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Preparation and characterization of fast-dissolving oral films for pediatric use

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Für A.

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List of abbreviations

ADI	Acceptable Daily Intake
API	Active Pharmaceutical Ingredient
a.u.	arbitrary unit
AV	acceptance value
BNFC	British National Formularium for Children
CAP	Caffeine for Apnea of Prematurity
cAMP	cyclic Adenosine-3', 5'-MonoPhosphate
ChemFET	Chemical Field Effect Transistor
CI	confidence interval
CMC	carboxymethyl cellulose sodium
cps	counts per second
CRS	Chemical Reference Substance
DSC	Differential Scanning Calorimetry
EDQM	European Directorate for the Quality of Medicines & HealthCare
EFSA	European Food Safety Authority
e.g.	for example
EMEA	European Medicines Agency
Equ.	Equation
EU	European Union
FAO	Food and Agriculture Organization
GMP	Good Manufacturing Practice
gtt.	drops
HPLC	High Performance Liquid Chromatography
HPMC	hydroxypropyl methyl cellulose
h	hour
ICH	International Conference on Harmonization
i.e.	that is
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint Expert Committee on Food Additives
MCG	Membrane Coating Granules
Me	methyl
min	minute
mV	millivolt
n.a.	not available
n.d.	not determined
NICU	Neonatal Intensive Care Unit
NIR-CI	Near Infrared Chemical Imaging
NOEL	No Observed Effect Level
NRF	Neues Rezeptur-Formularium
ODT	Orally Disintegrating Tablet
OTC	over-the-counter
p.a.	for analytical purpose
PDE	Permitted Daily Exposure
Ph. Eur.	European Pharmacopoeia
PLS	Partial Least Squares
ppm	parts per million
RDF	Rapidly Dissolving Film
RH	Relative Humidity
RMSEE	Root Mean Square Error of Estimation
rpm	rounds per minute
SD	Standard Deviation
s	second
SEM	Scanning Electron Microscope
sta	standard
Tg	Glass transition temperature
TGA-MS	Thermogravimetric analysis coupled with mass spectrometer
TMA	Thermomechanical analysis
UK	United Kingdom
USA	United States of America
USP	United States Pharmacopoeia
WHO	World Health Organization
®	registered trademark
™	trademark

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A INTRODUCTION AND AIM OF THE STUDY

1 Introduction

The oral cavity has been investigated as a site for drug delivery for a long period of time. In 1847 Sobrero found that nitroglycerine was absorbed from the oral cavity (Ponchel 1993). Since then various active substances have been investigated for local or systemic use (Kellaway 1990).

Drug delivery through the oral cavity offers many advantages. The oral mucosa is conveniently and easily accessible and therefore allows uncomplicated application of dosage forms. Furthermore, the oral mucosa is robust against local stress or damage and shows fast cellular recovery after such incidents (Rathbone et al. 1994). Active substances can be administered locally to treat oral diseases like periodontal disease, bacterial and fungal infections or aphthous stomatitis (Ali et al. 2002, Nafee et al. 2003, Singh et al. 2008). A systemic action can be achieved via drug permeation through the mucosal endothelium. For systemic drug absorption, various dosage forms and devices, e.g. buccal patches, buccoadhesive discs and mechatronic delivery devices have recently been developed (El-Samaligy et al. 2004, Chayed and Winnik 2007, Perioli et al. 2008, Scholz et al. 2008). The use of buccal patches allows drug absorption to be terminated immediately upon simple removal of the patch.

The aforementioned advantages of drug administration via the oral cavity offer new possibilities in the administration of drugs to “problematical” subpopulations like children and the elderly. These patients have special drug administration requirements as they are often unable to swallow solid dosage forms (e.g. tablets, capsules). Poor taste can also lead to medication being refused or spat out. Furthermore, the pediatric subpopulation is a very heterogeneous group. According to the reflection paper “Formulations of choice for the paediatric population” by the EMEA (Committee for medicinal products for human use 2005) the pediatric population is divided into six groups: preterm newborn infants, term newborn infants, infants/toddlers, pre-school children, school children and adolescents. As such, there are differences in the suitability of various dosage forms for the different pediatric age groups. However, it is not only the suitability of a dosage form that has to be taken into consideration. Dosing regimen, applicability, efficacy and safety must also be taken into account, but the missing clinical trials in children demonstrate the lack of relevant data. Therefore, it is not surprising that many drugs, especially in the preterm and term newborn infant groups, are used off-label and unlicensed ('t Jong et al. 2002, Conroy et al. 2000, O’Donnell et al. 2002, Pandolfini and Bonati 2005). For these two age-groups solid dosage forms are inappropriate due to an inability to swallow. The parenteral route is commonly used if the child is severely ill or still very young. In the case of repulsion of oral liquids, drugs are primarily administered via the rectal route to achieve systemic

effects but this route is not favored in some cultures. In particular, for the preterm and term infants liquid dosage forms (e.g. solution, drops, emulsions, suspensions) for peroral use are recommended (Committee for medicinal products for human use, 2005).

The poor stability of aqueous liquids is problematic. Substances like benzalkonium chloride, benzyl alcohol or parabens are commonly used as preservatives. Many such substances are known to be potentially allergenic which is a problem often underestimated. Moreover, preservatives can be toxic due to immature metabolic pathways in children.

Fast-dissolving solid drug dosage forms for application onto the oral cavity for the pediatric population seem to be very appropriate, especially in preterm and term newborn infants. The delivery of drugs via the oral mucosa offers easy application, prevents drug degradation by gastrointestinal fluids, avoids first-pass metabolism and potentially improves bioavailability (American Academy of Pediatrics Committee on Drugs 2007) with rapid drug absorption and fast onset of drug action.

