck V and the Texture Analyser due to the small size of the tablets and expected low results correlated to low compaction forces.

The commonly used tablet tester MultiCheck V could detect values starting from approximately 0.8 N (**Figure 19**, **Figure 20** and **Figure 21**). The hardness of tablets compressed with 0.4 kN of all formulations were not detectable except for Emdex® + Prosolv® SMCC with a tablet height of 3.0 mm. The following ranking can be concluded starting with the hardest tablets: Emdex® + Prosolv® SMCC > Parteck® M100 + Vivapur® 102 > Parteck® M100 > Prosolv® ODT > Flowlac® 100.

The microcrystalline cellulose incorporated in Emdex® + Prosolv® SMCC and Parteck® M100 + Vivapur® 102 provided a high plastic deformation connected to a high compactibility of the mixture and a higher hardness of the tablets. The silicified MCC of Prosolv® SMCC further increases the compaction performance of Emdex® due to a higher surface area compared to MCC as well as the incorporated starch. Obviously this was not the case for Prosolv® ODT. It is possible that Parteck® M100 and Vivapur® 102 enabled higher binding capacities in comparison to the incorporated excipients in Prosolv® ODT. The hardness of Parteck® M100 can be explained by its high surface area. Lactose as the component of Flowlac® 100 exhibited the lowest binding properties due to its mainly crystalline structure [181].

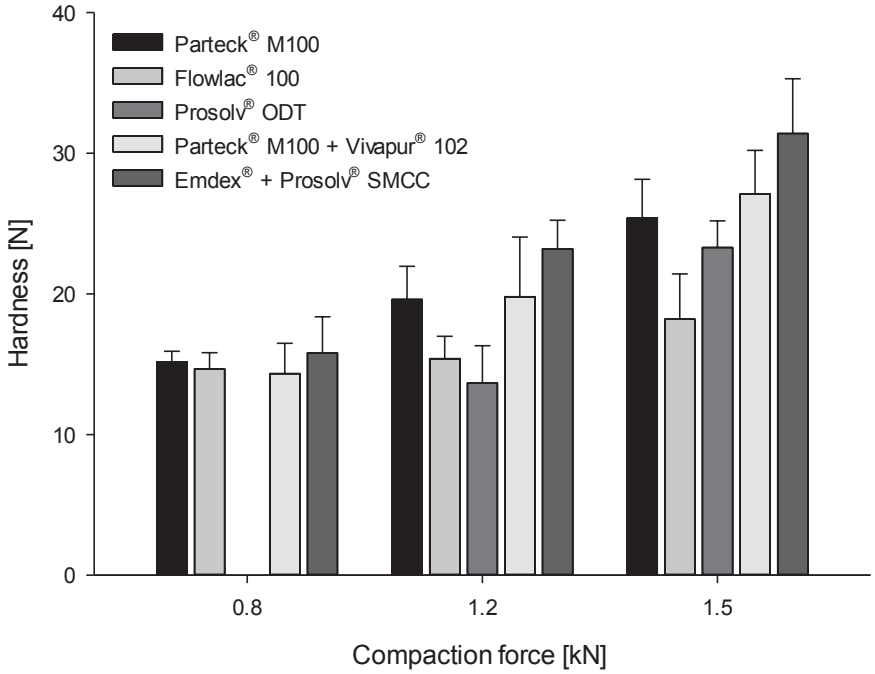


Figure 19. Tablet hardness using MultiCheck V. Comparison of all formulations with 2.0 mm tablet height x 3 mm diameter. Arithmetic mean ± S.D. (n = 10).

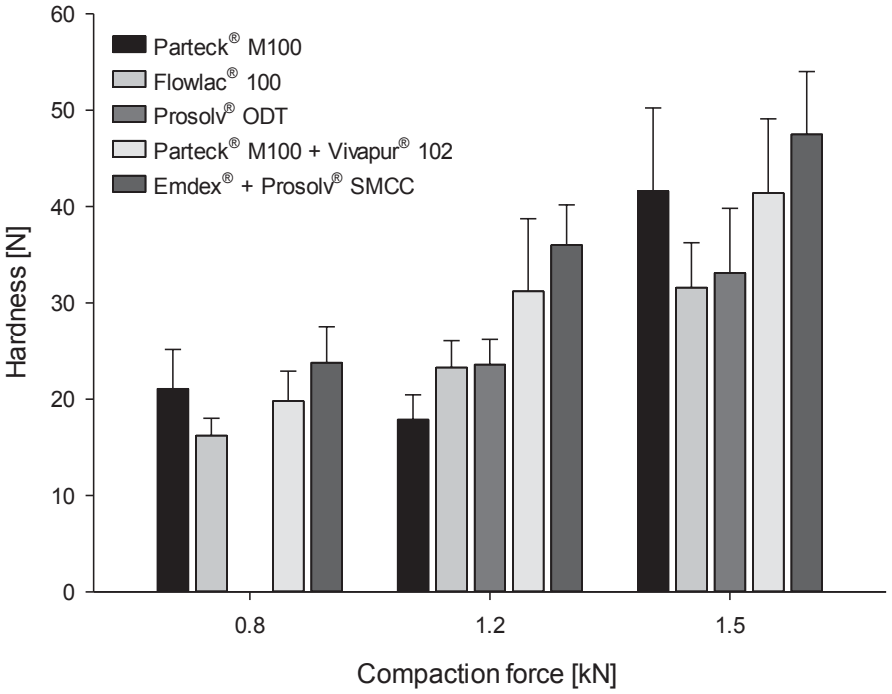


Figure 20. Tablet hardness using MultiCheck V. Comparison of all formulations with 2.5 mm tablet height x 3 mm diameter. Arithmetic mean ± S.D. (n = 10).

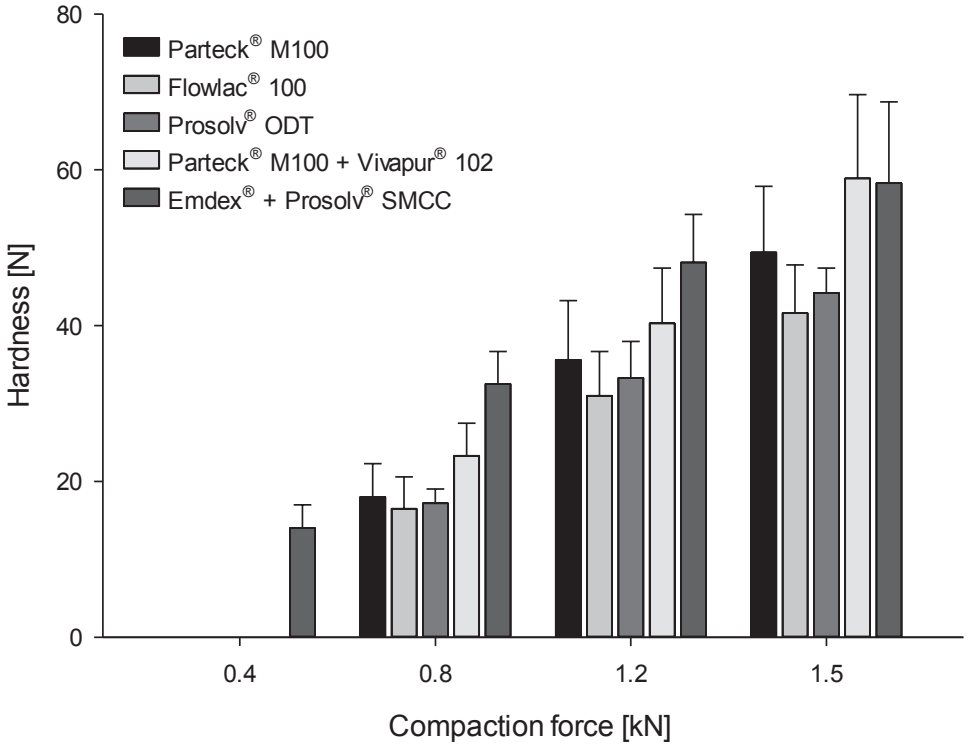


Figure 21. Tablet hardness using MultiCheck V. Comparison of all formulations with 3.0 mm tablet height x 3 mm diameter. Arithmetic mean ± S.D. (n = 10).

As an alternative method the Texture Analyser (6.2.2.12) was used to measure the hardness of tablets. This method enabled the hardness detection of all tablets even when manufactured with a compaction force of 0.4 kN. The comparison of both methods for Emdex® + Prosolv® SMCC is shown in **Figure 22**. The values of the Texture Analyser are slightly smaller than those of the Multicheck V with the same tablet batch, but are within the standard deviations. This is accompanied with the high sensitivity of the Texture Analyser and the slower measurement principle leading to an earlier detection of the breaking point of the tablet at lower forces.

As a conclusion, both methods could be applied for the analysis of MTs. The Texture Analyser could be preferred for very gently compressed tablets in the focus of the production of ODFs. A drawback are longer testing times for each tablet and full batches as the detection is not performed automatically in contrast to the Multicheck V.

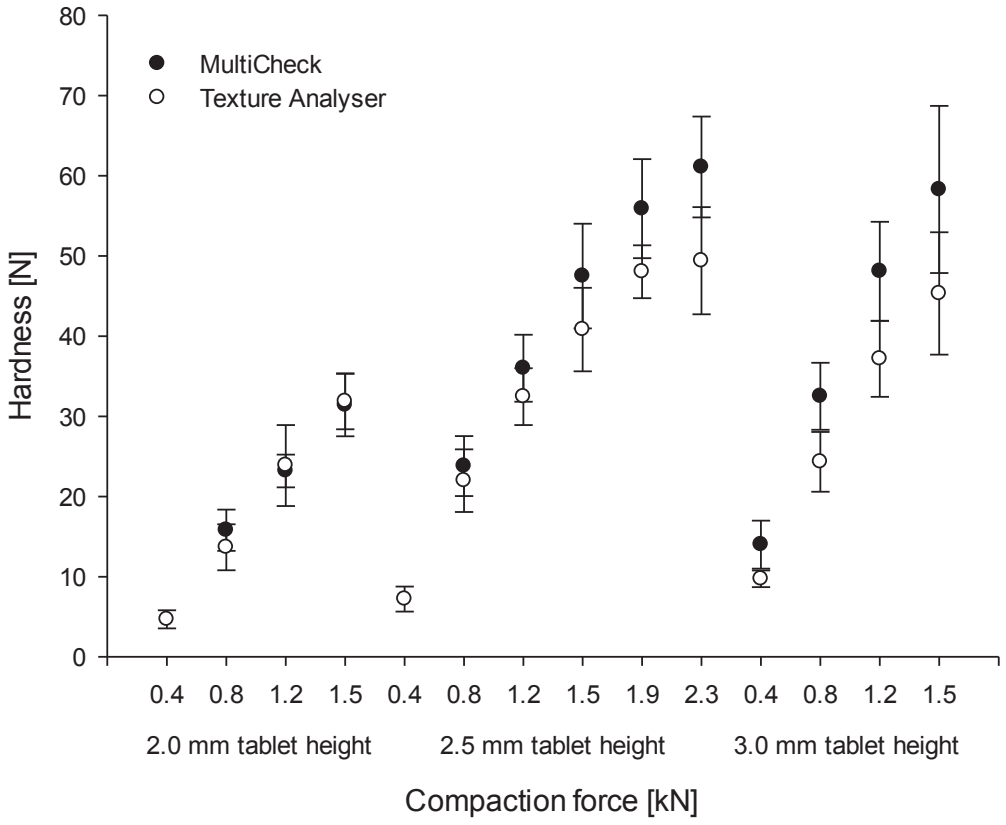


Figure 22. Tablet hardness using MultiCheck V and Texture Analyser. Comparison for Emdex® + Prosolv® SMCC. Arithmetic mean ± S.D. (n = 10).

The mechanical stability was furthermore investigated using a friabilator (6.2.2.14) according to Ph. Eur. 8.0 2.9.7. (**Figure 23**). The friability was below 1.0% for all formulations and batches except for Prosolv® ODT compressed with 0.4 kN. A high number of these tablets broke in the abrasion drum. Choosing a minimum pressing force of 0.8 kN resulted in a strongly decreased abrasion of all tablets.

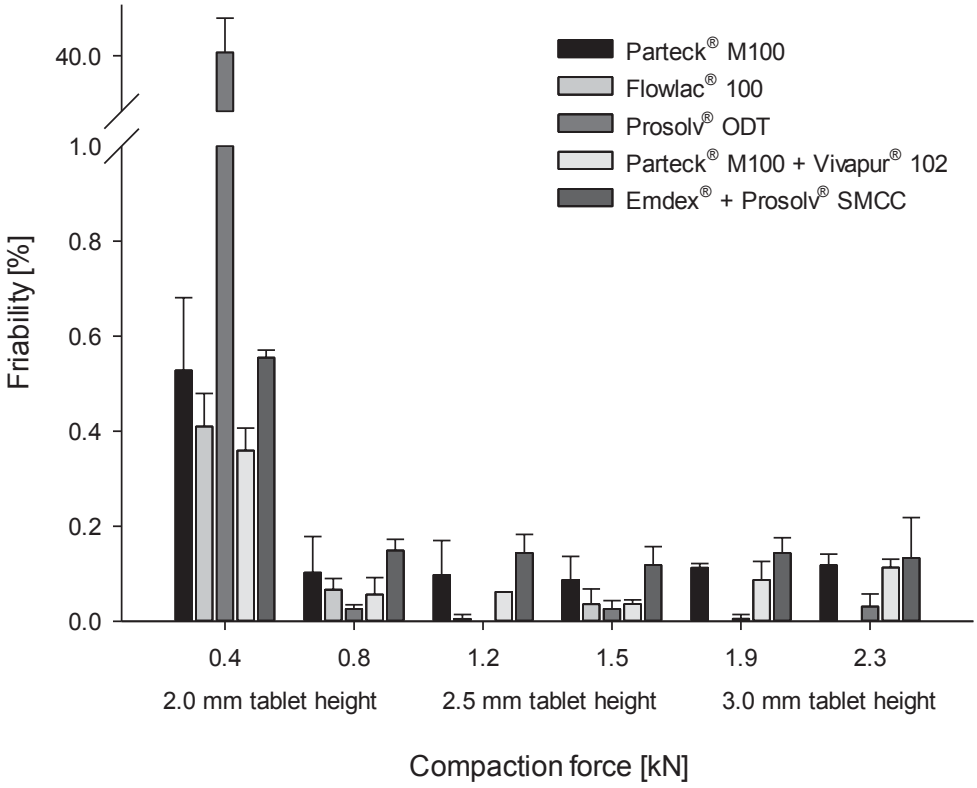


Figure 23. Friability. Summary of all formulations for a tablet height of 2.5 mm. Arithmetic mean ± S.D. (n = 3).

3.2.1.3. Disintegration

The Ph. Eur. defines a fast disintegration for ODFs within 180 s using the method 8.0 2.9.1. The disintegration was tested using the DisiTest 50 and a common basket equipped with a sieve of 10 mesh (distance of 2 mm between wires) (6.2.2.13.1). Due to the small size, 3 mm MTs can pass the sieve before the disintegration is completed. Thus, different methods were evaluated in comparison to the specified procedure of the Ph. Eur. A smaller sieve with 40 mesh (distance of 0.6 mm between wires) was applied (6.2.2.13.1) as well as the Texture Analyser (6.2.2.13.2).

Figure 24, **Figure 25** and **Figure 26** show the results of disintegration of all formulations and different compaction forces in accordance with the Ph. Eur.. The following ranking can be concluded starting with the lowest disintegration time: Parteck® M100 > Flowlac® 100 > Prosolv® ODT > Emdex® + Prosolv® SMCC > Parteck® M100 + Vivapur® 102. Formulations only consisting of water-soluble mannitol and lactose disintegrated wihtin 60 s even at higher compaction forces. The addition of microcrystalline cellulose in the formulation Parteck® M100 + Vivapur® 102 resulted in delayed disintegration times and higher standard deviations, which exceeded the specifications of ODFs when compressed with 1.5 kN or higher.

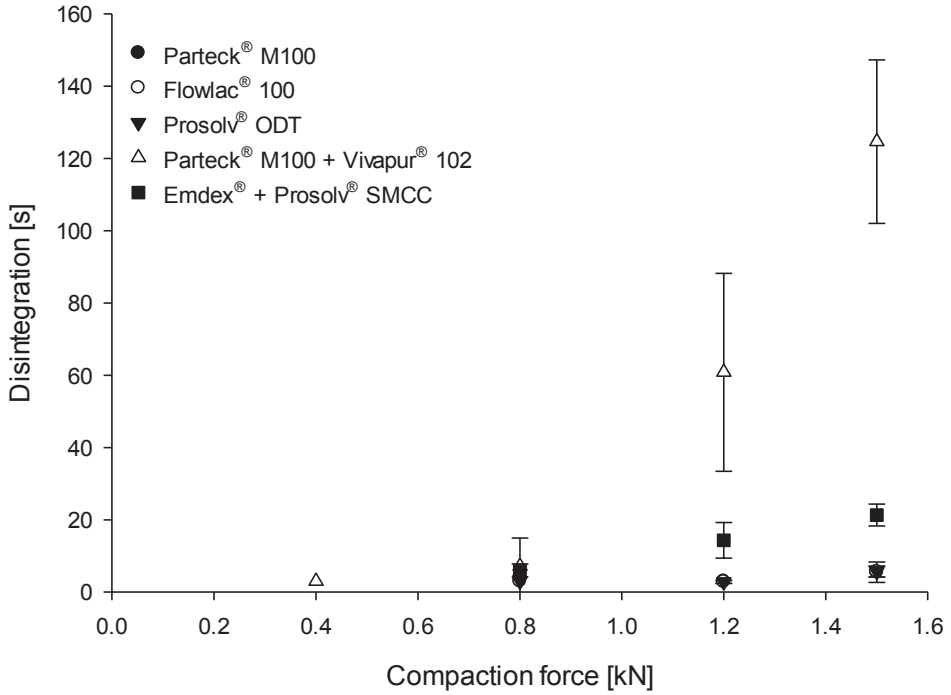


Figure 24. Disintegration according to European Pharmacopoeia. Comparison for all formulations with 2.0 mm tablet height. Arithmetic mean ± S.D. (n = 6).

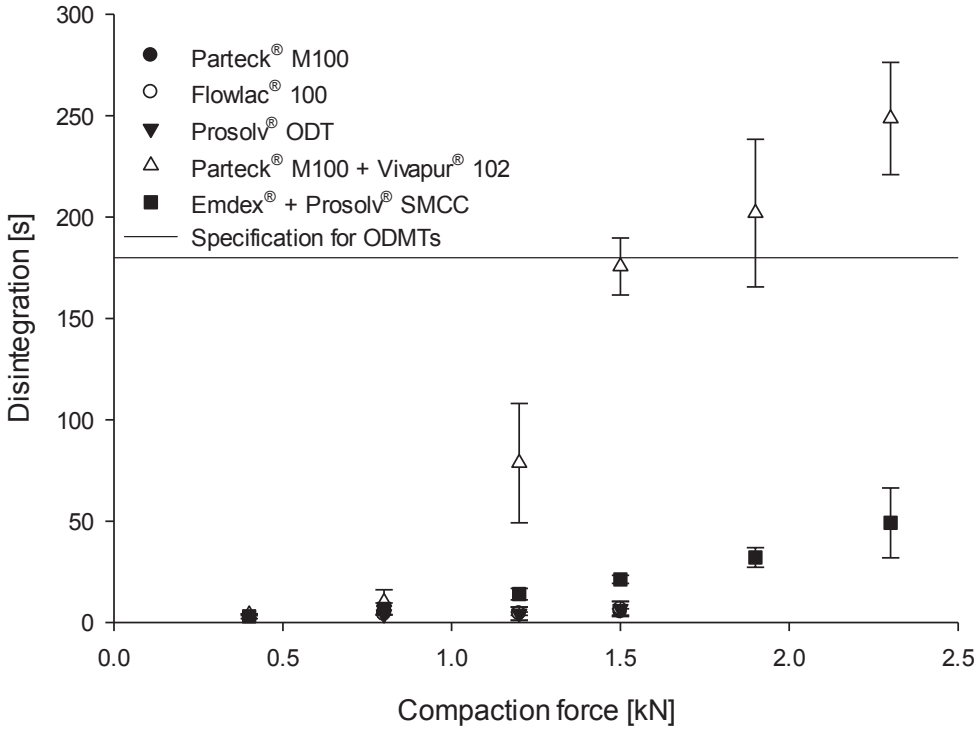


Figure 25. Disintegration according to European Pharmacopoeia. Comparison for all formulations with 2.5 mm tablet height. Arithmetic mean ± S.D. (n = 6).

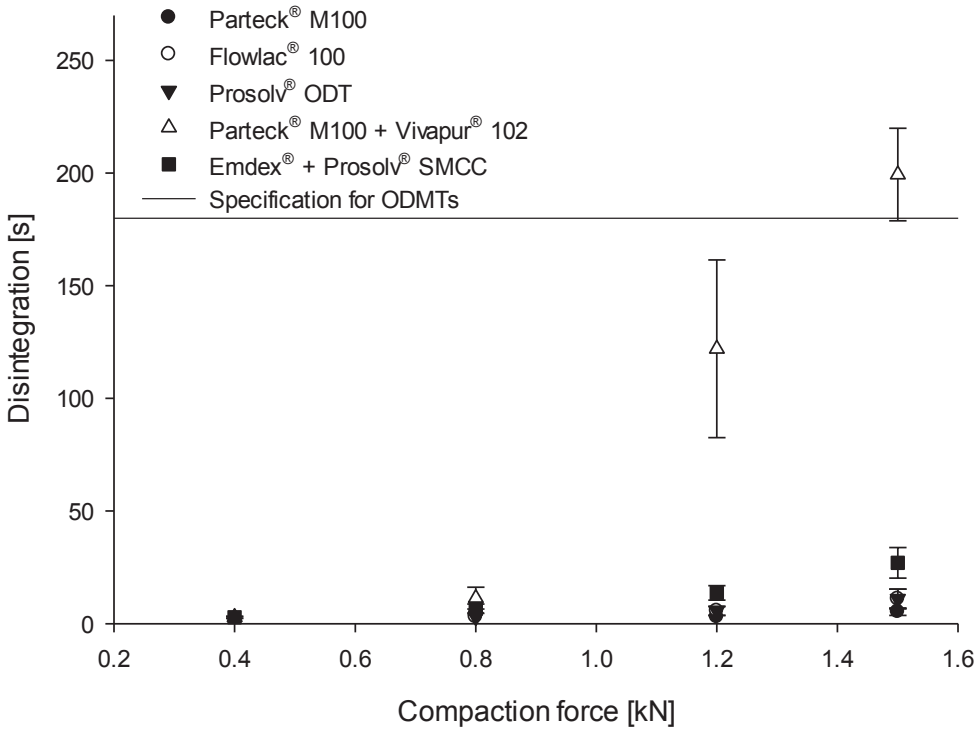


Figure 26. Disintegration according to European Pharmacopoeia. Comparison for all formulations with 3.0 mm tablet height. Arithmetic mean ± S.D. (n = 6).

Figure 27 compares all applied methods for tablets produced out of Flowlac® 100. Using a 40 mesh sieve instead of 10 mesh within the DisiTest 50 led to higher disintegration times, as the MTs needed more time to shrink to a size of less than 0.6 mm or lower to pass the sieve. Both methods used an unrealistic large amount of water of 900 ml. For a better *in vivo* prediction of the disintegration time two other procedures were tested. The Texture Analyser was used simulating the disintegration of the ODMT in 2 ml water. The tablet is not completely immersed in water and is pushed with a punch of low force simulating the tongue [183]. The subsequent disintegration time is much higher compared to the DisiTest 50. The endpoint is reached only when the complete tablet is softened. This method can be preferably used as a discriminating tool between batches of different compaction forces and mechanical stabilities. The simulating wetting test (6.2.2.13.3) describes the wetting time of a tablet with 200 µl blue solution. A fast wetting of the surface of the tablet is important for a short disintegration. The values are higher compared to the DisiTest 50 as there is no mechanical stress applied on the tablet and an only low volume of the colored solution used. A limitation of this method was the subjective endpoint determination which can lead to varying results between different analysts.

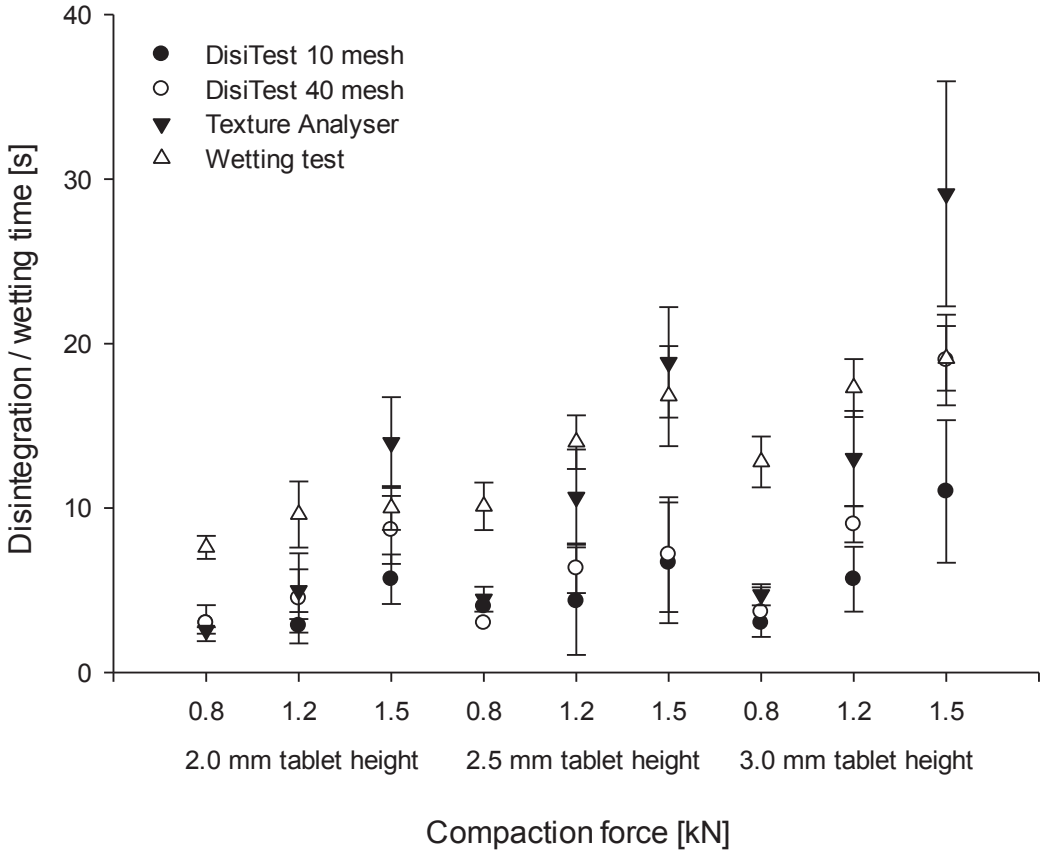


Figure 27. Comparison of disintegration methods and wetting time. Results for Flowlac® 100 measured with the DisiTest with a 10 and 40 mesh sieve, the Texture Analyser and the wetting test. Arithmetic mean ± S.D. (n = 6).

3.2.1.4. Summary

The production of ODMTs required powder mixtures with a high flowability and compactibility providing tablets with a high mechanical stability but fast disintegration. A clear correlation was found for the flowability of powders and the resulting standard deviations for the tablet weight during the process. Different analytical equipment for the mechanical stability of 3 mm MTs was tested. No critical differences were identified for tablets with a minimum hardness of 10 N. Tablets below 10 N exhibited too high values for the friability. Stronger deviations were obtained for different methods for the disintegration in dependency on the mechanical stress and amount of water.

Table 5 summarizes and ranks all formulations regarding their tableting characteristics for ODMTs. **Figure 28** summarizes the disintegration time in dependency on the hardness of tablets measured with the Texture Analyser as the most discriminating tool.

Parteck® M100 revealed as the most suitable excipient for the direct compression of ODMTs combining a high hardness with a fast disintegration.

Table 5
Ranking of characteristics of formulations for tableting (1 = best, 5 = worst).

	Parteck® M100	FlowLac® 100	Prosolv® ODT	Parteck® M100 + Vivapur® 102	Emdex® + Prosolv® SMCC
Flow function (ff)	2	4	5	3	1
Mechanical stability	3	5	4	2	1
Fast disintegration	1	2	3	5	4

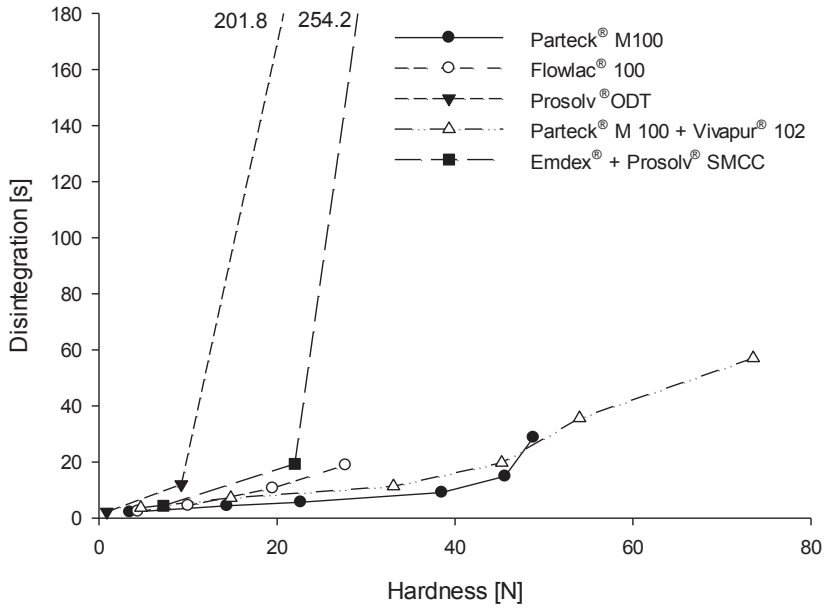


Figure 28. Effect of increasing hardness on disintegration for all formulations (n = 6).

3.2.2. Evaluation of drug-polymer combinations

In view of an effective taste masking of PZQ within an orodispersible or fast disintegrating and dissolving MT, PZQ can be embedded in a polymer matrix, e.g. via spray-drying or extrusion [2]. The pharmacological challenge is to reach a delayed release of PZQ in saliva, but simultaneously achieve an immediate release in gastric and intestinal media for a high drug absorption. A mixture of water soluble and water insoluble polymers: Eudragit® RL PO, RS PO Kollidon® SR; or using pH dependent polymers such as Eudragit® L100, S100 or E PO, can provide the adequate release profile for this taste masking approach [97, 184].

In order to rationally select the optimal polymer for the desired dissolution performance, the API-polymer miscibility was investigated and demonstrated via solvent evaporation in a small scale setup. The solubility and miscibility of an API within the named polymers has a significant effect on the stability and subsequent reproducibility of the API release of the solid dispersion [185].

A well-known technique determining the miscibility is measuring the glass transition temperature T_g using DSC [186]. A miscible API-polymer system is described as a single homogenous phase at a molecular level [185]. Thus, typically one single T_g is detected in contrast to separated phases exhibiting individual T_g values [187]. This method allows the rapid selection of suitable carriers for PZQ.

In terms of the desired drug dissolution, the small scale film preparation via solvent evaporation described by Wyttenbach *et al.* [188] is an effective tool for a rapid polymer screen. It is a time and resource saving technique for an efficient investigation of the polymer performance regarding taste making, solubility kinetics or supersaturation. Thus, this method was applied to PZQ with the chosen polymers.

3.2.2.1. Miscibility

The miscibility of PZQ with the polymers Eudragit® E PO, RL PO, RS PO, L100, S100 and Kollidon® SR were preliminary tested using DSC. Pure PZQ obtained a melting point at 140°C in the first heating cycle and a T_g at 39°C in accordance to previous studies [52]. All physical mixtures showed only one T_g within the second heating cycle (**Figure 29**). Thus, all binary drug-polymer mixtures appeared to be miscible in the investigated range [186].

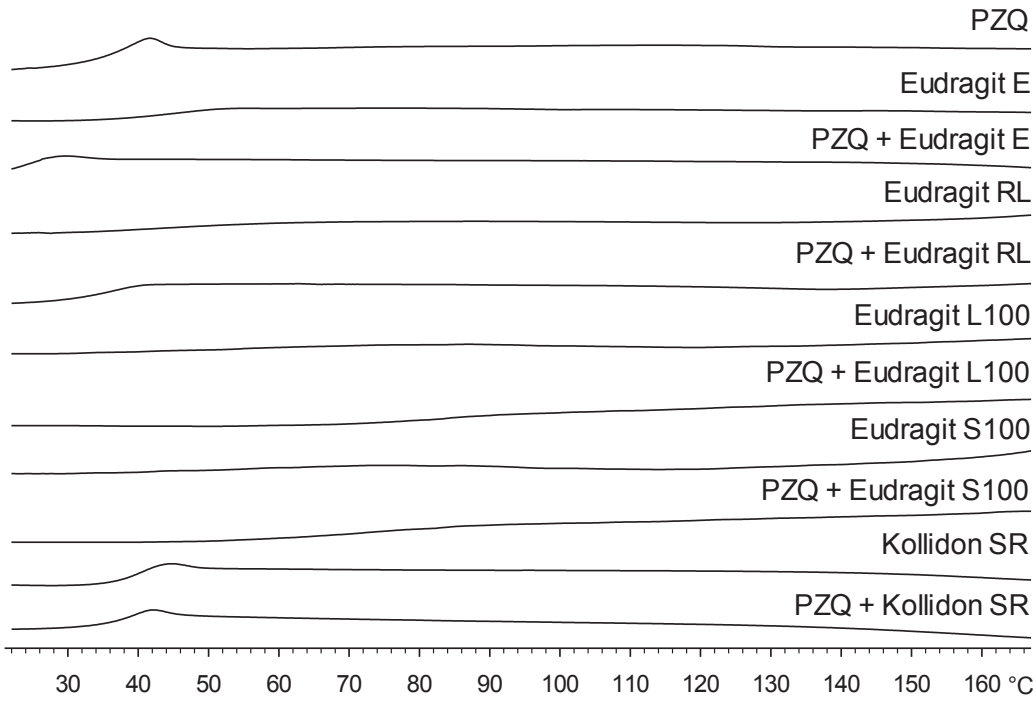
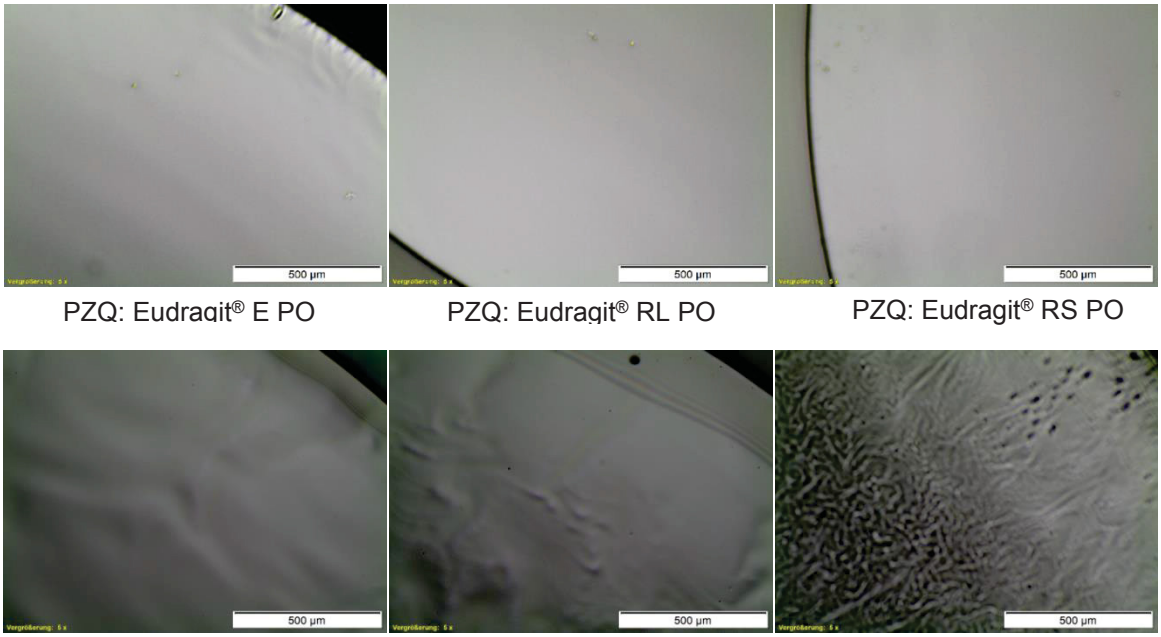


Figure 29. Miscibility of PZQ-polymer systems. Endothermic thermograms of differential scanning calorimetry of second heating cycle of crystalline praziquantel (PZQ), pure polymers and physical mixtures with PZQ in the ratio 1:1 (w/w).

3.2.2.2. Small scale film preparation

The 96-well plate screen facilitated precise and reproducible preparation of amorphous films in a small scale as reported in earlier studies [188]. Drug-polymer films with PZQ were successfully generated in this study with a drug load of 33.3%. None of the films showed crystalline PZQ under the microscope (**Figure 30**). Films with Eudragit® E PO, RL PO, RS PO, L100 and S100 demonstrated smooth surfaces. Films with Kollidon® SR showed a heterogeneous morphology indicating a poor molecular dispersion of PZQ in the films. DSC measurements indicated a miscibility of the API-polymer combination, but this technique has several limitations. The T_g of PZQ and Kollidon® SR are both at approximately 40°C for the pure compounds. A successful differentiation between the T_g values less than 10°C apart from each other in a physical mixture is hardly detectable [187]. Thus, the heterogeneous films could have been a result of an immiscibility of this API-polymer combination.



PZQ: Eudragit® E PO | PZQ: Eudragit® RL PO | PZQ: Eudragit® RS PO

PZQ: Eudragit® L100 | PZQ: Eudragit® S100 | PZQ: Kollidon® SR

Figure 30. Microscopic pictures of drug-polymer films with praziquantel (PZQ) and all polymers.

The 96-well plate screen was used to efficiently differentiate between the polymers Eudragit® E PO, RL PO, RS PO, L100, S100 and Kolllidon® SR regarding a delayed release effect of PZQ in SSF for a promising taste masking effect. Their applicability for an immediate release or supersaturation of PZQ was evaluated in the gastrointestinal media spSGF and FaSSIF.

The pH–independent thermodynamic solubility of crystalline PZQ in all media was 0.22 mg/ml. The preparation of amorphous films of pure PZQ reached an up to 3-fold supersaturation in all media (**Figure 31**, **Figure 32** and **Figure 33**) due to a decreased lattice energy and improved wettability and solubility of the amorphous drug molecules in the film [175].

Eudragit® E PO, RS PO, S100 and L100 significantly decreased the release of PZQ measured in SSF (**Figure 31**) in accordance with their insolubility in water ($p < 0.05$) [189]. The dissolved concentration of the drug after 30 s was more than 6 times lower with Eudragit® E PO and RS PO and more than 7 times lower with Eudragit® S100 in comparison to the pure PZQ assuming a carrier-controlled release. Eudragit® RL PO and Kollidon® SR did not affect the dissolution profile of PZQ ($p > 0.05$). Eudragit® RL PO is highly permeable and in this way might not affect the release of the amorphous drug [190]. It has a higher content of ammonium groups in comparison to Eudragit® RS PO resulting in a higher hydrophilicity, water uptake and provides a higher chain mobility for drug release [191]. Kollidon® SR consists of polyvinyl acetate and water soluble povidone forming pores for drug release. Through fast hydration of the polymer matrix and mechanical stress by the glass balls in this setup the amorphous PZQ showed a fast release from the films. Moreover, a possible immiscibility of PZQ with the polymer as described for the appearance of the films could have influenced the drug release.