Drug absorption through membranes depends on the drug concentration at the surface of the mucosa, the vehicle for drug delivery, the contact time with the mucosa, the constitution of mucosal tissue, the degree of ionization of the drug, the pH of the absorption site, the size of the molecule and the relative lipid solubility (American Academy of Pediatrics Committee on Drugs 2007). These parameters have to be taken into account when formulating dosage forms for oral mucosal delivery. The drug concentration at the surface can be increased by varying the solubility of the drug. The drug partitioning can be influenced by environmental changes such as pH modifications. The permeability coefficient values are often low, so the use of permeation enhancers is beneficial. The contact time at the mucosa may be prolonged by the use of mucoadhesive polymers such as chitosans (Langoth et al. 2006), poly(ethylene glycol)-tethered copolymers (Serra et al. 2006) or alginates (Juliano et al. 2004). The size of the drug delivery system (e.g. buccal patches) determines the contact area and can be varied depending on the physiological conditions. Other issues such as continual secretion and swallowing of saliva are unique problems and also need to be considered during formulation development. The salivary flow and/or movements of the tongue and cheeks may influence the area and time of contact between the drug and the mucosa and thus the rate of absorption. Limited loading capacities restrict the use of oral mucosal dosage forms to include only potent drugs which are extensively absorbed from the oral cavity (Ponchel 1993).

As bioadhesive systems have long residence times in the oral cavity and may lead to an unpleasant mouth feel, they are assumed to be inappropriate for use in young children. Consequently, this work focuses on the development of a fast-dissolving oral dosage form. Fast-dissolving technology platforms include orally disintegrating tablets (ODTs) and oral lyophilisates, both of which are listed in the ‘tablets’ monograph of the

European Pharmacopoeia, and the rapidly disintegrating drug-loaded films (RDFs), which are not yet included in the pharmacopoeias. They are designed to dissolve/disintegrate in the mouth within a few seconds without additional water and the need to swallow. The ODTs show a high porosity, low density and low mechanical strength (Mishra and Amin 2007). Oral lyophilisates are manufactured by freeze-drying (Zydis®, Quicksolv®). ODTs such as FlashDose® are prepared by a molding process, whereas WOWTAB® and Flashtab® are prepared by granulation followed by compression and OralSolv®, DuraSolv® and AdvaTab™ are directly compressed (Sandri et al. 2006). Recently, the RDFs have gained popularity as dosage forms for breath fresheners. Meanwhile the pharmaceutical industry has recognized their potential for delivering medicinal products and has launched several products for the OTC market using this formulation technology. The fast-dissolving film is placed onto the patient's tongue where it is instantly wet by saliva, hydrated and adheres to the mucosa. The film then disintegrates and dissolves, releasing the drug for absorption by the mucosa. The fast-dissolving oral films are hardly described and investigated in literature, but seem to be an ideal dosage form for use in young children, especially in preterm and term newborn infants. They combine the greater stability of a solid form and the good applicability of a liquid. Due to their fast-dissolving behavior and subsequent adherence to the mucosa it is almost impossible to spit them out after application onto the tongue. Because of the novelty of fast-dissolving oral films, no monograph exists in the pharmacopoeias. Due to the lack of standard methodology for preparation and analysis new procedures need to be developed and evaluated within this work.

2 Aim of the study

The main objective of this work was the development of a novel, fast-dissolving product on the technology platform of a small and thin drug-loaded film, also called an oral wafer.

Due to the lack of any pharmacopoeial monographs or relevant literature, detailed development and evaluation of methods for characterization of the prepared films was carried out.

Caffeine was chosen as model drug for which a need for child-appropriate drug formulations exists. The drug was selected based on a high frequency of prescribing, an appropriate indication and age for this new dosage form, an adequate dosage on the basis of the limited drug-loading capacity of the wafers and adequate bioavailability of the drug after application. Caffeine as base or citrate salt seemed to be appropriate.

In the development of the oral wafers the toxicological suitability of the excipients should be given special attention.

Familiarization with the manufacturing technology including the drying process and cutting of the oral wafers was crucial. Furthermore, storage conditions for these films were considered. The suitability of proven methods for the characterization of the prepared films were tested. There was a need to develop more appropriate methods for film characterization to aid decision-making during drug development and quality control. Furthermore, the stability of the obtained films needed to be thoroughly investigated.

B GENERAL PART

1 Absorption from the oral cavity

The oral cavity is divided into two parts, the outer and the interior oral vestibules. The outer oral vestibule includes the space between the cheeks, lips and teeth, whereas the interior oral vestibule is located behind the teeth.

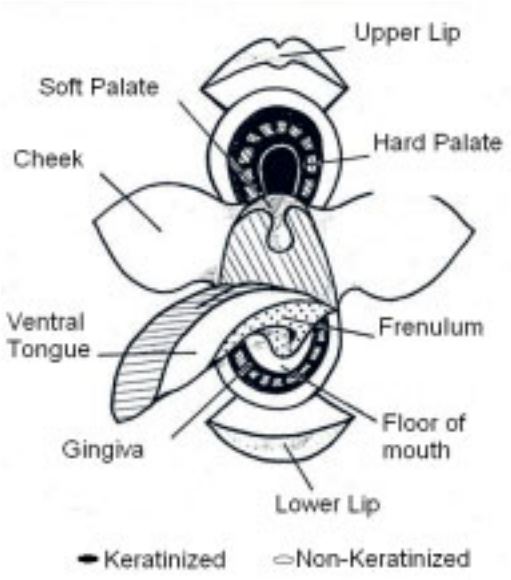


Figure 1: The oral cavity (modified from R.Gurny, H. E.Junginger. Bioadhesion - Possibilities and Future Trends).

The oral mucosa consists of different cell layers, the squamous stratified epithelium, the basement membrane, the lamina propria and the submucosa which contains blood vessels and nerves. It also contains sensory receptors such as taste receptors of the tongue. The buccal mucosa (non-keratinized) consists of a lining mucosa whereas the masticatory (keratinized) mucosa is found on the gingiva. The keratinized mucosa encompasses the gingiva and the hard palate (Figure 1). It contains ceramides and acylceramides which are assumed to be mainly responsible for the barrier function. The non-keratinized mucosa encompasses the soft palate, the sublingual and buccal region (Figure 1). It does not contain acylceramides and only a small amount of ceramides (Shojaei 1998). Consequently, permeability decreases in the order: sublingual > buccal > palatal (Sudhakar et al. 2006). As a result three categories of drug delivery are classified: (a) sublingual, lining the floor of the mouth and being systemic, (b) buccal, lining the cheeks and also being systemic and (c) local delivery directly into the oral cavity.

The thickness of the mucosa varies as well. The buccal mucosa is about 500 – 800 µm thick and the gingival mucosa (includes hard and soft palate, floor of the

mouth, ventral tongue and the gingiva) is about 100 – 200 µm thick. Turnover time is 3 – 8 days and 14 – 24 days respectively.

Three major salivary glands (parotid, submaxillary and sublingual) secrete saliva into the oral cavity. The production of saliva is influenced by the sympathetic and the parasympathetic system. The parotid and submaxillary glands produce an aqueous secretion, whereas the sublingual gland produces mainly viscous (mucus containing) saliva. Saliva has a weak buffer capacity, mainly from bicarbonate, but phosphate and proteins also play a role, with a pH around neutral (5.5 to 7.5) varying depending on whether saliva secretion is stimulated (Rathbone et al. 1994). The salivary film is very thin being only 70 to 100 µm in thickness. Humans typically produce 1 L/day, the resting flow is about 0.5 mL/min and can increase up to > 7 mL/min following maximal stimulation of the parasympathetic system. (Smart 2005)

The saliva has a variety of functions: the protection of soft tissues from abrasion, the continuous remineralization of the teeth, the neutralization of acid in the oral cavity and esophagus, the lubrication and cleansing of the oral mucosa and assistance in bolus formation. Furthermore, it plays an antibacterial role in the modulation of the oral flora and the saliva initiates the digestion of fat and starch (Smart 1993). The saliva is a complex fluid consisting of 99 % water, plus organic and inorganic materials such as electrolytes, mucus, antibacterial compounds and various enzymes.

Different groups of metabolizing enzymes are present in the oral cavity. They are divided into the following categories: (a) oxidases, (b) reductases, (c) lipoxygenases and cyclooxygenases, (d) phosphatases, (e) carbohydrases, (f) nucleases, (g) esterases and (h) peptidases. The enzymes originate from the buccal epithelial cells, except the phosphatase and carbohydrases which are present in the saliva (Yamahara and Lee 1993). In summary, the enzymatic activity in the oral cavity is low and drug inactivation neither rapid nor extensive.

The oral epithelium is a permeation barrier although the sublingual and buccal areas offer drug absorption. Mainly, the mucus layer, the keratinized layer, the intercellular lipids, the basement membrane and the lamina propria are responsible for the barrier function. Furthermore, there are so-called “membrane-coating-granules” (MCG) present (Harris and Robinson 1992). They form part of the permeability barrier of the oral epithelia as well. The MCG are found in intermediate cell layers of many stratified epithelia. They are spherical or oval organelles that are 100 to 300 nm in diameter and have been widely reported in both keratinized and non-keratinized epithelia and epidermis. MCG contain polar lipids, glycoprotein and hydrolytic enzymes. The polar lipids are supposed to be precursors of the non-polar lipids which represent the permeability barrier of the keratinized epithelia.

The drug absorption capability of the oral mucosa lies generally between that of the skin and intestinal mucosa. The blood flow is faster and richer than in the skin (Hao and Heng 2003), but only a much smaller surface area is available for drug delivery.

There are two possible routes for drug absorption: the transcellular (intracellular, passing through the cell) and the paracellular (intercellular, passing around the cell) route. Another classification involves passage through non-polar (lipid elements) and polar (hydrophilic material through aqueous pores) routes (Gandhi and Robinson 1994). The permeation mainly occurs by the paracellular route, but the route taken depends on the physicochemical properties of the drug. Small molecules, predominantly lipophilic, are absorbed most rapidly, whereas large hydrophilic molecules are generally poorly absorbed. Hydrophilic molecules take the paracellular route, compared to lipophilic molecules which take the transcellular route. The permeability decreases as the molecule size increases.

The passage across the oral mucosa follows a first order simple diffusion process. Although passive diffusion is the main mechanism of drug absorption, specialized transport mechanisms have been reported to exist: active transport, pinocytosis and passage through aqueous pores, but all of them play an insignificant role. (Smart 2005)

Another aspect is the improvement of the permeation of drugs by penetration enhancers. This is predominantly necessary for large molecules. The penetration enhancers encompass a large group of different substances. The most common are fatty acids, bile salts and surfactants such as sodium dodecyl sulphate, but it has been shown that ionic molecules may damage oral mucosa. The penetration enhancers vary in their mechanisms of action, they can change mucus rheology, increase the fluidity of membrane lipid bilayers, affect the components involved in the formation of intercellular junctions, overcome the enzymatic barrier or increase the thermodynamic activity of drugs (Ganem-Quintanar et al. 1997). For the choice of penetration enhancer, its physicochemical properties, the structural organization with the membrane as well as the variations of the oral mucosa should be taken into consideration and the penetration enhancers have to be included in the formulations for oral delivery.

Besides all the mentioned advantages as a matter of course the disadvantages should be mentioned as well. One major disadvantage is the small total area of about 100 cm^2. Furthermore, only a limited amount of drug can be incorporated due to the limited thickness of a buccal delivery device to avoid an unpleasant mouth feel and some methods of penetration enhancement (i.e. iontophoresis) cannot be applied in the oral cavity (De Vries et al. 1991).

The attempt to determine the absorption of drugs from the oral cavity has been investigated for many years. In 1967 Beckett and Triggs described the buccal absorption test (Beckett and Triggs 1967). It is a simple, non-invasive method in which a known quantity of drug in a solution is taken into the mouth, swirled around and expelled. The drug concentration of the solution is measured before and after expectoration and the amount absorbed calculated. The main disadvantage is that it is not able to provide any information on absorption in specific mucosal systems because all parts of the oral mucosa are contacted with the solution. Beckett et al. investigated the absorption of many substances such as amphetamines, carboxylic acids or barbiturates. Among other things he found out that the absorption of barbiturates increased as the pH decreased and the concentration of unionized barbiturates increased (Beckett and Moffat 1971). Later he investigated the absorption of steroids across biological membranes (Beckett and Pickup 1975). In 1971 Dearden and Tomlinson presented a new absorption model (Dearden and Tomlinson 1971b) with the investigation on p-substituted acetanilides (Dearden and Tomlinson 1971a). Tucker (Tucker 1988) advanced the existing tests by taking a small sample of solution in the oral cavity every few minutes without removing the residual solution. Therewith he achieved a more sophisticated variant with only 15 to 20 minutes testing time. Still, the uncertainty about the absorption rates from the different areas in the oral cavity remained.