Eudragit® E PO demonstrated an immediate release of the drug in spSGF in accordance with its cationic characteristics (**Figure 32**). The ternary amino groups of this copolymer based on

dimethylaminoethyl methacrylate, butylmethacrylate and methyl methacrylate got protonated and led to a fast dissolution of the polymeric film [192]. It enabled the highest degree of supersaturation after 5 min (1.21 mg/ml), followed by Eudragit® RL PO after 60 min and Eudragit® RS PO after 120 min. Eudragit® L100 and S100 proved their enteric properties. They are anionic polymers with free carboxyl groups (approximately 50% in Eudragit® L100 and 33.3% in Eudragit® S100) leading to a pH dependent solubility in water: above pH 5.5 for Eudragit® L100 and pH 7 for Eudragit® S100 [189].

Among all polymers tested, Eudragit® E PO showed the highest degree of supersaturation for PZQ in FaSSIF (1.05 mg/ml) within this setup followed by Eudragit® L100 (**Figure 33**). Similar results for Eudragit® E PO have been reported in the literature based on the best molecular dispersion of the drug in this polymer, improved wettability and the hydrodynamics of this dissolution setup [188]. The slight differences between spSGF and FaSSIF for the pH–independent polymers Eudragit® RL PO and RS PO could be caused by different compositions of the salts in the buffers as it was reported by Bodmeier *et al.* [193].

Eudragit® E PO revealed as the best additive for combining taste masking and immediate release properties for PZQ and was chosen for further extrusion and spray-drying.

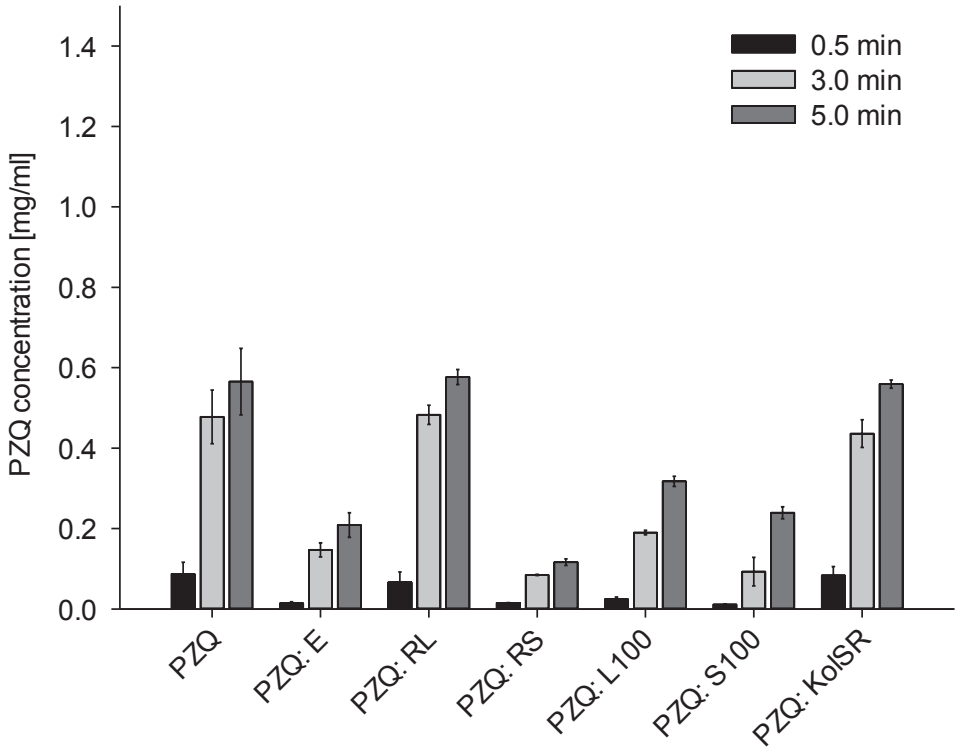


Figure 31. Dissolved praziquantel (PZQ) in drug-polymer-films in simulated salivary fluid at pH 6.8 after 0.5, 3.0 and 5.0 min. Arithmetic mean ± S.D. (n=3)

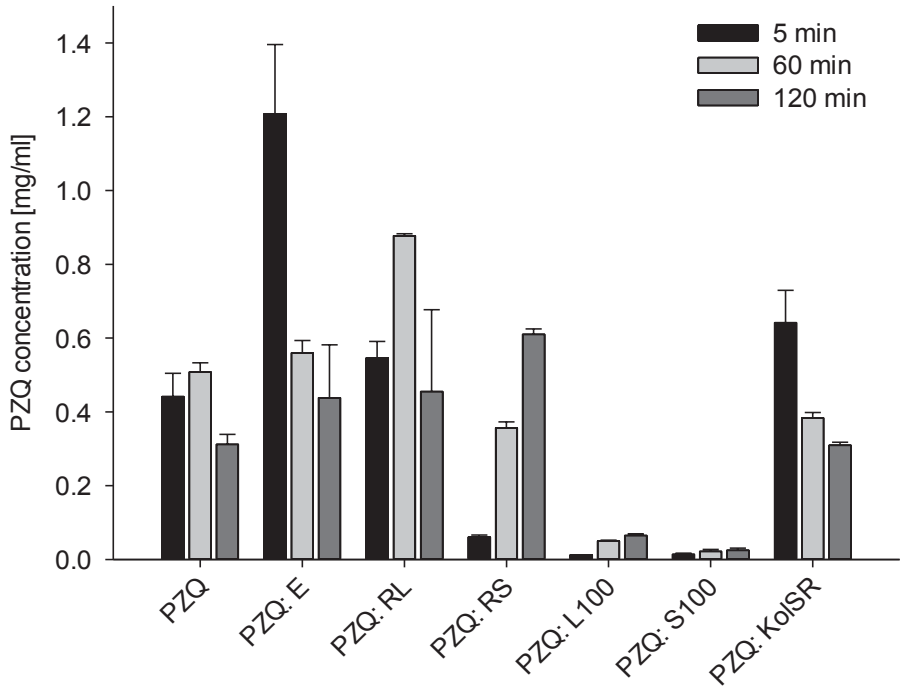


Figure 32. Dissolved praziquantel (PZQ) in drug-polymer-films in simulated gastric fluid at pH 1.2 after 5, 60 and 120 min. Arithmetic mean ± S.D. (n=3)

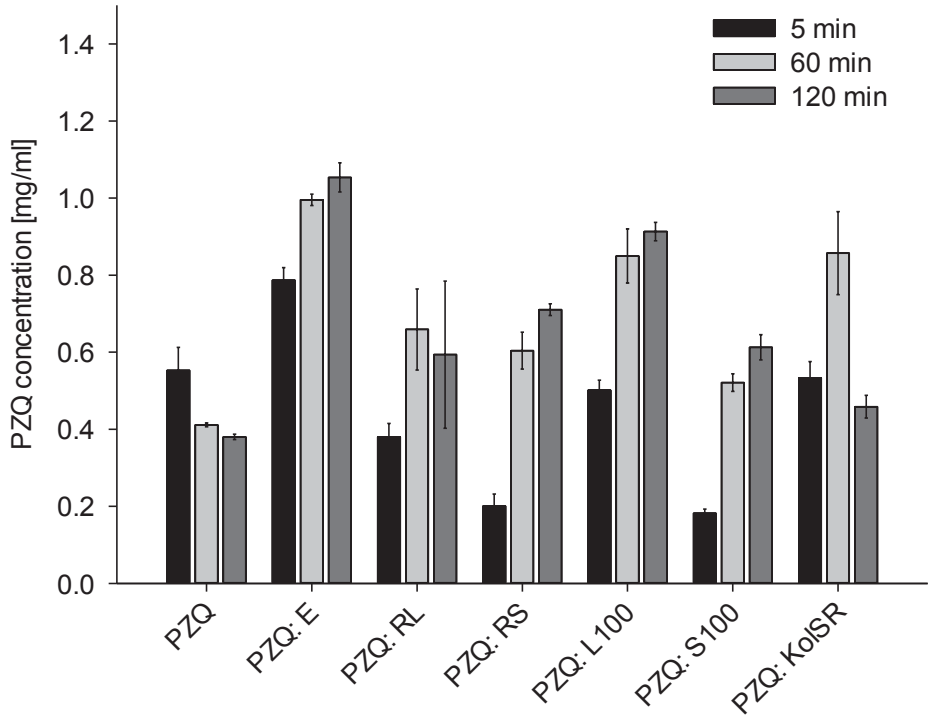


Figure 33. Dissolved praziquantel (PZQ) in drug-polymer-films in fasted state simulated intestinal fluid at pH 6.5 after 5, 60 and 120 min. Arithmetic mean ± S.D. (n=3)

3.2.3. Extrusion for taste masking

Extrusion has recently been identified as an effective tool for taste masking of APIs. It is described as a solvent-free, robust and continuous process to disperse an API in a polymer or lipid matrix [106]. Based on the previous findings (3.2.2.2) extrusion was conducted as HME with Eudragit® E PO. In addition SLE was applied with glyceryl monostearate (GMS) as an additional taste masking agent to the polymer.

The applicability of SLE for taste masking of PZQ was investigated by Witzleb *et al.* with various lipids [115, 118, 194]. They demonstrated a smooth reproducible extrusion process when milling the needle-shaped PZQ before blending with the lipids. A successful taste masking was proven in a randomized palatability study using cats. In the study, GMS was superior to other di- and triglycerides in terms of less electrostatic charging during the process and exhibiting a dissolution of more than 80% after 2 h in biorelevant media. Various studies focusing on SLE exhibited a slow drug release of formulations with lipidic carriers as the dissolution is diffusion controlled [112, 113, 115, 120]. A more rapid drug release could be achieved when adding release modifiers such as polyethylene glycol [121] or disintegrants [114].

The purpose of this study was to prepare extrudates with a polymer and a multiparticulate system consisting of polymer and lipid enabling taste masking properties and providing an immediate dissolution. The drug release was systematically analyzed in simulated media for the saliva, the gut and the intestine. Moreover the polymorphic transformations of the incorporated lipid was monitored at different process temperatures and over storage. Lipids are complex chemical and physical compositions showing polymorphic transformations upon heating and rapid cooling [119]. The altering of the physical modification was analyzed using DSC, XRPD and drug release studies.

3.2.3.1. Drug-free evaluation

The thermal process window of Eudragit® E PO with the Three Tec extruder was evaluated from 70 to 150°C: 70 to 120°C for heating zone 1 and 100 to 150°C for heating zones 2 and 3 (6.2.1.4, **Table 14**). All settings resulted in transparent extrudates. Extrusion temperatures from 70 to 120°C were easily feasible. Lowering the extrusion temperature resulted in too high screw torque values, whereas higher process temperatures led to a low viscosity mass, sticking to the die and lowering extrudate diameters. The extrusion temperature did not affect the DSC profiles of the pure polymer directly after production and storage for 4 weeks at 5 ± 3°C, 25°C with 60% r. h. and 40°C with 75% r. h. (**Figure 34**). The glass transition temperature (T_g) was determined as 42 – 48°C.

The extrudates were ground in a vibrational ball mill (6.2.1.5). The yield after grinding ranged from 54 to 62%. The cohesive powder highly sticked to the grinding bowl and was very electrostatic leading to the loss of powder. The undesired electrostatic charging of materials results in an uncontrolled behavior and adhesion not only during milling, but also during sieving, mixing and weighing. As a consequence the accuracy of the dose and homogeneity of the final product could be an issue.

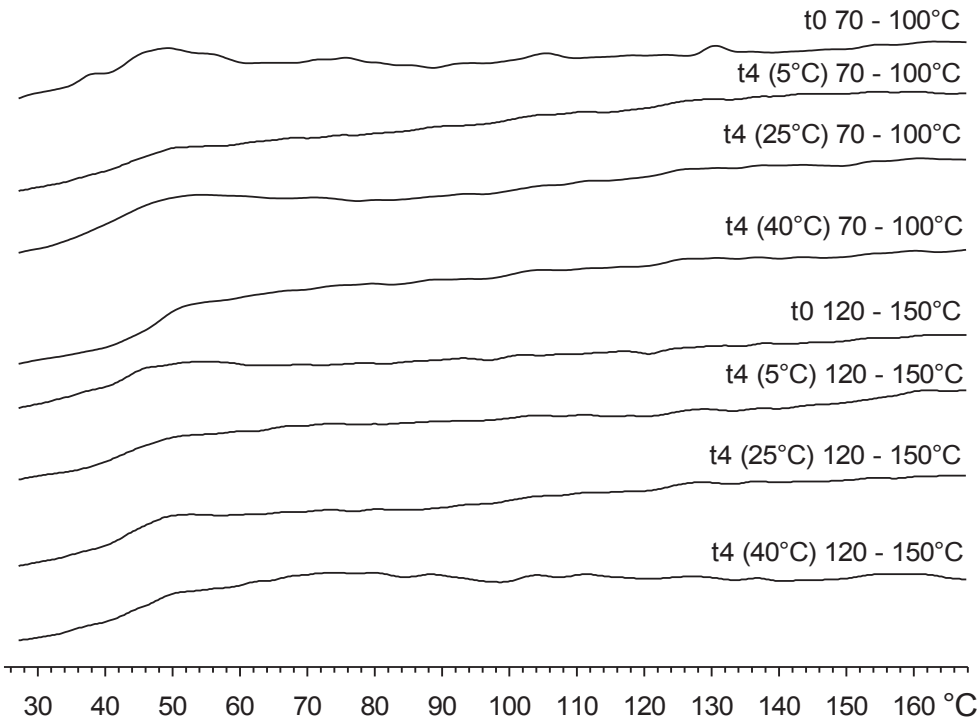


Figure 34. Evaluation of extrusion temperature for Eudragit® E PO. Endothermic thermograms of differential scanning calorimetry of extrudates prepared at 70 – 100°C or 120 to 150°C (t0) and stored for 4 weeks at 5 ± 3°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity.

GMS was added to Eudragit® E PO for extrusion providing additional taste masking capabilities for PZQ. It improved the flowability of the powder blend and enabled a constant feeding of the extruder. Simultaneously, it served as a glidant decreasing the applied shear and friction stress in the process. This resulted in lower torque values and enabled a broader thermal process window for extrusion including temperatures below the melting range of the lipid, defined as SLE [108].

Prior to extrusion, the melting range and solidification behavior of GMS was investigated using DSC analysis (**Figure 35**). Pure and untreated GMS showed one melting peak from 65 to 85°C representing the thermodynamically stable beta form. Complete melting and subsequent cooling led to the formation of a second polymorph structure with a low melting peak at 36 to 44°C identified as the unstable alpha form. As expected, the observed alpha structures underwent transition to the stable beta phase during storage at 15 to 25°C. As already described, this effect can lead to stability issues during storage of the final drug product. Thus, various temperatures as intended for extrusion (**Table 14**) were applied to GMS using DSC to identify the process temperatures for solidification of the alpha modification. Heating GMS until 70°C did not result in the appearance of the second melting peak in contrast to higher temperatures from 100°C (**Figure 35**). Thus, it was concluded that the alpha form is not supposed to crystallize after melting up to 70°C.

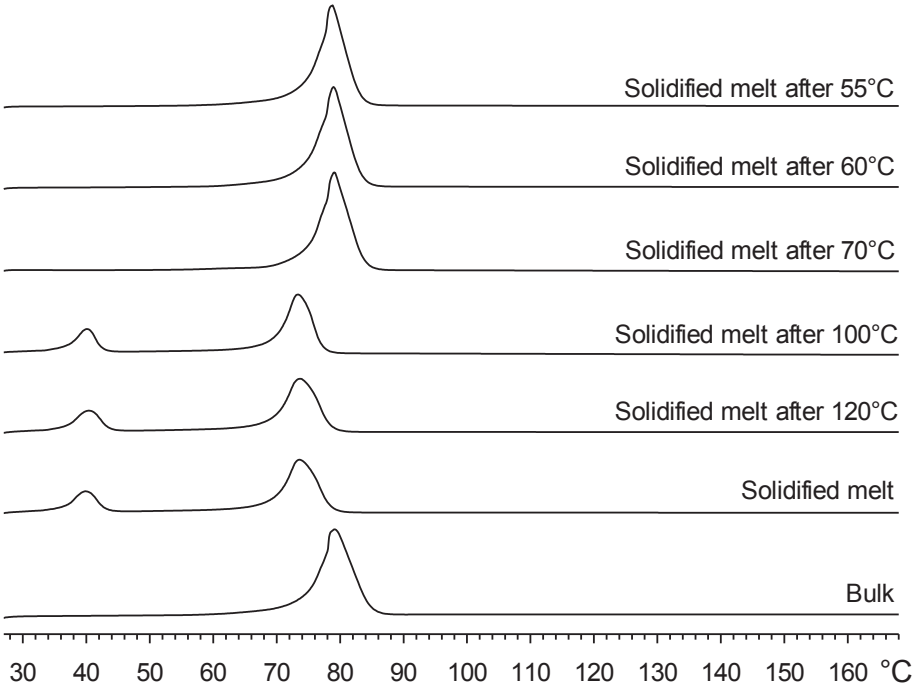


Figure 35. Evaluation of extrusion temperature for GMS. Endothermic thermograms of differential scanning calorimetry of pure glyceryl monostearate in first heating cycle, solidified melt as reference and solidified melts in dependence on former labelled heating temperature.

The DSC observations for pure GMS were applied for physical mixtures with Eudragit® E PO (0.3:1 w/w) (**Figure 36**). Heating the powder mixture once showed two distinct thermal events: the T_g of Eudragit® E PO at 46°C and melting of GMS at 65 to 85°C. In accordance with the data for pure GMS, heating until 70°C did not generate the unstable alpha form of GMS.

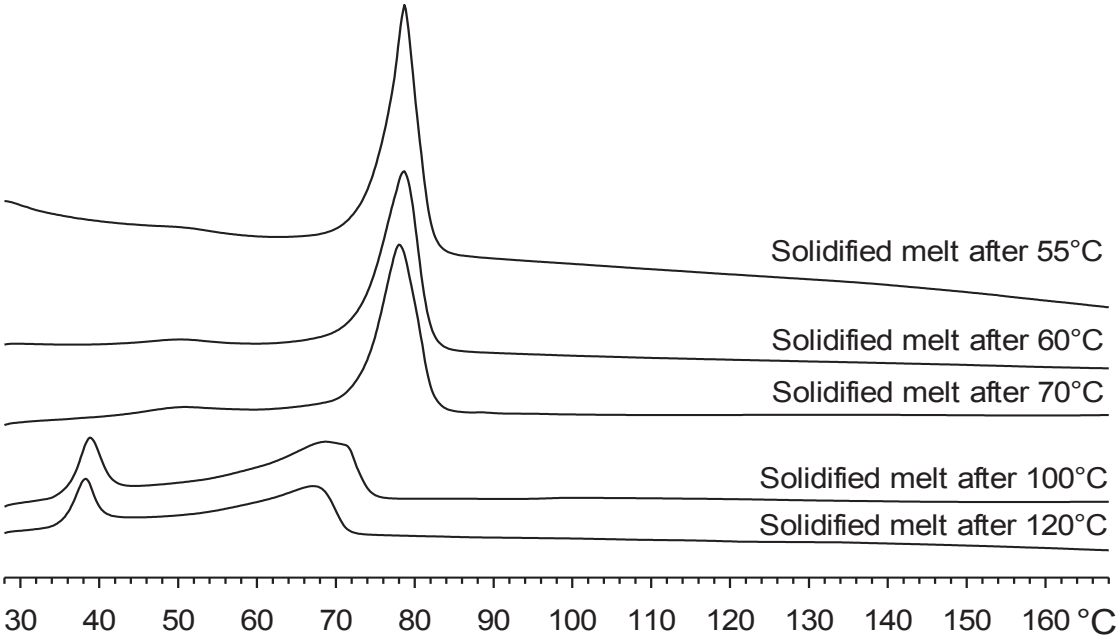


Figure 36. Evaluation of extrusion temperature for Eudragit® E PO and glyceryl monostearate. Endothermic thermograms of differential scanning calorimetry of solidified melts in dependence on heating temperature.

Extrusion of the multiparticulate system of Eudragit® E PO and GMS with a ratio of 1: 0.3 (w/w) was conducted from 45 to 120°C: 45° to 70°C for heating zone 1 and 55 to 120°C in heating zones 2 and 3 (6.2.1.4, **Table 14**). Settings below and above the melting range of GMS were applied to evaluate the impact of the extrusion temperature on the morphology and stability of the extrudates. Decreasing the extrusion temperature below 45°C resulted in too high values regarding the screw torque. Temperature settings from 45 to 70°C were feasible and easily extrudable. Higher temperatures resulted in a low viscosity of the molten extrudate, sticking to the die. All extrudates prepared with maximum 70°C during the process showed an opaque and rough surface as the lipid was only softened but not completely molten and the polymer remained completely solid. For higher temperatures up to 120°C white strands with smooth surfaces were obtained due to completely molten and solidified lipid.

All extrudates were ground in a vibrational ball mill (6.2.1.5). The yield after grinding ranged from 87 to 97%. GMS performed as antistatic agent during milling. In contrast to extrudates of pure Eudragit® E PO, the powder did not stick to the grinding bowl. GMS decreased the friction between milled particles and between the powder and the milling bowl due to its glidant properties. Thus, the formulation was easily collectable and free-flowing which is of particular importance for the downstream of the process [125, 195]. An efficient and cost effective formulation development in the pharmaceutical industry in terms of blending, tableting or capsule filling is essentially affected by the flow properties of the powder material [196, 197].

DSC measurements of all ground extrudates prepared at different process temperatures were conducted directly after extrusion (**Figure 37**). Besides the T_g of Eudragit® E PO at approximately 45°C and the melting peak of GMS at 65 to 80°C, all DSC thermograms exhibited the formation of the unstable alpha modification.

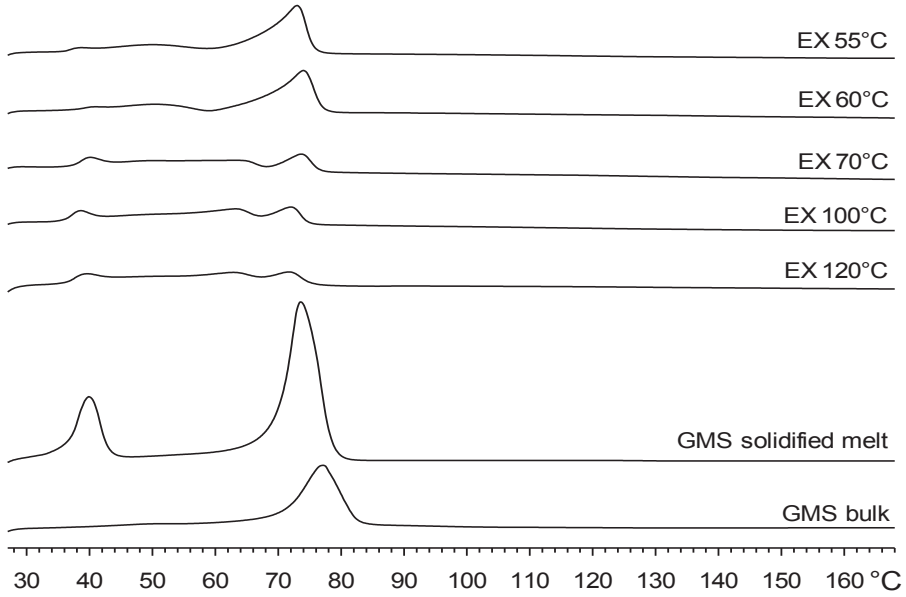


Figure 37. Analytics of extrudates (EX). Endothermic thermograms of differential scanning calorimetry of pure glyceryl monostearate (GMS) and EX of Eudragit® E PO with GMS 1: 0.3 (w/w) prepared at different process temperatures.

The partly melting of GMS occurred at lower extrusion temperatures than determined in DSC measurements of the physical mixtures. Thus, the solidification of the alpha form cannot only be attributed to the influence of the extrusion temperature. During the extrusion process the lipid underwent mechanical and thermal stress: heat energy as demonstrated in DSC measurements and additionally shear- and friction forces inducing melting of the lipid. Furthermore, cooling of extrudates was rapid in comparison to conditions in the DSC analysis which supports the formation of the alpha modification [118]. Windbergs *et al.* reported extrusion without blooming of a lipid when keeping the process temperature and especially the temperature at which the extrudate passes the die between both melting ranges of the alpha- and the beta-form as the partly molten lipid directly solidified into the stable beta-form [119]. For GMS, this was tested with the temperature set up of 50°C for heating zone 1 and 60°C for heating zones 2 and 3, but could not be confirmed. Reasons for this contradictory result are the use of different extruders leading to varying friction and shear forces influencing the amount of molten lipid in the formulation. Moreover, within this study screw speeds of 300 rpm were applied in contrast to 30 rpm at Windbergs *et al.* [119]. The higher screw speed probably resulted in a lower residence time and faster cooling of extrudates. The development of the unstable alpha form could be avoided applying lower screw speeds and controlled cooling of the extrudates. Nevertheless, the amount of the alpha modification was lower for extrusion temperatures up to 60°C (**Figure 37**). Thus, with regard to minimizing stability issues for formulations with PZQ, the extrusion temperature should be kept as low as possible.

The transformation of the alpha modification of GMS to the stable beta form was observed for all samples stored at 25 and 40°C. The intensity of the lower melting peak decreased or completely disappeared. Storage at 5 ± 3°C avoided the change of the DSC profiles within 4 weeks. The formulations prepared at 50 – 60°C and 70 – 100°C are shown as examples in **Figure 38**.

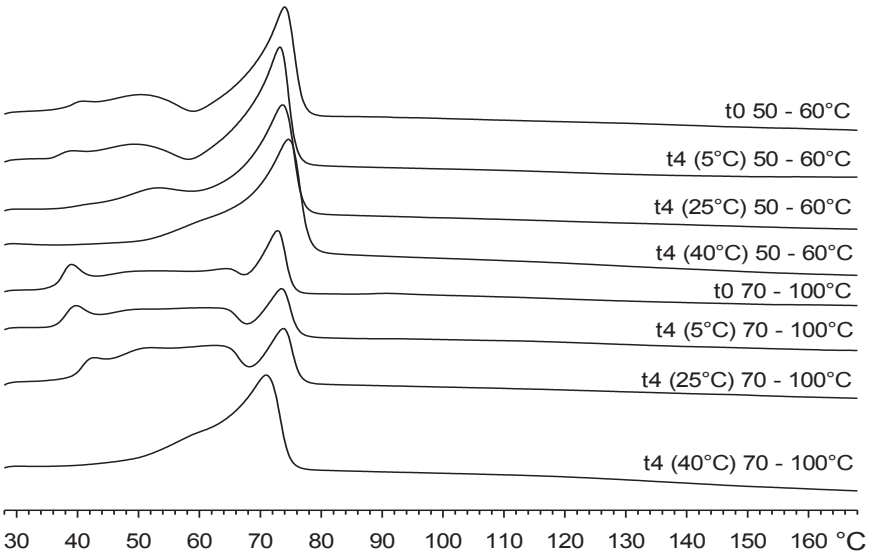
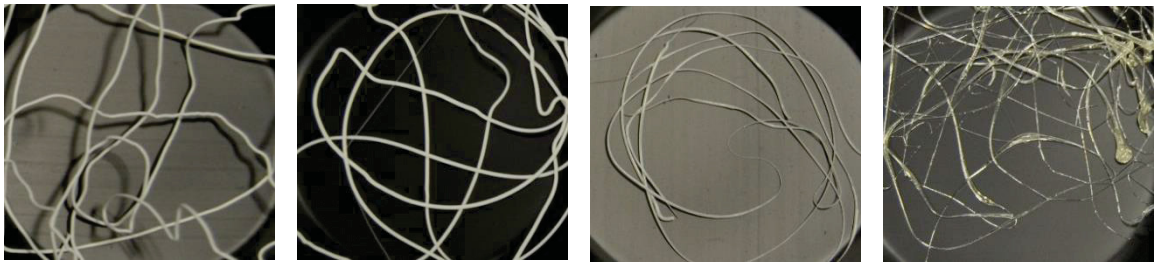


Figure 38. Analytics of extrudates. Endothermic thermograms of differential scanning calorimetry of Eudragit® E PO with glyceryl monostearate 1: 0.3 (w/w) prepared at 50 – 60°C and 70 – 100°C directly after production (t0) and after storage for 4 weeks at temperatures at 5 ± 3°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity.

In conclusion, extrusion within this setup with Eudragit® E PO and GMS (1:0.3 w/w) resulted in polymorphism of the lipid for all process temperatures during storage at 25 and 40°C. Conducting extrusion below the melting range of the lipid did not avoid the formation of the unstable alpha form of the lipid, but could minimize it. The relevance for formulations with PZQ were tested as next step.

3.2.3.2. Extrusion with API

Extrusion with PZQ was performed only with the polymer Eudragit® E PO and the mixture of Eudragit® E PO with GMS (1:0.3 w/w), with two drug loads each. Due to the needle shaped PZQ the physical mixtures were ground in a mortar prior to extrusion until a homogenous mixture was obtained to prevent interference with the extrusion process. Witzleb *et al.* reported blocking of the dies during extrusion with needle-shaped PZQ due to accumulated material in the barrel [194].
Extrusion with PZQ and Eudragit® E PO was conducted with 33.3% and 50.0% drug load and process temperatures from 70 to 125°C based on the results of the placebo evaluation (6.2.1.4, **Table 14**). As observed for the pure polymer, the mixture exhibited a narrow thermal processing window. A temperature setting of 70 to 100°C was easily feasible. Lowering the extrusion temperature below 70°C resulted in too high screw torque values, whereas extrusion temperatures above 100°C with the PZQ led to a nonextrudable, low viscosity mass. Increasing the PZQ content further complicated the process. Therefore, extrusion with 50% PZQ was only conducted with a temperature setting of 70 to 100°C. Opaque elastic extrudates were obtained for all extrudates except for the sample of 33.3% PZQ extruded with the highest temperature of 125°C in zone 3 exhibiting a glassy appearance (**Figure 39**).



EX 50% 70-100°C EX 33.3% 70-100°C EX 33.3% 80-120°C EX 33.3% 80-125°C

Figure 39. Photographs of extrudates (EX) with praziquantel and Eudragit® E PO with the labelled drug load and extrusion temperatures.

DSC measurements revealed a decreasing content of crystalline PZQ by increasing the extrusion temperature (**Figure 40**). However, partly crystalline PZQ was detected as well when conducting extrusion at 125°C showing poor extrudability but a glassy extrudate. In terms of practicability and the physical state of PZQ, extrusion at higher temperatures of 80 to 125°C was not advantageous. Focusing on taste masking the crystalline form of PZQ is preferred over the amorphous dispersion as the latter is related to a faster and higher solubility [175]. XRPD patterns confirmed, that PZQ was embedded in the polymer matrix in crystalline state (**Figure 43**). The patterns of the pure drug and the extrudates showed characteristic peaks of the API: 3.94, 6.23, 6.60, 7.90, 8.20, 15.40, 16.40, 17.00, 20.10 and 25.60° in accordance to literature [52, 194].

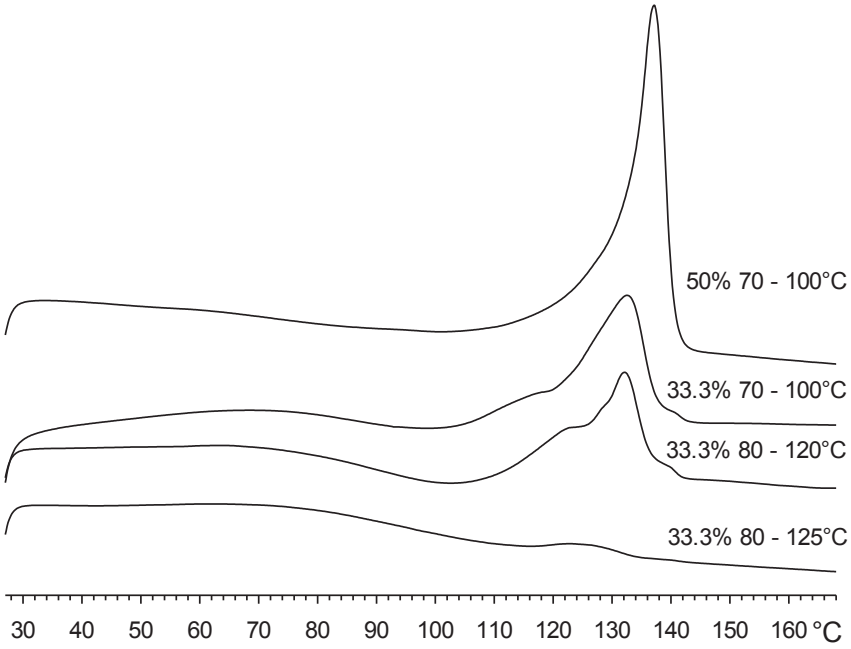


Figure 40. Analytics of extrudates (EX). Endothermic thermograms of differential scanning calorimetry of EX with praziquantel and Eudragit® E PO with the labelled drug load and extrusion temperatures.

The drug loads of the extrudates were verified with low deviations regarding the content uniformity (< 0.5% RSD). The extrudates were ground in a vibrational ball mill (6.2.1.5). The yield after this process step was 83% for 50.0% drug load and 61% for 33.3% drug load. The cohesive powder was highly stuck to the grinding bowl and was high electrostatically charged resulting in a loss of powder.

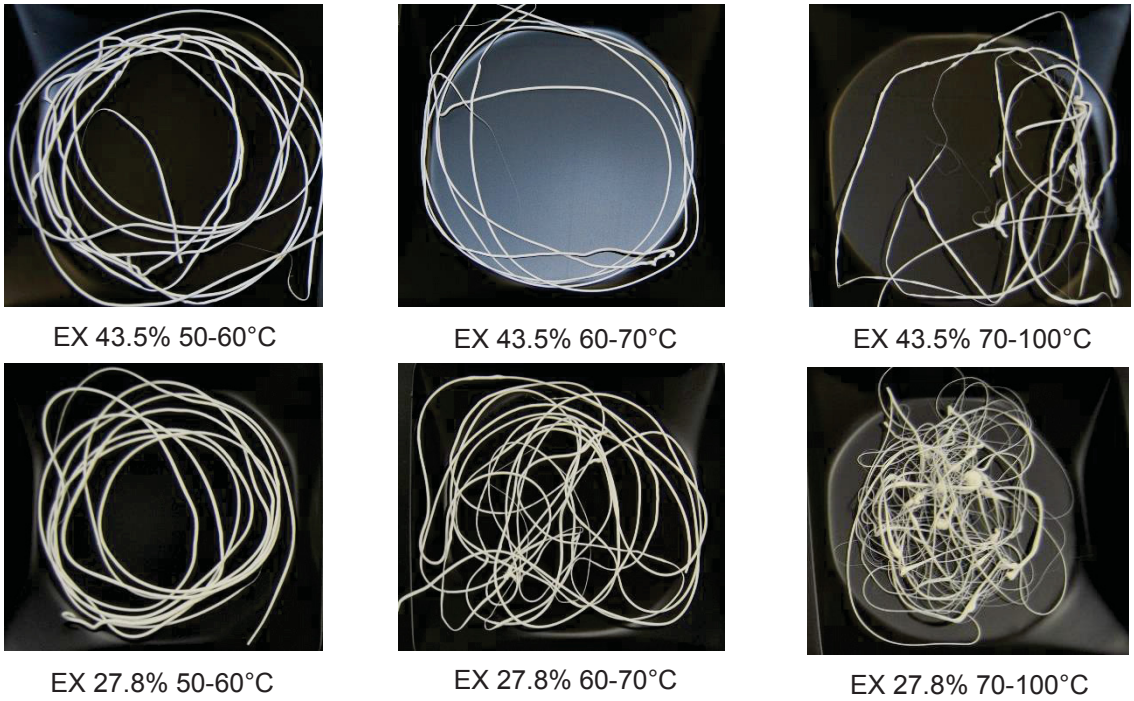


Figure 41. Photographs of extrudates (EX) with praziquantel, Eudragit® E PO and glyceryl monostearate with the labelled drug load and extrusion temperatures.

The addition of GMS as an additional taste masking agent improved the flowability of the physical mixture with PZQ and Eudragit® E PO and enabled a constant feeding of the extruder as already experienced in placebo evaluations. Simultaneously, it served as a glidant for extrusion decreasing the applied shear and friction stress in the extruder. Lower process temperatures of 50°C in heating zone 1 and 60°C in heating zones 2 and 3 below the melting range of the lipid were feasible with both drug loads of 43.5 and 27.8% providing even more gentle process conditions for PZQ. All extrudates were opaque due to GMS (**Figure 41**). Samples of lower extrusion temperatures (50 – 70°C) showed a rough surface as the incorporated GMS was only softened and not completely molten. At higher temperatures (70 – 100°C) the lipid was molten resulting in white solidified extrudates with smooth surfaces and lower screw torques during the process related to a lower viscosity of the mixture.

Crystalline PZQ was detected in all formulations with GMS independent of the process temperature using DSC and XRPD measurements (**Figure 42** and **Figure 43**). The drug loads of the extrudates were verified with low deviations regarding content uniformity (< 2.3% RSD). The process yield of milling these brittle extrudates with GMS was increased (93 – 96%). GMS decreased the friction between milled particles and between the powder and the milling bowl due to its glidant properties. Thus, the ground extrudates did not stick to the wall and were not electrostatically charged resulting in a visually good flowability. The particle sizes ranged from 3 to 400 µm with a median of 23 – 39 µm for all compositions.

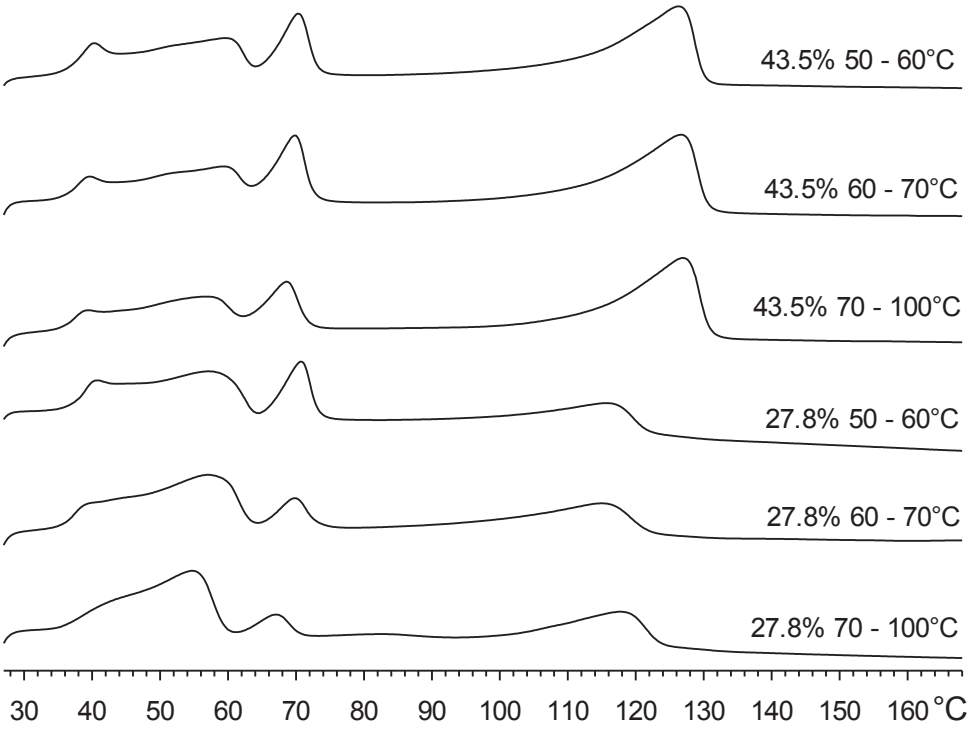


Figure 42. Analytics of extrudates (EX). Endothermic thermograms of differential scanning calorimetry of extrudates with praziquantel, Eudragit® E PO and glyceryl monostearate with the labelled drug load and extrusion temperatures.