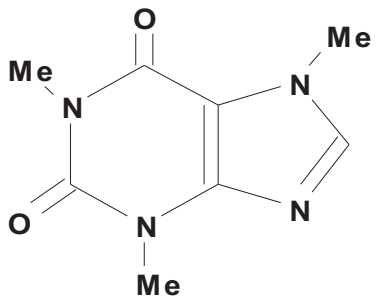
Enhancements with disc methods (Rathbone and Hadcraft 1991) have been described in the literature as well. Some investigators used perfusion cells from humans or animals to determine the remaining amount of drug (Rathbone 1991). They were clamped or otherwise attached to the intact mucosa. Another approach were delivery systems like ointments, gels, tablets or patches, but contact area and drug concentration were often unknown (Harris and Robinson 1992).

For *in vitro* experiments the permeability of excised tissue was measured by diffusion cells (De Vries et al. 1991). The most common example for a diffusion cell is the Franz cell.

Dosage forms for drug delivery into the oral cavity have not long been in existence and have been investigated for the delivery of peptides (Voorspoels et al. 1996, Tiwari et al. 1999, Kitano et al. 1998, Shin et al. 2000, Veuillez et al. 2001). Due to degradation by gastrointestinal fluids and high first-pass metabolism peptides are not effective when given by the oral route and make them interesting for the use in the oral cavity.

2 Therapeutic aspects of caffeine

Caffeine is a methylxanthine which naturally occurs in coffee, tea, guarana, mate, cacao or cola nuts and is the most consumed pharmacologically active substance in the world. Generally, it is a psychoactive stimulant and activates the central nervous system, increases the activity of the heart (increase of pulse), increases blood pressure, dilates the bronchi, acts as a diuretic, activates gut motility and decreases the blood flow of the brain and gut. Furthermore, caffeine appears to be useful in the treatment of some types of headache. The pharmacological action of caffeine relies on the binding to A_1 and A_{2A} adenosine receptors, antagonizing the effect of adenosine which is an inhibitor of respiration (Potts and Anderson 2006). Additionally, caffeine inhibits the enzymatic degradation of cAMP (cyclic adenosine-3',5'-monophosphate).



Chemical IUPAC Name	1,3,7-trimethylpurine-2,6-dione
Chemical Formula	$C_8H_{10}N_4O_2$
Average Molecular Weight	194.19 g/cm³
State	Solid
Melting Point	238 °C
Experimental Water Solubility	22 mg/ml

Table 1: Chemical structure and physicochemical properties of caffeine (source: DrugBank)

In pediatrics caffeine is especially used for neonates. There are two indications for caffeine therapy in the newborn: to treat the symptomatic apnea of prematurity and as an aid for weaning from the ventilator of ventilated infants starting before the extubation (Stephenson 1997). The incidence of apnea increases with decreasing birth weight and gestational age (Potts and Anderson 2006), so it is extremely common in infants of less than 34 weeks gestation and almost universal among infants less than 1000 grams birth weight (Stephenson 1997).

Apnea of prematurity is defined as cessation of breathing for at least 20 seconds, or less when accompanied by bradycardia, cyanosis and decreased response in an infant of less than 37 weeks gestational age (Calhoun 1996). There are many causes for apnea including breathing difficulties, infections, hypoglycemia, congenital heart diseases, anemia, epileptic seizures, drugs given to the mother before birth or to the child after birth and many more (Stephenson 1997).

In addition to caffeine, theophylline is used in the treatment of apnea as well. In a number of studies the use of caffeine versus theophylline has been compared and an equal efficacy of both in the treatment of apnea and bradycardia has been demonstrated. Caffeine is preferred because of its wider therapeutic index, a favorable

pharmacokinetic profile compared to theophylline, more predictable plasma concentrations, earlier onset of action and fewer side effects. Due to the long half-life of caffeine it allows dosing once a day in comparison to theophylline which has to be administered three times a day (Calhoun 1996).

For the treatment of both, apnea and peri-extubation management, the same dose of 10 mg per kg body weight caffeine base, which is equivalent to 20 mg per kg caffeine citrate, is recommended. In a study, it could be proven that a higher dose of 20 mg per kg per day is more effective than a lower dose of 5 mg per kg per day for weaning from ventilation (Steer et al. 2004). Normally an oral or intravenous loading dose of 10 to 12.5 mg per kg body weight and a maintenance dose of 2.5 to 5 mg per kg per 24 hours of caffeine base are given (Aranda et al. 1980). This maintenance dose can be administered either orally or as intravenous infusion once daily.

In a recent study (Barnscheid 2007) the demand for child-appropriate drug formulations in pediatric wards of German hospitals were evaluated. In this study the data were collected according to different aspects. Within the prescription the drug, the dosage form, the dose/concentration, the quantity, the dosage instruction, the age, the weight, the sex and the indication were asked for. The data showed that caffeine is one of the most frequently prescribed drug substances whether listed in the prescriptions (63 % relative frequency of all pediatric wards) or single doses (17.340 estimated in one year).

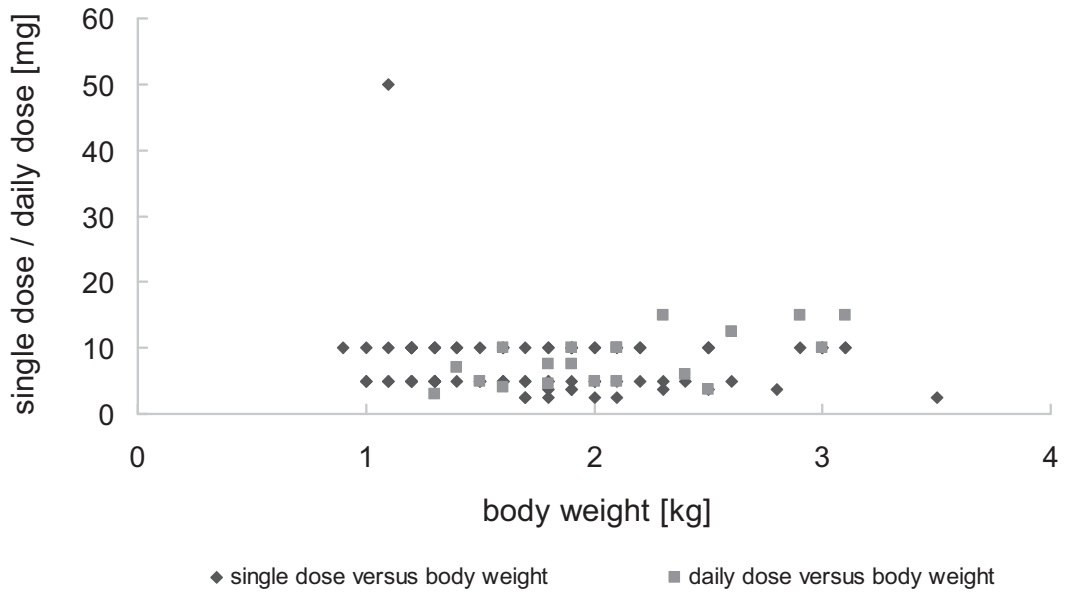


Figure 2: Single and daily doses [mg] of caffeine base versus body weight [kg] from a study in German pediatric wards (modified from Barnscheid 2007).

The results from the study showed that independent from the body weight the single doses of caffeine base range from 2.5 mg to 10 (- 50) mg and the daily doses range between 3 and 15 mg. Normally it would be assumed that with increasing body weight the single and daily doses increase as well, but this assumption could not be proved within the study. This fact shows the difficulty in the treatment of the pediatric population.

Caffeine is rapidly and almost completely absorbed from the gastro-intestinal tract. A study (Kamimori et al. 2002) showed that the rate of drug absorption from the buccal cavity was significantly faster than from the gastro-intestinal tract. In addition, the obtained amounts are comparable with each other.

The biotransformation of orally or intravenously administered caffeine occurs via hepatic cytochrome P-450 monooxygenases (Al-Alaiyan et al. 2001). Caffeine, a trimethylxanthine, is metabolized to theophylline, a dimethylxanthine, but also methylation of theophylline to caffeine occurs in preterm infants. Furthermore four other pathways are known, but the demethylation of caffeine is the predominant pathway (Potts and Anderson 2006) in neonates. A large variability in the elimination rate of caffeine was noted which is problematic in the clinical setting. The half-life in neonates ranges from 40 to 230 hours (Potts and Anderson 2006) according to the intraindividual variations. The large variability results from the immature activity of the hepatic enzymes in the preterm infants. The half-life decreases with proceeding development of the hepatic system to a half-life of 3.5 hours in adults.

The most common side-effects during caffeine intake are a contribution of gastro-esophageal reflux due to a reduction of the lower esophageal sphincter pressure, which may contribute to recurrent apneas, an increase in gastric acid secretion, an increase in urine flow and a natriuresis. One study reported an association between caffeine and a fall in blood glucose.

Caffeine toxicity in children is extremely rare, only four cases have been reported (Kulkarni and Dorand 1979). Two children died and two others survived.

Long-term effects and safety of caffeine therapy in premature infants are not sufficiently known (Millar and Schmidt 2004). Therefore the Caffeine for Apnea of Prematurity (CAP) trial (Schmidt 2005) should provide answers to the question whether caffeine influences growth, neurological development and childhood behavior. Meantime the results from the CAP trial resolved the longstanding uncertainty about the efficacy and safety of methylxanthine therapy in preterm infants. Caffeine improved the rate of survival without neurodevelopmental disability, reduced the incidences of cerebral palsy, but had no significant effects on the mortality, severe hearing loss or bilateral blindness (Schmidt et al. 2007). These observations justify the overall benefits of methylxanthine therapy and outweigh any potential risks.

Coming to the complex of problems of dosage forms for the pediatric population, it must be mentioned that about 50 % of all medicinal products for the pediatric population and up to 90 % especially in neonatal intensive care units (NICU) are either unlicensed or used off-label (Kleist 2001). Unlicensed preparation is the manufacturing of (pediatric) dosage forms that do not have regulatory approval (e.g. in-house production of ointments in hospital pharmacy) and off-label use is the use of a drug beyond the regulatory permitted scope of application. Licensing aims to ensure safety, quality and efficacy. The most common reason for off-label use is the dosage. Given that caffeine is the most commonly prescribed drug in NICU, it is the most frequently unlicensed and off-label used drug in NICU as well ('t Jong et al. 2001).

The formulations are manufactured or modified by the hospital pharmacy due to the lack of medicinal products. The WHO published a list (WHO 2007) in which caffeine citrate is one of the essential medicines for children and not available in an appropriate dosage form. In Australia only one unlicensed product for caffeine is available (O'Donnell et al. 2002), in the United Kingdom the British National Formulary for Children (BNFC) gives advice for the preparation of caffeine citrate for neonates (BMJ Publishing Group Ltd 2006). Within the German "Neues Rezeptur-Formularium" (NRF) a solution of caffeine citrate for pediatrics is described (Neues Rezeptur-Formularium 2005). Therein caffeine citrate is preferably used because of its better solubility in comparison to caffeine base. Details about the stability of the formulation are given. Due to the bitter taste of caffeine the use of sugar syrup and cherry flavor are recommended, but unfortunately both are preserved. Only one licensed drug product is available in USA, either as injection or as oral solution, named CAFCIT®. Detailed information about the ingredients are not available, however, both solutions are preservative free. Recently, a product containing caffeine citrate (caffeine solution 5 mg/mL) was launched in the UK.

Caffeine is known as a bitter substance which is a disadvantage. Naturally, bitter taste suggests danger and protects organisms from poisoning, hence natural toxins like alkaloids taste bitter. Children are more sensitive to bitter tastes than adults; hence they dislike coffee or beer for example. They prefer significantly higher concentrations of sweeteners and salts when compared to adults. During adolescence taste preferences change. Adults may believe that bitter medicines are more efficacious and aid recovery but children differ from that opinion. Unfortunately clinical studies for new drug products are performed predominantly with adults, accordingly the taste preferences of children are not considered. However, a better tasting medicine is easier to administer to children and should be brought more into focus. During the development of the new dosage form containing caffeine, the need for a pleasant taste was paid particular attention.

3 Oral wafers

Oral films, also called oral wafers in the related literature, are a group of flat films which are administered into the oral cavity. There are three different subtypes: flash-release, mucoadhesive melt-away and mucoadhesive sustained-release wafers. In general, the wafer systems possess an area of 2 – 8 cm², can be formed in every conceivable manner and are between 20 and 500 µm thick. The obtainable drug-load varies with the physicochemical properties of the drug and is up to 15 mg. Due to the naturally given absorption the place of application is limited to the tongue, the gingiva, the buccal region and the upper palate. As previously mentioned systemic or local action can be achieved and many advantages support the use of oral wafers.