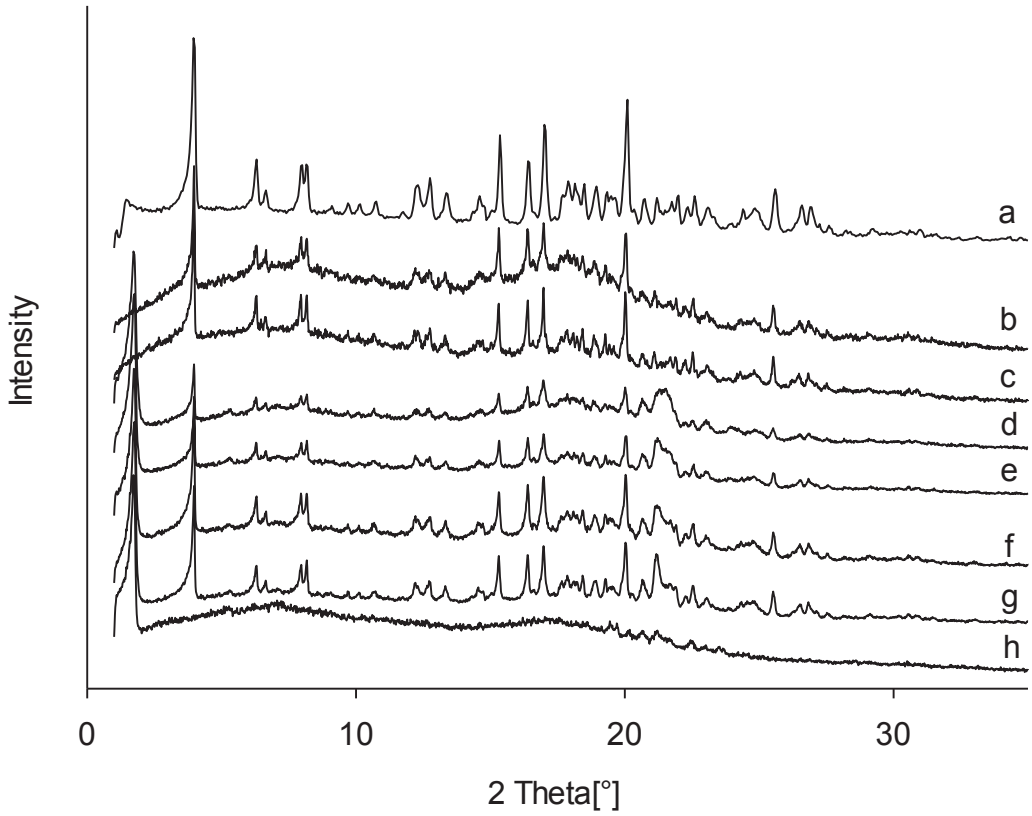


Figure 43. XRPD patterns of ground extrudates. a) crystalline praziquantel (PZQ) as reference; (b) 33.3% PZQ: Eudragit® E PO (E); (c) 50% PZQ:E; (d) 27.8% PZQ:E: glyceryl monostearate (GMS) extruded at 70-100°C; (e) 27.8% PZQ:E:GMS at 50-60°C; (f) 43.5% PZQ:E:GMS at 70-100°C ;(g) 43.5% PZQ:E:GMS at 70-100°C; (h) E:GMS.

Non-sink *in vitro* dissolution studies were conducted to evaluate the impact of extrusion temperatures and the addition of GMS on the taste masking properties in simulated salivary fluid (SSF, pH 6.8), kinetic solubility in simulated gastric (spSGF, pH 1.2) and intestinal media (FaSSIF, pH 6.5). They were set up for formulations of PZQ with Eudragit® E PO for both drug loads prepared at the lowest extrusion temperatures from 70 to 100°C in comparison to the glassy extrudate prepared up to 125°C. Furthermore, analysis was performed for formulations with Eudragit® E PO and GMS for both drug loads prepared at 50 to 60°C and 70 to 100°C. The other formulations with extrusion temperatures from 60 to 70°C were excluded as DSC and XRPD measurements revealed no differences to formulations extruded at 70 to 100°C. The results were compared to pure PZQ which has a pH-independent thermodynamic solubility of 0.22 mg/ml in all media.

An appreciable delayed release of PZQ in SSF was observed for all extrudates (**Figure 44**). Extrusion of PZQ with the Eudragit® E PO at maximum 100°C significantly decreased the drug release up to 2.7-fold in the first min and 1.8-fold after 5 min ($p < 0.001$) correlating with the pH-dependent solubility of Eudragit® E PO and maintaining crystalline PZQ [198]. A higher extrusion temperature, resulting in partly amorphous PZQ, increased the solubility kinetic with no difference to pure PZQ after 5 min ($p = 0.658$).

The addition of GMS enhanced the delayed release effects significantly up to 11-fold ($p < 0.001$) due to its insolubility in water [199]. Increasing the drug load or the extrusion temperature did not affect the drug release in SSF for both drug loads with GMS ($p > 0.05$). This has already been reported, as the applied extrusion temperatures up to 100°C had no impact on the physical state of PZQ [113]. Addition of GMS is important to the composition as this lipid intensifies the matrix-controlled drug release properties of the ground extrudate.

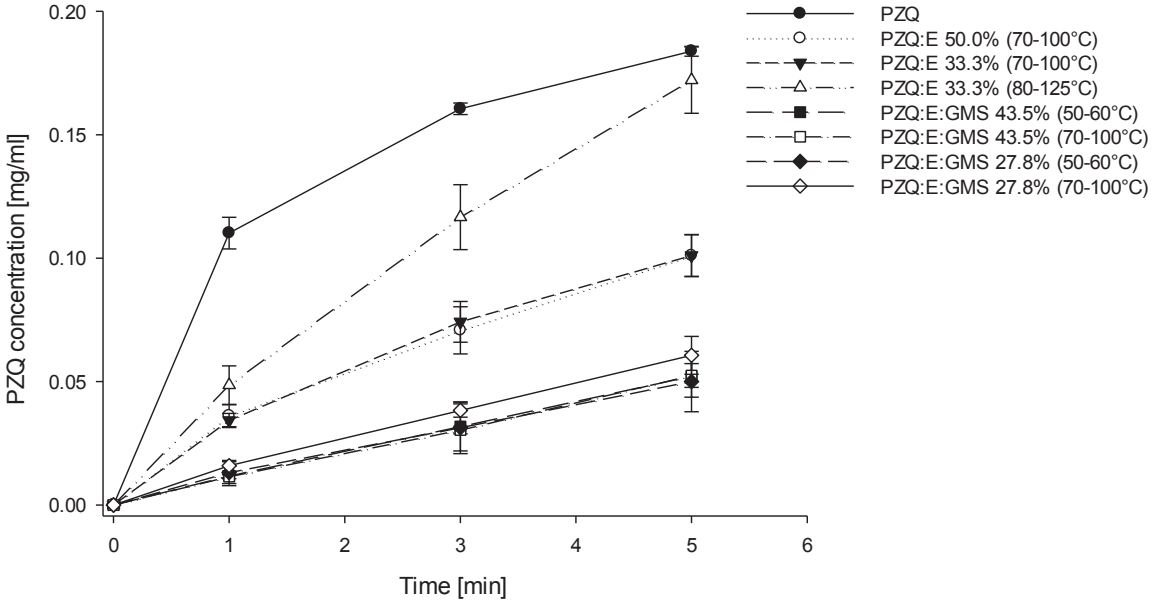


Figure 44. Non-sink dissolution profiles in SSF. Comparison of pure praziquantel (PZQ) and extrudates with polymer (E) and lipid (GMS). Drug load and extrusion temperatures are labelled. Arithmetic mean ± S.D. (n=3).

All preparations increased the kinetic solubility of PZQ in spSGF and FaSSIF (**Figure 45** and **Figure 46**). Starting with the lowest concentration, the extrudates were ranked as follows: PZQ:E:GMS 43.5% (50-60°C) < PZQ:E:GMS 43.5% (70-100°C) < PZQ:E 50.0% (70-100°C) < PZQ:E:GMS 27.8% (50-60°C) < PZQ:E:GMS 27.8% (70-100°C) < PZQ:E 33.3% (70-100°C) < PZQ:E 33.3% (80-125°C). Increased solubility was obtained with increasing amount of Eudragit® E PO acting as a solubilizer for PZQ. Drug release in spSGF is a drug-controlled mechanism, as the cationic polymer quickly dissolves due to protonation of the tertiary groups [192]. Thus, the concentration is dominated by the dissolution of the poorly soluble crystalline PZQ or in the case of the glassy extrudate of 125°C the partly amorphous PZQ. The latter exhibited an unstable high supersaturation in spSGF (8.5-fold after 5 min), but fast recrystallization and a 5.5-fold supersaturation in FaSSIF after 60 min. These observations were in accordance to the results of the amorphous drug-polymer films described in 3.2.2.2 revealing the same recrystallization of amorphous PZQ in spSGF and degree of supersaturation in FaSSIF.

A higher amount of polymer and lipid increased the wettability of the drug. The solubility and dissolution rate enhancing effect of Eudragit® E PO has been demonstrated in various studies [189, 200]. In this case, a higher extrusion temperature above the melting point of the lipid slightly

improved the kinetic solubility of PZQ at a drug load of 27.8%. The lower viscosity of the mixture in the extrusion process could have led to an improved molecular distribution of PZQ in the polymer and lipid matrix [201]. This affects the solubilization of PZQ in those media in which Eudragit® E PO is more soluble. The difference between extrusion temperatures for a drug load of 43.5% was not significant ($p > 0.05$).

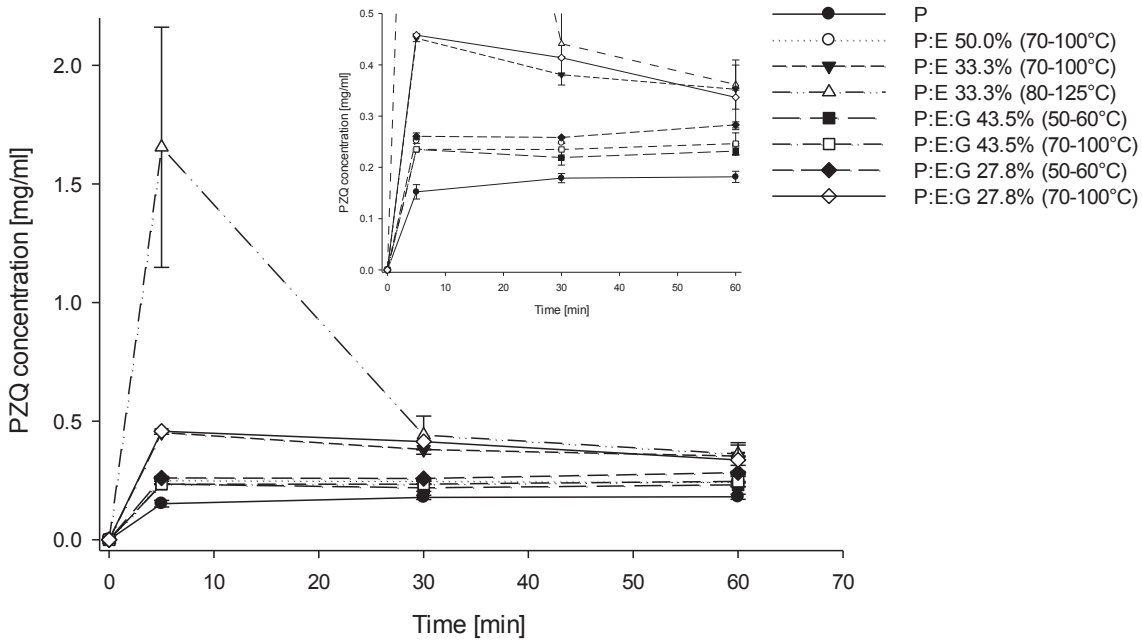


Figure 45. Non-sink dissolution profiles in spSGF of pure praziquantel (P) and extrudates with polymer (E) and lipid (G). Drug load and extrusion temperatures are labelled. Arithmetic mean ± S.D. (n=3).

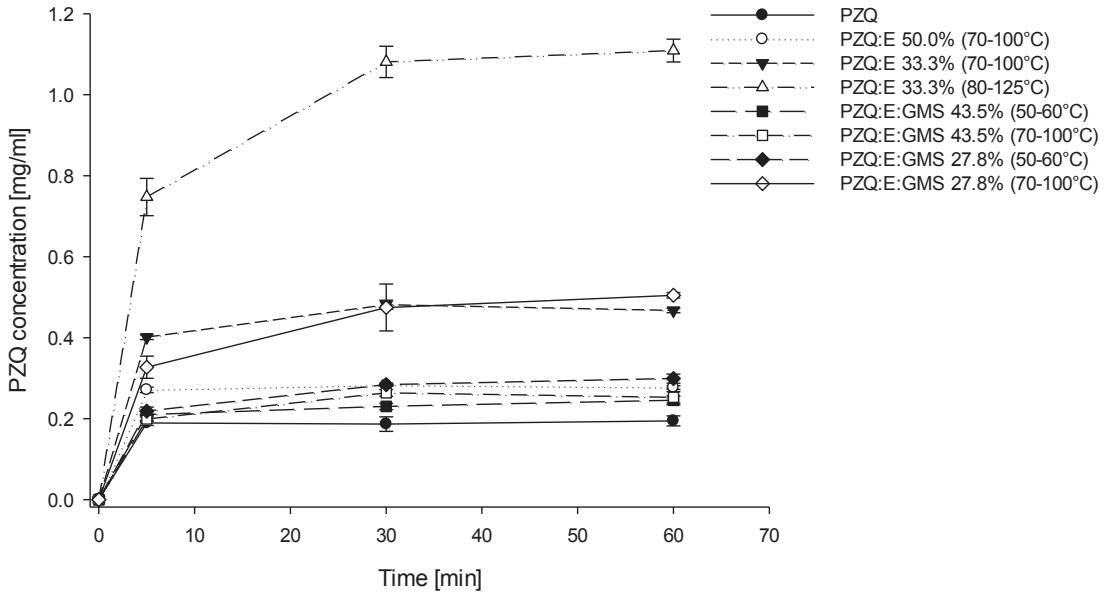


Figure 46. Non-sink dissolution profiles in FaSSIF of pure praziquantel (PZQ) and extrudates with polymer (E) and lipid (GMS). Drug load and extrusion temperatures are labelled. Arithmetic mean ± S.D. (n=3).

The formulation PZQ:E:GMS 43.5% (50-60°C) is the most favorable one regarding practicability of the extrusion process including milling, taste masking and additionally provided a high drug load. The extrudate PZQ:E 33.3% (80-125°C) provided the highest solubility kinetics for PZQ which can be interesting in terms of solubility and bioavailability enhancement as PZQ belongs to BCS class 2 [48]. However, the efficacy of PZQ is more likely dependent on the exposure time than on the maximum concentration level in the plasma. Furthermore, the absorption of PZQ is rapid and nearly complete leading to a relatively low benefit for the bioavailability when providing a supersaturation in gastrointestinal media [48].

3.2.3.3. Stability over storage

Ground extrudates were stored for 8 weeks at 5 ± 3°C, 25°C with 60% r. h. and 40°C with 75% r. h. (6.2.2.17) to evaluate changes in the formulations performance.
XRPD-patterns of formulations only with PZQ and Eudragit® E PO (**Figure 47**) revealed no changes during storage as PZQ was incorporated in its crystalline state. XRPD-patterns with PZQ, Eudragit® E PO and GMS (**Figure 48** and **Figure 78** in appendix) revealed a polymorph transition of GMS independent on the applied extrusion temperature and the drug load. This was confirmed by DSC measurements (**Figure 79** and **Figure 80** in appendix). As shown for placebo formulations, lower melting alpha structures transformed to the higher melting and stable beta modification during storage.

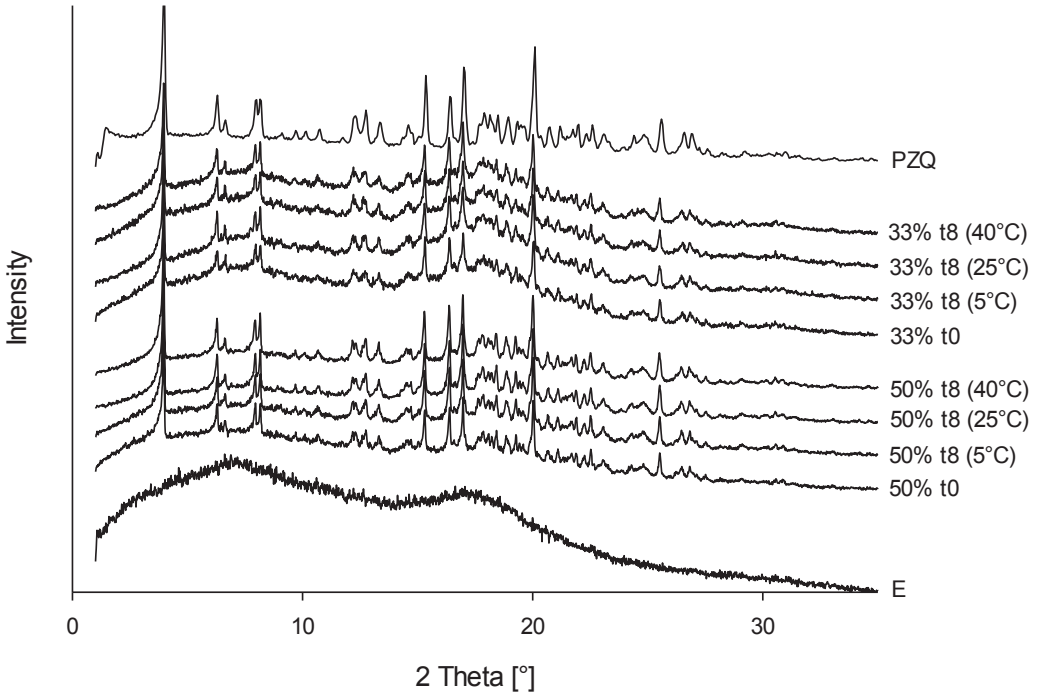


Figure 47. XRPD patterns of praziquantel (PZQ), Eudragit® E PO (E) and ground extrudates prepared with 33.3% or 50.0% drug load directly after production (t0) and after storage for 8 weeks (t8) at 5 ± 3°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity.

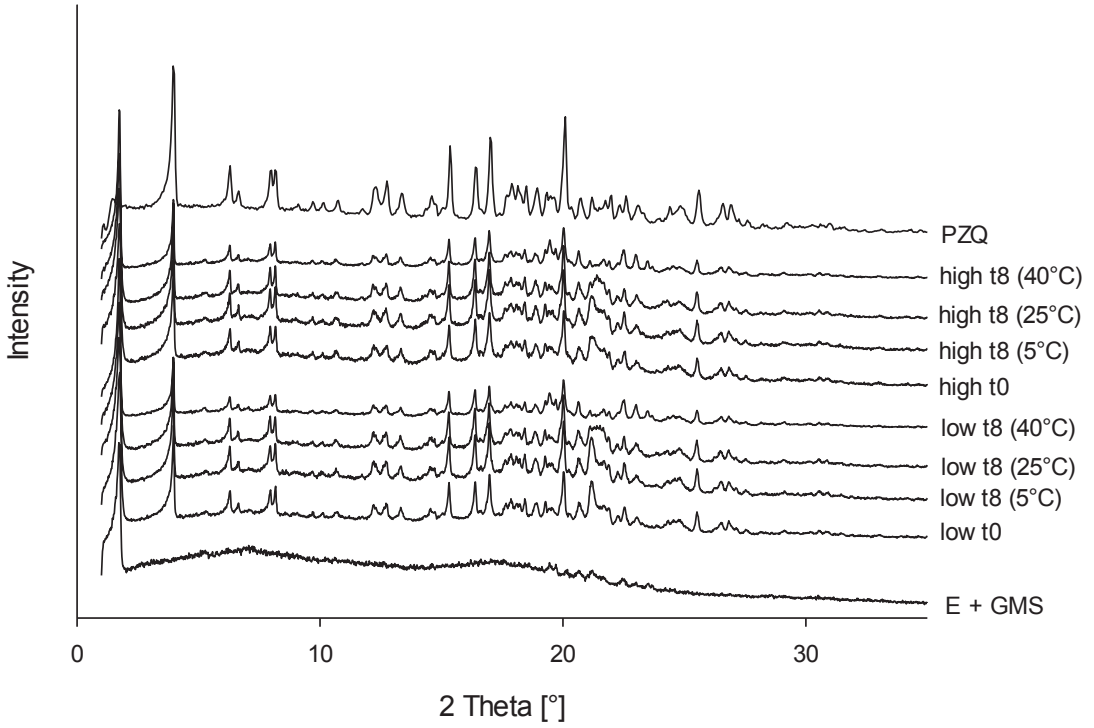


Figure 48. XRPD patterns of praziquantel (PZQ), Eudragit® E PO and glyceryl monostearate (E + GMS) 1:0.3 (w/w) and ground extrudates prepared with 43.5% PZQ at low (50–60°C) and high (70-100°C) extrusion temperatures directly after production (t0) and after storage for 8 weeks (t8) at 5 ± 3°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity.

All formulations exhibited changes in non-sink dissolution profiles in SSF over storage as demonstrated for 50% and 44% drug load (**Figure 49**). Ground extrudates with PZQ and Eudragit® E PO exhibited fusion and sticking of particles during storage due to the electrostatic charging and low T_g (< 30°C) of the mixtures. This led to a higher particle size and decreased surface area resulting in a lower dissolution. This was especially observed in SSF where the polymer did not dissolve within this short set up of 5 min and was less crucial in spSGF (**Figure 50**) and FaSSIF (**Figure 81** in appendix), where Eudragit® E PO dissolved.

Formulations with GMS showed polymorph alteration during storage with increasing temperature. The formed beta modification exhibits a larger density in packing than the alpha modification decreasing the dissolution in SSF (**Figure 49**) [119]. Furthermore, previous studies explained a lower dissolution after storage by an increased contact angle and subsequent lower wetting of the particles due to transformation of the lipid [114, 118]. As observed for formulations only with the polymer, this was less crucial in media where the excipients are more likely to dissolve. There were no significant changes regarding the dissolved concentration of PZQ measured in spSGF for the ground extrudates with a drug load of 43.5% prepared at 50-60°C extrusion temperature ($p > 0.05$). In the view of further processing to a final drug product, this extrudate was identified as the most stable formulation, showing no dissolution changes in spSGF and only minor changes in SSF under non-sink-conditions.

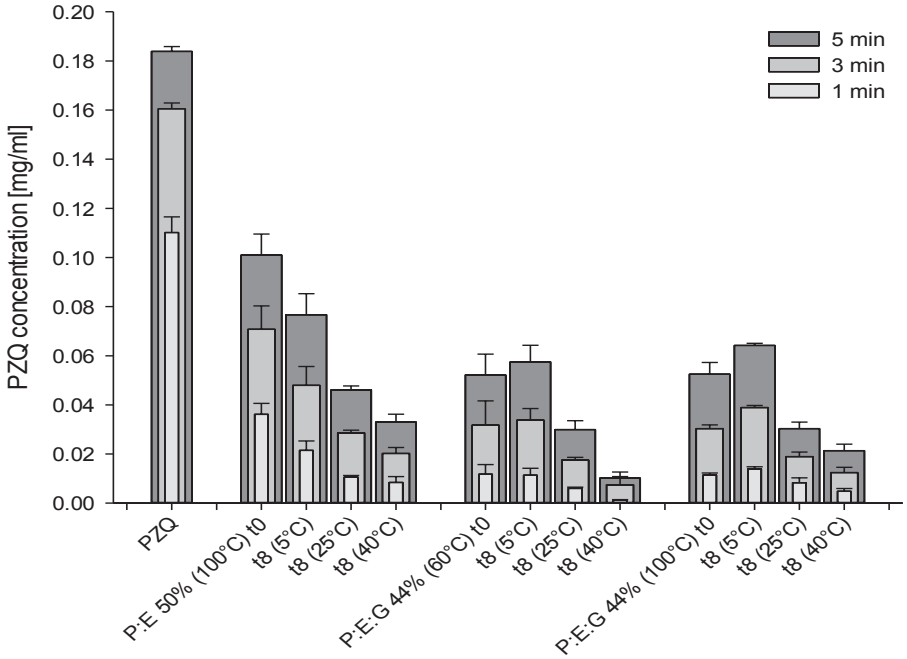


Figure 49. Stability of drug release characteristics. Praziquantel (PZQ) release in SSF of extrudates with polymer (E) and lipid (G) with the labelled drug load and extrusion temperatures directly after production (t0) and after storage for 8 weeks (t8) at 5°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity. Arithmetic mean ± S.D. (n=3).

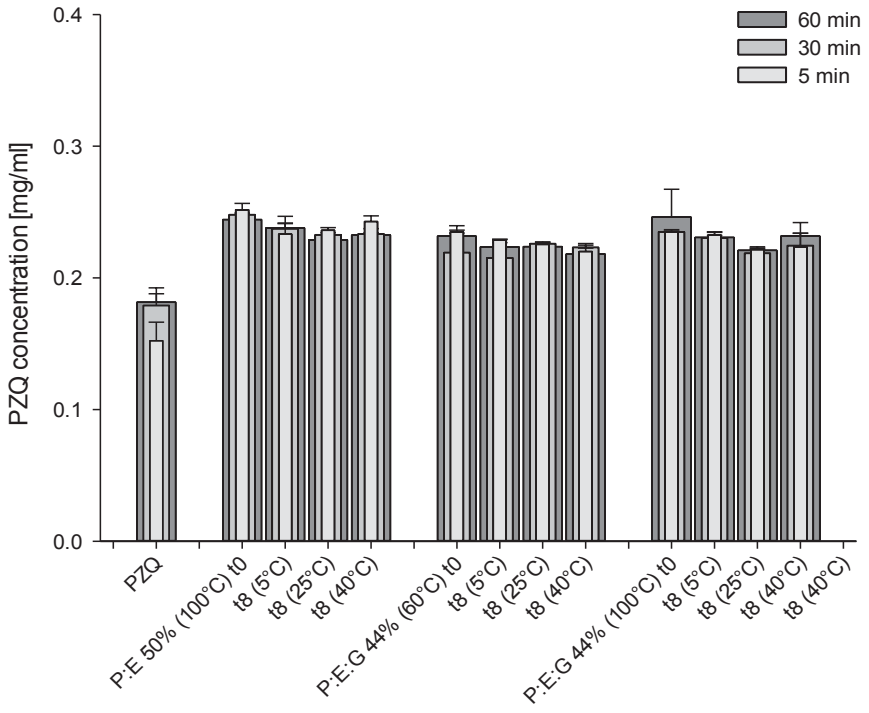


Figure 50. Stability of drug release characteristics. Praziquantel (PZQ) release in spSGF of extrudates with polymer (E) and lipid (G) with the labelled drug load and extrusion temperatures directly after production (t0) and after storage for 8 weeks (t8) at 5°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity. Arithmetic mean ± S.D. (n=3).

3.2.4. Spray-drying for taste masking

3.2.4.1. Introduction

Besides extrusion, spray-drying is a well-known continuously performable process for an efficient encapsulation of an API, applicable for the purpose of solubility enhancement and taste masking in oral solid formulations [2]. Spray-drying of a solution of API and additives like polymers or lipids is known to be an efficient process to generate a molecular dispersion of an API in various carrier systems [122, 124]. Paudel *et al.* [124] recently summarized frequently used carriers in formulating spray-dried dispersions including polymers, polyethylene oxides, lipids and carbohydrates. Furthermore, the addition of a third component to the liquid feed facilitates a more convenient spray-drying process (e.g. surfactant) and downstream of the powder (e.g. disintegrant, binder, glidant) [124].

Based on the previous results (3.2.2.2) and for comparison with prepared extrudates, spray-drying was conducted with Eudragit® E PO and GMS as taste masking carriers. The most frequently used lipids for spray-drying are Gelucire 44/14 and 50/13. Challenging for the spray-drying process are their low melting ranges (44 and 50°C) and sticky and tacky nature [124]. This could be less crucial for GMS exhibiting a higher melting range (65 to 80°C, 3.2.3.1).

The purpose of this study was to prepare spray-dried powders with the polymer and a multiparticulate system consisting of polymer and lipid. The drug release was systematically analyzed as described for ground extrudates. Moreover, the polymorphic transformations of the incorporated lipid and PZQ were monitored over storage using DSC, XRD and drug release studies. Finally, the applicability of the process was assessed in comparison to HME and SLE.

3.2.4.2. Drug-free evaluation

Spray-drying of Eudragit® E PO, GMS and mixtures of both (1:0.3 w/w) out of ethanol were evaluated with 60 and 80°C inlet temperature resulting in outlet temperatures of 42 to 43°C and 50 to 56°C (6.2.1.3, **Table 13**). Lowering the inlet temperature below 60°C was not sufficient for an effective drying of the particles. Higher temperatures than 80°C were applicable for the pure polymer, but not feasible for formulations with GMS as the sticky solidified particles covered the glass walls of the drying chamber and cyclone and were not collectable. Both process temperatures of 60 and 80°C exhibited the same DSC profiles directly after production for the pure GMS, pure Eudragit® E PO and mixtures of both excipients (**Figure 51**).

Interestingly, spray-drying of the pure GMS did not result in the formation of the lower melting alpha modification of GMS (described before in 1.8 and 3.2.3.1), but was observed for the mixture with Eudragit® E PO despite using and exhibiting the same process conditions (inlet, outlet temperature, atomizing air, air flow and liquid dosing speed). This was surprising at first glance, as the drying rate and subsequent solidifying of GMS particles was assumed to be faster than for droplets with additional Eudragit® E PO. The pure GMS solution had a lower feed concentration accompanied with smaller droplet sizes after atomization by the nozzle in the drying chamber and smaller dry powder particles. Thus, the evaporation rate was high leading to a shorter drying time [123]. As

explained before (3.2.3.1), rapid solidifying results in the formation of the alpha modification of GMS. However, as soon as the drying is completed, any heat transfer from the air increases the solid particle temperature. This effect could have prevented the formation of the alpha modification [119]. In contrast, spray-drying of the higher concentrated feed solution with Eudragit® E PO and GMS accompanied with larger droplets, particle density and dried particle sizes resulted in the formation of the alpha form.

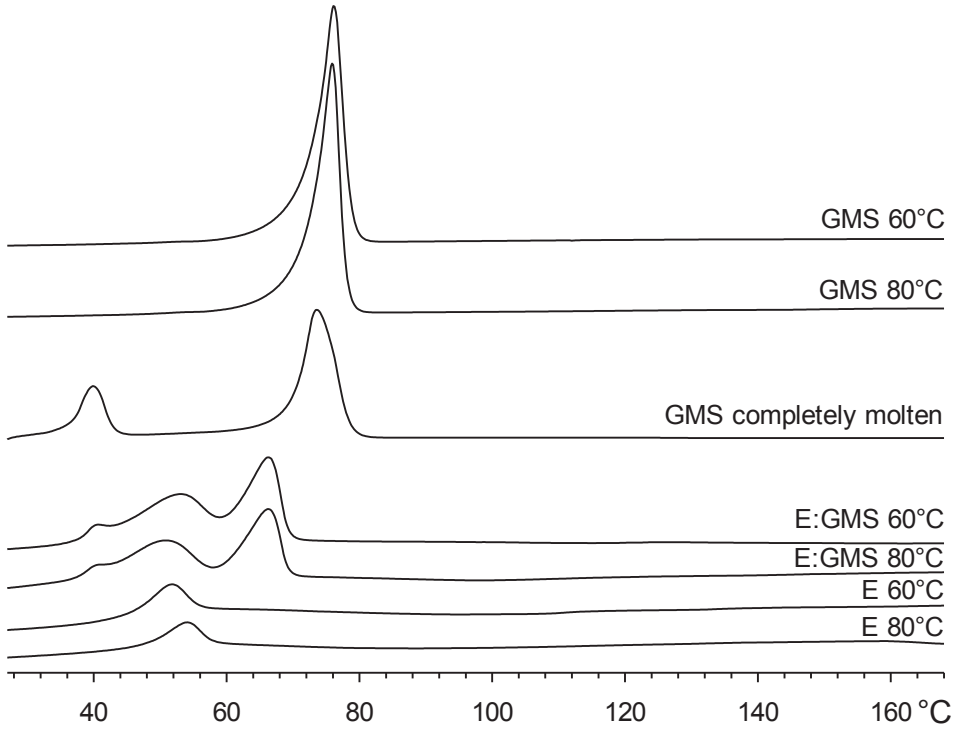


Figure 51. Analytics of spray-dried powders. Endothermic thermograms of differential scanning calorimetry of spray-dried Eudragit® E PO (E) and glyceryl monostearate (GMS) at 60 and 80°C inlet temperature directly after production and completely molten GMS for comparison.

DSC thermograms of spray-dried particles with Eudragit® E PO and GMS are similar to the results of ground extrudates (**Figure 38**) showing both melting peaks of GMS and in between the T_g of the polymer from 40 to 70°C. As demonstrated for ground extrudates, the unstable and lower melting alpha modification transformed to the stable and higher melting beta form during storage (**Figure 52**). The melting peak of the alpha form disappeared in the DSC profile.

As observed for extrusion, the pure polymer was very electrostatic leading to a high loss of powder. The yield ranged from 30 to 41%. The main amount of the powder sticked to the drying chamber and the cyclone. Moreover, very fine particles (< 2 µm) can pass directly into the exhaust air [148]. Stickiness is a well-known issue with spray-dried polymers resulting in low yields [123]. The addition of GMS improved the optical flowability of the final powder and decreased the electrostatic charge of the material. This resulted in a higher yield of the process from 38 to 48%. The particle sizes ranged from 3 to 30 µm for Eudragit® E PO and 3 to 17 µm for mixtures with the lipid. The particle size of spray-dried powders correlated to the droplet size depending on the nozzle size, feed concentration, dosing, as well as on the atomizing air flow rate and was limited by the residence

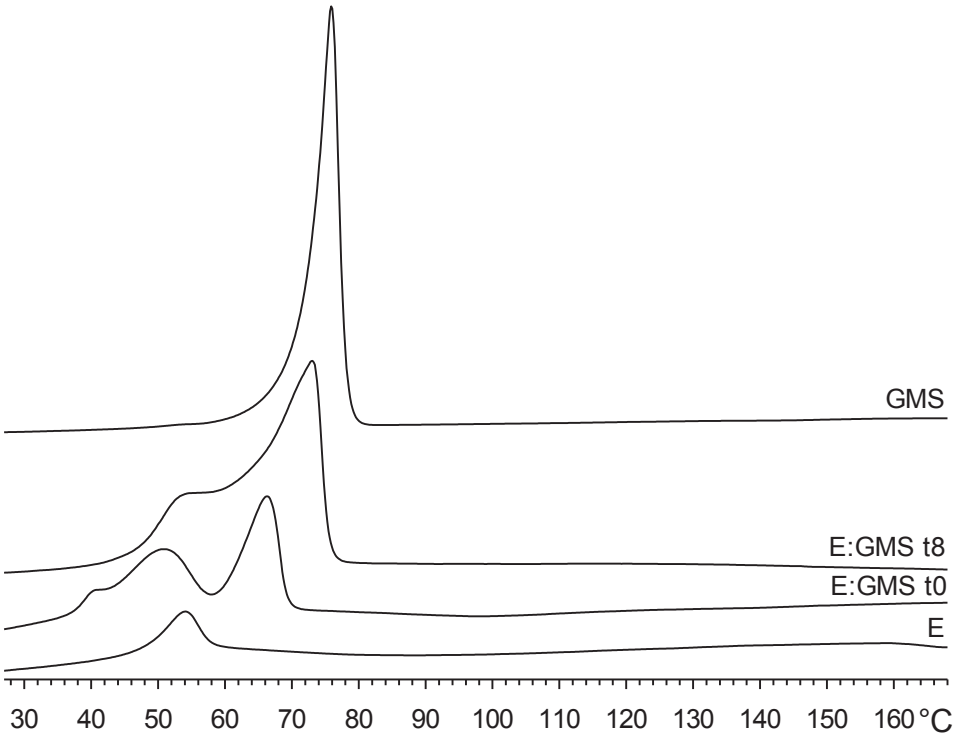


Figure 52. Stability analytics of spray-dried powders. Endothermic thermograms of differential scanning calorimetry of spray-dried Eudragit® E PO (E) with glyceryl monostearate (GMS) at 60 °C inlet temperature directly after production (t0) and after storage for 8 weeks (t8) at 25°C with 60% relative humidity.

time in the drying chamber of the lab scale spray-dryer used in this study [162]. All powders consisted of very fine spherical particles (**Figure 53**). The pure polymer exhibited particles with wrinkled surfaces which is well-known for polymers [162]. The evaporation of the ethanol at the surface of the atomized droplets in the drying chamber was fast compared to the diffusion of the incorporated polymer in the droplets. This lead to the formation of a shell at the surface due to a local high concentration and viscosity. During further drying the shell collapsed resulting in the wrinkled surface [162]. GMS acted as a plasticizer leading to a smoother surface of the spray-dried particles.

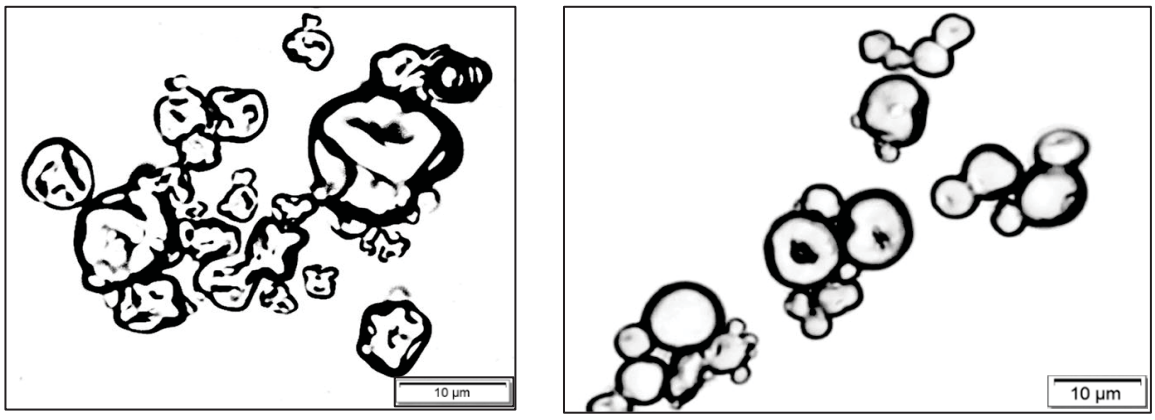


Figure 53. Microscopic pictures of spray-dried particles; left: Eudragit® E PO, right: Eudragit® E PO + GMS (1:0.3 w/w).