Depending on the type of the wafer different properties can be utilized. While flash-release wafers dissolve in a maximum of 60 seconds and immediately release the drug, the mucoadhesive melt-away subtype sticks to the mucosa, totally dissolves within minutes and continuously releases the drug over this time. The mucoadhesive sustained release wafers stick to the mucosa as well, but remain there for up to several hours. For the duration of the application time the drug release is sustained and the wafer must be removed at the end of application.

Additionally, it should be mentioned that drug release occurs upon disintegration and dissolution which cannot be differentiated in flash-release wafers.

subtype / property	Flash release wafer	Mucoadhesive melt-away wafer	Mucoadhesive sustained release wafer
Area [cm²]	2 – 8	2 – 7	2 – 4
Thickness[µm]	20 – 70	50 – 500	50 – 250
Structure	Film: single layer Foam: 3D structure	Single or multilayer system	Multi layer system
Excipients	Soluble, highly hydrophilic polymers	Soluble, hydrophilic polymers	Low/Non-soluble polymers
Drug phase	Solid solution	Solid solution or suspended drug particles	Suspension and/or solid solution
Place of application	Tongue, (upper palate)	Gingiva or buccal region	Gingival, (other regions in the oral cavity)
Dissolution	Maximal 60 seconds	Disintegration in a few minutes, forming gel which dissolves	Maximal 8 – 10 hours
Site of action	Systemic or local	Systemic or local	Systemic, (local)

Table 2: Types of wafers and their properties (modified from LTS Lohmann Therapy Systems, Wafer Systems 2005).

Normally, the wafers allow a multidirectional drug transport. Otherwise by using a backing layer even unidirectional drug transport can be achieved (Gandhi and Robinson 1994). The smaller the size and the thickness, the more convenient are the wafers. The film thickness after drying should not exceed 70 µm to avoid an unpleasant mouth feel (Zerbe et al. 2003).

The manufacturing process for the wafers in the pharmaceutical industry is divided into different steps. Generally, the mass is prepared first under the control of temperature and steering speed. Afterwards, the wafers are coated and dried in a drying tunnel, once again the temperature, air circulation and line speed are controlled. Then follows a slitting and in the last step the wafers are punched, pouched and sealed. Other ways of manufacturing oral wafers are spraying process (Davidson and Kehoe 2005) or extrusion, in particular hot-melt extrusion (Repka et al. 2003, Tumuluri et al. 2008).

	trade name	*manufacturer*
(a)	Eclipse Flash Strips	Wrigley's
(b)	Cool Mint/Fresh Burst Listerine Pocket Paks	Pfizer
(c)	Neocitran® Thin Strips™ Cough and Nighttime Cough	Novartis
(d)	Health Strips	Mattel/Momentus Solutions, LLC.™
(e)	Thera Flu Thin Strips Multi Symptom and Long Acting Cough	Novartis
(f)	Triaminic Thin Strips Long Acting Cough and Runny Nose	Novartis
(g)	Suppress Cough herbal	Innozen
(h)	Suppress Cough	Innozen
(i)	Chloraseptic Sore Throat Relief Strips	Prestige Brands/Zengen (Innozen)
(j)	Ora Film Pain Relieving Strips	Apothecus Pharmaceutical Corp.
(k)	Sudafed PE™ Quick Dissolve Strips Cherry Menthol	Pfizer/Johnson & Johnson

Table 3: Survey of pharmaceutical wafers launched in the recent years.

Recently, some pharmaceutical companies recognized the utility of oral wafers and launched several products with active ingredients. Originally, the oral wafers were used as breath fresheners (a+b). Within the listed preparations most contain psycho stimulants such as dextrometorphane, diphenhydramine or phenylephrine (c, e, f, h and k), which are used in the treatment of cough and cold. Furthermore vitamins (d) or local anesthetics like benzocaine (i) are included. The rest of the listed preparations either contain menthol as the active ingredient or it is not declared.

There are some wafers containing caffeine as well, but these are not pharmaceutical products and derived from dubious sources. They have no indication in the pharmaceutical sense and contain between 8 and 25 mg per film strip.

The mechanism of drug transport of the incorporated API through the mucosal membranes was thoroughly described in a former chapter. A main assumption for the drug absorption is the adhesion of the oral wafer to the mucosa. This bioadhesion process is characterized by a two step process (Gandhi and Robinson 1994). The first step is the initial contact between the two surfaces. The second step includes the formation of secondary bonds due to non-covalent interactions. Between the two surfaces, the membrane and the adhesive, an interfacial layer is formed. This interface causes bond formation. Bonding occurs mainly through physical and weak chemical bonds. Mucosal-adhesive materials are mainly hydrophilic polymers which contain numerous hydrogen bond-forming groups (Smart 1993). The presence of hydroxyl, carboxyl or amine groups on the molecules increase adhesion and they are called ‘wet’ adhesives (Smart 2005). They are activated by moistening and will adhere nonspecifically to many surfaces and form a slippery mucilage due to hydration. Typical examples are the polyacrylic acids and the polysaccharides such as chitosan and the cellulose derivatives. However, the anionic (e.g. sodium carboxymethyl cellulose, sodium alginate) and cationic macromolecules (e.g. chitosan) are stronger mucoadhesives than non-ionic polymers, such as hydroxypropyl methyl cellulose, hydroxypropyl cellulose or polyvinyl alcohol are.

Several theories of bioadhesion are proposed to explain the mechanisms of attachment of adhesives. These theories encompass the electronic theory, the adsorption theory, the wetting theory, the diffusion theory and the fracture theory (Smart 1993). To measure the bioadhesive strength several methods for both, *in vivo* and *in vitro*, have been reported in the literature (Kellaway 1990). For instance, the Texture Analyzer is often used to determine bioadhesive properties of oral wafers.

C RESULTS AND DISCUSSION

1 Formulation development of oral wafers

1.1 Patent survey

Rapidly dissolving films are a novel dosage form hardly mentioned in the scientific world. RDFs are described in the patent literature and thus, an intensive patent study was conducted which was aimed at finding out compositions and manufacturing of RDFs. The results from the patent search indicated that although a variety of excipients are used, some specific functional components for casting oral films are required. These main components include the film forming agents, the plasticizing agents, surfactants and solvents. Other excipients used are stabilizers, disintegrants, emulsifiers, bulk fillers, mouth feel improvers, cooling agents, flavors, fragrances, thickening agents, preservatives and salivary stimulating agents as well as sweeteners. They are included in the formulation depending on the required properties. Furthermore, depending on the drug used, the excipients have to be adjusted to achieve desired properties such as a pleasant taste of the oral wafers.

1.2 Film forming agents

Initially, for the present study the number of excipients used was reduced to a minimum to evaluate differences in the film forming agents. Later, taste improvement was addressed and therefore sweeteners, flavor agents and dyes were added. All substances were chosen on the basis of being harmless to the pediatric population.

Most commonly used film formers are cellulose derivatives such as sodium carboxymethyl cellulose or hydroxypropyl methyl cellulose, for example LTS Lohmann Therapy Systems (Zerbe et al. 2003) uses hydroxypropyl methyl cellulose and tesa Labtec (Labtec 1999) hydroxypropyl cellulose. In a patent of Pfizer, formerly Warner Lambert, a relatively seldom used polymer, pullulan, is incorporated as the film forming agent (Kulkarni et al. 2004). Pullulan is a linear homopolysaccharide of glucose. Every third glucose units is α-(1→6) linked forming a maltotriose substructure. Pullulan is secreted by the fungus *Aureobasidium pullulans* and is used as a food ingredient. The enzyme pullulanase specifically hydrolyzes the α-(1→6) linkages in pullulan and converts the polysaccharide almost quantitatively to maltotriose (Leathers 2003). Pullulan is a highly hydrophilic polymer generally regarded as safe (EFSA 2004) and dissolves rapidly in water.

In the literature, efforts were made to replace pullulan as a highly soluble and edible polymer for use in oral films because it is rarely available and expensive. Recently, starches (Lydzinski et al. 2003) and modified starches (Shin et al. 2006, Sorg et al.

2004), maltodextrin (Dzija et al. 2003), have been investigated for substitution of pullulan.

polymer	*preformulation*	*stability testing*	*optimized formulations*
Walocel® C 30 PA 09	x	x	
Walocel® C 2.000 PA 07	x	x	
Walocel® C 2.000 PA 09	x	x	
Walocel® C 40.000 PA 09	x		
Walocel® HM 6 PA 2910	x	x	
Walocel® HM 50 PA 2910	x	x	
Walocel® HM 4.000 PA 2910	x		
Gelatin		x	x
Instacoat® P-4			
Klucel® GF	x		
Kollicoat® IR	x		
Metolose® 65SH-1500	x	x	
Mowiol® 4-88		x	x
Pharmacoat® 615	x		
Pullulan	x	x	x

Table 4: Film formers used for oral films in the present study in different development phases.

1.3 Choice of excipients

In general pharmaceutical excipients are regarded as “inactive” and therefore safe for human use, but this may not always be the case. Substances which are nontoxic for adults can be harmful for the pediatric population and are only usable with age or dose restrictions. A variety of adverse effects resulting from “inactive” ingredients have been described in the literature (American Academy of Pediatrics Committee on Drugs 1997). Such problems with pharmaceutical excipients have been reported for propylene glycol, polyethylene glycol and benzyl alcohol (Breitkreutz 2004). Deaths have even been linked to their use. Aspartame, a dipeptide of aspartic acid and a methyl ester of phenylalanine, is commonly used as sweetener in pharmaceutical products, but is hazardous in children who suffer from phenylketonuria. Current issues are impurities of glycerol and propylene glycol as well as residual solvents such as ethanol (Whittaker et al. 2009). As a consequence glycerol and propylene glycol are still only allowed to be produced under GMP conditions. It has been reported that an exceeding of the limit for ethanol (for an average man weighing 70 kg) occurred in children (Whittaker et al. 2009).

As previously mentioned, the polymers and all excipients were chosen with respect to suitability for pediatric use to avoid any damage which could arise due to the immature metabolic systems. Therefore, the labeling of “inactive” ingredients seems to be mandatory. In the present study all used substances were checked for their ADI value

which is one of the introduced limits. The ADI value is the acceptable daily intake of a substance (given in milligram per kilogram body weight) that a person can ingest over a lifetime without appreciable health risk. However, ADI values are fixed for adults and cannot be transferred to children, ill people, pregnant women or the elderly. In particular, young children have a reduced metabolic capacity and excretion. Therefore an interpolation of the values is difficult and has to be assessed case by case. The values for the ADI are based on experiments with animals. The animals are given varying amounts of the substance under investigation. The highest level at which no health effect is observed is determined, that is the no observed effect level (NOEL). This NOEL is divided by a safety factor (usually 100) to give the ADI value. The safety factor depends on the extrapolation between species, individual differences within a species, a toxicity factor for short-time exposure as well as a factor for severe toxicity (Breitkreutz 2004). Basically the ADI values are established by the WHO/FAO Joint Expert Committee on Food Additives (JECFA).

substance	***ADI** value [mg/kg body weight]*	*substance*	***ADI** value [mg/kg body weight]*
polymers		*other excipients in films*	
gelatin	not limited	ethanol 96 %	no data available
		aspartame	0 – 40
hydroxypropyl cellulose	not specified	Brij® 35	no data available
		κ-carrageenan	not specified
hydroxypropyl methyl cellulose	not specified	citric acid	not limited
		dyes	
		yellow: E102	0 – 7.5
		E124	no data available
		red: E124	no data available
		flavors:	
Kollicoat® IR	no data available	cherry	no data available
		orange	no data available
		glycerol 85 %	not specified
polyvinyl alcohol	0 – 50	locust bean gum	not specified
		mannitol	not specified
pullulan	not specified	Tween® 80	0 – 25
		potassium sorbate	0 – 12.5 unconditional acceptance 12.5 – 25 conditional acceptance
sodium alginate	0 – 50	saccharin	0 – 5
		sorbitol	not limited
sodium carboxymethyl cellulose	not specified	sucralose	0 – 15
		xanthan gum	not specified

Table 5: List of ADI values of used substances incorporated in oral wafers (source: JECFA 2008).

1.4 Film formulations

According to the results of the patent search different formulations for oral films were included in the investigations.