3.2.4.3. Spray-drying with API

Spray-drying with PZQ was conducted with Eudragit® E PO or with Eudragit® E PO and GMS in the ratio 1: 0.3 (w/w) and applying different drug loads. The physical mixtures were dissolved in ethanol and spray-dried with an inlet temperature of 60°C based on the placebo evaluation and for gentle process conditions for PZQ.

Spray-drying of PZQ with Eudragit® E PO was performed with 33.3 and 50% drug load as conducted during extrusion. The yield after this process step was 50% for 33.3% PZQ and 48% for 50% PZQ. The drug load of both formulations was verified with low deviations regarding the content uniformity (< 1%). The main drawback of the spray-dried material was the low visual flowability and electrostatic charge. The powder highly tended to agglomerate. This became apparent during storage at ambient temperature (**Figure 54**).

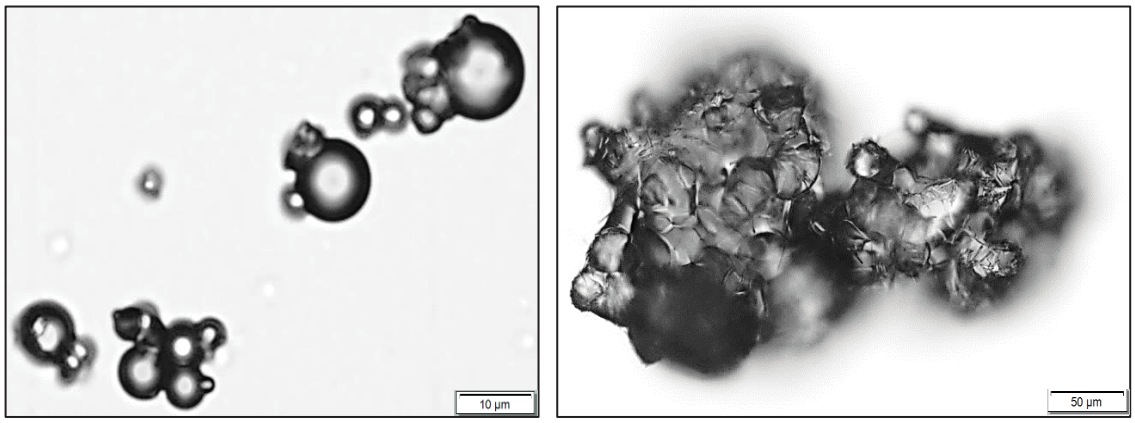


Figure 54. Microscopic pictures of spray-dried particles with 50% PZQ and Eudragit® E PO directly after production (left) and three days storage at ambient temperature (right).

The spherical particles exhibited particle sizes from 1 to 15 µm. XRPD patterns revealed an amorphous state of PZQ for both drug loads directly after production (**Figure 55**).

The addition of GMS as an additional taste masking agent improved the visual flowability of the spray-dried powder. Drug loads of 43.5 and 27.8% were prepared. The process yield for spray-drying was 51% for 43.5% drug load and 44% for 27.8% drug load as some of the powder remained in the drying chamber and the cyclone. The drug load was confirmed by a high content uniformity (< 1.5% RSD). The yield can be increased in larger scale setups as the relative constant loss of powder on the walls is a decreasing fraction of the total production amount [149]. Spherical, non-electrostatic particles were obtained with particle sizes from 2 to 18 µm (**Figure 56**). PZQ was in amorphous state after spray-drying for both drug loads (**Figure 55**). The characteristic peak at 21.01° was determined as crystalline structures of GMS for both drug loads.

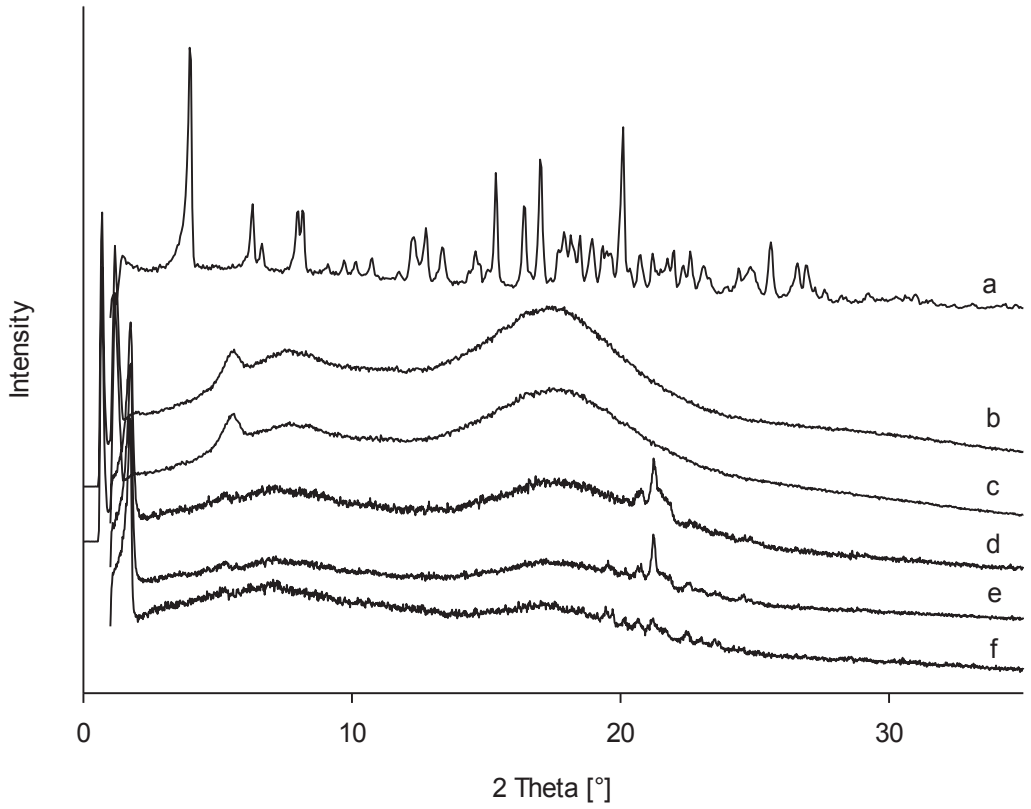


Figure 55. XRPD patterns spray-dried powders. (a) crystalline praziquantel (PZQ); (b) 33.3% PZQ:Eudragit® E PO (E); (c) 50.0% PZQ:E; (d) 27.8% PZQ:E:glyceryl monostearate (GMS); (e) 43.5% PZQ:E:GMS; (f) E:GMS.

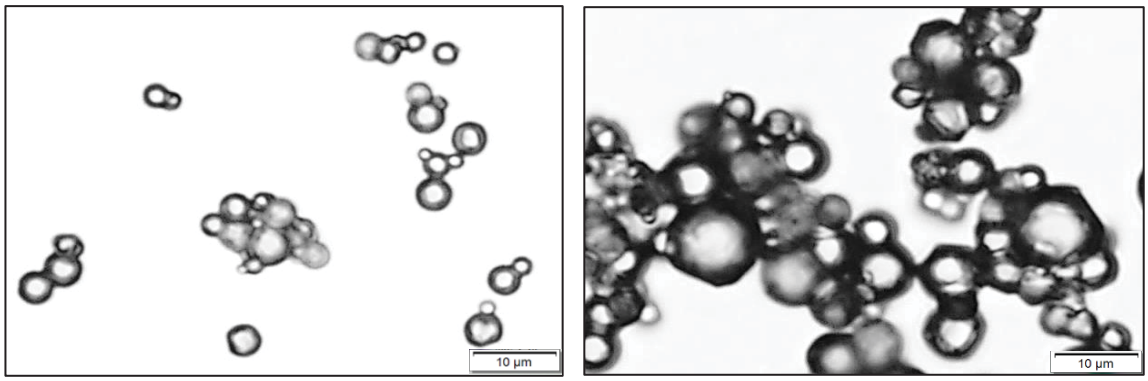


Figure 56. Microscopic pictures of spray-dried particles with 43.5% (left) PZQ or 27.8% (right) and Eudragit® E PO and GMS directly after production.

Non-sink *in vitro* dissolution studies were conducted to evaluate the impact of the additional GMS on drug release and solubility kinetics of PZQ in spray-dried formulations. The dissolution was performed in SSF (pH 6.8), in spSGF (pH 1.2) and FaSSIF (pH 6.5). They were set up for formulations only with Eudragit® E PO with 33.3 and 50% PZQ and with Eudragit® E PO and GMS with 27.8 and 43.5% PZQ. The results were compared with the pure PZQ exhibiting a pH-independent thermodynamic solubility of 0.22 mg/ml in all three media.

A delayed release of PZQ in SSF in view of taste masking was observed for formulations with Eudragit® E PO only and with GMS with 27.8% drug load (**Figure 57**). Spray-drying with Eudragit® E PO significantly decreased the drug release up to 3-fold in comparison to pure PZQ ($p < 0.05$). This is based on the low solubility of the polymer above pH 6 [189]. There was no significant difference for the drug loads of 33.3 and 50% ($p > 0.189$). Formulations with GMS showed a higher drug release than powders with the polymer only. GMS led to a faster and higher solubilization of PZQ. Nevertheless, the spray-dried powder with a drug load of 27.8% still delayed the drug release significantly about 1.7-fold after 5 min ($p = 0.013$). In view of taste masking, the addition of GMS in the spray-drying process was not advantageous in comparison to formulations only with the polymer.

All preparations increased the kinetic solubility in spSGF and FaSSIF due to the amorphous state of PZQ. **Figure 58** illustrates the results for spSGF. Formulations only with the polymer showed a high supersaturation of PZQ in spSGF after 5 min (1.13 mg/ml for both drug loads, 5.6-fold). However, PZQ demonstrated a recrystallization ending up in 2-fold supersaturation after 60 min. These results are in accordance with the dissolution of amorphous drug-polymer films (3.2.2.2) and partly amorphous extrudates (3.2.3.2). Interestingly, the formulations with GMS demonstrated a stable 3–fold supersaturation for 60 min in spSGF and up to 5-fold supersaturation in FaSSIF. Thus, the addition of GMS for spray-drying was advantageous in terms of a high solubility for an immediate release in simulated gastric and intestinal media.

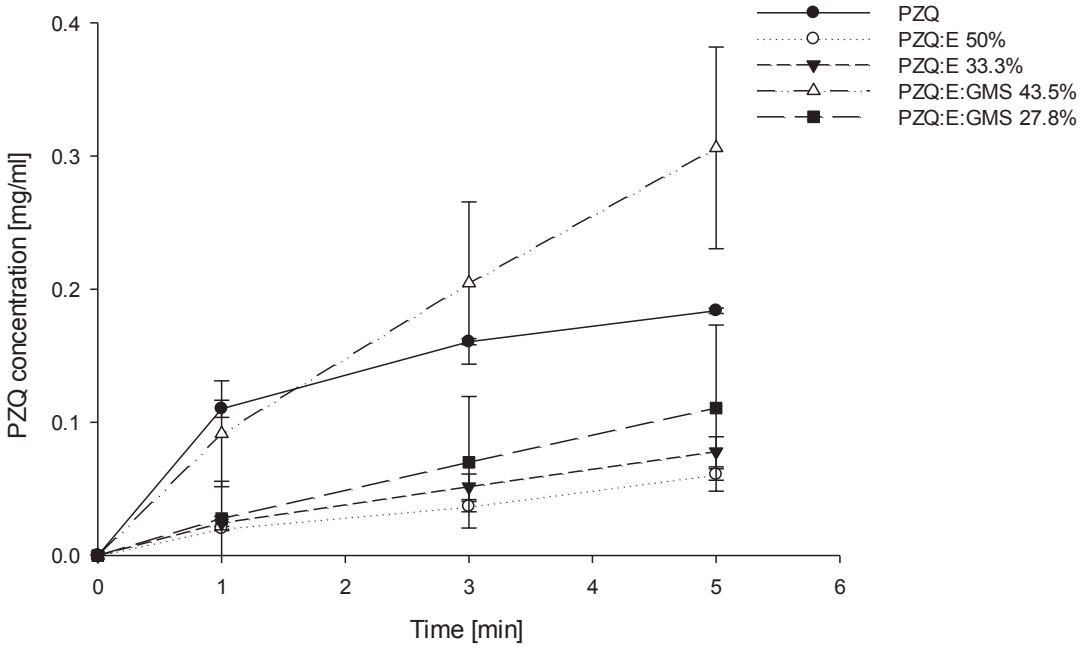


Figure 57. Non-sink dissolution profiles in SSF of pure praziquantel (PZQ) and spray-dried powders with polymer (E) and lipid (GMS) with the labelled drug load. Arithmetic mean ± S.D. (n=3).

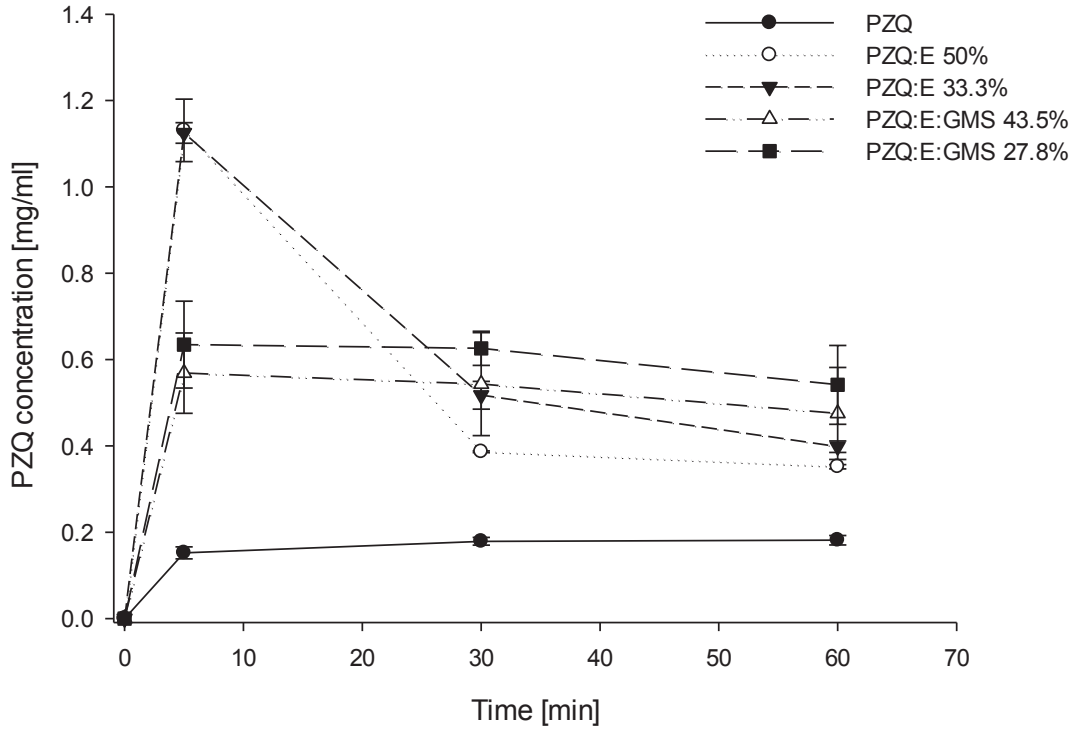


Figure 58. Non-sink dissolution profiles in spSGF of pure praziquantel (PZQ) and spray-dried powders with polymer (E) and lipid (GMS) with the labelled drug load. Arithmetic mean ± S.D. (n=3).

3.2.4.4. Stability over storage

Spray-dried powders with Eudragit® E PO and GMS with both drug loads were stored for 8 weeks at 5 ± 3°C, 25°C with 60% r. h. and 40°C with 75% r. h. (6.2.2.17). Changes in the physical state of PZQ and both excipients were evaluated and the effect on the performance of the formulations regarding the drug release. Formulations with the polymer only were excluded, as the further processing of the powders was not feasible due to the electrostatic and agglomerated powder characteristics.

XRPD patterns of both formulations demonstrated changes in the physical state of the spray-dried powders, especially when stored at 40°C with 75% r. h. (**Figure 59**). Recrystallization of GMS was observed for both drug loads during storage. As described before, the unstable alpha form transformed to the stable beta form of the lipid. Characteristic peaks of the lipid from 19 to 24° appeared. In addition, further peaks arose at 9.5, 12.1, 15.2 and 16.6° among many others, indicating the recrystallization of PZQ. The polymorphism of the lipid could have promoted the recrystallization of PZQ in the spray-dried formulations. As there was a lack of characteristic signals of the bulk PZQ (e.g. 6.23, 6.60, 7.90 or 8.20) the storage of spray-dried material could have led to a new polymorphic form.

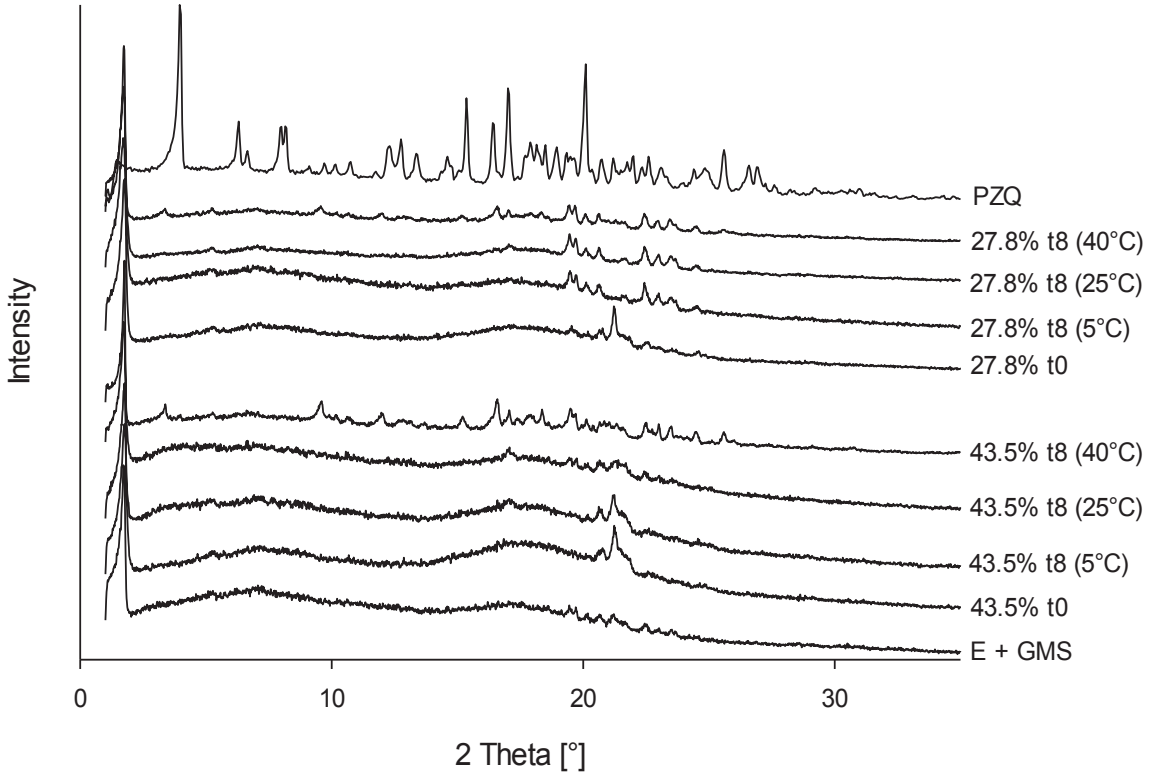


Figure 59. Stability analytics of spray-dried powders. XRPD patterns of praziquantel (PZQ), Eudragit® E PO with glyceryl monostearate (GMS) and spray-dried powders prepared with 27.8% or 43.5% PZQ directly after production (t0) and after storage for 4 weeks at 5 ± 3°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity.

Both formulations exhibited changes in the drug release at discrete timepoints in SSF over storage based on the polymorph alteration of the lipid and PZQ (**Figure 60**). The formed beta modification of GMS exhibits a larger density in packing than the alpha modification, decreasing the drug release in SSF [119]. Furthermore, previous studies explained a lower drug release after storage by an increased contact angle and subsequent lower wetting of the particles due to transformation of the lipid [114, 118].

The polymorph transformations of PZQ and the lipid were accompanied with high variations of the drug release in spSGF and FaSSIF. The solubility of PZQ in the spray-dried formulations was still higher than the bulk material after storage. This was probably due to the presence of partly amorphous PZQ. In addition, the polymer and the lipid in the dissolution medium lowered the surface tension of the medium and enabling a better wetting of PZQ [51].

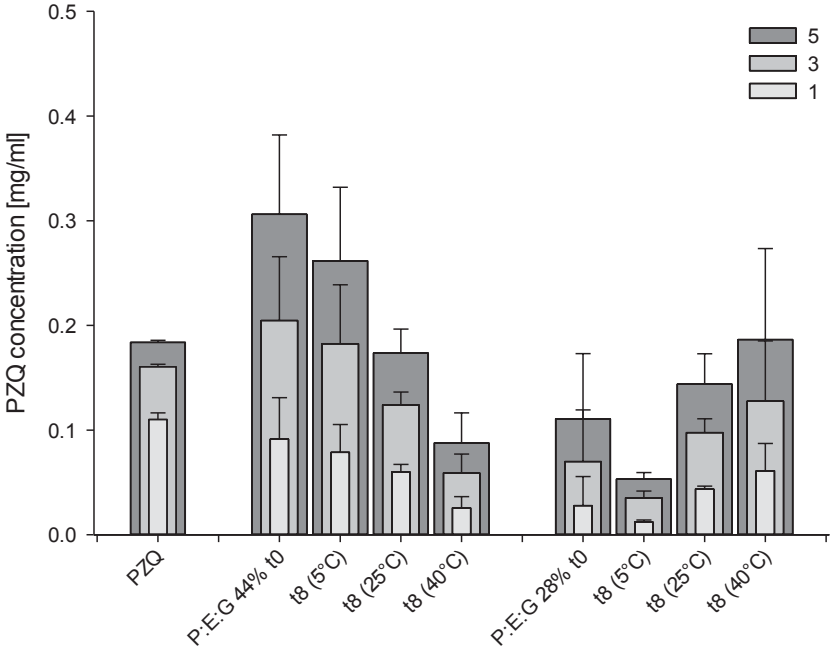


Figure 60. Stability of drug release characteristics. Praziquantel (PZQ) release in SSF of spray-dried powders with polymer (E) and lipid (G) directly after production (t0) and after storage for 8 weeks (t8) at 5°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity. Arithmetic mean ± S.D. (n=3).

3.2.4.5. Comparison of extrusion and spray-drying

Extrusion and spray-drying are useful manufacturing techniques for the encapsulation or embedding of PZQ in a polymer or multiparticulate matrix with polymer and lipid. Due to the different manufacturing process principles, the particle sizes differed strongly between spray-dried powders (1 – 16 µm) and ground extrudates (3 – 400 µm). The particle size of spray-dried powders correlated to the droplet size depending on the nozzle size, feed concentration and dosing, as well as on the atomizing air flow rate and was limited by the short residence time in the drying chamber of the lab scale spray-dryer used in this study [162]. Spray-dried powders consisted of very fine spherical particles with a visually low flowability. In contrast, the brittle extrudates exhibited more angular ground particles with an improved flowability which is of particular importance for the downstream of both processes [125, 195]. An efficient and cost effective formulation development in pharmaceutical industry in terms of blending, tableting or capsule filling is essentially affected by the flow properties of the powder material [196, 197]. The differences in the yield of both methods is as well accompanied to the process principle. The addition of GMS improved the yield in both processes, as the final powder was less sticky and electrostatic. Moreover, in terms of further processing, formulations with GMS exhibited an easier handling than formulations only with Eudragit® E PO.

In contrast to extrudates, spray-dried powders revealed amorphous PZQ and only crystalline structures of GMS for both drug loads within the optimized thermal processing windows. For formulations with GMS spray-dried powders exhibited a higher API release in SSF in comparison to extrudates, physical mixtures and pure PZQ (**Figure 61**). Moreover, the smaller particle size led to faster solubility kinetics and higher solubility, a well-known reported impact [173, 174]. A higher amount of the insoluble polymer in SSF in relation to PZQ could counteract and slow down the release, but extrudates of the same composition were still superior due to incorporated crystalline PZQ. The importance of taste masking for PZQ was demonstrated using the BATA-model (3.1.2.2.1). PZQ showed a very low taste threshold of 0.03 mg/ml. A solubilized content of 0.06 mg/ml PZQ was identified as aversive and the IC_{50} value (concentration inhibiting 50% of response of pure water as the reference solution). Concentrations up to 0.01 mg/ml PZQ did not show a significant difference to pure water in the BATA-model. Only extrudates of both drug loads provided a delayed release of the API below 0.01 mg/ml after 1 min and below 0.06 mg/ml after 5 min within this setup. Pure PZQ, physical mixtures as well as the spray-dried formulations quickly reached concentrations above 0.06 mg/ml. Thus, spray-drying with Eudragit® E PO and GMS was not as appropriate as extrusion to encapsulate PZQ in view of taste masking.

All investigated physical mixtures showed a slightly increased solubility of PZQ in spSGF and FaSSIF in the presence of Eudragit® E PO and GMS in comparison to the pure PZQ as they provide an improved wetting of the crystalline PZQ surface ($p < 0.05$) [51]. Ground extrudates showed a slight, but significant supersaturation (1.3-fold) in comparison to physical mixtures and pure PZQ ($p < 0.05$). The amorphous PZQ in spray-dried powders enabled the highest supersaturation in spSGF (3-fold) and FaSSIF (5-fold) ($p < 0.05$).

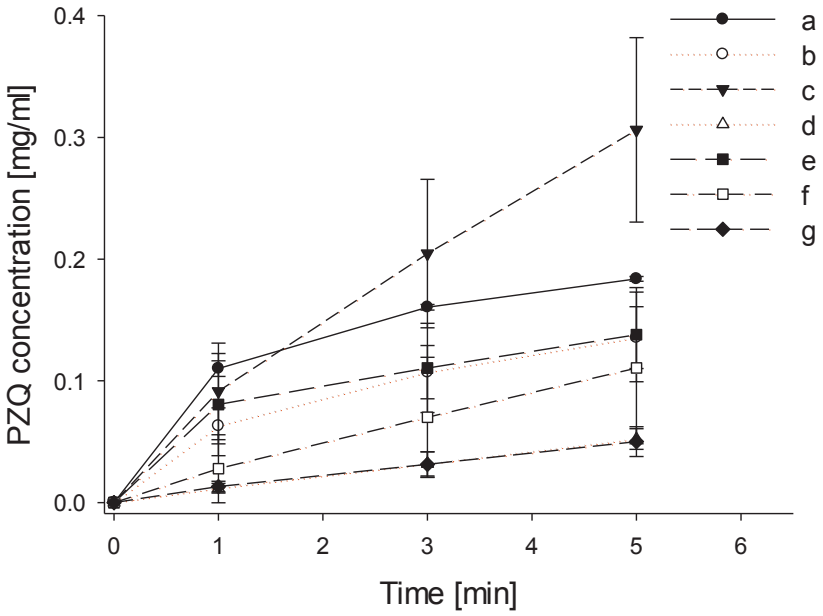


Figure 61. Non-sink dissolution profiles of formulations with praziquantel (PZQ) and Eudragit® E PO: glyceryl monostearate 1:0.3 (w/w) in SSF. (a) crystalline PZQ; (b) physical mixtures with 43.5% PZQ; (c) spray-dried powders with 43.5% PZQ; (d) ground extrudates with 43.5% PZQ; (e) physical mixtures with 27.8% PZQ, (f) spray-dried powders with 27.8% PZQ; (g) ground extrudates with 27.8% PZQ. Arithmetic mean ± S.D. (n=3).

Amorphous solid dispersions are thermodynamically metastable systems that tend to recrystallize during storage [202]. In fact, phase transformation of PZQ was observed in the spray-dried formulations (**Figure 59**) showing polymorphism in contrast to the extrudates (**Figure 47**, **Figure 48** and **Figure 78**) leading to altered drug release characteristics. Whereas the released concentration of PZQ in SSF decreased strongly in stored spray-dried powders, there are only minor changes in the kinetic solubility of ground extrudates where PZQ was incorporated in its crystalline form (**Figure 62**). There were no significant changes regarding the released concentration of PZQ for the ground extrudates measured in spSGF ($p > 0.05$) in contrast to spray-dried powders (**Figure 63**). All XRPD-patterns revealed a change of the physical state of GMS, confirmed by DSC measurements, for both preparation techniques.

In conclusion, extrusion was superior to spray-drying utilizing the same compositions with Eudragit® E PO and GMS. GMS efficiently improved the taste masking capabilities of the extrudates and facilitated gentle process conditions for PZQ. Solid lipid extrusion enabled a promising decreased release effect of PZQ in SSF correlating to an efficient taste masking. Moreover, it demonstrated a fast and reproducible drug release in simulated gastric and intestinal media through encapsulation of the crystalline PZQ. Based on the *in vivo* taste assessment in the BATA model, identifying a taste threshold of 0.03 mg/ml for PZQ, it could be assumed that only the extrudates would reach sufficient taste masking with Eudragit® E PO and GMS. As expected, maintaining the crystalline state of PZQ was advantageous in terms of delayed release formulations in SSF and stability.

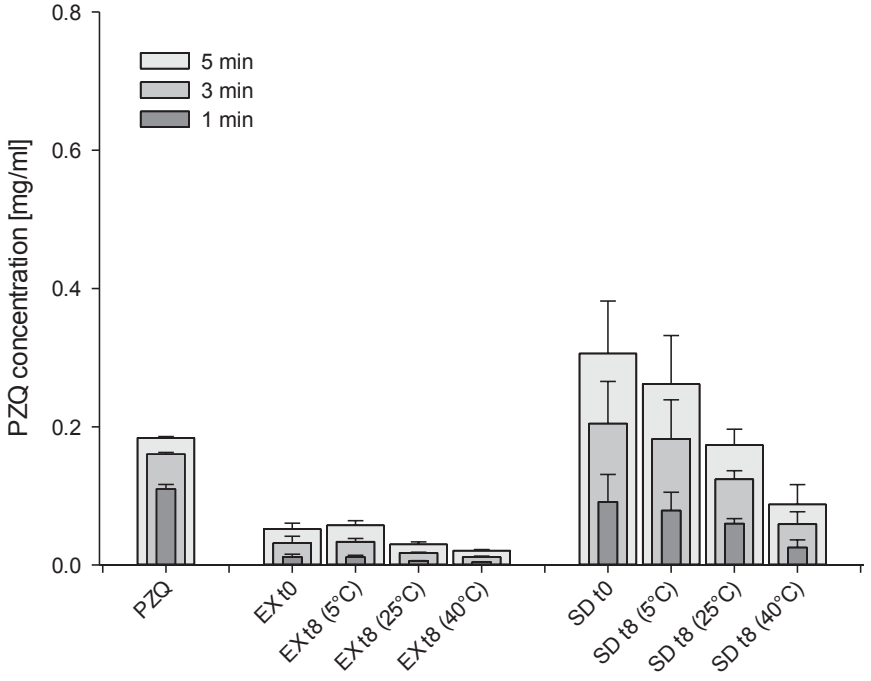


Figure 62. Stability of drug release characteristics in SSF. Ground extrudates (EX) and spray-dried powders (SD) in comparison to crystalline praziquantel (PZQ) directly after production (t0) and after storage for 8 weeks (t8) at 5°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity. Arithmetic mean ± S.D. (n=3).

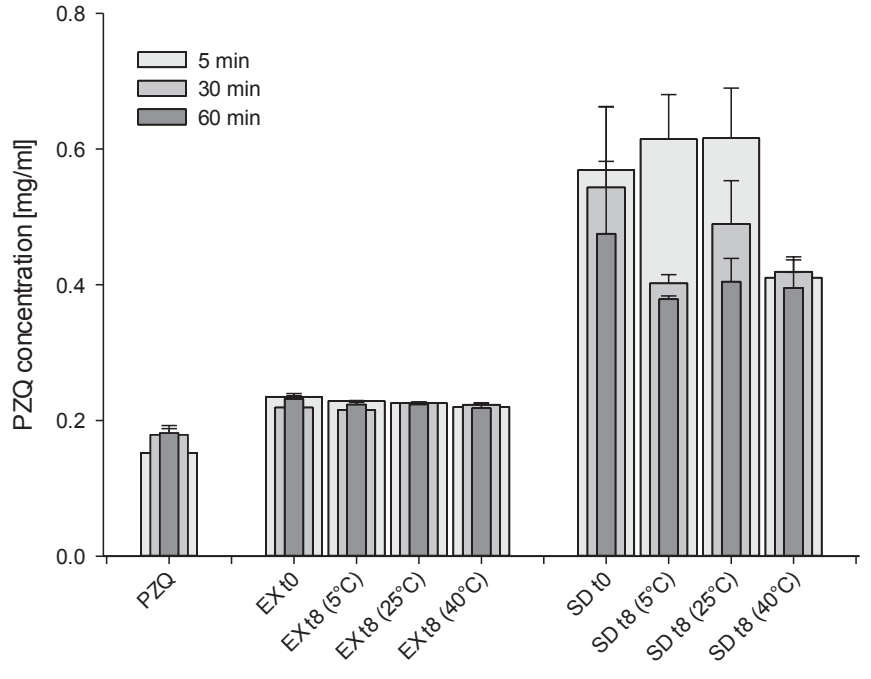


Figure 63. Stability of drug release characteristics in spSGF. Ground extrudates (EX) and spray-dried powders (SD) in comparison to crystalline praziquantel (PZQ) directly after production (t0) and after storage for 8 weeks (t8) at 5°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity. Arithmetic mean ± S.D. (n=3).

3.2.5. Tableting of taste masked powders

As described in 1.2 and 3.2.1 uncoated fast dissolving MTs were chosen as the most suitable dosage form for children offering a high flexibility regarding dosing and an easy administration [25-27].

As described and evaluated in 3.2.1, the production of MTs required powder mixtures with a high flowability and compactibility, providing tablets with a high mechanical stability but fast disintegration. In this study, Parteck® M100 revealed as the most suitable excipient for direct compression of ODMTs combining high compaction properties and a fast disintegration. Thus, this excipient was chosen as a filler for the preparation of taste masked MTs. As the flowability and compressibility can be limited when tableting spray-dried or extruded formulations, the use of co-processed excipients can be of advantage. Thus, Ludiflash® as a directly compressible filler and binder for ODFs based on mannitol was evaluated.

Based on the previous findings in 3.2.3 and 3.2.4, formulations with Eudragit® E PO and GMS and a drug load of 43.5% PZQ were selected for tableting of taste masked fast dissolving MTs.

3.2.5.1. Tableting

MTs according to the compositions in **Table 6** were prepared via direct compression (6.2.1.6). The small particle size and low bulk density of spray-dried powders generally results in a poor flowability [123]. Further compaction or granulation can be applied to improve the flowability and handling of the powders for processing to tablets or other solid dosage forms [123]. In this study spray-dried powders were not granulated or compacted before tableting as amorphous systems are thermodynamically unstable systems. The mechanical stress could have a high impact on the physical state of the API [123].

The results of tablet weight, height, hardness and content uniformity are summarized in **Table 7**. All tablet formulations made from extrudates, SD-Ludi-MT and the ODMT met the specifications of weight and content uniformity of the Ph. Eur. as the AV value was below 15. All other MTs of spray-dried powders and physical mixtures demonstrated low variations regarding the tablet weight but too high variations of the PZQ content. Spray-dried powders and the micronized raw material of PZQ exhibited smaller particle sizes in comparison to the ground extrudates. Thus, separation of different particle sizes could have led to the variations regarding the API content. Mixing was performed with the Turbula T2C as described in 6.2.1.1 subjecting the powder to rotation, translation and inversion in periodically motions [203]. During the rotation of the vessel, particles can roll on the wall of the vessel or on the free surface formed by the powder bed. Because of the size difference of spray-dried particles and fillers, surface aggregation can lead to inhomogeneity of the blend [204]. The subsequent mixing is effected by convection, diffusion at longer mixing times and shear [205]. During mixing and further processing various particle sizes can be accompanied with segregation through sifting as small particles can pass the void space between large particles [206]. Thus, ground extrudates with higher particle sizes were superior to spray-dried and physical mixtures regarding blend homogeneity for tableting.

Table 6
Percentage composition of formulations for tableting of mini-tablets (MT).

Formulation		PZQ: E: GMS (1:1:0.3 w/w)	Ludiflash®	Parteck® M100	Kollidon® CL-SF	K_2CO_3	PRUV®
MT of extrudates	**EX-MT**	100.00					
	EX-Kol-MT	97.55			2.45		
	EX-Ludi-MT-1	75.00	23.75		1.25		
	EX-Ludi-MT-2	49.00	49.00				2.00
	EX-Man-MT-1	75.00		22.55	2.45		
	EX-Man-MT-2	49.00		46.55	2.45		2.00
	EX-K_2CO_3-MT	49.00		44.55	2.45	2.00	2.00
MT of spray-drying	**SD-Ludi-MT**	49.00	49.00				2.00
	SD-Man-MT			46.55	2.45		
	SD-K_2CO_3-MT			44.55		2.00	
MT of physical mixtures	**PM-Ludi-MT**	49.00	49.00				2.00
	PM-Man-MT			46.55	2.45		
	PM-K_2CO_3-MT			44.55		2.00	
ODMT		21.30 PZQ		75.25	2.45		2.00

Table 7
Physical characterization of mini-tablets.

Formulation	Weight [mg]	Height [mm]	Hardness [N]	Content [%]	AV value
EX-MT	19.6 ± 0.3	2.78 ± 0.09	23 ± 5	94.7 ± 0.8	5.7
EX-Kol-MT	19.8 ± 0.3	2.77 ± 0.06	22 ± 4	95.2 ± 1.1	5.9
EX-Ludi-MT-1	19.8 ± 0.4	2.72 ± 0.14	31 ± 4	96.1 ± 2.1	7.6
EX-Ludi-MT-2	19.9 ± 0.4	2.61 ± 0.02	30 ± 4	98.1 ± 3.5	8.9
EX-Man-MT-1	19.8 ± 0.4	2.64 ± 0.05	33 ± 6	96.1 ± 2.1	9.5
EX-Man-MT-2	19.8 ± 0.4	2.65 ± 0.03	39 ± 5	97.3 ± 3.1	8.6
EX-K_2CO_3-MT	19.8 ± 0.3	2.65 ± 0.02	30 ± 6	97.6 ± 3.0	8.1
SD-Ludi-MT	20.0 ± 0.5	2.64 ± 0.02	36 ± 5	101.3 ± 4.6	8.3
SD-Man-MT	19.9 ± 0.6	2.67 ± 0.04	28 ± 4	93.5 ± 9.2	27.0
SD-K_2CO_3-MT	20.1 ± 0.4	2.64 ± 0.04	36 ± 14	100.0 ± 11.3	25.6
PM-Ludi-MT	19.7 ± 0.7	2.75 ± 0.01	35 ± 15	95.0 ± 4.8	14.9
PM-Man-MT	20.3 ± 0.6	2.66 ± 0.02	35 ± 7	100.6 ± 9.7	19.5
PM-K_2CO_3-MT	20.0 ± 0.6	2.72 ± 0.02	27 ± 7	95.1 ± 5.3	16.2
ODMT	19.7 ± 0.3	2.50 ± 0.01	29 ± 8	96.6 ± 2.9	9.0

The disintegration of the prepared formulations was tested in water according to PH. Eur. 8.0 2.9.1 (6.2.2.13.1) and additionally in spSGF as the included polymer Eudragit® E PO is soluble below pH 5 and swellable at higher pH values. As expected, disintegration in spSGF was faster for all formulations in comparison to water (**Figure 64**). There was no significant difference regarding the disintegration in spSGF among MTs prepared from extrudates ($p > 0.05$) except for EX-Ludi-MT-2 demonstrating a significant higher disintegration time of 4.6 ± 0.8 min ($p < 0.05$). The other formulations including extrudates observed mean values from 2.0 to 3.3 min in spSGF. The immediate solubilization of the polymer resulted in a fast disintegration of the tablets. Both media revealed a faster or equal disintegration of tablets made from extrudates in comparison to physical mixtures using the same amount of filler. Tablets of spray-dried powders showed the slowest disintegration in both media based on the smaller particle size of the raw material. Parteck® M100 demonstrated a faster disintegration than Ludiflash® ($p < 0.05$) accompanied with the included Kollicoat® SR 30D. However, both fillers decreased the disintegration time in water with increasing percentages along with a lower amount of Eudragit® E PO ($p < 0.05$). MTs of pure extrudates did not disintegrate in water within 60 min. The addition of 2.45% Kollidon® CL-SF (EX-Kol-MT) facilitated a faster disintegration of 22.3 ± 3.3 min. Adding K_2CO_3 to accelerate disintegration based on previous studies in literature [207] was not successful for all formulations ($p > 0.05$).
None of the formulations met the specification of ODTs (disintegration within 3 min in water) due to the high amount of incorporated Eudragit® E PO. EX-Man-MT-2 demonstrated a disintegration in water of 4.3 ± 0.7 min and 2.0 ± 0.3 min in spSGF.

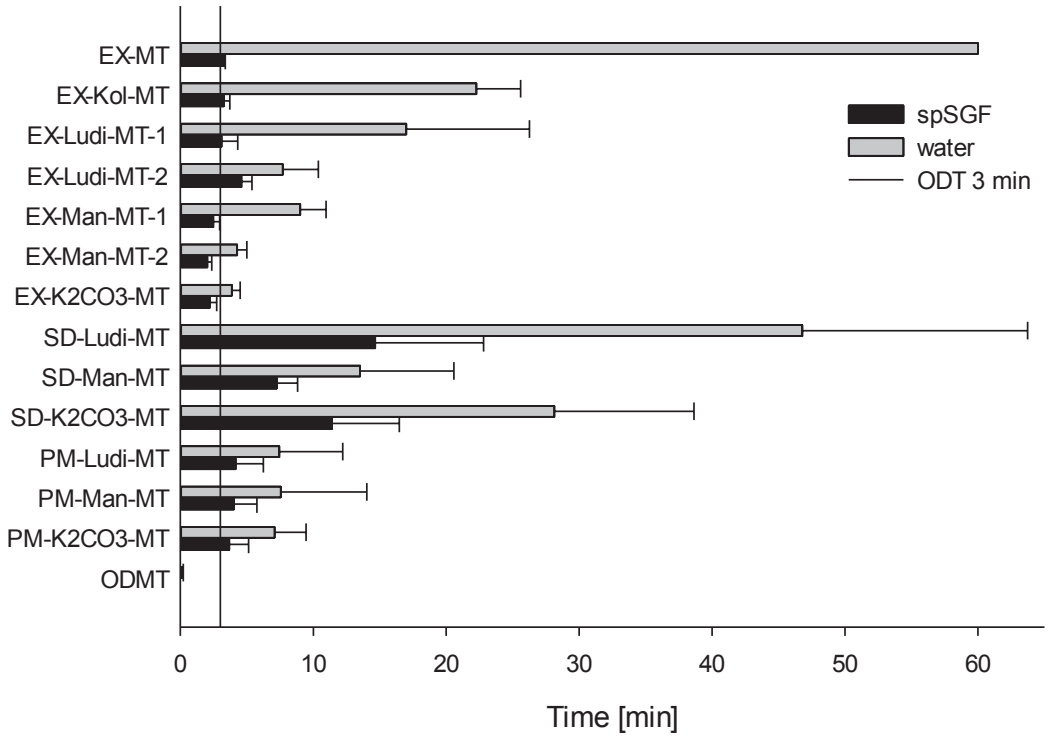


Figure 64. Disintegration of all mini-tablet formulations in water and spSGF.

The dissolution of all formulations was evaluated in SSF, spSGF and FaSSIF. All MTs demonstrated delayed release profiles of PZQ in SSF for a promising taste masking effect compared to the reference ODMT formulation without polymer and lipid. Tablets prepared from ground extrudates were superior to physical mixtures and spray-dried powders (**Figure 65**). There was no significant difference for the relative drug release (**Figure 66**) among all formulations made from extrudates despite higher drug loads and filler composition ($p > 0.05$). All showed a drug release of less than 0.5% in 5 min correlating to a released mass of less than 0.04 mg. Increasing the drug load resulted only in a slight tendency (< 0.01 mg) of a higher absolute drug release as the amount of polymer and lipid simultaneously increased (**Figure 82** in appendix). These achievements indicate a high taste masking effect [2].

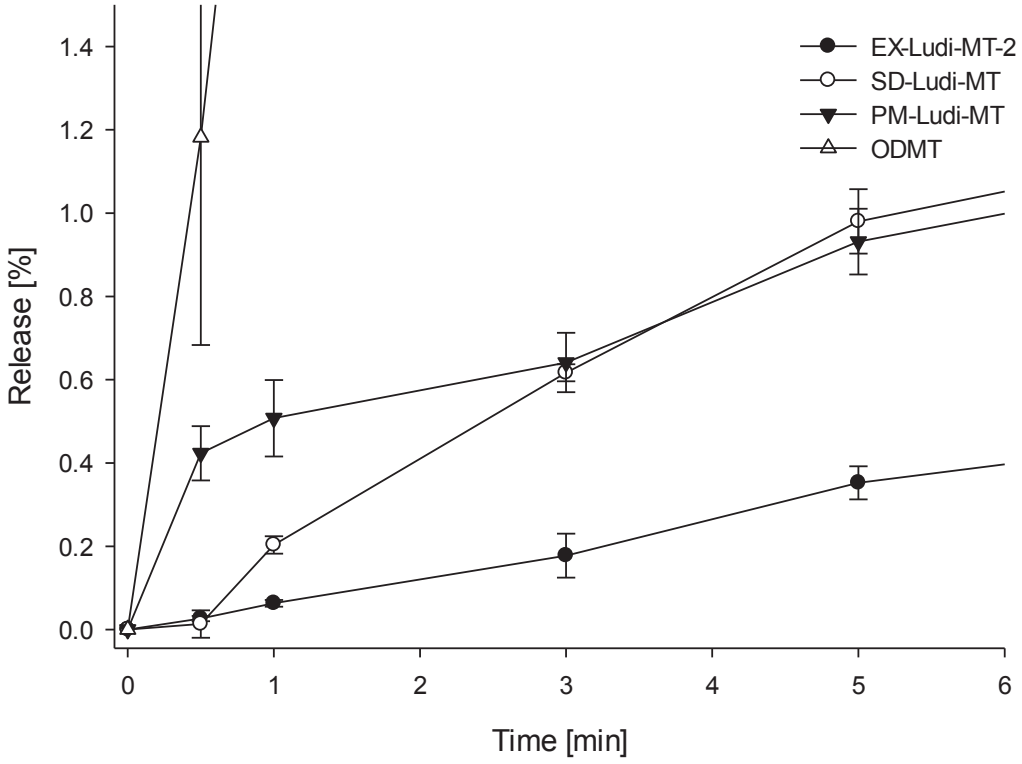


Figure 65. Drug release of mini-tablets with Ludiflash® in SSF. Arithmetic mean ± S.D. (n=6).

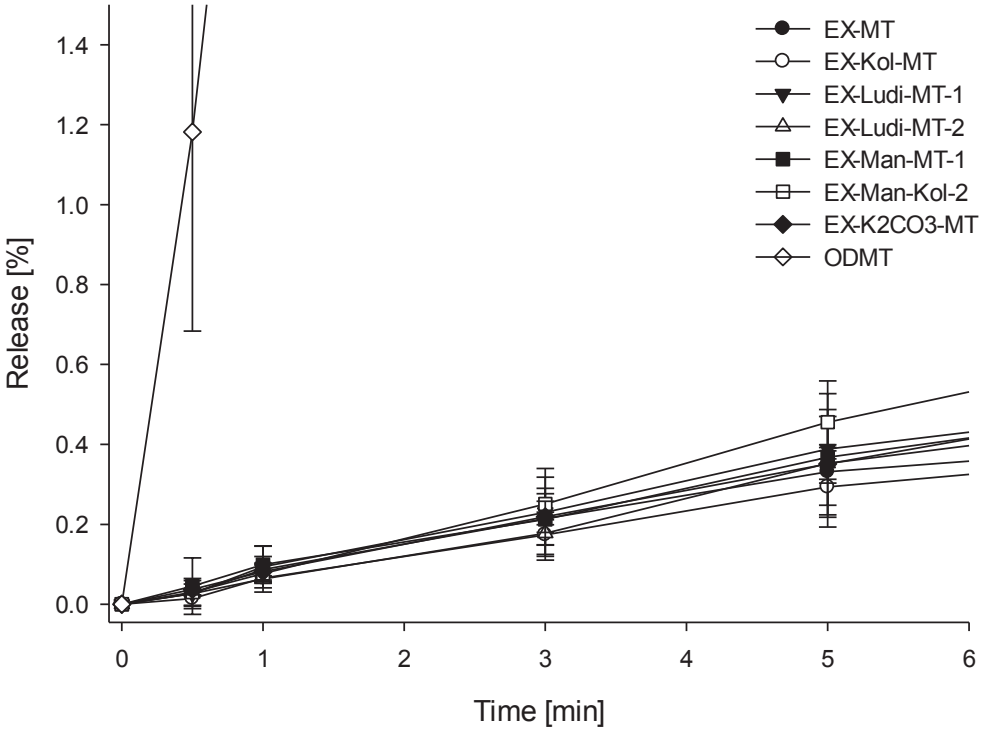


Figure 66. Drug release of mini-tablets made from extrudates in SSF. Arithmetic mean ± S.D. (n=6).

The drug release of MTs made from extrudates, spray-dried formulations and physical mixtures in spSGF demonstrated huge differences (**Figure 67**). Extrudate formulations enabled a fast and complete drug release of 100% after 60 min, whereas spray-dried formulations and physical mixtures released less than 30% of the incorporated PZQ. This is mainly attributed to the different disintegration time of the MTs. Moreover, formulations with extrudates exhibited a higher drug release than the ODMT reaching approximately 62% API release after 60 min. The addition of Eudragit® E PO and GMS could have evenly improved the solubilisation of PZQ under sink-conditions due to an improved wettability [208]. Due to amorphous PZQ in spray-dried formulations, one could have expected an immediate and complete drug release of these formulations. In contrast, even low compression forces during tableting and a low crushing strength below 40 N resulted in a matrix-controlled release of the API, even slower than for physical mixtures.

Comparing different fillers and drug loads for MTs made from extrudates (**Figure 68**), Parteck® M100 enabled the fastest dissolution in spSGF, followed by pure extrudates and formulations with Ludiflash®. Accompanied with the included Kollicoat® SR 30D EX-Ludi-MT-2 showed a slower disintegration and subsequent slower dissolution than all other MTs prepared from extrudates.

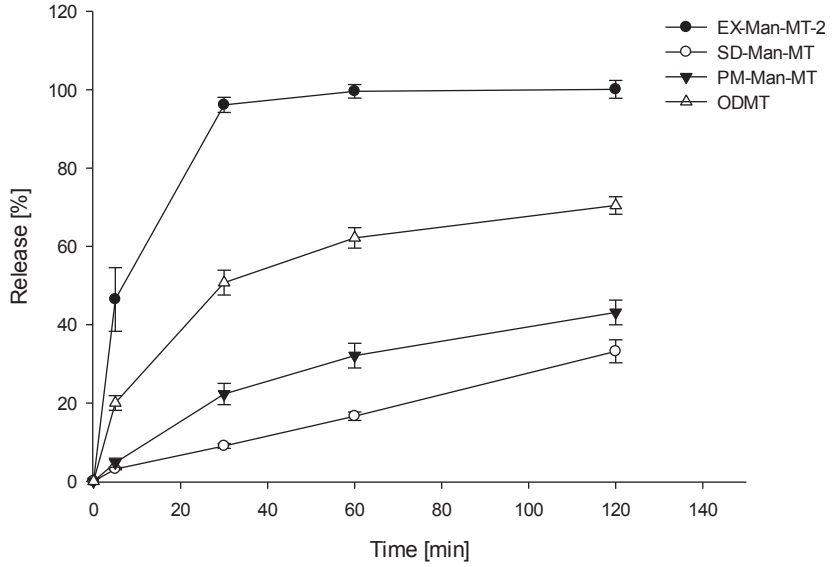


Figure 67. Drug release of mini-tablets with Parteck® M100 in spSGF. Arithmetic mean ± S.D. (n=6).

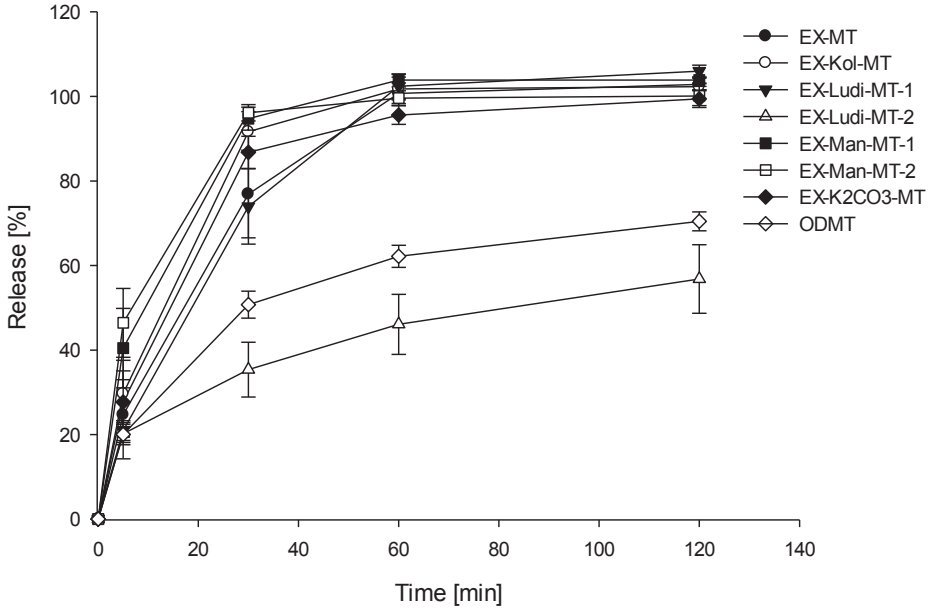


Figure 68. Drug release of mini-tablets made from extrudates in spSGF. Arithmetic mean ± S.D. (n=6).

For further comparison of all formulations, the mean dissolution time (MDT) was calculated (**Figure 69**). The MDT is an index of the dissolution rate of a drug over time and describes the conceptional mean residence time of the drug in the drug product [209]. Thus a low MDT is connected to a fast release of PZQ and subsequent solubilisation enabling the required absorption. The MDT in spSGF could be ranked as follows: EX-Man-MT-2 < EX-Man-MT-1 < EX-Kol-MT < EX-K_2CO_3-MT < EX-MT < EX-Ludi-MT-1 < ODMT < EX-Ludi-MT-2 < PM-K_2CO_3-MT < PM-Man-MT < SD-K_2CO_3-MT < SD-

Man-MT < PM-Ludi-MT < SD-Ludi-MT. Formulations made from extrudates were superior to spray-dried and physical mixtures. Parteck® M100 as a filler provided a faster drug release than Ludiflash®.

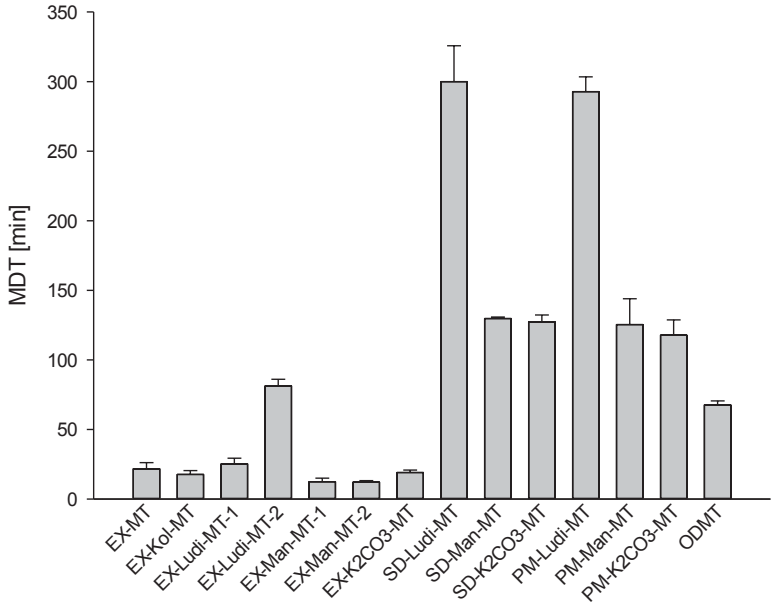


Figure 69. Mean dissolution time off all formulations in spSGF. Arithmetic mean ± S.D. (n=6).

The same results were observed for dissolution in FaSSIF (**Figure 83, Figure 84 and Figure 85** in appendix). MTs made from extrudates were superior to spray-dried powders and physical mixtures. Mannitol demonstrated the fastest dissolution among the tested fillers and drug loads. The mean dissolution time in FaSSIF could be ranked as follows: EX-Man-MT-2 < EX-K_2CO_3-MT < EX-Man-MT-1 < PM-Man-MT < EX-Ludi-MT-1 < PM-K_2CO_3-MT < EX Kol-MT < EX-MT < EX-Ludi-MT-2 < SD-Man-MT < SD-K_2CO_3-MT < PM-Ludi-MT < SD-Ludi-MT.

3.2.6. Summary & outlook

Eudragit® E PO was identified as the most suitable polymer combining taste masking capabilities and immediate release properties for PZQ. Among the applied encapsulation techniques, extrusion was superior to spray-drying utilizing the same compositions with Eudragit® E PO and GMS. GMS efficiently improved the taste masking capabilities of the extrudates and facilitated gentle process conditions for PZQ. Solid lipid extrusion enabled a promising decreased release effect of PZQ in SSF correlating to an efficient taste masking. Moreover, it demonstrated a fast and reproducible dissolution in simulated gastric and intestinal media through encapsulation of the crystalline PZQ. Based on the *in vivo* taste assessment in the BATA model identifying a taste threshold of 0.03 mg/ml for PZQ, it could be assumed that only the extrudates would reach a taste masking with Eudragit® E PO and GMS. This needs to be confirmed *in vivo*. As expected, maintaining the crystalline state of PZQ was advantageous in terms of delayed release formulations and stability. The demonstrated extrusion process can be easily scaled up and adapted to a variety of APIs including heat-sensitive and volatile compounds with subsequent processing to drug products. Moreover, Eudragit® E PO and GMS are excipients with low safety concerns applicable in drug dosage forms for adults, children and animals [210, 211].

The ground extrudates provided an adequate powder performance for subsequent direct compression to fast disintegrating mini-tablets in comparison to spray-dried formulations and physical mixtures. The MTs made from extrudates with Parteck® M100 as a filler demonstrated an exceptional low drug release in SSF combined with a fast and complete dissolution in spSGF. Even under sink-conditions the solubilising effect of Eudragit® E PO and GMS was present compared to an ODMT with PZQ. The developed MTs provide a high flexibility of dosing applicable for a broad range of patients including children, adults and animals.

4. Summary

Within this study suitable taste masking strategies were identified and compared for the pharmaceutical development of oral liquid and solid dosage forms for paediatrics for the bitter and aversive compound praziquantel (PZQ). The work was based on the current high need for a suitable and acceptable taste masked formulation for humans, including children from 6 months age, and picky animals such as cats. Based on the chemical and physical characteristics of PZQ appropriate taste masking techniques were chosen and applied. The focus was set on the complexation, encapsulation and embedding of PZQ in liquid or solid dosage forms to facilitate an efficient taste masking during oral uptake.

Within liquid formulations the taste masking efficiency of maltodextrins (MDs) was evaluated in comparison to cyclodextrins (CDs) in solution. As a prerequisite for the successful analysis of these multicomponent liquid systems, different non-human taste assessment tools were examined for PZQ. The electronic tongue and the rodent brief-access taste aversion (BATA) model were chosen as the most useful methods based on current literature. The evaluated sensors of the electronic tongue in this study were not applicable for PZQ due to the non-ionic characteristic and the low solubility of PZQ in water. None of the tested sensors provided conclusive responses for PZQ and hence for further evaluation of multicomponent systems. In contrast, the rats in the BATA model demonstrated a concentration-dependent sensitivity to the PZQ aversiveness, leading to an IC_{50} value of 0.06 mg/ml. During the experiment, a maximum concentration of 0.01 mg/ml of PZQ in water was identified as well tolerated with no significant difference to pure water. Based on these findings the taste masking efficiency of MDs and CDs were evaluated in the BATA model revealing the MD as efficient as both CDs for PZQ. In the view of a final dosage form MDs would be preferable over CDs due to various reasons such as excipient costs and safety.

Within solid formulations mini-tablets (MTs) were chosen as the most suitable and flexible dosage form for the majority of patients including children. To efficiently inhibit the interaction of PZQ with the taste buds during oral uptake of the MTs, PZQ was encapsulated or embedded in a carrier system. First, via a solvent casting method various polymers were evaluated for the combined approach of a taste masking effect in simulated salivary fluid and a fast solubilization of PZQ in simulated gastric and intestinal media. Second, among various taste masking approaches, extrusion and spray-drying were applied for PZQ with a subsequent processing of the formulations to mini-tablets (MTs). Extrusion of PZQ with Eudragit® E PO and glyceryl monostearate enabled an exceptional delayed drug release in simulated salivary fluid and an increased solubility kinetic of the drug in simulated gastric and intestinal media in comparison to spray-dried formulations and physical mixtures. This was observed for powder formulations as well as for subsequent manufactured MTs.

In conclusion, the developed ready-to-use MTs are superior to the solution with MD in terms of administration, stability, transport and use. They provide an easy ingestion including for patients with swallowing difficulties and a high flexibility regarding dosing.

5. Zusammenfassung

Ziel dieser Arbeit war die Erarbeitung und der Vergleich verschiedener Geschmacksmaskierungsstrategien für flüssige und feste orale Darreichungsformen für Kinder für den bitter schmeckenden Wirkstoff Praziquantel (PZQ). PZQ wurde auf Basis des derzeitig hohen Bedarfs an einer geeigneten geschmacksmaskierten Formulierung sowohl für den Menschen (Kinder ab sechs Monaten inbegriffen) als auch für Tiere, die die Einnahme von schlecht schmeckenden Substanzen verweigern (z.B. Katzen), ausgewählt. Basierend auf den chemischen und physikalischen Eigenschaften des Wirkstoffes wurden geeignete Formulierungen entwickelt. Der Fokus lag auf der Komplexierung, Verkapselung und Einbettung von PZQ in flüssigen und festen Darreichungsformen um eine effiziente Geschmacksmaskierung während der oralen Arzneimitteleinnahme zu ermöglichen.

Im Hinblick auf flüssige Darreichungsformen wurde der Einsatz von Maltodextrinen (MD) als Alternative zu Cyclodextrinen (CD) in Lösung getestet. Als Voraussetzung für die erfolgreiche Analyse dieser Formulierungen wurden zunächst verschiedene nicht-humane Methoden zur Geschmackserfassung von PZQ untersucht. Die elektronische Zunge und das Ratten-Modell „brief-access taste aversion“ (BATA) wurden auf Basis der aktuellen Literatur ausgewählt. Aufgrund der geringen Wasserlöslichkeit und nicht-ionischen Eigenschaft von PZQ waren die Ergebnisse der in dieser Studie getesteten Sensoren der elektronischen Zunge nicht erfolgreich. Keiner der getesteten Sensoren führte zu einem konzentrationsabhängigen Signal für das reine PZQ, wonach auch Mehrkomponenten-Systeme mit dieser Technik nicht ausgewertet werden konnten. Im Gegenteil dazu, zeigten die Ratten im BATA-Modell eine konzentrationsabhängige Empfindlichkeit gegenüber PZQ. Der IC_{50}-Wert lag bei einer Konzentration von 0.06 mg/ml. Bis zu einer Konzentration von 0.01 mg/ml war der Geschmack PZQ sehr gut verträglich mit keinem signifikanten Unterschied zu Wasser. Basierend auf diesen Ergebnissen wurden mit dem BATA-Modell Lösungen mit MD und CD im Hinblick auf ihre Geschmacksmaskierung für PZQ untersucht. Es zeigte sich, dass das eingesetzte MD den Geschmack von PZQ ebenso gut überdecken konnte wie die CD. Dies ist auch für die endgültige Darreichungsform von Vorteil, da MD sichere und kostengünstigere Hilfsstoffe im Vergleich zu CD sind.

Im Hinblick auf orale feste Darreichungsformen wurden Minitabletten (MT) als die geeignetste und flexibelste Dosierungsform für die Mehrheit der Patienten einschließlich Kinder ausgewählt. Um die Interaktion von PZQ mit den Geschmacksknospen im Mund während der Einnahme der Tabletten zu verhindern, wurde PZQ in ein Trägersystem verkapselt bzw. eingebettet. Verschiedene Polymere wurden für den kombinierten Ansatz aus Geschmacksmaskierung bei trotzdem schneller Freisetzung im Magen-Darm-Trakt evaluiert. Anschließend wurden über Extrusion und Sprühtrocknung Formulierungen mit PZQ, Eudragit® E PO als Polymer und Glycerolmonostearat als Lipid hergestellt und zu MT weiterverarbeitet. Die Extrusion von PZQ mit Eudragit® E PO und Glycerylmonostearat ermöglichte eine besonders niedrige Arzneimittelfreisetzung in simulierter Speichelflüssigkeit und eine erhöhte Löslichkeitskinetik von PZQ in simulierten Magen- und Darmmedien im Vergleich zu sprühgetrockneten Formulierungen und physikalischen Mischungen.

Dies wurde sowohl bei Pulverformulierungen als auch bei nachfolgend hergestellten MT beobachtet.
Abschließend sind die entwickelten MT der Lösung mit MD überlegen im Hinblick auf die Einnahme, Stabilität, den Transport und die Handhabung. Sie ermöglichen eine simple Verabreichung für Patienten mit Schluckbeschwerden und eine hohe Flexibilität für die Dosierung von verschiedenen Altersklassen.

6. Experimental part

6.1. Materials

Table 8
Model compound Praziquantel (PZQ).

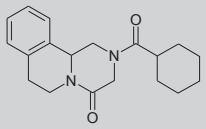
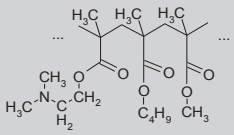
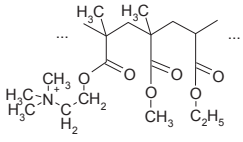
Chemical structure	Solubility in water [mg/ml] at 22°C	Melting point (T_M) [°C]	Glass transition temperature (T_G) [°C]	Supplier
	0.22	140	39	Merck

Table 9
Excipients for complexation of Praziquantel.

Excipient	Product	Molecular weight M_w [g/mol]	Supplier
Maltodextrin	Maltodextrin dextrose equivalent 13.0 – 17.0	2432.1	Sigma Aldrich
Maltodextrin	Kleptose® linecaps 17	12635.0	Roquette
Maltodextrin	Glucidex® 17 D	10976.0	Roquette
Hydroxypropyl-beta-Cyclodextrin	Kleptose® HPB	1387.2	Roquette
Sulfobutyl-eter-beta-Cyclodextrin	Captisol®	2163.0	CyDex

Table 10
Polymers and lipid.

Excipient	Chemical structure	Charge	Application	Solubility	T_G, T_M [°C]	Supplier
Eudragit® E PO		Cationic copolymer	Protective, taste masking	< pH 5, Swellable and permeable > pH 5	42.0	Evonik
Eudragit® RL PO		Cationic copolymer	Sustained release	Insoluble, pH independent swelling, high permeability	56.4	

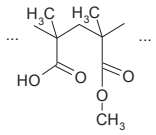
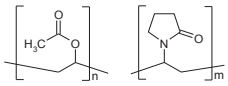
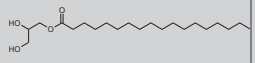
Eudragit® RS PO				Insoluble, pH independent swelling, low permeability	58.9	
Eudragit® L100		Anionic copolymer	Gastro-resistance and GI-targeting	> pH 6	> 130	
Eudragit® S100				> pH 7	> 130	
Kollidon® SR		Non-ionic Kollidon	Sustained release	pH independent	42.4	BASF
Glyceryl mono stearate (GMS)		Non-ionic, HLB 3.8	Emulsifying, binding, retarding	Insoluble	65 - 80	Cremer Oleo

Table 11
Excipients for tableting.

Excipient	Ingredients	Application	Supplier
Ludiflash®	D-Mannitol, Kollidon® CL-SF, Kollicoat® SR 30D	Filler, binder	BASF
Parteck® M100	Mannitol	Filler, binder	Merck
FlowLac® 100	Lactose monohydrate	Filler, binder	Meggle
Prosolv® ODT	Mannitol, microcrystalline cellulose, colloidal silicon dioxide, fructose, crospovidone	Filler, binder	JRS Pharma
Vivapur® 102	Microcrystalline cellulose	Filler, binder	JRS Pharma
Emdex®	Glucose monohydrate	Filler, binder	JRS Pharma
Prosolv® SMCC 50	Microcrystalline cellulose, colloidal silicon dioxide	Filler, binder	JRS Pharma
Aerosil® 200	Colloidal silicon dioxide	Filler	Evonik
Kollidon® CL-SF	Crospovidone	Super disintegrant	BASF
PRUV®	Sodium stearyl fumarate	Glidant	JRS Pharma

Table 12
Excipients for buffer preparation and analytics.

Excipient	**Supplier**
Sodium chloride (NaCl)	Merck
Sodium dihydrogen phosphate hydrate (NaH_2PO_4 * H_2O)	Merck
Calcium chloride dihydrate ($CaCl_2$ * $2H_2O$)	Merck
Magnesiumchloride hexahydrate ($MgCl_2$ * $6H_2O$)	Merck
Potassium carbonate sesquihydrate (K_2CO_3 * $1.5H_2O$)	Merck
DiSodium hydrogen phosphate heptahydrate (Na_2HPO4 * $7H_2O$)	Merck
Hydrochloric acid 1N (HCl)	Merck
Sodium hydroxide pellets and 1N (NaOH)	Merck
Simulated intestinal fluid (SIF)	Biorelevant
Chininhydrochloride dihydrate	Buchler
Potassium chloride	Grüssing
Tartaric acid	Applichem
Erioglaucine disodium salt (Brilliant Blue FCF)	Sigma Aldrich
Ethanol absolute (LiChrosolv® grade)	Merck
Acetonitrile (LiChrosolv® grade)	Merck KGaA
Milli-Q®-water	Merck KGaA

6.2. Methods

6.2.1. Preparation methods

6.2.1.1. Complexation with maltodextrins and cyclodextrins

The complexation efficiency of PZQ with MDs and CDs was compared using a phase solubility study. Maltodextrin DE 13.0 – 17.0 of Sigma Aldrich, Kleptose® linecaps 17 and Glucidex® were chosen as maltodextrins and Kleptose® HPB (hydroxypropyl-beta-cyclodextrin) and Captisol® (sulfobutylether-beta-cyclodextrin) as CDs.

Aqueous solutions of the MDs and CDs were prepared in volumetric flasks with Milli®-Q-water at concentrations of 2, 5, 10, 15 and 20 mM. Each concentration was set up three times for each excipient. After addition of excess amounts of PZQ (10-fold based on the thermodynamic solubility), the resulting suspensions were stirred for seven days at ambient temperature and filtered through 0.45 µm PTFE membrane filters (VWR Chemicals, Leuven, Belgium). The first 2 ml of the filtrate were suspended. The filtrate was analyzed using HPLC (6.2.2.1) regarding the solubilized drug content to determine the apparent stability constant and the complexation efficiency.

6.2.1.1. Mixing

Physical mixtures (approximately 10 g) of PZQ and CDs (3.1.1.2) or the polymers Eudragit® E PO, RL PO, RS PO, L100, S100 and Kollidon® SR (3.2.2.1) and GMS (3.2.3 and 3.2.4) were prepared by homogenous blending in a mortar for 10 min immediately before usage.

Powders for tableting (3.2.1.1 and 3.2.5.1) were weighed in glass bottles and mixed with the blender Turbula T2C (W. A. Bachofen, Basel, Switzerland) for 20 min at 20 rpm. The glidant was added afterwards and the mixture was blended again for another 2 min.

6.2.1.2. Small scale film preparation

Drug-polymer films were prepared in a quartz 96-well plate (Hellma Analytics, Mühlheim, Germany) based on Wyttenbach *et al.* [188]. Stock solutions of PZQ and each polymer were prepared separately in methanol: 5.5 mg/ml PZQ and 11.0 mg/ml polymer. 60 µl of the PZQ stock solution and 60 µl of a polymer stock solution were mixed in each well with a positive displacement pipette (Rainin®, VWR, Leuven, Belgium) for a final drug load of 33.3% in 120 µl. For control samples wells contained pure PZQ or pure polymer. All samples were prepared in triplicate. PZQ-polymer films were generated by fast evaporation under vacuum in a desiccator (100 mbar for 30 min). The films were visually controlled regarding appearance of crystals using a BX60 Olympus microscope (Olympus, Japan) and polarized light.

The release of PZQ in the films was analyzed in various media (6.2.2.10) directly after production.

6.2.1.3. Spray-drying

Spray-drying was performed with a 4M8-TriX Formatrix Spray Dryer (ProCepT, Zelzate, Belgium) and a binary nozzle of 1.2 mm.

Solutions of PZQ with Kleptose® HPB and Captisol® were spray-dried out of water (prepared in 6.2.1.1., 200 ml) as well as solutions of PZQ with polymer and lipid (3.2.4) out of ethanol (150 ml). PZQ and Eudragit® E PO were dissolved in ethanol absolute for a final feed concentration of 5% and a drug:polymer weight ratio of 1:1 and 1:2. Glycerol monostearate was dissolved in the solution as antistatic agent with 30% (w/w) based on the amount of the polymer.

Process parameters were as described in **Table 13**. The dosing was set to 2 ml/min. The settings were adapted for a high yield of the dried powder and a low outlet temperature for spray dried powders with the lipid to prevent melting. Only the dried powder in the collecting vessel below the cyclone was used for further processing and the calculation of the process yield with the following equation:

$$\text{Yield } [\%] = \frac{\text{Amount of collected sample}}{\text{Initial powder amount}} \cdot 100 \qquad \text{Eq. 7.}$$

Table 13
Process parameters for spray-drying.

Process parameter	PZQ with Cyclodextrins	PZQ with Eudragit® E and GMS	
Inlet temperature [°C]	80	60	80
Outlet temperature [°C]	52-54	42-43	50-56
Cyclone temperature [°C]	41-43	31-33	40-44
Atomizing air [l/min]	10	10	
Air speed [%]	80	80	
Manual air flow [m^3/min]	0.175	0.15	
Pressure drop in the cyclone [mbar]	38-42	63-65	

6.2.1.4. Extrusion

Extrusion was conducted with the co-rotating twin-screw extruder (Three Tec Extruder ZE 5, Seon, Switzerland) with a not segmented screw of 5 mm in diameter and a length of 10 cm. The barrel was split in three heating zones (HZ) and one feeding zone. A die plate of 1 mm was used. The screw speed was set to 300 rpm. The feeding zone was cooled with a cryostat (WK4600 LAUDA, LAUDA DR. R. WOBSER, Lauda-Königshofen, Germany) at 12.5°C and 0.5 bar. The cryostat temperature was set to 17.5°C for temperatures below 80°C in heating zone 1 to prevent the formation of condensation water in the feeding zone. The temperatures of the heating zones were varied from minimum 45 – 55°C to maximum 120 – 150°C (**Table 14**) operating below and above the melting range of PZQ, Eudragit® E PO and GMS. The settings were limited by the maximum screw torque of 2 Nm. The batch sizes were set to 10 g for placebo formulations (3.2.3.1) and 25 g

for formulations with PZQ (3.2.3.2) for analyzing the ground extrudates and further processing to tablets.

Table 14
Temperature settings for extrusion in heating zones (HZ) and corresponding cryostat temperature.

Formulation	HZ 1 [°C]	HZ 2 [°C]	HZ 3 [°C]	Cryostat temperature [°C]
Eudragit® E PO	70	100	100	17.5
	80	120	120	12.5
	100	130	130	12.5
	120	150	150	12.5
Eudragit® E PO + GMS	45	55	55	17.5
	50	60	60	17.5
	60	70	70	17.5
	70	100	100	17.5
	80	120	120	12.5
PZQ + Eudragit® E PO	70	100	100	17.5
	80	120	120	12.5
	80	120	125	12.5
PZQ + Eudragit® E PO + GMS	50	60	60	17.5
	60	70	70	17.5
	70	100	100	17.5

6.2.1.5. Milling

The extrudates were milled with a vibrational ball mill (Pulverisette 23, Fritsch, Idar-Oberstein, Germany) and two grinding balls of 10 mm. The grinding bowl and balls consisted of zirconium oxide. Milling was performed for 4 min with 30 oscillations per s ensuring a negligible heat exposure during the process. The powder was sieved through 400 µm to separate large particles. The process yield was calculated with the following equation:

$$\text{Yield } [\%] = \frac{\text{Amount of milled powder}}{\text{Amount of extrudate strand}} \cdot 100 \qquad \text{Eq. 8.}$$

6.2.1.6. Tableting

Tableting according to chapter 3.2.1.1 was performed with the instrumented rotary tablet press Pressima (Romaco Kilian, EU-B/D, Cologne, Germany) and biconcave 13-tip mini-tableting tools (Notter, Ölbronn-Dürrn, Germany) (**Figure 70**). The speed of rotation of the die table was set to 10 rpm. All excipients were directly compressed into biconvex MTs with a diameter of 3 mm. The batch sizes were set to 2 kg. Tablet heights were adjusted to 2.0, 2.5 and 3.0 mm with an electronic calliper. Different compression forces from 0.4 to 2.3 ± 0.04 kN were applied and monitored with the software MS 2300 Pressima (Kilian, Cologne, Germany).

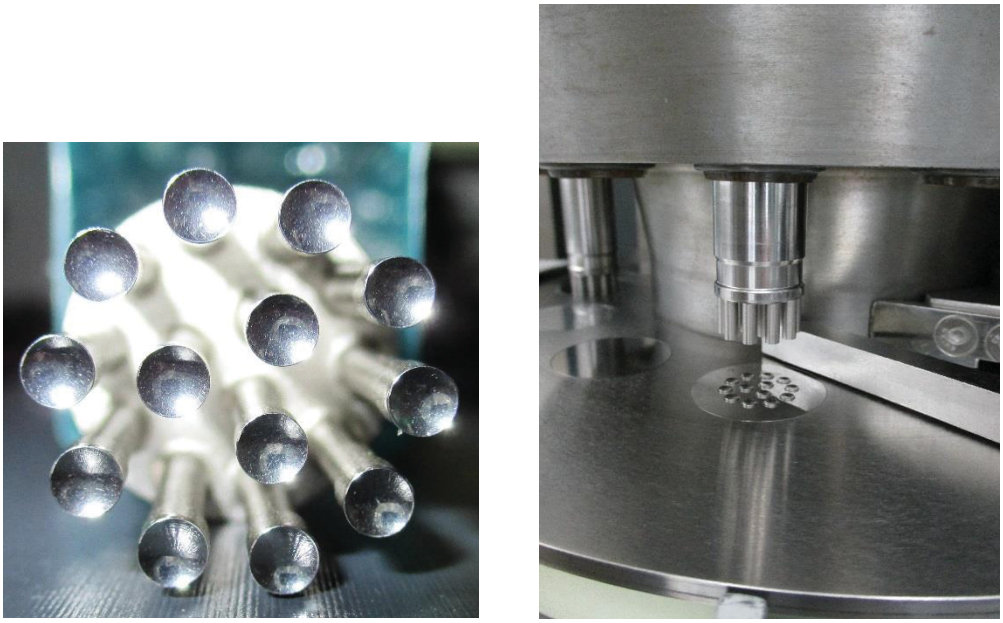


Figure 70. Biconcave 13-tip tableting tools for 3 mm mini-tablets.

Tableting of spray-dried powders and ground extrudates according to chapter 3.2.5.1 was performed with an eccentric tablet press EK0 (Korsch, Berlin, Germany) equipped with one tableting biconcave tip for a biconvex 3 mm MT (Notter, Ölbronn-Dürrn, Germany). The powder mixtures (25 – 30 g) were directly compressed. Tablet mass was adjusted to 20 mg with a compression force of 1 kN and 3.5 kN monitored by the software PMA3 (Pharmapress Messwert Analyse, Korsch, Berlin, Germany).

6.2.2. Analytical methods

6.2.2.1. HPLC and UPLC method

The solubilized drug content was determined via high performance liquid chromatography (HPLC) using an Agilent 1100 (Agilent Technologies, Santa Clara, USA) and UPLC® (ACQUITY, Waters, Ireland, Dublin). The detection was performed using UV-Vis spectroscopy at a wavelength of 210 nm. The analysis was done with a Waters Symmetry® column (Waters Symmetry® Shield RP 18, 150 x 4.6 mm 3.5 µm) for the HPLC and a Waters ACQUITY column (Waters ACQUITY UPLC® BEH Shield RP 18, 50 x 2.1 mm 1.7 µm) for the UPLC®. The column temperature was set to 22°C. Eluents were Milli®-Q-water and acetonitrile in a validated gradient method with a flow rate of 1.5 (HPLC) or 0.85 (UPLC®) ml/min and a run time of 32 (HPLC) and 4.5 (UPLC®) min. The retention time for PZQ was 9.6 (HPLC) and 1.3 (UPLC®) min. The injection volume was set to 5 (HPLC) and 3 µl (UPLC®). The samples were diluted with Milli®-Q-water/acetonitrile 55/45 (v/v) prior to the analysis for a target concentration of PZQ between 0.015 and 0.045 mg/ml. For quantification of the samples of an unknown concentration of PZQ a calibration curve was generated using five different concentrations (n = 3) of the pure PZQ in water/acetonitrile 55/45 (v/v): 0.009, 0.015, 0.03, 0.045 and 0.06 mg/ml (R^2 = 0.999).

6.2.2.2. Complexation efficiency according to Higuchi & Connors

The solubilized concentration of PZQ in all filtrates with Maltodextrin DE 13.0 – 17.0 of Sigma Aldrich, Kleptose® linecaps 17 and Glucidex® as MDs, Kleptose® HPB (HP-β-CD) and Captisol® (SBE-β-CD) as CDs was determined via HPLC according to 6.2.2.1 (n = 3). The drug content was plotted against the concentration of the MDs and CDs. According to Higuchi and Connors [151] the apparent stability constant was calculated (n = 1) from the slope and S_0 (intrinsic solubility of the drug):

$$K_{1:1} = \text{slope}/(S_0\ (1 - \text{slope})\ ) \qquad \text{Eq. 9.}$$

As a more accurate method and to compare the affinity of both drugs for MDs and CDs the CE was determined independent from the intrinsic solubility of the drug (n = 1) [150]:

$$CE = \text{slope}/(1 - \text{slope}) \qquad \text{Eq. 10.}$$

6.2.2.3. Light microscopy

The Microscope Olympus BX60-F3 (Olympus, Japan) was used for the analysis of the drug-polymer films (3.2.2.2) regarding crystals using polarized light and the particle size distribution of spray-dried powders with CDs (3.1.1.2) or polymer and lipid (3.2.4) as well as for extrudate strands and ground extrudates with polymer and lipid (3.2.3). The used microscope lenses were 5-fold (5x, MPlan, 0.1), 10-fold (10x, Plan, 0.25) and 50-fold (50x, SLMPlan, 0.45).

6.2.2.4. Particle size distribution

The particle size distribution according to horizontal Feret (distance between two parallel tangentials) of spray-dried powders and ground extrudates (n = 100) was determined with the Microscope Olympus BX60-F3 (Olympus, Japan) and the Software Olympus Stream Essentials (Version 1.9.4).

6.2.2.5. Differential scanning calorimetry (DSC)

The DSC was used for thermal analysis of PZQ as a pure substance, in physical mixtures with MDs or CDs, with polymers Eudragit® E PO, RS PO, RL PO, L100, S100 and Kollidon® SR and with the lipid GMS. It was used to identify PZQ in its crystalline or amorphous state detecting the melting point or the T_g and to indicate miscibility of PZQ with the polymers.
Thermograms were recorded using the Mettler Toledo DSC1 and the results were analyzed with the STARe software (Mettler Toledo, Gießen, Germany).
Samples of approximately 6 mg (n = 3) were weighed in aluminum pans with pierced lids and heated twice with a heating rate of 10 K/min from 0 to 170°C under a nitrogen flow of 50 ml/min in the oven and 150 ml/min around it. Modulated DSC measurements were performed with a heating rate of 2 K/min from 0 to 170°C (heated once) and a modulated temperature amplitude of 0.5 K for 15 – 45 seconds.
The miscibility of PZQ with the polymers (3.2.2.1) was tested with physical mixtures prepared at ratios 3:1, 1:1 and 1:3 (w/w, drug:polymer) by homogenous blending. Miscibility of the drug and a polymer was indicated with the presence of a single T_g within the second heating cycle of the DSC in contrast to two distinct T_g indicating immiscibility [187].

6.2.2.6. X-ray powder diffraction (XRPD)

Spray-dried powders and ground extrudates with PZQ and CDs, as well as with Eudragit® E and GMS were analyzed via XRPD in comparison to PMs with the same drug and carrier weight ratio prepared by homogenous blending (n = 3).
XRPD of the powders was carried out with a STADI P diffractometer (STOE & Cie, Darmstadt, Germany). Diffraction patterns were obtained at ambient temperature with Cu as radiation source, 40 kV voltage and 40 mA current. The 2θ range was set from 0 to 65 with a step size of 0.015 and a step time of 15 s.
For the physical mixtures and spray-dried powders of PZQ and CDs a short method was used with a 2θ range from 0 to 36 with a step size of 0.03 and a step time of 30 s.

6.2.2.7. Scanning electron microscopy (SEM)

The morphological analysis of PZQ spray-dried with CDs was carried out via SEM with a Zeiss Leo 1530 (Zeiss, Oberkochen, Germany) with a field emission cathode under high vacuum. Magnification was used from 200 to 1000 fold and acceleration voltage from 0.2 to 30 kV.

6.2.2.8. Drug load verification

The drug load of the physical mixtures, spray-dried samples, the extrudates and the ground extrudates were verified in triplicate via UPLC® (6.2.2.1) with the following equation:

$$\text{drug load } [\%] = (\text{weight of PZQ in sample})/(\text{total weight of sample}) \cdot 100 \quad \text{Eq. 11.}$$

Samples of approximately 10 mg were dissolved in a suitable solvent (ethanol for mixtures with polymer, otherwise water/acetonitrile 55/45 (v/v)) and further diluted with water/acetonitrile 55/45 (v/v) for a target concentration of PZQ between 0.015 and 0.045 mg/ml. All samples were filtered through 0.45 µm PTFE membrane filters (VWR Chemicals, Leuven, Belgium) prior to the analysis via UPLC®.

6.2.2.9. Dissolution under non-sink conditions

Dissolution of spray-dried powders and ground extrudates was set up in three different media to simulate the oral uptake of the drug: SSF [87], spSGF and FaSSIF (**Table 15**). Non-sink conditions were chosen to effectively discriminate between formulations regarding a delayed release of PZQ in SSF for taste masking and a higher and faster kinetic solubility of PZQ in spSGF and FaSSIF. This was analyzed in dependency on the added excipients (CDs, polymer and lipid) and the preparation method of the powders in comparison to the pure crystalline PZQ and physical mixtures. The analysis was conducted in snap cap vials with 2 ml medium and a magnetic stirring bar (12.7 mm Spinwedge®, VWR, Leuven, Belgium). The used amount of powder for each formulation always contained 2.3 mg API (5-fold excess of PZQ based on its thermodynamic solubility). Samples of 0.1 ml were taken manually after 1, 3, and 5 min in SSF and after 5, 30 and 60 min in spSGF and FaSSIF and immediately filtered through 0.45 µm PTFE membrane filters (VWR Chemicals, Leuven, Belgium) and afterwards diluted with acetonitrile and water (45:55 v/v). The solubilized drug concentration was determined via UPLC as described in 6.2.2.1. Each formulation was tested in triplicate.

Table 15
Composition and characteristics of dissolution media.

	Simulated salivary fluid	**Simulated gastric fluid sine pepsin**	**Fasted state simulated intestinal fluid**
Compounds	1.017 g/l NaCl 0.273 g/l $NaH_2PO_4 \cdot H_2O$ 0.228 g/l $CaCl_2 \cdot 2H_20$ 0.061 g/l $MgCl_2 \cdot 6H_2O$ 0.603 g/l $K_2CO_3 \cdot 1.5\ H_2O$ 0.204 g/l $Na_2HPO_4 \cdot 7H_2O$	2.0 g/l NaCl 1N HCl	6.186 g/l NaCl 3.954 g/l $NaH_2PO_4 \cdot H_2O$ 0.420 g/l NaOH
pH	6.8	1.2	6.5
Osmolarity [mOsmol/kg]	60	180	275

6.2.2.10. Dissolution of drug-polymer-films in the 96-well plate

The release of PZQ from the drug-polymer films according to 6.2.1.2 was analyzed directly after preparation. The dissolution was carried out in a microplate incubator at 37°C (THERMOstar, BMG LABTECH, Offenburg, Germany) in three different media: SSF, spSGF and FaSSIF (**Table 15**). The 96-well plate and the media were preconditioned at 37°C. Two glass balls (2-3 mm, VWR BDH Prolabo, Leuven, Belgium) were placed in each well prior to the medium for stirring during orbital shaking of the incubator. 200 µl medium were added to each well and then the orbital shaking was set to 700 rpm. Samples of 50 µl were taken manually after 0.5, 3.0 and 5.0 min in SSF and 5, 30 and 60 min in spSGF and FaSSIF, immediately filtered through a 0.45 µm PTFE 96-well filter plate (AcroPrep®, Pall Corporation, Ann Arbor, USA) with a Heraeus® Multifuge® 3SR+ centrifuge (Thermo Scientific, Waltham, USA) and afterwards diluted with acetonitrile and water (45:55 v/v). The solubilized drug content was determined via UPLC® (6.2.2.1).

6.2.2.11. Flowability and compressibility of powder mixtures

Measurements for flow properties and compressibility of powder mixtures were performed with the FT4 powder rheometer (freeman technology, Gloucestershire, UK) equipped with a 25 mm x 10 ml vessel. The vessel was filled with each powder mixture with approximately 4 to 6 g, to a maximum of 10 mm below the top of the vessel. A conditioning cycle was performed before each test to facilitate a homogenously packed powder bed. Within this cycle, a blade moves downwards and upwards using a helix to remove any precompaction or air holes. Measuring the flow function coefficient is utilized with a shear head inducing vertical and rotational stresses. The shear head moves downwards inserting its blades into the powder until a preliminary compression (normal stress) of the powder of 3 kPa and a shear plane is reached (pre-shearing). Afterwards, the head starts rotating overcoming the resistance of the powder and inducing the maximum shear stress until the powder starts to shear. The measurement is repeated with 5 lower normal stresses down to 1 kPa. The measured shear stresses are plotted against the applied normal stresses. A line is fitted through the data points and extrapolated to the shear stress at a normal stress of 0 kPa. Two Mohr circles are drawn tangential to this line where the circumferences pass through the origin and the pre-shear value. The flow function is calculated as follows and classified as described in **Table 16**.

$$\mathrm{ffc} = \frac{\sigma_C}{\sigma_1} \qquad \text{Eq. 12}$$

The value σ_C is defined as the greater of the two normal stresses where the smaller Mohr circle intercepts the axis, whereas σ_1 is the greater of the two normal stresses where the larger Mohr circle intercepts the axis.

Table 16
Classification of flow properties according to Jenike.

Flow function coefficient (ffc)	Classification
> 10	Easy-flowing
4 – 10	Free-flowing
2 – 4	Cohesive
< 2	Very cohesive and non-flowing

The compressibility is analyzed with a vented piston applying normal stresses from 1 to 15 kPa on the powder bed for a defined time. The enclosed air can escape at the surface of the powder bed as the piston is constructed from a stainless steel mesh. The result is expressed as the percentage change in volume calculated by the travelled distance of the piston. A high compressibility is usually associated with large amounts of enclosed air and a very cohesive powder with smaller particle sizes. A low compressibility can be explained by minimal entrapped air in a non-cohesive powder with large particles.

6.2.2.12. Crushing of tablets

The hardness of tablets of 3.2.1.2 was tested with the compact tablet tester MultiCheck V (Erweka, Heusenstamm, Germany) according to Ph Eur. 8.0 2.9.8 in comparison to the Texture Analyser (TA-XT plus, Stable Micro Systems, Winopal, Elze, Germany). Due to the small size of the 3 mm MTs and the applied low compression forces both methods were evaluated.
The Texture Analyser was equipped with a punch of 5 mm in diameter (Winopal, Elze, Germany) based on the detection of the hardness of pellets [212]. All tablets were positioned on the band height under the punch for comparison with the MultiCheck V. The test speed was set to 0.1 mm/s. A force-distance-diagram was recorded with the Exponent Software (Winopal, Elze, Germany). 10 tablets of each formulation were tested with both methods.
The hardness of tablets of 3.2.5.1 made of spray-dried powders or ground extrudates were analyzed only with the compact tablet tester Multicheck V (n = 10).

6.2.2.13. Disintegration of tablets

The disintegration of MTs was analyzed according to Ph. Eur. 8.0 2.9.1 Disintegration of tablets and capsules. Due to the small size of the 3 mm mini-tablets different methods were evaluated.

6.2.2.13.1. Disintegration with DisiTest50

3 mm MTs of all tablet heights were tested regarding their disintegration with the DisiTest 50 (Pharmatron, Thun, Switzerland) in accordance with the Ph. Eur.. 6 tablets of each formulation and tablet height were analyzed. The common sieve of the basket of the disintegration tester has 10 mesh accompanied with a distance of 2 mm between wires of the sieve. Therefore MTs can pass the sieve before the disintegration is completed. As a second method a sieve with 40 mesh meaning a distance of 0.6 mm between wires usually applied for granules was used (**Figure 71**). The tests were performed in water at 37°C.

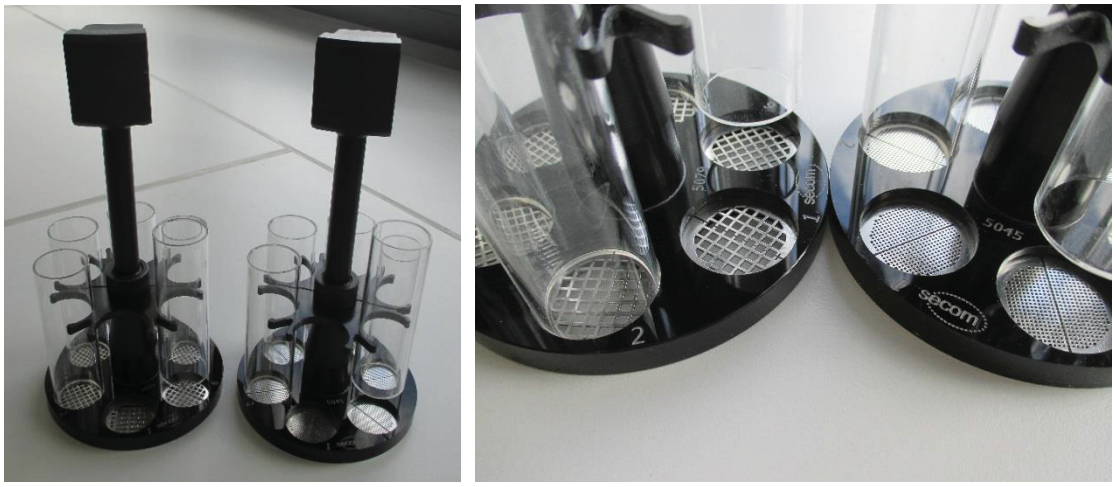


Figure 71. Disintegration baskets for the DisiTest 50 with 10 (left) and 40 (right) mesh.

6.2.2.13.2. Disintegration with Texture Analyser

As an additional method the disintegration of MTs was determined with the Texture Analyser (TA-XT plus, Stable Micro Systems, Winopal) [213]. It was equipped with a punch with a diameter of 5 mm (Winopal). The MT was fixed to the bottom of the punch with its central cylinder to be able to compare all tablet heights of all formulations. A petri dish with a diameter of 5.5 cm filled with 2 ml water and a filter paper of 5 cm in diameter was positioned below the punch. The punch moved down until the tablet reached the filter paper activating the test speed of 2 mm/s and a constant force of 0.4 N. A distance-time-diagram was recorded with the Exponent Software (Winopal) showing an increase of the distance until the tablet is completely disintegrated resulting in a plateau. The intersection point of the increasing line and the plateau marks the disintegration time of the MT which was calculated by the Exponent Software. 6 tablets of each formulation and tablet height were tested.

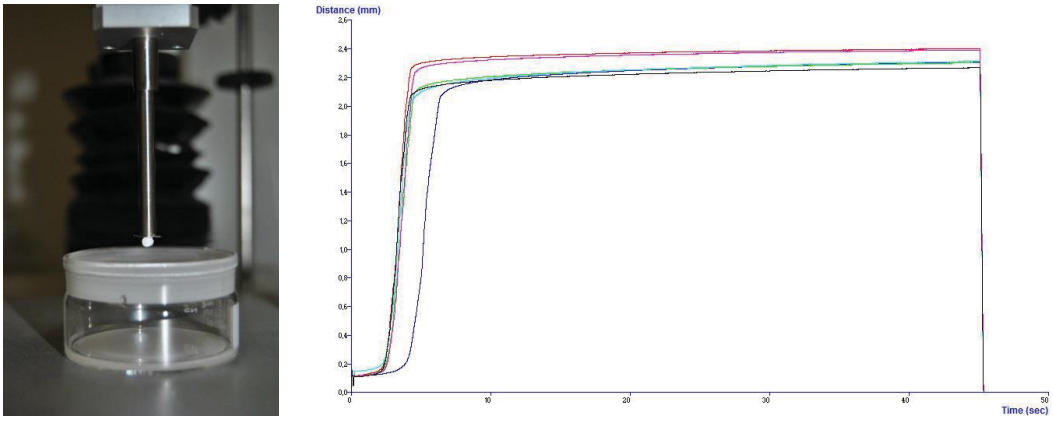


Figure 72. Disintegration set up with the Texture Analyser (left) and resulting distance-time-diagrams (right).

6.2.2.13.3. Simulating wetting test

The simulating wetting test describes the wetting time of MTs with water without any mechanical stress [33]. The test was performed in a UV 96-well plate (Corning®, New York, USA) with 20 µl of a 0.1% Brilliant Blue solution and a filter paper (Whatman®, GE Healthcare, Freiburg, Germany) in each well. One tablet was placed in each well covered only on the curved bottom with blue solution. The time was recorded until the blue solution completely wetted the tablet. 6 tablets of each formulation and tablet height were tested.

6.2.2.14. Friability of tablets

The friability of tablets was determined according to Ph. Eur. 8.0 2.9.7. An equivalent amount of MTs for a minimum of 6.5 g were placed in the drum of the friabilator (Erweka, Heusenstamm, Germany) and rotated 100 times with 25 rpm. The tablets were dedusted and weighed again after the procedure. The abrasion is defined as the difference between the masses. The test was performed 3 times for each formulation and tablet height. An abrasion below 1.0% meets the specifications of to the Ph. Eur..

6.2.2.15. Content analysis of tablets

The content analysis was determined for MTs with PZQ made from physical mixtures, spray-dried powders or ground extrudates according to Ph. Eur. 8.0 2.9.6 Uniformity of content of single-dose preparations and 2.9.40 Uniformity of dosage units.
10 tables of each production were accurately weighed and dissolved in 10 ml ethanol and kept in an ultrasonic bath for 10 min until the tablet was completely suspended. The resulting suspension was filtrated through 0.45 µm PTFE membrane filters (VWR Chemicals, Leuven, Belgium) and diluted with acetonitrile and water (45:55 v/v) for the content determination via UPLC (6.2.2.1).
According to Ph. Eur. 8.0 2.9.6 the formulation complies if not more than one individual content is outside of 85 to 115% of the average content. According to Ph. Eur. 8.0 2.9.40 the formulations are in specifications if the acceptance value (AV value) is below 15.

6.2.2.16. Dissolution of tablets under sink conditions

Dissolution under sink conditions was adapted from Ph. Eur. 8.0 2.9.3 with an Erweka DT80 (Erweka, Heusenstamm, Germany) equipped with small dissolution vessels of maximum 400 mL and equivalent paddles. As dissolution media 100 ml SSF, spSGF or FaSSIF were used. 6 tablets were accurately weighed and tested for each medium. The medium temperature was maintained at 37°C. At predetermined time points, 1 ml samples were withdrawn, filtered through 0.45 µm PTFE membrane filters (VWR Chemicals, Leuven, Belgium) and analyzed with UPLC (6.2.2.1).
The mean dissolution time (MDT) [214] enables the comparison of all dissolution profiles with the following equation based on $\dot{t}_i$ (midpoint of time period during which the fraction ΔMi has been released) and ΔM_i (released drug amount):

$$\mathrm{MDT} = \frac{\sum_{I=1}^{n} \bar{t}_1 * \Delta M_i}{\sum_{i=1}^{n} \Delta M_i} \qquad \text{Eq. 13.}$$

6.2.2.17. Stability analysis

For stability testing the physical mixtures, spray-dried powders and ground extrudates were stored at 5°C, at 25°C with 60% r. h. and 40°C with 75% r. h. for 8 weeks in snap cap vials.

6.2.3. Analytical methods for taste assessment

6.2.3.1. Electronic tongue

The electronic tongue TS-5000Z (Insent Inc., Atsugi-chi, Japan) was used as an *in vitro* taste assessment tool to evaluate the taste intensity of PZQ. The system is composed of a sensor unit with a sample table with two circles of sample positions, two sensor heads with up to eight sensors at a robot arm and a data recording system.
Using a potentiometric measurement principle, it is equipped with an Ag/AgCl reference electrode and separately attached lipid membrane sensors showing different taste perceptions: saltiness, sourness, astringency, sweetness, umami and bitterness. The commercially available sensors (Insent Inc., Atsugi-chi, Japan) included in this study were SB2AC0, SB2AN0 and SB2BT0 (cationic and neutral bitter compounds), SB2AAE (umami), SB2CT0 (saltiness) and SB2CA0 (sourness). As the detection of non-ionic and slowly water soluble substances like PZQ is limited self-developed sensors were evaluated named as A to G (**Table 17**). The self-developed sensors were prepared using Polyvinyl chloride (Sigma-Aldrich, Steinheim, Gemany) as polymer, isopropylmyristate (Cognis GmbH, Duesseldorf, Germany) as plasticizer, either tetra-dodecyl ammonium bromide (TB, Sigma-Aldrich), trioctylmethyl ammonium chloride (TC, Alfa Aesar, Karlsruhe, Germany) or bis(2-ethylhexyl) phosphate (BP, Sigma-Aldrich, Steinheim, Germany) as artificial lipids, oleic acid (Fluka Analytical, Steinheim, Germany) and either hydroxypropyl-ß-cyclodextrin (HPßCD, Roquette, Lestrem, France) or a cyclodextrin oligomer (CDO, HHU, Duesseldorf, Germany) as ionophores according to Immohr *et al.* [176]. All sensors were preconditioned in a standard solution (30 mM

potassium chloride and 0.3 mM tartaric acid in distilled water) for one day. Prior to each measurement a sensor check was performed.
Quinine hydrochloride with a concentration of 0.5 mM was used as an external reference to monitor the results of each sensor over time. This is recommended as the sensor response is affected by the environment, e.g. the temperature, and the age of the sensor [76]. PZQ was dissolved in distilled water for 4 different concentrations: 0.01 (0.0032 mg/ml), 0.05 (0.016 mg/ml), 0.1 (0.032 mg/ml) and 0.5 mM (0.16 mg/ml) to generate a calibration curve for a reliable drug detection of all sensors.

Table 17
Labelling and membrane composition of the applied self-developed sensors.

Sensor labeling	Ionophore	Artificial lipid	Oleic acid
A	ßCD	TB	
B	HPßCD	TC	
C	CDO	TC, BP	x
D	HPßCD	TC	
E	HPßCD	TC, BP	
F		TB	x
G	HPßCD	TB	x

The measurement circle started with three washing steps in a washing solution. For positively charged sensors this was conducted in the standard solution, for negatively charged sensors 100 mM hydrochloric acid and ethanol 30% (w/w) were used. Afterwards a sample was analyzed regarding its taste for 30 s followed by two short washing steps of 3 s and the detection of the aftertaste for 30 s. The aftertaste depicts the change of the membrane potential due to absorption (CPA) of the compound to the lipid membrane of the sensor. This was followed by washing steps ending in the next circle. Each sample was measured 5 times in a randomized order, but always starting with the reference solution to monitor the sensor response.
Univariate data analysis was applied for comparison of all sensors and concentrations of PZQ. The results are displayed as a change of the membrane potential in mV. They were calculated in relation to the reference solution. The first two runs of each sample were discarded as they were considered as preconditioning of the sensors. Based on the last three results of each concentration the mean and standard deviations were calculated for the taste and aftertaste.

6.2.3.2. Brief-Access Taste Aversion (BATA) model

Another taste assessment tool is the rodent Brief-Acess Taste Aversion model (BATA) [61, 65]. PZQ was tested in this model regarding its aversiveness and bitter taste.
As a first step, solutions of PZQ in deionized water with 6 different concentrations: 0.005, 0.01, 0.03, 0.05, 0.10 and 0.20 mg/ml were tested as a calibration to evaluate the feasibility and the response of the rodents to PZQ. A stock solution of 0.2 mg/ml was prepared and diluted with deionized water.
Afterwards solutions of PZQ with the MD Kleptose® linecaps and the CDs Kleptose® HPB and Captisol® were tested in three different concentrations based on the first results of the calibration

of the drug: 0.06, 0.2 and 0.4 mg/ml for the MD and 1.5 mg/ml as the highest concentration for the CDs. The solutions were prepared as described in 6.2.1.1 and analyzed as described in 6.2.2.1.

The taste assessment was carried out with three groups of ten adult male Sprague-Dawley rats (Charles-River, Kent, UK) in accordance with Animals (Scientific Procedures) Act 1986 (Project Licence PPL 70/7668). They were housed in pairs in standard cages with 21 ± 2°C and 50 ± 10% humidity. The procedure consisted of training and testing days for the rats during the 12 hours light phase of a day. The experimental procedure is described in detail and validated in [64]. Preliminary to testing of the samples each rat received a water-deprivation for 22 hours. Each sample was presented in a sipper tube randomly for 4 times to each rat for 8 seconds on each testing day, resulting in a final number of 80 values per sample. Between samples was a water rinse for 2 s. Deionized water was used as a reference. The recording started when the rat licked at the presented sipper tube for the first time. After each testing period of maximum 40 minutes per day the rats received tap water for one hour for rehydration. The taste of each sample was assessed by the number of licks electronically recorded by a lickometer Davis MS-160 (DiLog Instruments, Tallahassee, Florida, USA).

The data was analyzed regarding the average number of licks as a function of the concentration of PZQ for the complete testing period and separately for each testing day and each rat. The IC_{50} value that describes the concentration of PZQ inhibiting 50% of the maximum lick numbers compared to the reference was calculated as described by Soto [64]. For comparison between formulations the percentage of inhibition of licks was calculated with the following equation and classified as described in **Table 18**:

$$\%\ \text{inhibition of licks} = \frac{N_0\text{licks}_{\text{reference}} - N_0\text{licks}_{\text{concentration API}}}{N_0\text{licks}_{\text{reference}}} \text{ x } 100 \qquad \text{Eq. 14.}$$

Table 18
Classification of number of licks in the Brief Access Taste Aversion model.

Classification	% lick inhibition
Fully tolerated	0
Well tolerated	1 – 30
Tolerated	30 – 50
Aversive/ untolerated	50 – 75
Highly aversive/ highly untolerated	> 75

7. Appendix

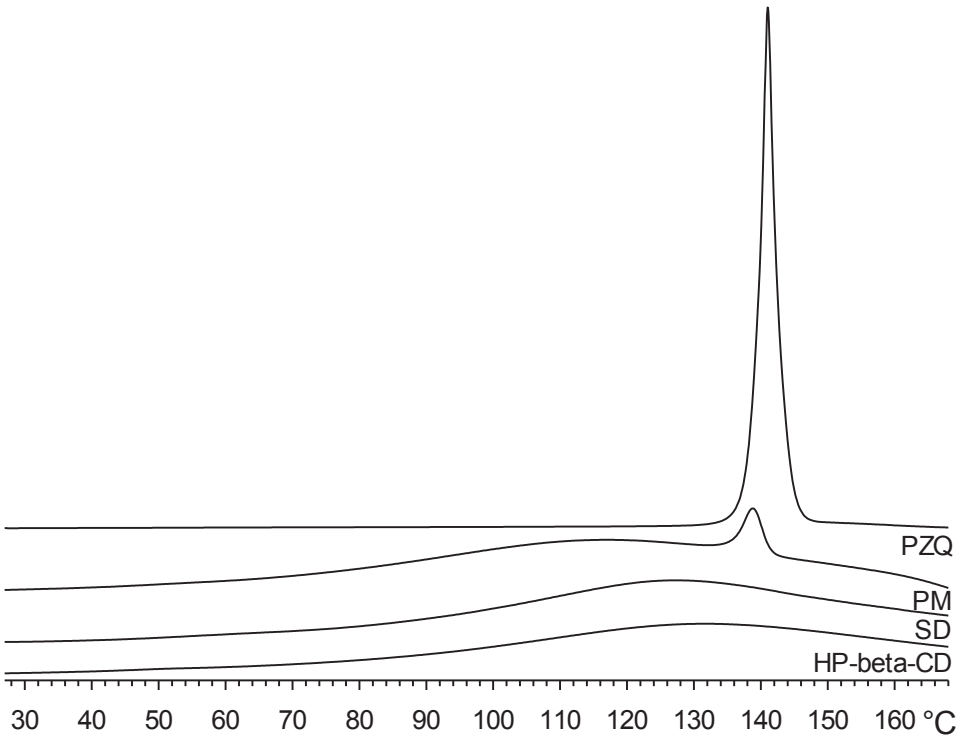


Figure 73. Endothermic thermograms of differential scanning calorimetry of crystalline praziquantel (PZQ), physical mixtures (PM) sulfobutylether-beta-cyclodextrin (SBE-β-CD) and spray-dried (SD) powders.

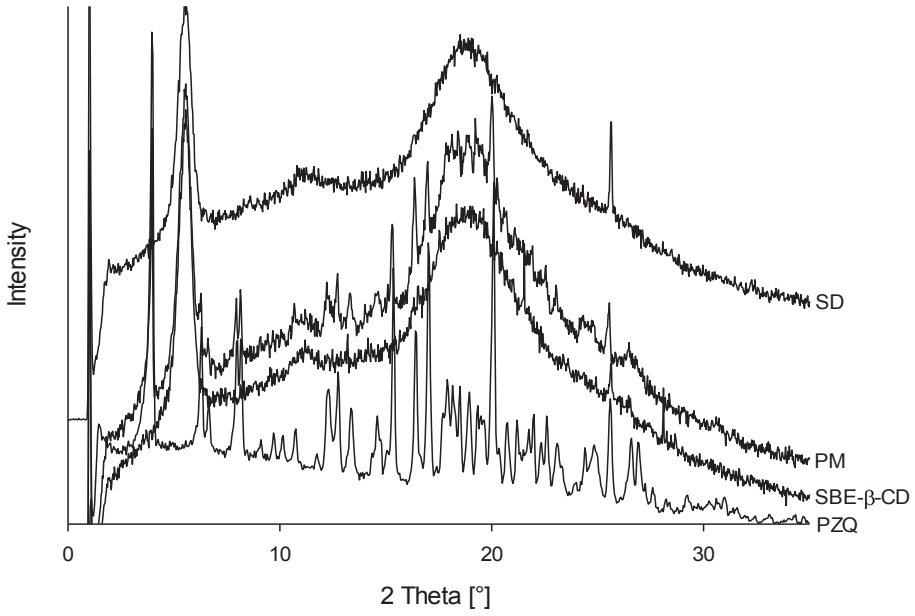


Figure 74. X-ray powder diffraction patterns of praziquantel (PZQ) with sulfobutylether-beta-cyclodextrin (SBE-β-CD) (right), physical mixture (PM) in the ratio 1:1 (Mol/Mol) and spray-dried (SD) powder.

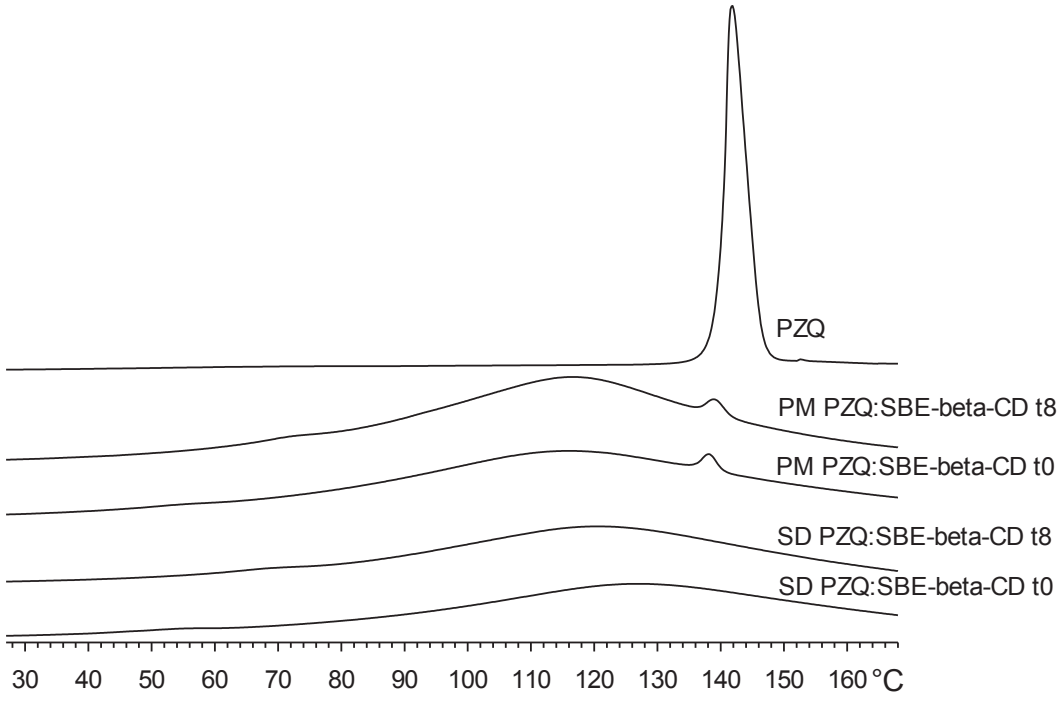


Figure 75. Endothermic thermograms of differential scanning calorimetry of crystalline praziquantel (PZQ), physical mixture (PM) with sulfobutylether-beta-cyclodextrin (SBE-β-CD) and spray-dried (SD) powder directly after production (t0) and after storage of 8 weeks (t8) at 40°C and 75% r. H..

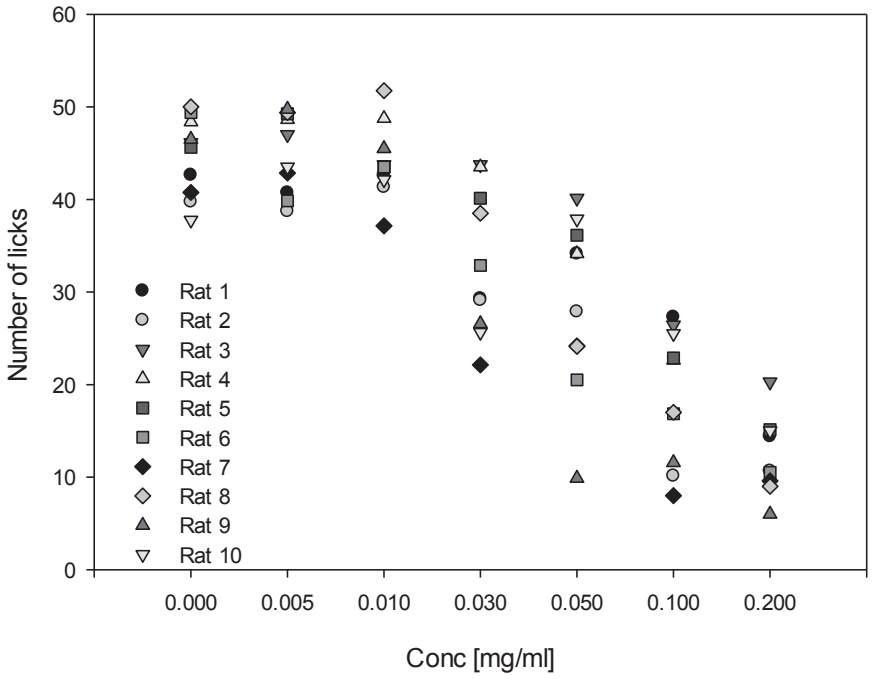


Figure 76. Calibration for BATA model. Recorded number of licks in BATA model as a function of PZQ concentration in water and differentiation between individual rats. Arithmetic mean (n = 8).

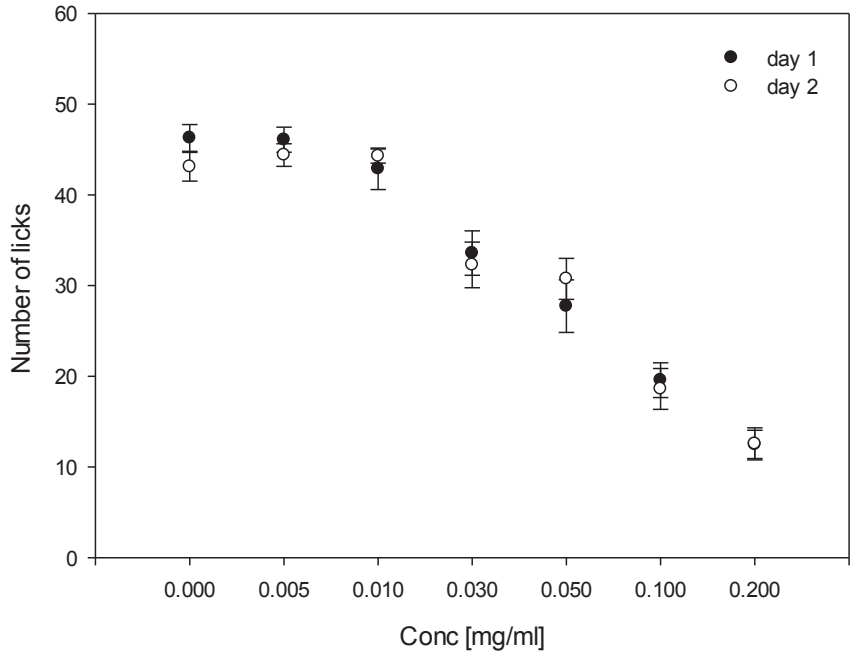


Figure 77. Calibration for BATA model. Recorded number of licks in BATA model as a function of PZQ concentration in water and differentiation between test day 1 and test day 2, arithmetic mean (n = 40) ± SEM.

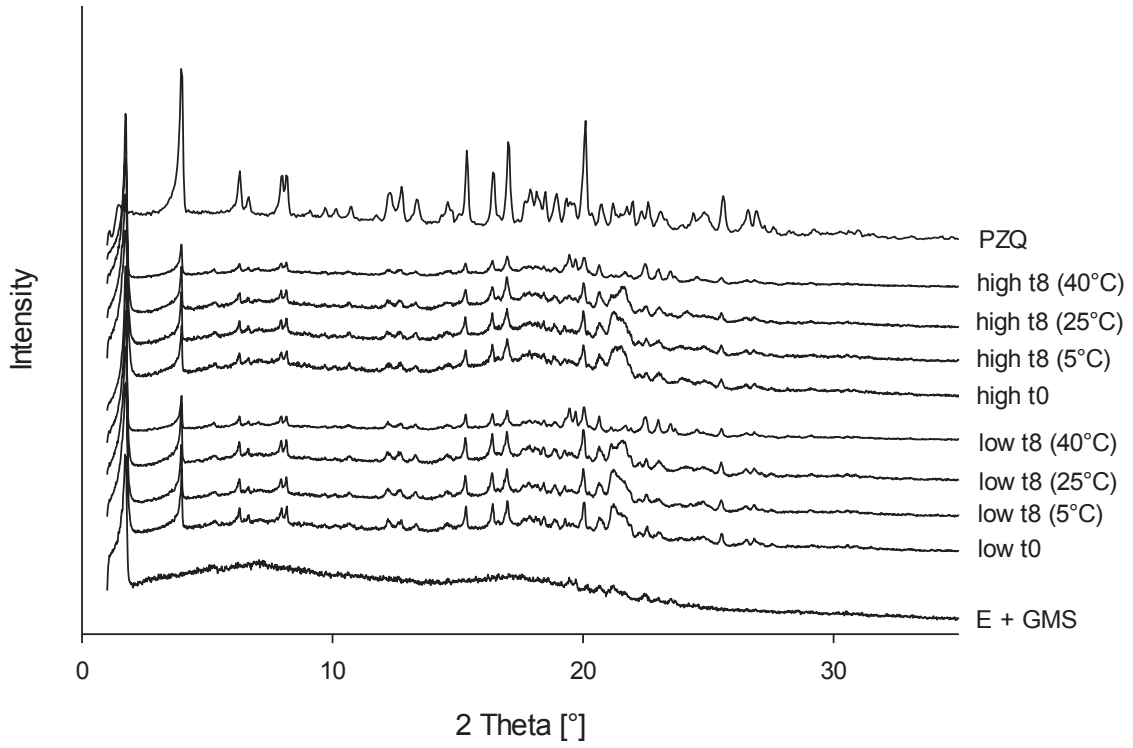


Figure 78. XRPD patterns of praziquantel (PZQ), Eudragit® E PO and glyceryl monostearate (E + GMS) 1:0.3 (w/w) and ground extrudates prepared with 27.8% PZQ at low (50–60°C) and high (70-100°C) extrusion temperatures directly after production (t0) and after storage for 8 weeks at 5 ± 3°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity.

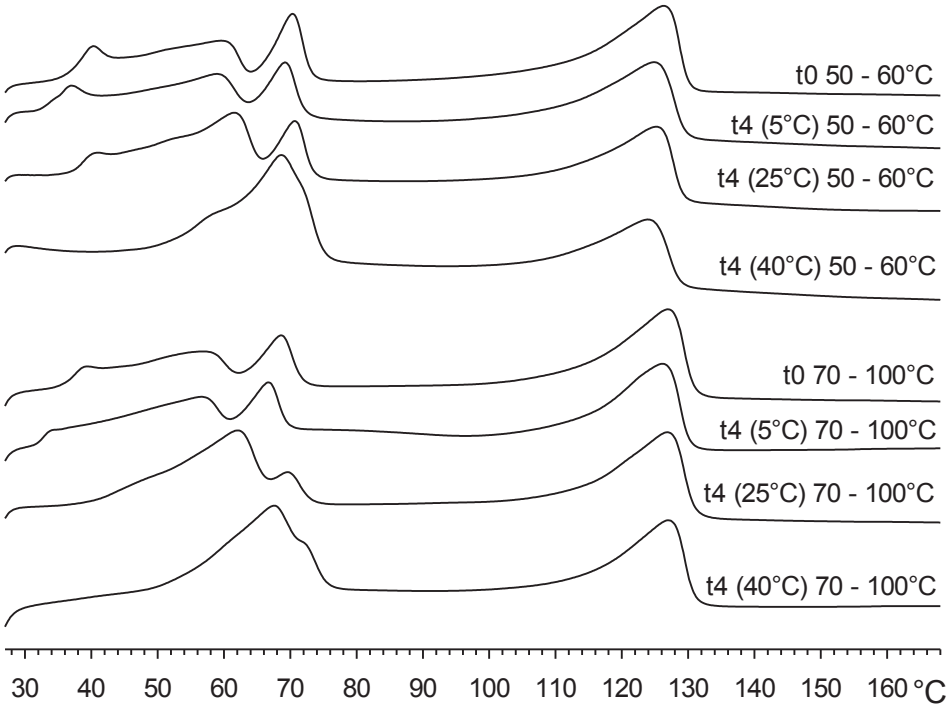


Figure 79. Stability analytics of extrudates (EX) with 43.5% praziquantel (PZQ). Endothermic thermograms of differential scanning calorimetry of EX with PZQ, Eudragit® E PO and glyceryl monostearate prepared at low (50-60°C) and high (70-100°C) extrusion temperatures directly after production (t0) and after storage for 8 weeks (t8) at 5 ± 3°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity.

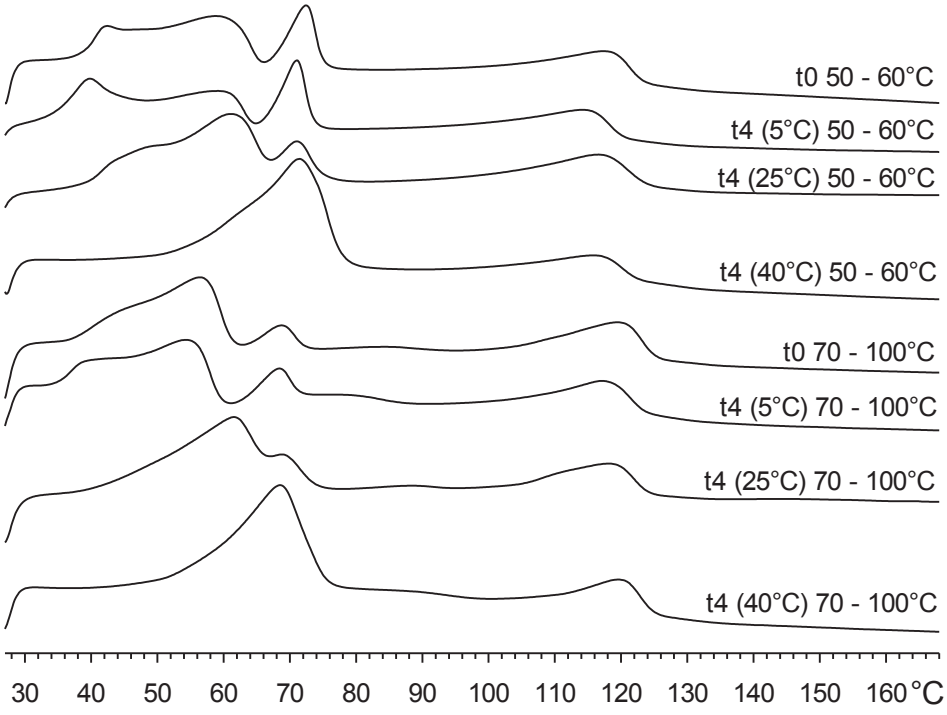


Figure 80. Stability analytics of extrudates (EX) with 27.8% praziquantel (PZQ). Endothermic thermograms of differential scanning calorimetry of EX with PZQ, Eudragit® E PO and glyceryl monostearate prepared at low (50-60°C) and high (70-100°C) extrusion temperatures directly after production (t0) and after storage for 8 weeks (t8) at 5 ± 3°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity.

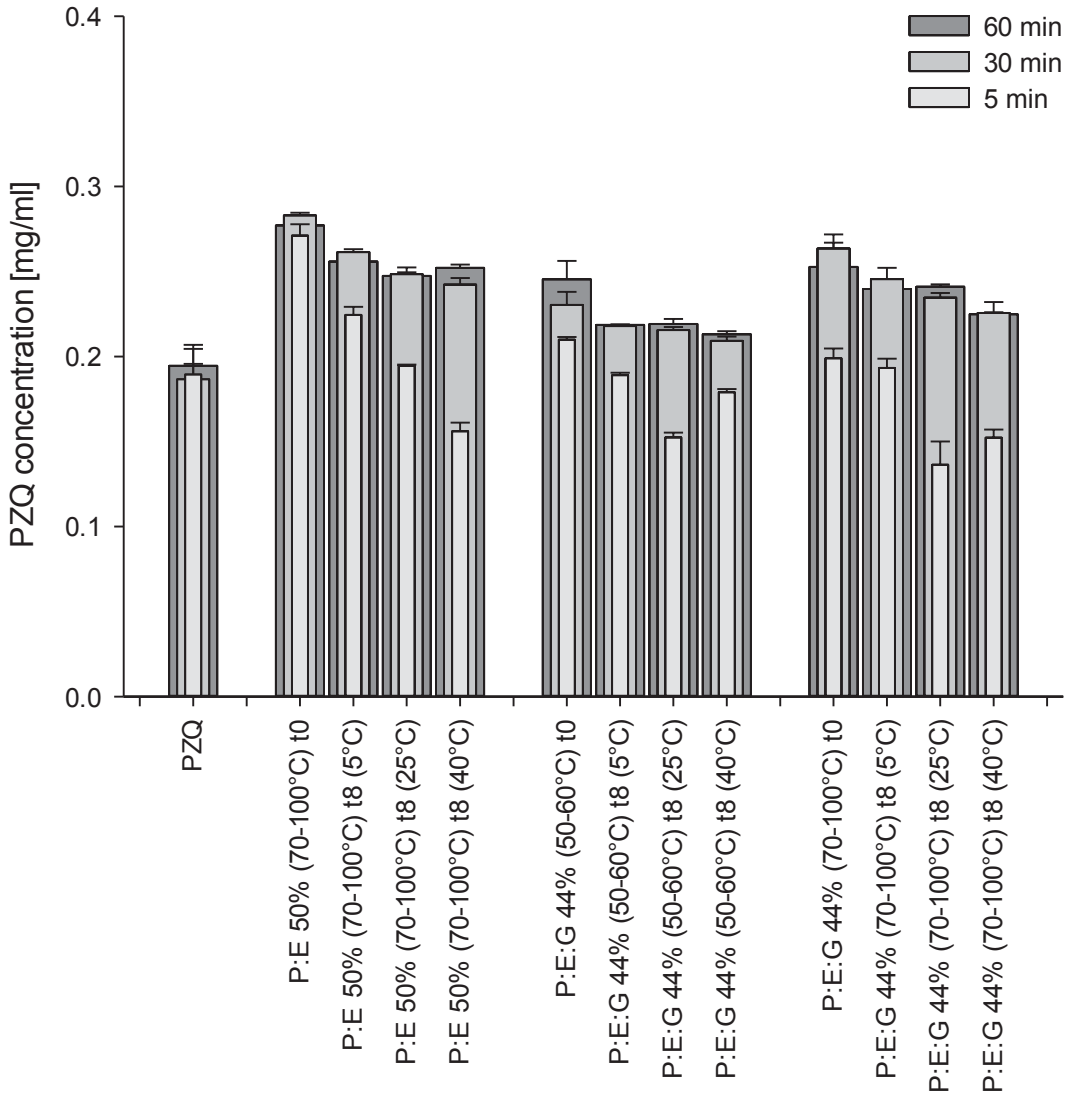


Figure 81. Stability of drug release characteristics. Praziquantel (PZQ) release at discrete time points in FaSSIF of extrudates with polymer (E) and lipid (G) with the labelled drug load and extrusion temperatures directly after production (t0) and after storage for 8 weeks (t8) at 5°C, 25°C with 60% relative humidity and 40°C with 75% relative humidity. Arithmetic mean ± S.D. (n=3).

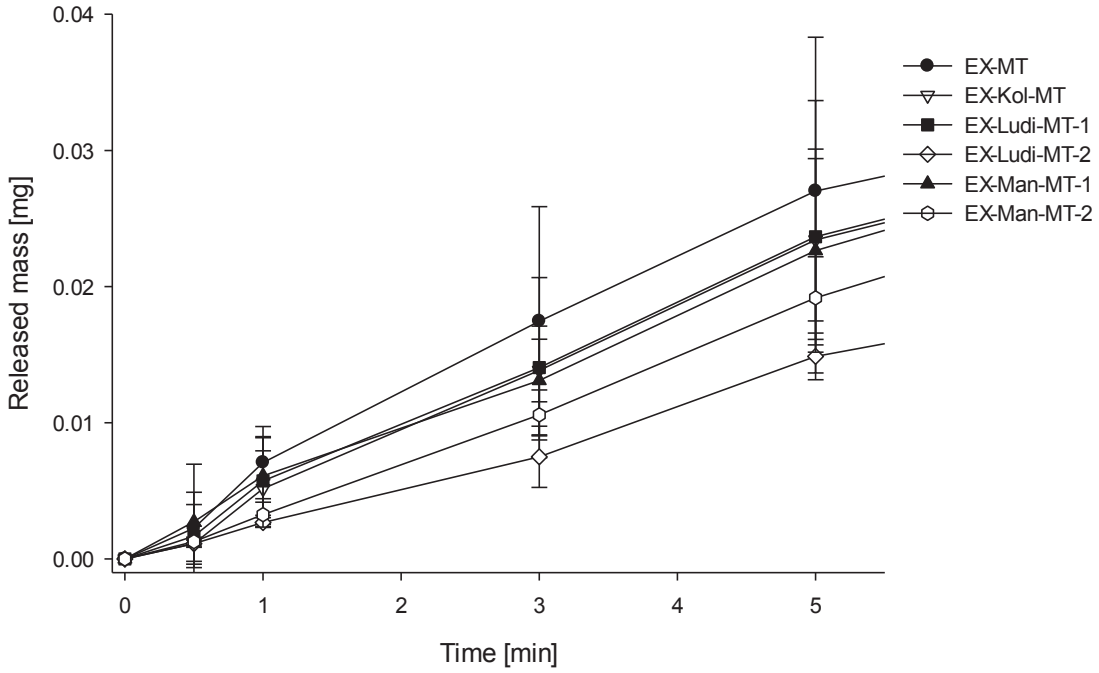


Figure 82. Drug release of mini-tablets made from extrudates in SSF. Arithmetic mean ± S.D. (n=6).

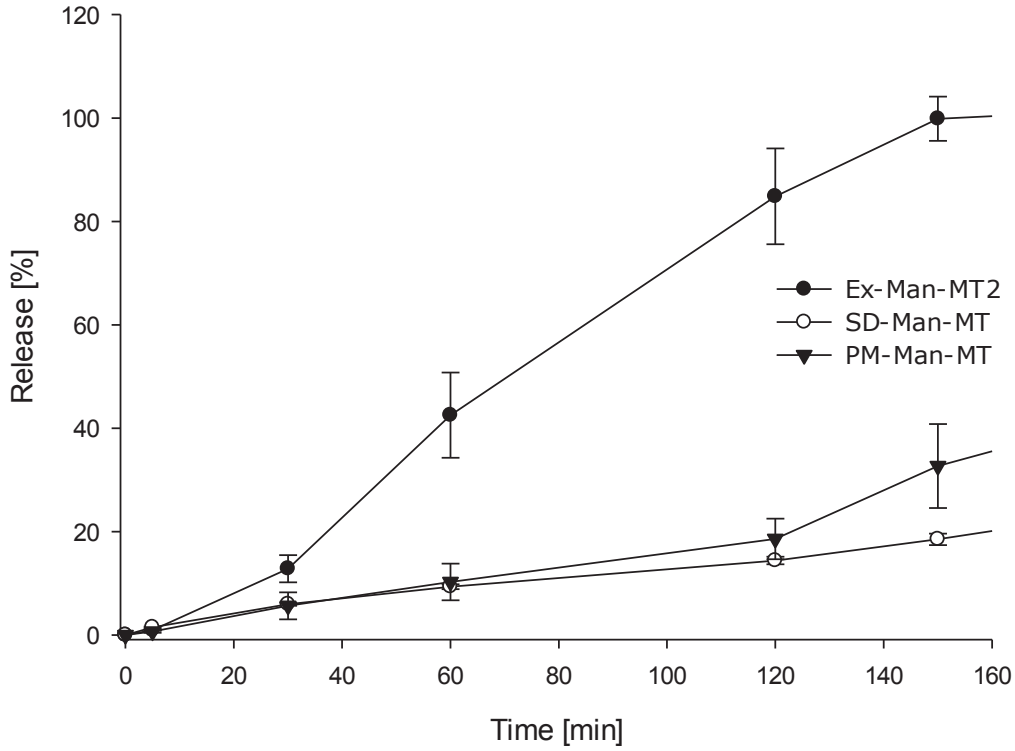


Figure 83. Drug release of mini-tablets with Parteck® M100 in FaSSIF. Arithmetic mean ± S.D. (n=6).

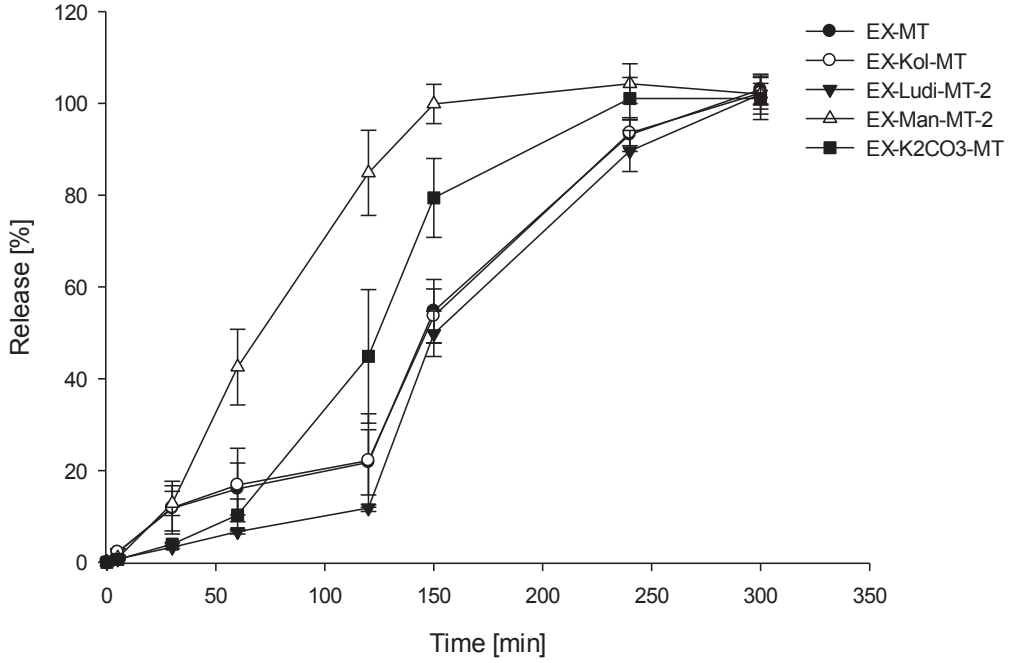


Figure 84. Drug release of mini-tablets made from extrudates in FaSSIF. Arithmetic mean ± S.D. (n=6).

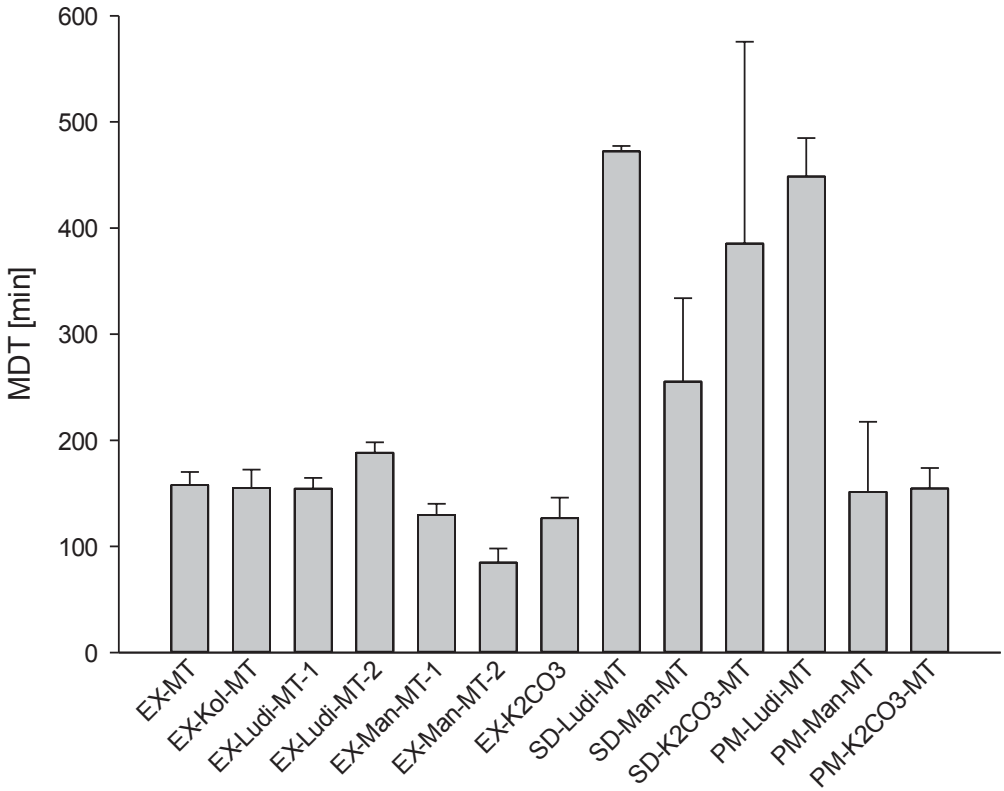


Figure 85. Mean dissolution time of all mini-tablet formulations in FaSSIF. Arithmetic mean ± S.D. (n=6).

Weighting of the authorship of the scientific publications

The following publications were included in this thesis:

1. Münster, M., Mohamed-Ahmed, A.H.A., Immohr, L.I., Schoch, C., Schmidt, C., Tuleu, C., Breitkreutz, J., 2017. *Comparative in vitro and in vivo taste assessment of liquid praziquantel formulations.* Int. J. Pharm. 529, 310-318.
   DOI: 10.1016/j.ijpharm.2017.06.084.

The following persons have made a significant contribution to the publication “Comparative in vitro and in vivo taste assessment of liquid praziquantel formulations” published in the International Journal of Pharmaceutics on the 8th of July 2017: Magdalena Münster, Abeer H. A. Mohamed-Ahmed, Laura I. Immohr, Corinna Schoch, Carsten Schmidt, Catherine Tuleu and Jörg Breitkreutz.

Author / co-author	**Idea [%]**	**Study design [%]**	**Experimental [%]**	**Evaluation [%]**	**Manuscript [%]**
Magdalena Münster	20	55	56	40	40
Abeer H. A. Mohamed-Ahmed	0	10	38	20	15
Laura I. Immohr	0	10	6	10	15
Corinna Schoch	20	5	0	10	10
Carsten Schmidt	20	5	0	5	5
Catherine Tuleu	20	10	0	10	10
Jörg Breitkreutz	20	5	0	5	5

The results of the manuscript are divided in three chapters: phase solubility study, measurements with the electronic tongue (e-tongue) and measurements using the rodent brief-access taste aversion (BATA) model.
The topic and the idea were formulated and discussed with equal shares by M. Münster, C. Schoch, C. Schmidt, C. Tuleu and J. Breitkreutz. The research plan was developed mainly by M. Münster. A. H. A. Mohamed-Ahmed and C. Tuleu contributed to the study design of the experiments using the BATA model. L. I. Immohr supported the study plan for the e-tongue. The other authors contributed to the finalization of the complete research plan. The experimental part of the phase solubility study and the measurements with the e-tongue were conducted by M. Münster. The self-developed sensors for the e-tongue were contributed by L. I. Immohr who in addition assisted during e-tongue measurements. The experimental part using the BATA model was performed by A. H. A. Mohamed-Ahmed. The evaluation and interpretation of the results for this manuscript was mostly done by M. Münster. A. H. A. Mohamed-Ahmed, C. Schoch and C. Tuleu were included in the discussion of the findings of the experiments using the BATA model. L. I. Immohr supported the

interpretation of the results of the e-tongue. C. Schoch, C. Schmidt and J. Breitkreutz contributed to the finalization of the findings of the complete manuscript. The draft of the manuscript in terms of content was formulated by M. Münster. A. H. A. Mohamed-Ahmed, L. I. Immohr and C. Tuleu substantially contributed to the content concerning the BATA model and the e-tongue. C. Schoch, C. Schmidt and J. Breitkreutz contributed to the complete manuscript.

2. Münster. M., Schoch, C., Schmidt, C., Tuleu, C., Breitkreutz, J., 2017. *Multiparticulate system combining taste masking and immediate release properties for the aversive compound praziquantel.* Eur. J. Pharm. Sci. 109, 446-454.
   DOI: 10.1016/j.ejps.2017.08.034

The following persons have made a significant contribution to the publication “Multiparticulate system combining taste masking and immediate release properties for the aversive compound praziquantel” published in the European Journal of Pharmaceutical Sciences on the 5th of September 2017: Magdalena Münster, Corinna Schoch, Carsten Schmidt and Jörg Breitkreutz.

Author / co-author	**Idea [%]**	**Study design [%]**	**Experimental [%]**	**Evaluation [%]**	**Manuscript [%]**
Magdalena Münster	25	70	100	60	70
Corinna Schoch	25	10	0	20	15
Carsten Schmidt	25	10	0	10	5
Jörg Breitkreutz	25	10	0	10	10

The topic and the idea were formulated and discussed with equal shares by M. Münster, C. Schoch, C. Schmidt and J. Breitkreutz. The study design was developed by M. Münster. All other authors contributed to the finalization of the complete research plan. The experimental part was completely conducted by M. Münster. The evaluation and interpretation of the results for this manuscript was mostly done by M. Münster. C. Schoch, C. Schmidt and J. Breitkreutz were included in the discussion of the findings of the experiments and supported the interpretation of the results. All authors contributed to the finalization of the findings of the complete manuscript. The draft of the manuscript in terms of content was formulated by M. Münster. C. Schoch, C. Schmidt and J. Breitkreutz substantially contributed to the finalization of the complete manuscript.

8. References

[1] Breitkreutz J., Boos J., *Paediatric and geriatric drug delivery*, Exp. Opin. Drug Deliv., 4 (2007) 37-45.

[2] Walsh J., Cram A., Woertz K., Breitkreutz J., Winzenburg G., Turner R., Tuleu C., *Playing hide and seek with poorly tasting paediatric medicines: Do not forget the excipients*, Adv. Drug Deliv. Rev., 73 (2014) 14-33.

[3] Matsui D., *Current issues in pediatric medication adherence*, Paediatr. Drugs, 9 (2007) 283-288.

[4] Ernest T.B., Craig J., Nunn A., Salunke S., Tuleu C., Breitkreutz J., Alex R., Hempenstall J., *Preparation of medicines for children - a hierarchy of classification*, Int. J. Pharm., 435 (2012) 124-130.

[5] EMA, *Reflection paper on collecting and reporting information on off-label use in pharmacovigilance EMA/293194/2016*, available online at: http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2016/04/WC500205499.pdf (accessed 17.07.2017), (2016).

[6] EMA, *Report on the survey of all paediatric uses of medicinal products in Europe*, EMA/794083/2009, (2010).

[7] EU, *EC 1901/2006 on medicinal products for paediatric use*, 1901/2006 (2007).

[8] Breitkreutz J., *European perspectives on pediatric formulations*, Clin. Ther., 30 (2008) 2146-2154.

[9] Batchelor H., Salunke S., Tuleu C., *Formulating better medicines for children-reflections*, Int. J. Pharm., 492 (2015) 301-303.

[10] Salunke S., Tuleu C., *The STEP database through the end-users eyes—USABILITY STUDY*, Int. J. Pharm., 492 (2015) 316-331.

[11] Liu F., Ranmal S., Batchelor H.K., Orlu-Gul M., Ernest T.B., Thomas I.W., Flanagan T., Kendall R., Tuleu C., *Formulation factors affecting acceptability of oral medicines in children*, Int. J. Pharm., 492 (2015) 341-343.

[12] Slavkova M., Breitkreutz J., *Orodispersible drug formulations for children and elderly*, Eur. J. Pharm. Sci., 75 (2015) 2-9.

[13] EMA, *Reflection paper: formulations of choice for the paediatric population*, EMEA/CHMP/PEG/194810/2005, (2006).

[14] EMA, *Guideline on Pharmaceutical Development of Medicines for Paediatric Use*, EMA/CHMP/QWP/805880/2012 Rev. 2, European Medicines Agency (2014).

[15] WHO, *Report of the informal expert meeting on dosage forms of medicines for children*, World Health Organization (2008).

[16] WHO, *Better medicines for children*, World Health Organization (2007).

[17] Drumond N., van Riet-Nales D.A., Karapinar-Çarkit F., Stegemann S., *Patients' appropriateness, acceptability, usability and preferences for pharmaceutical preparations: Results from a literature review on clinical evidence*, Int. J. Pharm., 521 (2017) 294-305.

[18] Klingmann V., Seitz A., Meissner T., Breitkreutz J., Moeltner A., Bosse H.M., *Acceptability of Uncoated Mini-Tablets in Neonates-A Randomized Controlled Trial*, J. Pediatr., 167 (2015) 893-896.

[19] Mitra B., Chang J., Wu S.J., Wolfe C.N., Ternik R.L., Gunter T.Z., Victor M.C., *Feasibility of mini-tablets as a flexible drug delivery tool*, Int. J. Pharm., 525 (2017) 149-159.

[20] FDA, *Guidance for Industry Orally Disintegrating Tablets*, U.S. Department of Health and Human Services Food and Drug Administration, (2008).

[21] Ranmal S.R., Cram A., Tuleu C., *Age-appropriate and acceptable paediatric dosage forms: Insights into end-user perceptions, preferences and practices from the Children's Acceptability of Oral Formulations (CALF) Study*, Int. J. Pharm., 514 (2016) 296-307.

[22] Kimura S., Uchida S., Kanada K., Namiki N., *Effect of granule properties on rough mouth feel and palatability of orally disintegrating tablets*, Int. J. Pharm., 484 (2015) 156-162.

[23] Gryczke A., Schminke S., Maniruzzaman M., Beck J., Douroumis D., *Development and evaluation of orally disintegrating tablets (ODTs) containing Ibuprofen granules prepared by hot melt extrusion*, Colloids Surf B Biointerfaces, 86 (2011) 275-284.

[24] Lennartz P., B. M.J., *Minitabletting: improving the compactability of paracetamol powder mixtures*, Int. J. Pharm., 173 (1998) 75-85.

[25] Rumondor A.C.F., Harris D., Flanagan F., Biyyala V., Johnson M.A., Zhang D., Patel S., *Minitablets: Manufacturing, characterization methods, and future opportunities*, Am. Pharmaceut. Rev., available online at http://www.americanpharmaceuticalreview.com/Featured-Articles/190921-Minitablets-Manufacturing-Characterization-Methods-and-Future-Opportunities/ (accessed 05.02.2018) (2016).

[26] Hagen E., Løding F.S., Mattsson S., Tho I., *Use of interactive mixtures to obtain mini-tablets with high dose homogeneity for paediatric drug delivery*, J. Drug Deliv. Sci. Technol., 34 (2016) 51-59.

[27] Nart V., Beringhs A.O., Franca M.T., de Espindola B., Pezzini B.R., Stulzer H.K., *Carnauba wax as a promising excipient in melt granulation targeting the preparation of mini-tablets for sustained release of highly soluble drugs*, Mater. Sci. Eng. C. Mater. Biol. Appl., 70 (2017) 250-257.

[28] Brabander C.D., Vervaet C., Fiermans L., Remon J.P., *Matrix mini-tablets based on starch, microcrystalline wax mixtures*, Int. J. Pharm., 199 (2000) 195-203.

[29] Roberts M., Vellucci D., Mostafa S., Miolane C., Marchaud D., *Development and evaluation of sustained-release Compritol 888 ATO matrix mini-tablets*, Drug Dev. Ind. Pharm., 38 (2012) 1068-1076.

[30] Lopes C.M., Lobo J.M., Pinto J.F., Costa P., *Compressed mini-tablets as a biphasic delivery system*, Int. J. Pharm., 323 (2006) 93-100.

[31] Aleksovski A., Luštrik M., Šibanc R., Dreu R., *Design and evaluation of a specific, BI-phase extended release system based on differently coated mini tablets*, Eur. J. Pharm. Sci., 75 (2015) 114-122.

[32] Tissen C., Woertz K., Breitkreutz J., Kleinebudde P., *Development of mini-tablets with 1mm and 2mm diameter*, Int. J. Pharm., 416 (2011) 164-170.

[33] Stoltenberg I., Breitkreutz J., *Orally disintegrating mini-tablets (ODMTs) - A novel solid oral dosage form for paediatric use*, Eur. J. Pharm. Biopharm., 78 (2011) 462-469.

[34] Spomer N., Klingmann V., Stoltenberg I., Lerch C., Meissner T., Breitkreutz J., *Acceptance of uncoated mini-tablets in young children: results from a prospective exploratory cross-over study*, Arch. Dis. Child., 97 (2012) 283-286.

[35] Klingmann V., Spomer N., Lerch C., Stoltenberg I., Frömke C., Bosse H.M., Breitkreutz J., Meissner T., *Favorable Acceptance of Mini-Tablets Compared with Syrup: A Randomized Controlled Trial in Infants and Preschool Children*, J. Pediatr., 163 (2013) 1728-1732.

[36] Thomson S.A., Tuleu C., Wong I.C.K., Keady S., Pitt K.G., Sutcliffe A.G., *Minitablets: New Modality to Deliver Medicines to Preschool-Aged Children*, Pediatrics, 123 (2009) 235-238.

[37] Kluk A., Sznitowska M., Brandt B., Sznurkowska K., Plata-Nazar K., Mysliwiec M., Kaminskac B., Kotlowska H., *Can preschool-aged children swallow several minitablets at a time? Results from a clinical pilot study*, Int. J. Pharm., 15 (2015) 1-6.

[38] van Riet-Nales D.A., de Neef B.J., Schobben A.F.A.M., Ferreira J.A., Egberts T.C.G., Rademaker C.A.M., *Acceptability of different formulations in infants and preschool children.*, Arch. Dis. Child., 98 (2014) 725 - 731.

[39] van Riet-Nales D.A., Ferreira J.A., Schobben A.F.A.M., de Neef B.J., Egberts T.C.G., Rademaker C.A.M., *Methods of administering oral formulations and child accceptability*, Int. J. Pharm., 491 (2015) 261-267.

[40] Hayakawa Y., Uchida S., Namiki N., *Evaluation of the ease of taking mini-tablets compared with other tablet formulations in healthy volunteers*, Eur. J. Pharm. Sci., 84 (2016) 157-161.

[41] Mistry P., Batchelor H., *Evidence of acceptability of oral paediatric medicines: a review*, J. Pharm. Pharmacol., 69 (2017) 361-376.

[42] Hautala J., Airaksinen S., Naukkarinen N., Vainio O., Juppo A.M., *Evaluation of new flavors for feline mini-tablet formulations.*, J. Excipients and Food Chem., 5 (2014) 81-99.

[43] Bowles A., Keane J., Ernest T., Clapham D., Tuleu C., *Specific aspects of gastro-intestinal transit in children for drug delivery design*, Int. J. Pharm., 395 (2010) 37-43.

[44] Meyer T., Sekljic H., Fuchs S., Bothe H., Schollmeyer D., Miculka C., *Taste, a new incentive to switch to (R)-Praziquantel in Schistosomiasis Treatment*, PLoS Negl. Trop. Dis., 3 (2009) e357.

[45] Passerini N., Albertini B., Perissutti B., Rodriguez L., *Evaluation of melt granulation and ultrasonic spray congealing as techniques to enhance the dissolution of praziquantel*, Int. J. Pharm., 318 (2006) 92-102.

[46] WHO, 2017. World Health Organization. http://www.who.int/schistosomiasis/disease/en/ (accessed 30.05.2017)

[47] Stothard J.R., Sousa-Figueiredo J.C., Betson M., Bustinduy A., Reinhard-Rupp J., *Schistosomiasis in African infants and preschool children: let them now be treated!*, Trends Parasitol, 29 (2013) 197-205.

[48] Olliaro P., Delgado-Romero P., Keiser J., *The little we know about the pharmacokinetics and pharmacodynamics of praziquantel (racemate and R-enantiomer)*, J. Antimicrob. Chemother., 69 (2014) 863-870.

[49] WHO, 2017. World Health Organization. http://www.who.int/mediacentre/factsheets/fs115/en/ (accessed 30.05.2017)

[50] Pediatric Praziquantel Consortium. https://www.pediatricpraziquantelconsortium.org/schistosomiasis-children-major-public-health-problem (accessed 30.05.2017)

[51] Costa E.D., Priotti J., Orlandi S., Leonardi D., Lamas M.C., Nunes T.G., Diogo H.P., Salomon C.J., Ferreira M.J., *Unexpected solvent impact in the crystallinity of praziquantelpoly(vinylpyrrolidone) formulations. A solubility, DSC and solid-state NMR study*, Int. J. Pharm., 511 (2016) 983 - 993.

[52] Trastullo R., Dolci L.S., Passerini N., Albertini B., *Development of flexible and dispersible oral formulations containing praziquantel for potential schistosomiasis treatment of pre-school age children*, Int. J. Pharm., 495 (2015) 536-550.

[53] Reed D.R., Tanaka T., McDaniel A.H., *Diverse tastes: Genetics of sweet and bitter perception*, Physiol. Behav., 88 (2006) 215-226.

[54] Mennella J.A., Spector A.C., Reed D.R., Coldwell S.E., *The bad taste of medicines: overview of basic research on bitter taste*, Clin. Ther., 35 (2013) 1225-1246.

[55] Devillier P., Naline E., Grassin-Delyle S., *The pharmacology of bitter taste receptors and their role in human airways*, Pharmacol. Ther., 155 (2015) 11-21.

[56] Behrens M., Meyerhof W., *Bitter taste receptors and human bitter taste perception*, Cell. Mol. Life. Sci., 63 (2006) 1501-1509.

[57] Anand V., Kataria M., Kukkar V., Saharan V., Choudhury P.K., *The latest trends in the taste assessment of pharmaceuticals*, Drug Discov. Today, 12 (2007) 257-265.

[58] Pein M., Preis M., Eckert C., Kiene F.E., *Taste-masking assessment of solid oral dosage forms-A critical review*, Int. J. Pharm., 465 (2014) 239-254.

[59] Davies E.H., Tuleu C., *Medicines for children: a matter of taste*, J. Pediatr., 153 (2008) 599-604, 604 e591-592.

[60] Tuleu C., Breitkreutz J., *Educational paper: formulation-related issues in pediatric clinical pharmacology*, Eur. J. Pediatr., 172 (2013) 717-720.

[61] Mohamed-Ahmed A.H., Soto J., Ernest T., Tuleu C., *Non-human tools for the evaluation of bitter taste in the design and development of medicines: a systematic review*, Drug Discov. Today, 21 (2016) 1170-1180.

[62] Maniruzzaman M., Boateng J., Douroumis D., *Taste masking of bitter APIs by using hot melt extrusion (HME)*, AAPS J, 13 (2011).

[63] Slack J.P., Brockhoff A., Batram C., Menzel S., Sonnabend C., Born S., Galindo M.M., Kohl S., Thalmann S., Ostopovici-Halip L., Simons C.T., Ungureanu I., Duineveld K., Bologa C.G., Behrens M., Furrer S., Oprea T.I., Meyerhof W., *Modulation of bitter taste perception by a small molecule hTAS2R antagonist*, Curr. Biol., 20 (2010) 1104-1109.

[64] Soto J., *Assessing the feasibility of using an animal model for in vivo taste assessment of pharmaceutical compounds and formulations, doctoral thesis*, in: School of Pharmacy, University College London, UK, 2016.

[65] Soto J., Sheng Y., Standing J.F., Orlu Gul M., Tuleu C., *Development of a model for robust and exploratory analysis of the rodent brief-access taste aversion data*, Eur. J. Pharm. Biopharm., 91 (2015) 47-51.

[66] Contreras R.J., Carson C.A., Pierce C.E., *A novel psychophysical procedure for bitter taste assessment in rats*, Chem. Senses., 20 (1995) 305-312.

[67] Treesukosol Y., Boersma G.J., Oros H., Choi P., Tamashiro K.L., Moran T.H., *Similarities and differences between "proactive" and "passive" stress-coping rats in responses to sucrose, NaCl, citric acid, and quinine*, Chem. Senses., 39 (2014) 333-342.

[68] Glendinning J.I., Bloom L.D., Onishi M., Zheng K.H., Damak S., Margolskee R.F., Spector A.C., *Contribution of α-Gustducin to Taste-guided Licking Responses of Mice*, Chem. Senses., 30 (2005) 299-316.

[69] Bhat M.G., Jordt R.M., Khan M.A., Foley C.E., Gilbertson T.A., *Validation of a rat behavioral avoidance model from a drug delivery perspective*, Int. J. Pharm., 303 (2005) 31-36.

[70] Devantier H.R., Long D.J., Brennan F.X., Carlucci S.A., Hendrix C., Bryant R.W., Salemme F.R., Palmer R.K., *Quantitative assessment of TRPM5-dependent oral aversiveness of pharmaceuticals using a mouse brief-access taste aversion model*, Behav. Pharmacol., 19 (2008) 673-682.

[71] Rudnitskaya A., Kirsanov D., Blinova Y., Legin E., Seleznev B., Clapham D., Ives R.S., Saunders K.A., Legin A., *Assessment of bitter taste of pharmaceuticals with multisensor system employing 3 way PLS regression*, Analytica Chimica Acta, 770 (2013) 45-52.

[72] Noorjahan A., Amrita B., Kavita S., *In vivo evaluation of taste masking for developed chewable and orodispersible tablets in humans and rats*, Pharm. Dev. Technol., 19 (2014) 290-295.

[73] Clapham D., Kirsanov D., Legin A., Rudnitskaya A., Saunders K., *Assessing taste without using humans: rat brief access aversion model and electronic tongue*, Int. J. Pharm., 435 (2012) 137-139.

[74] Soto J., Winzenburg G., Turner R., Desset-Brèthes S., Sheng Y., Orlu-Gul M., Tuleu C., *Assessing the bitter taste of medicines: A comparison between rat taste panels (via the brief-access taste aversion (BATA) model) and human taste panels*, Int. J. Pharm., 511 (2016) 1127-1128.

[75] Pein M., Kirsanov D., Ciosek P., Del Valle M., Yaroshenko I., Wesoly M., Zabadaj M., Gonzalez-Calabuig A., Wroblewski W., Legin A., *Independent comparison study of six different electronic tongues applied for pharmaceutical analysis*, J. Pharm. Biomed. Anal., 114 (2015) 321-329.

[76] Woertz K., Tissen C., Kleinebudde P., Breitkreutz J., *A comparative study on two electronic tongues for pharmaceutical formulation development*, J. Pharm. Biomed. Anal., 55 (2011) 272-281.

[77] Woertz K., Tissen C., Kleinebudde P., Breitkreutz J., *Performance qualification of an electronic tongue based on ICH guideline Q2*, J. Pharm. Biomed. Anal., 51 (2010) 497-506.

[78] Khan R.R., Kang S.-W., *Highly Sensitive Multi-Channel IDC Sensor Array for Low Concentration Taste Detection*, Sensors, 15 (2015) 13201-13221.

[79] Woertz K., Tissen C., Kleinebudde P., Breitkreutz J., *Taste sensing systems (electronic tongues) for pharmaceutical applications*, Int. J. Pharm., 417 (2011) 256-271.

[80] Lorenz J.K., Reo J.P., Hendl O., Worthington J.H., Petrossian V.D., *Evaluation of a taste sensor instrument (electronic tongue) for use in formulation development*, Int. J. Pharm., 367 (2009) 65-72.

[81] Toko K., *Taste sensor with global selectivity*, Mater. Sci. Eng. C 4, (1996) 69-82.

[82] Eckert C., Lutz C., Breitkreutz J., Woertz K., *Quality control of oral herbal products by an electronic tongue - Case study on sage lozenges*, Sensors and Actuators B: Chemical, 156 (2011) 204-212.

[83] Kobayashi Y., Habara M., Ikezazki H., Chen R., Naito Y., Toko K., *Advanced taste sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores*, Sensors, 10 (2010) 3411-3443.

[84] Toko K., Matsuno T., Yamafuji K., *Multichannel taste sensor using electric potential changes in lipid membranes*, Biosensors and Bioelectronics, 9 (1994) 359-364.

[85] Woertz K., Tissen C., Kleinebudde P., Breitkreutz J., *Rational development of taste masked oral liquids guided by an electronic tongue*, Int. J. Pharm., 400 (2010) 114-123.

[86] Eckert C., Pein M., Breitkreutz J., *Lean production of taste improved lipidic sodium benzoate formulations*, Eur. J. Pharm. Biopharm., 88 (2014) 455-461.

[87] Pimparade M.B., Morott J.T., Park J., Kulkarni V., Majumdar S., Murthy S.N., Lian Z., Pinto E., Bi V., Durig T., Murthy R., Vanaja K., Repka M.A., *Development of taste masked caffeine citrate formulations utilizing hot melt extrusion technology and in vitro-in vivo evaluations*, Int. J. Pharm., 487 (2015) 167-176.

[88] Preis M., Pein M., Breitkreutz J., *Development of a taste-masked orodispersible film containing dimenhydrinate*, Pharmaceutics, 4 (2012) 551-562.

[89] Woertz K., Tissen C., Kleinebudde P., Breitkreutz J., *Development of a taste-masked generic ibuprofen suspension: Top-down approach guided by electronic tongue measurements*, J. Pharm. Sci., 100 (2011) 4460-4470.

[90] Zheng J.Y., Keeney M.P., *Taste masking analysis in pharmaceutical formulation development using an electronic tongue*, Int. J. Pharm., 310 (2006) 118-124.

[91] Legin A., Rudnitskaya A., Clapham D., Seleznev B., Lord K., Vlasov Y., *Electronic tongue for pharmaceutical analytics: quantification of tastes and masking effects*, Analytical and Bioanalytical Chemistry, 380 (2004) 36-45.

[92] Tokuyama E., Matsunaga C., Yoshida K., Mifsud J.-C., Irie T., Yoshida M., Uchida T., *Famotidine orally disintegrating tablets bitterness comparison of original and generic products*, Chem. Pharm. Bull., 57 (2009) 382-387.

[93] Wesoły M., Zabadaj M., Amelian A., Winnicka K., Wróblewski W., Ciosek P., *Tasting cetirizine-based microspheres with an electronic tongue*, Sensors and Actuators B: Chemical, 238 (2017) 1190-1198.

[94] Eckert C., Pein M., Reimann J., Breitkreutz J., *Taste evaluation of multicomponent mixtures using a human taste panel, electronic taste sensing systems and HPLC*, Sensors and Actuators B: Chemical, 182 (2013) 294-299.

[95] Haraguchi T., Yoshida M., Kojima H., Uchida T., *Usefulness and limitations of taste sensors in the evaluation of palatability and taste-masking in oral dosage forms*, Asian J. Pharm. Sci., 11 (2016) 479-485.

[96] Sohi H., Sultana Y., Khar R.K., *Taste masking technologies in oral pharmaceuticals: recent developments and approaches*, Drug Dev. Ind. Pharm., 30 (2004) 429-448.

[97] Gittings S., Turnbull N., Roberts C.J., Gershkovich P., *Dissolution methodology for taste masked oral dosage forms*, J. Control. Release, 173 (2014) 32-42.

[98] Ayenew A., Puri V., Kumar L., Bansal A.K., *Trends in pharmaceutical taste masking technologies.*, Recent Pat. Drug. Deliv. Formul., 3 (2009) 26 - 39.

[99] Douroumis D., *Practical approaches of taste masking technologies in oral solid forms*, Exp. Opin. Drug Deliv., 4 (2007) 417-426.

[100] Richey R.H., Hughes C., Craig J.V., Shah U.U., Ford J.L., Barker C.E., Peak M., Nunn A.J., Turner M.A., *A systematic review of the use of dosage form manipulation to obtain required doses to inform use of manipulation in paediatric practice*, Int. J. Pharm., 518 (2017) 155-166.

[101] Mura P., *Analytical techniques for characterization of cyclodextrin complexes in the solid state: a review*, J. Pharm. Biomed. Anal., 113 (2015) 226-238.

[102] Maniruzzaman M., Boateng J.S., Snowden M.J., Douroumis D., *A review of hot-melt extrusion: process technology to pharmaceutical products*, ISRN Pharm, 2012 (2012) 436763.

[103] Breitenbach J., *Melt extrusion: from process to drug delivery technology*, Eur. J. Pharm. Biopharm., 54 (2002) 107-117.

[104] Crowley M.M., Zhang F., Repka M.A., Thumma S., Upadhye S.B., Battu S.K., McGinity J.W., Martin C., *Pharmaceutical applications of hot-melt extrusion: part I*, Drug Dev. Ind. Pharm., 33 (2007) 909-926.

[105] Stankovic M., Frijlink H.W., Hinrichs W.L., *Polymeric formulations for drug release prepared by hot melt extrusion: application and characterization*, Drug Discov Today, 20 (2015) 812-823.

[106] Maniruzzaman M., Boateng S.J., Chowdhry B.Z., Snowden M.J., Douroumis D., *A review on the taste masking of bitter APIs: hot-melt extrusion (HME) evaluation*, Drug Dev. Ind. Pharm., 40 (2014) 145-156.

[107] Maniruzzaman M., Boateng J.S., Bonnefille M., Aranyos A., Mitchell J.C., Douroumis D., *Taste masking of paracetamol by hot-melt extrusion: An in vitro and in vivo evaluation*, Eur. J. Pharm. Biopharm., 80 (2012) 433-442.

[108] Breitkreutz J., El-Saleh F., Kiera C., Kleinebudde P., Wiedey W., *Pediatric drug formulations of sodium benzoate: II. Coated granules with a lipophilic binder*, Eur. J. Pharm. Biopharm., 56 (2003) 255-260.

[109] Krause J., Thommes M., Breitkreutz J., *Immediate release pellets with lipid binders obtained by solvent-free cold extrusion*, Eur. J. Pharm. Biopharm., 71 (2009) 138-144.

[110] Schulze S., Winter G., *Lipid extrudates as novel sustained release systems for pharmaceutical proteins*, J. Control. Release, 134 (2009) 177-185.

[111] Breitkreutz J., Bornhöft M., Wöll F., Kleinebudde P., *Pediatric drug formulations of sodium benzoate: I. Coated granules with a hydrophilic binder*, Eur J Pharm Biopharm, 56 (2003) 247-253.

[112] Reitz C., Kleinebudde P., *Solid lipid extrusion of sustained release dosage forms*, Eur. J. Pharm. Biopharm., 67 (2007) 440-448.

[113] Michalk A., Kanikanti V.R., Hamann H.J., Kleinebudde P., *Controlled release of active as a consequence of the die diameter in solid lipid extrusion*, J. Control. Release, 132 (2008) 35-41.

[114] Vaassen J., Bartscher K., Breitkreutz J., *Taste masked lipid pellets with enhanced release of hydrophobic active ingredient*, Int. J. Pharm., 429 (2012) 99-103.

[115] Witzleb R., Kanikanti V.R., Hamann H.J., Kleinebudde P., *Solid lipid extrusion with small die diameters--electrostatic charging, taste masking and continuous production*, Eur. J. Pharm. Biopharm., 77 (2011) 170-177.

[116] Jannin V., Cuppok Y., *Hot-melt coating with lipid excipients*, Int. J. Pharm., 457 (2013) 480-487.

[117] Sudke S., Sakarakar D., *Lipids-An Instrumental Excipient In Pharmaceutical Hot-Melt Coating*, Lipids, 5 (2013) 607-621.

[118] Witzleb R., Mullertz A., Kanikanti V.R., Hamann H.J., Kleinebudde P., *Dissolution of solid lipid extrudates in biorelevant media*, Int. J. Pharm., 422 (2012) 116-124.

[119] Windbergs M., Strachan C.J., Kleinebudde P., *Understanding the solid-state behaviour of triglyceride solid lipid extrudates and its influence on dissolution*, Eur. J. Pharm. Biopharm., 71 (2009) 80-87.

[120] Vithani K., Maniruzzaman M., Slipper I.J., Mostafa S., Miolane C., Cuppok Y., Marchaud D., Douroumis D., *Sustained release solid lipid matrices processed by hot-melt extrusion (HME)*, Colloids Surf B Biointerfaces, 110 (2013) 403-410.

[121] Gures S., Kleinebudde P., *Dissolution from solid lipid extrudates containing release modifiers*, Int. J. Pharm., 412 (2011) 77-84.

[122] Sosnik A., Seremeta K.P., *Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers*, Adv. Colloid Interface Sci., 223 (2015) 40-54.

[123] Singh A., den Mooter G.V., *Spray drying formulation of amorphous solid dispersions*, Adv. Drug Deliv. Rev., 100 (2015) 27-50.

[124] Paudel A., Worku Z.A., Meeus J., Guns S., den Mooter G.V., *Manufacturing of solid dispersions of poorly water soluble drugs by spray drying: Formulation and process considerations*, Int. J. Pharm., 453 (2013) 253-284.

[125] Demuth B., Nagy Z.K., Balogh A., Vigh T., Marosi G., Verreck G., Van Assche I., Brewster M.E., *Downstream processing of polymer-based amorphous solid dispersions to generate tablet formulations*, Int. J. Pharm., 486 (2015) 268-286.

[126] Yi E.J., Kim J.Y., Rhee Y.S., Kim S.H., Lee H.J., Park C.W., Park E.S., *Preparation of sildenafil citrate microcapsules and in vitro/in vivo evaluation of taste masking efficiency*, Int. J. Pharm., 466 (2014) 286-295.

[127] Bora D., Borude P., Bhise K., *Taste masking by spray-drying technique*, AAPS Pharm. Sci. Tech., 9 (2008) 1159-1164.

[128] Tung N.T., Tran C.S., Nguyen T.L., Hoang T., Trinh T.D., Nguyen T.N., *Formulation and biopharmaceutical evaluation of bitter taste masking microparticles containing azithromycin loaded in dispersible tablets*, Eur. J. Pharm. Biopharm., in press, corrected proof (2017) http://dx.doi.org/10.1016/j.ejpb.2017.1003.1017.

[129] Szente L., Szemán J., Sohajda T., *Analytical characterization of cyclodextrins: History, official methods and recommended new techniques*, J. Pharm. Biomed. Anal., 130 (2016) 347-365.

[130] Jambhekar S.S., Breen P., *Cyclodextrins in pharmaceutical formulations I: structure and physicochemical properties, formation of complexes, and types of complex*, Drug Discov. Today, 21 (2016) 356-362.

[131] Rajewski R.A., Stella V.J., *Pharmaceutical Applications of Cyclodextrins. 2. In Vivo Drug Delivery*, J. Pharm. Sci., 85 (1996) 1142-1168.

[132] Arrua E.C., Ferreira M.J., Salomon C.J., Nunes T.G., *Elucidating the guest-host interactions and complex formation of praziquantel and cyclodextrin derivatives by (13)C and (15)N solid-state NMR spectroscopy*, Int. J. Pharm., 496 (2015) 812-821.

[133] Loftsson T., Brewster M.E., *Pharmaceutical applications of cyclodextrins. I. Drug solubilization and stabilization*, J. Pharm. Sci., 85 (1996) 1017-1025.

[134] Del Valle E.M.M., *Cyclodextrins and their uses: a review*, Process Biochemistry, 39 (2004) 1033-1046.

[135] Szejtli J., Szente L., *Elimination of bitter, disgusting tastes of drugs and foods by cyclodextrins*, Eur. J. Pharm. Biopharm., 61 (2005) 115 - 125.

[136] Desai S., Poddar A., Sawant K., *Formulation of cyclodextrin inclusion complex-based orally disintegrating tablet of eslicarbazepine acetate for improved oral bioavailability*, Materials Science and Engineering: C, 58 (2016) 826-834.

[137] Stella V.J., He Q., *Cyclodextrins*, Toxicol. Pathol., 36 (2008) 30-42.

[138] Duchêne D., Bochot A., *Thirty years with Cyclodextrins*, Int. J. Pharm., 514 (2016) 58-72.

[139] Irie T., Uekama K., *Pharmaceutical Applications of Cyclodextrins. III. Toxicological Issues and Safety Evaluation*, J. Pharm. Sci., 86 (1997) 147-162.

[140] EMA, *Background review for cyclodextrins used as excipients EMA/CHMP/333892/2013*, in, available online at:

http://www.ema.europa.eu/docs/en_GB/document_library/Report/2014/12/WC500177936.pdf (accessed 17.06.2017), European Medicines Agency, 2014.

[141] De Schaepdrijver L., Marien D., Rhimi C., Voets M., van Heerden M., Lammens L., *Juvenile animal testing of hydroxypropyl-beta-cyclodextrin in support of pediatric drug development*, Reprod. Toxicol., 56 (2015) 87-96.

[142] Preis M., Eckert C., Häusler O., Breitkreutz J., *A comparative study on solubilizing and taste-masking capacities of hydroxypropyl-β-cyclodextrin and maltodextrins with high amylose content*, Sensors and Actuators B: Chemical, 193 (2014) 442-450.

[143] Chronakis I.S., *On the Molecular Characteristics, Compositional Properties, and Structural-Functional Mechanisms of Maltodextrins: A Review*, Critical Reviews in Food Science and Nutrition, 38 (1998) 599-637.

[144] Marchal L.M., Beeftink H.H., Tramper J., *Towards a rational design of commercial maltodextrins*, Trends Food Sci. Technol., 10 (1999) 345-355.

[145] Carbinatto F.M., Ribeiro T.S., Colnago L.A., Evangelista R.C., Cury B.S.F., *Preparation and Characterization of Amylose Inclusion Complexes for Drug Delivery Applications*, J. Pharm. Sci., 105 (2016) 231-241.

[146] Luo Z., Zou J., Chen H., Cheng W., Fu X., Xiao Z., *Synthesis and characterization of amylose-zinc inclusion complexes*, Carbohydr. Polym., 137 (2016) 314-320.

[147] Ribeiro A.C., Rocha Â., Soares R.M.D., Fonseca L.P., da Silveira N.P., *Synthesis and characterization of acetylated amylose and development of inclusion complexes with rifampicin*, Carbohydr. Polym., 157 (2017) 267-274.

[148] Kong L., Ziegler G.R., *Molecular encapsulation of ascorbyl palmitate in preformed V-type starch and amylose*, Carbohydr. Polym., 111 (2014) 256-263.

[149] EFSA, *Conclusion on the peer review of the pesticide risk assessment of the active substance maltodextrin*, in, EFSA J. 11, 3007., European Food Safety Agency, 2013.

[150] Loftsson T., Hreinsdottir D., Masson M., *The complexation efficiency*, J. Incl. Phenom. Macrocycl. Chem., 57 (2007) 545–552.

[151] Higuchi T., Connors K.A., *Phase solubility techniques*, Adv. Anal. Chem. Instrum., 4 (1965) 117-212.

[152] Loftsson T., Jarho P., Masson M., Jarvinen T., *Cyclodextrins in drug delivery*, Exp. Opin. Drug Deliv., 2 (2005) 335-351.

[153] de Oliveira C.X., Ferreira N.S., Mota G.V., *A DFT study of infrared spectra and Monte Carlo predictions of the solvation shell of Praziquantel and beta-cyclodextrin inclusion complex in liquid water*, Spectrochim. Acta. A Mol. Biomol. Spectrosc., 153 (2016) 102-107.

[154] de Jesus M.B., de Matos Alves Pinto L., Fraceto L.F., Takahata Y., Lino A.C., Jaime C., de Paula E., *Theoretical and experimental study of a praziquantel and beta-cyclodextrin inclusion complex using molecular mechanic calculations and H1-nuclear magnetic resonance*, J. Pharm. Biomed. Anal., 41 (2006) 1428-1432.

[155] Becket G., Schep L.J., Tan M.Y., *Improvement of the in vitro dissolution of praziquantel by complexation with L-, ß-, and y-cyclodextrins*, Int. J. Pharm., 179 (1999) 65-71.

[156] Maragos S., Archontaki H., Macheras P., Valsami G., *Effect of cyclodextrin complexation on the aqueous solubility and solubility/dose ratio of praziquantel*, AAPS Pharm. Sci. Tech., 10 (2009) 1444-1451.

[157] El-Arini S.K., Leuenberger H., *Dissolution Properties of Praziquantel-β-cyclodextrin Systems*, Pharm. Dev. Technol., 1 (1996) 307-315.

[158] Cugovčan M., Jablan J., Lovrić J., Cinčić D., Galić N., Jug M., *Biopharmaceutical characterization of praziquantel cocrystals and cyclodextrin complexes prepared by grinding*, J. Pharm. Biomed. Anal., 137 (2017) 42-53.

[159] Dufour G., Bigazzi W., Wong N., Boschini F., de Tullio P., Piel G., Cataldo D., Evrard B., *Interest of cyclodextrins in spray-dried microparticles formulation for sustained pulmonary delivery of budesonide*, Int. J. Pharm., 495 (2015) 869-878.

[160] Anton N., Jakhmola A., Vandamme T.F., *Trojan microparticles for drug delivery*, Pharmaceutics, 4 (2012) 1-25.

[161] Mohtar N., Taylor K.M.G., Sheikh K., Somavarapu S., *Design and development of dry powder sulfobutylether-β-cyclodextrin complex for pulmonary delivery of fisetin*, Eur. J. Pharm. Biopharm., 113 (2017) 1-10.

[162] Vehring R., *Pharmaceutical Particle Engineering via Spray drying*, Pharm. Res., 25 (2008) 999-1022.

[163] Tsapis N., Bennett D., Jackson B., Weitz D.A., Edwards D.A., *Trojan particles: large porous carriers of nanoparticles for drug delivery*, Proc. Natl. Acad. Sci. U S A, 99 (2002) 12001-12005.

[164] Vehring R., Foss W.R., Lechuga-Ballesteros D., *Particle formation in spray drying*, J. Aero. Sci., 38 (2007) 728-746.

[165] El-Arini S.K., Giron D., Leuenberger H., *Solubility Properties of Racemic Praziquantel and Its Enantiomers*, Pharm. Dev. Technol., 3 (1998) 557-564.

[166] Liu Y., Wang X., Wang J.K., Ching C.B., *Structural characterization and enantioseparation of the chiral compound praziquantel*, J. Pharm. Sci., 93 (2004) 3039-3046.

[167] Ma S.X., Chen W., Yang X.D., Zhang N., Wang S.J., Liu L., Yang L.J., *Alpinetin/hydroxypropyl-beta-cyclodextrin host-guest system: preparation, characterization, inclusion mode, solubilization and stability*, J. Pharm. Biomed. Anal., 67-68 (2012) 193-200.

[168] Wang J., Cao Y., Sun B., Wang C., *Characterisation of inclusion complex of trans-ferulic acid and hydroxypropyl-beta-cyclodextrin*, Food Chemistry, 124 (2011) 1069-1075.

[169] Spamer E., Müller D.G., Wessels P.L., Venter J.P., *Characterization of the complexes of furosemide with 2-hydroxypropyl-ß-cyclodextrin and sulfobutyl ether-7-ß-cyclodextrin*, Eur. J. Pharm. Sci., 16 (2002) 247-253.

[170] Williams Iii R.O., Mahaguna V., Sriwongjanya M., *Characterization of an inclusion complex of cholesterol and hydroxypropyl-β-cyclodextrin*, Eur. J. Pharm. Biopharm., 46 (1998) 355-360.

[171] Pose-Vilarnovo B., Perdomo-Lopez I., Echezarreta-Lopez M., Schroth-Pardo P., Estrada E., Torres-Labandeira J.J., *Improvement of water solubility of sulfamethizole through its complexation with ß- and hp-ß-cd*, Eur. J. Pharm. Sci., 13 (2001) 325-331.

[172] Sri K.V., Kondaiah A., Ratna J.V., Annapurna A., *Preparation and characterization of quercetin and rutin cyclodextrin inclusion complexes*, Drug Dev. Ind. Pharm., 33 (2007) 245-253.

[173] Hancock B.C., Zografi G., *Characteristics and Significance of the Amorphous State in Pharmaceutical Systems*, J. Pharm. Sci., 86 (1997) 1-12.

[174] Leuner C., Dressman J., *Improving drug solubility for oral delivery using solid dispersions*, Eur. J. Pharm. Biopharm., 50 (2000) 47-60.

[175] Vasconcelos T., Sarmento B., Costa P., *Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs*, Drug Discov. Today, 12 (2007) 1068-1075.

[176] Immohr L.I., Turner R., Pein-Hackelbusch M., *Impact of sodium lauryl sulfate in oral liquids on e-tongue measurements*, Int. J. Pharm., 515 (2016) 441-448.

[177] Tissen C., *Entwicklung und Charakterisierung von geschmacksmaskierten, multipartikulären, festen Arzneiformen. Dissertation*, in: Mathematisch-Naturwissenschaftliche Fakultät, Heinrich-Heine-Universität Düsseldorf, 2011.

[178] Immohr L.I., Pein-Hackelbusch M., *Development of stereoselective e-tongue sensors considering the sensor performance using specific quality attributes – a bottom up approach*, Sensors and Actuators B, 253 (2017) 868-878.

[179] Roquette, Kleptose linecaps product information, available online at: https://www.roquette.com/media-center/resources/pharma-leaflet-taste-masking-kleptose-linecaps/ (accessed 07.03.17).

[180] Behrens M., Meyerhof W., *Mammalian bitter taste perception*, Results Probl. Cell. Differ., 47 (2009) 203-220.

[181] Bolhuis G.K., Chowhan Z.T., Materials for Direct Compaction, in: Pharmaceutical Powder Compaction Technology, Marcel Dekker Inc., New York, 1996, pp. 419-500.

[182] Jenike A.W., Storage and flow of solids, Univ. Utah, Salt Lake City, 1964.

[183] Hermes M.F.K., *Kindgerechte, niedrigdosierte Zubereitungen mit Enalaprilmaleat. Dissertation*, in: Mathemathisch-Naturwissenschaftliche Fakultät, Heinrich-Heine-Universität Düsseldorf, 2012.

[184] Grund J., Koerber M., Walther M., Bodmeier R., *The effect of polymer properties on direct compression and drug release from water-insoluble controlled release matrix tablets*, Int. J. Pharm., 469 (2014) 94-101.

[185] Baghel S., Cathcart H., O'Reilly N.J., *Theoretical and experimental investigation of drug-polymer interaction and miscibility and its impact on drug supersaturation in aqueous medium*, Eur. J. Pharm. Biopharm., 107 (2016) 16-31.

[186] Meng F., Trivino A., Prasad D., Chauhan H., *Investigation and correlation of drug polymer miscibility and molecular interactions by various approaches for the preparation of amorphous solid dispersions*, Eur. J. Pharm. Sci., 71 (2015) 12-24.

[187] Meng F., Dave V., Chauhan H., *Qualitative and quantitative methods to determine miscibility in amorphous drug-polymer systems*, Eur. J. Pharm. Sci., 77 (2015) 106-111.

[188] Wyttenbach N., Janas C., Siam M., Lauer M.E., Jacob L., Scheubel E., Page S., *Miniaturized screening of polymers for amorphous drug stabilization (SPADS): rapid assessment of solid dispersion systems*, Eur. J. Pharm. Biopharm., 84 (2013) 583-598.

[189] Liu H., Taylor L.S., Edgar K.J., *The role of polymers in oral bioavailability enhancement; a review*, Polymer, 77 (2015) 399-415.

[190] Nollenberger K., Albers J., *Poly(meth)acrylate-based coatings*, Int. J. Pharm., 457 (2013) 461-469.

[191] Siepmann F., Siepmann J., Walther M., MacRae R.J., Bodmeier R., *Polymer blends for controlled release coatings*, J. Control. Release, 125 (2008) 1-15.

[192] Joshi S., Petereit H.U., *Film coatings for taste masking and moisture protection*, Int. J. Pharm., 457 (2013) 395-406.

[193] Bodmeier R., Guo X., Sarabia R.E., Skultety P.F., *The influence of buffer species and strength on diltiazem HCl release from beads coated with the aqueous cationic polymer dispersions, Eudragit RS, RL 30D*, Pharm. Res., 13 (1996) 52-56.

[194] Witzleb R., Kanikanti V.R., Hamann H.J., Kleinebudde P., *Influence of needle-shaped drug particles on the solid lipid extrusion process*, Powder Technol., 207 (2011) 407-413.

[195] Shah U.V., Karde V., Ghoroi C., Heng J.Y., *Influence of particle properties on powder bulk behaviour and processability*, Int. J. Pharm., 518 (2016) 138-154.

[196] Capece M., Silva K.R., Sunkara D., Strong J., Gao P., *On the relationship of inter-particle cohesiveness and bulk powder behavior: Flowability of pharmaceutical powders*, Int. J. Pharm., 511 (2016) 178-189.

[197] Sun C.C., *Setting the bar for powder flow properties in successful high speed tableting*, Powder Technol., 201 (2010) 106-108.

[198] Xu J., Bovet L.L., Zhao K., *Taste masking microspheres for orally disintegrating tablets*, Int. J. Pharm., 359 (2008) 63-69.

[199] http://www.petercremerna.com/products/849389119, 2017 (accessed 10.01.2017)

[200] Albers J., Alles R., Matthee K., Knop K., Nahrup J.S., Kleinebudde P., *Mechanism of drug release from polymethacrylate-based extrudates and milled strands prepared by hot-melt extrusion*, Eur. J. Pharm. Biopharm., 71 (2009) 387-394.

[201] Thiry J., Krier F., Evrard B., *A review of pharmaceutical extrusion: Critical process parameters and scaling-up*, Int. J. Pharm., 479 (2014) 227-240.

[202] Vasconcelos T., Marques S., das Neves J., Sarmento B., *Amorphous solid dispersions: Rational selection of a manufacturing process*, Adv. Drug Deliv. Rev., 100 (2016) 85-101.

[203] Obadele B.A., Masuku Z.H., Olubambi P.A., *Turbula mixing characteristics of carbide powders and its influence on laser processing of stainless steel composite coatings*, Powder Technol., 230 (2012) 169-182.

[204] Mayer-Laigle C., Gatumel C., Berthiaux H., *Mixing dynamics for easy flowing powders in a lab scale Turbula® mixer*, Chem. Eng. Res. Des., 95 (2015) 248-261.

[205] Williams J.C., *The segregation of particulate materials. A review*, Powder Technol., 15 (1976) 245-251.

[206] Oka S., Sahay A., Meng W., Muzzio F., *Diminished segregation in continuous powder mixing*, Powder Technol., 309 (2017) 79-88.

[207] Hughey J.R., Keen J.M., Miller D.A., Kolter K., Langley N., McGinity J.W., *The use of inorganic salts to improve the dissolution characteristics of tablets containing Soluplus(R)-based solid dispersions*, Eur J Pharm Sci, 48 (2013) 758-766.

[208] Shamma R.N., Basha M., *Soluplus®: A novel polymeric solubilizer for optimization of Carvedilol solid dispersions: Formulation design and effect of method of preparation*, Powder Technol., 237 (2013) 406-414.

[209] Tanigawara Y., Yamaoka K., Nakagawa T., Nakagawa M., Uno T., *Correlation between in vivo mean dissolution time and in vitro mean dissolution time of ampicillin products*, J. Pharm. Dyn., 5 (1982) 370-372.

[210] EFSA, *Scientific Opinion on the use of Basic Methacrylate Copolymer as a food additive*, in, EFSA J. 8, 1513., European Food Safety Agency, 2010.

[211] FDA, *Direct Food Substances Affirmed as Generally Recognized as Safe*, in, https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1324 (accessed 07.08.2017), Food and Drug Administration, 2016.

[212] Kleinebudde P., *Pharmazeutische Pellets durch Extrudieren/Sphäronisieren - Herstellung, Eigenschaften, Modifizierung. Habilitationsschrift*, in: Mathematisch-Naturwissenschaftliche Fakultät, Christian-Albrechts-Universität Kiel, 1997.

[213] Stoltenberg I., *Orodispersible Minitabletten – Entwicklung und Charakterisierung einer neuen festen Darreichungsform für die Pädiatrie. Dissertation*, in: Mathematisch-Naturwissenschaftliche Fakultät, Heinrich-Heine-Universität Düsseldorf, 2012.

[214] Rinaki E., Dokoumetzidis A., Macheras P., *The mean dissolution time depends on the dose/solubility ratio*, Pharm. Res., 20 (2003) 406-408.

9. Publications and contributions to conferences

9.1. Publications

Münster, M., Mohamed-Ahmed, A.H.A., Immohr, L.I., Schoch, C., Schmidt, C., Tuleu, C., Breitkreutz, J., 2017. Comparative in vitro and in vivo taste assessment of liquid praziquantel formulations. Int. J. Pharm. 529, 310-318.
DOI: 10.1016/j.ijpharm.2017.06.084.

Münster. M., Schoch, C., Schmidt, C., Tuleu, C., Breitkreutz, J., 2017. Multiparticulate system combining taste masking and immediate release properties for the aversive compound praziquantel. Eur. J. Pharm. Sci. 109, 446-454.
DOI: 10.1016/j.ejps.2017.08.034

9.2. Contribution to conferences

M. Münster, C. Pfeifer, J. Breitkreutz; “Development and Characterization of Orodispersible Mini-tablets Manufactured by Direct Compression”, 1st European Conference on Pharmaceutics – Drug Delivery, Reims, France, 13/14th April 2015

M. Münster, C. Schoch, C. Schmidt, J. Breitkreutz; “Development of a Taste Masked Paediatric Formulation for Praziquantel”, 10st World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Glasgow, UK, 04/07th April 2016

9.3. Oral presentations

M. Münster; “Paediatric drug delivery for bitter APIs”, Merck Student’s Day, Darmstadt, Germany, 11th November 2016

10. Danksagung

Die vorliegende Arbeit entstand unter der Leitung von Herrn Prof. Dr. Jörg Breitkreutz der Heinrich-Heine-Universität Düsseldorf und Frau Dr. Corinna Schoch der Firma Merck KGaA im Rahmen meiner Tätigkeit als Doktorandin bei Merck KGaA in der Abteilung Pharmaceutical Technologies, Chemical & Pharmaceutical Development.

Mein besonderer Dank gilt meinem Doktorvater, Prof. Dr. Jörg Breitkreutz, für seine Betreuung der letzten 4 Jahre während der aktiven Arbeiten meiner Promotion und darüber hinaus während des Schreibens dieser Arbeit. Ich danke ihm sehr für seine fachliche Unterstützung, Diskussionsbereitschaft und wertvollen Hinweise mit seinem Blick auf die Wissenschaft, die mich stets vorangebracht haben.

Ebenfalls danke ich Prof. Dr. Dr. h.c. Peter Kleinebudde für die Übernahme des Koreferats und des Gutachtens, die zum Gelingen dieser Arbeit beigetragen haben.

Ganz besonders möchte ich mich bei Dr. Corinna Schoch für ihre fachliche und auch nicht-fachliche Unterstützung in den letzten Jahren bedanken. In jeder noch so stressigen Phase, in Höhen und Tiefen, sowie in spontanen Diskussionen zwischen Schreibtisch und Labor hatte sie immer ein offenes Ohr und wertvolle Anregungen für mich, die meine Fähigkeiten wissenschaftlich zu Denken und zu Arbeiten maßgeblich weiterentwickelt haben.

Mein Dank gilt weiterhin Dr. Carsten Schmidt für seine Betreuung, Rückendeckung, gemeinsame Gespräche und Diskussionsrunden, die mein Blickfeld noch zusätzlich erweitert haben.

Dr. Markus Weigandt danke ich für die Möglichkeit in Pharmaceutical Technologies zu promovieren und an nationalen und internationalen Seminaren und Kongressen teilzunehmen.

Besonders hervorzuheben ist die schöne gemeinsame Zeit mit den Merck-Kollegen während und nach der Arbeit, die mein Leben neben der Promotion bereichert hat. Mein besonderer Dank gilt Mira Oswald und Melanie Hofmann für die zahlreichen gemeinsamen Stunden und Ratschläge, die mich immer wieder motiviert haben. Weiterhin danke ich meinen „PhD-Weggefährten“ Carolin Auch, Steffen Wöll, Markus Riehl, Christian Jede, Karsten Flügel und Stefan Schiller für Ihre ständige Hilfs- und Diskussionsbereitschaft auf der Arbeit, ebenso wie meinen lieben Kollegen Vicky Schmitt und Katrin Grieser. Ich bedanke mich bei allen Kollegen innerhalb von Pharmaceutical Technologies, die mir in meiner Promotion mit Rat und Tat zur Seite standen.

Mein Dank gilt auch den wissenschaftlichen Mitarbeitern des Instituts für Pharmazeutische Technologie und Biopharmazie für die sehr nette Aufnahme in den Arbeitskreis bei meinen Besuchen in Düsseldorf und stetige Hilfsbereitschaft bei jeglichen Fragestellungen. Im Besonderen gilt dieser Dank auch Isabell Immohr und Prof.'in Dr. Miriam Pein-Hackelbusch.

Meinen Eltern danke ich für ihre Unterstützung, Geduld und ihr Vertrauen. Ohne diesen Rückhalt wären Studium und Promotion nicht möglich gewesen.