A	B	C
polymer	polymer	polymer
(caffeine anhydrous)	potassium sorbate	caffeine anhydrous
(caffeine citrate)	sucralose	(sucralose)
sorbitol	mannitol	(sorbitol)
glycerol 85 %	glycerol 85 %	glycerol 85 %
citric acid	xanthan gum	citric acid
Tween® 80	locust bean gum	(Tween® 80)
Brij® 35	carrageenan	(ethanol 96 %)
ethanol 96 %	Tween® 80	water
water	water	(aspartam)
		(saccharin)
		(sodium bicarbonate)
		(dyes)
		(flavors)

Table 6: Basic film formulations which were modified from patent literature (A and B) and further developed (C) for use in the present study (the detailed film formulations see D 2.1.1).

Formulation A is modified from a patent of LTS Lohmann Therapy Systems (Zerbe et al. 2003), formulation B is a variation of the patented composition of Warner Lambert (Kulkarni et al. 2004) and only used for the drug-free films made from pullulan.

The oral films were prepared according to a standard scheme (Figure 62, D 2.1.2). For films made from pullulan and gelatin only water was used as the solvent due to their incompatibility with alcohol in any concentration. Otherwise the solvent used was a mixture of ethanol 96 % and water (1:1). The film forming agent was added in varying amounts according to the respective gel forming properties.

The polymers used differ in their chemical structure as well as in their physicochemical properties. Besides the aforementioned and described polymers different cellulose ethers were also used. Different types of carboxymethyl cellulose sodium (CMC): Walocel® C 30 PA 09, Walocel® C 2.000 PA 07, Walocel® C 2.000 PA 09 and Walocel® C 40.000 PA 09 which either differ in their viscosity of a 2 % aqueous solution or in their degree of substitution were used. Further, different types of hypromellose (hydroxypropyl methyl cellulose, HPMC) such as Metolose® 65SH-1500, PHARMACOAT® 615 and Walocel® HM 6 PA 2910, Walocel® HM 50 PA 2910 as well as Walocel® HM 4.000 PA 2910 were included. The HPMC only differ in their viscosity of a 2 % aqueous solution.

The oral films made of CMC were only castable if the polymer was dissolved in distilled water and ethanol was added in a final step. If a mixture of ethanol and water or only ethanol was used, the polymer precipitated.

Given that the film solution made from Instacoat® (sodium alginate in a mixture of talc, titanium dioxide and triacetin) and Klucel® GF (hydroxypropyl cellulose) could not be cast, these polymers were excluded from further studies. Additionally, oral films made from C40.000PA09 (CMC), HM4.000PA2910 and Pharmacoat® 615 (both HPMC) and Kollicoat® IR, a polyethylene glycol-polyvinyl alcohol copolymer, were also excluded. Independent from the film height, the films of C40.000PA09 could not be peeled off the release liner and cut into pieces. Films of HM4.000PA2910 and Pharmacoat® 615 had a poor appearance due to porous structure. Finally, the oral films of Kollicoat® IR had a poor taste which is a criterion for exclusion as application is intended in the oral cavity.

Carrageenan, locust bean gum and xanthan gum act as plasticizing agents and support the function of the film formers. Sorbitol and glycerol 85 % were used as plasticizers within the formulations. Citric acid was used as salivary stimulating agent, but also acts as a permeation enhancer (Sudhakar et al. 2006). Polysorbate 80, Tween® 80, and Brij® 35 (dodecyl-poly(ethylene oxide-23) ether) were used as surfactants. Potassium sorbate was only used in formulation B and acts as a preserving agent.

Different sweeteners were used, such as aspartame, saccharin and sucralose. Interestingly, caffeine is supposed to enhance the sweetness of some sweeteners including saccharin, but it has no effect on other sweeteners like aspartame (Schiffman and Gatlin 1993). For saccharin a bitter, metallic and astringent aftertaste is detectable by about 25 % of the population (Schiffman and Gatlin 1993). The higher the concentration, the stronger this aftertaste. Sucralose has palatable sweetness without unpleasant aftertaste. The amount of incorporated sweetener was calculated according to its relative sweetness compared to sucrose (Grueneberg 2006) to achieve equal levels of sweetness.

sweetener	relative sweetness	inserted amount [mg/film strip]
aspartame	200	2.10
saccharin	500	0.84
sucralose	600	0.70

Table 7: Sweeteners used, their relative sweetness and amount per oral film (6 cm²).

As caffeine is utilized as active ingredient and also exhibits a bitter taste, consideration was given to how the bitterness could be masked by pharmaceutical-technological options. In the patent literature carbonates have been described to mask bitterness (Rademacher et al. 2003). There is a patent existing in which sodium bicarbonate is combined with caffeine to mask bitterness (Ludwig and Krumme 2001). The amount of sodium bicarbonate used was half of the amount of the bitter tasting substance.

These specifications were adapted to the study formulations. Preliminary tests with API were carried out with formulation A. These trials led to development of formulation C, but only caffeine base was used as active ingredient due to the limited drug-load of the oral wafers. It should be mentioned that caffeine and its citrate salt tend to recrystallize in the polymer films, which was an issue during different investigations in the forthcoming chapters.

The addition of dyes and flavors was aimed at optimizing a pleasant taste of the manufactured oral wafers. Orange and cherry flavoring were selected as being preferred by the German population. Thus, orange flavored films were dyed with a mixture of yellow and red food colorings and cherry flavored films were dyed with red food coloring. During the drying process, part of the flavor volatilized and thus, could not be recognized by humans in the palatability study.

2 Physicochemical characterization of oral films

2.1 Pharmacopoeial status

Monographs of common dosage forms are provided by the pharmacopoeias (e.g. Ph. Eur., USP). Even though dosage forms for application in the oral cavity such as medicated chewing gums, oromucosal preparations, orodispersible tablets or oral lyophilisates are included, monographs and specifications for oral films of diverse dissolution kinetics have not yet been established. There are inadequate pharmaceutical technical procedures for analysis in development and quality control of oral films as well. For instance, disintegration and dissolution testing procedures may be provided, but the recommended conditions such as volumes of media do not reflect the natural conditions in the oral cavity. An aim of the present studies was to develop more appropriate methods and enhancements of common methods as well as the characterization of fast-dissolving oral films using these methods.

2.2 Morphological properties

2.2.1 Visual inspection

Properties such as homogeneity, color, transparency and surface of the oral films were evaluated. The polymer films were assessed in three-month intervals over a period of 12 months stored at two climatic conditions (25 °C / 60 % RH and 40 °C / 75 % RH) as described in ICH guideline Q1A (ICH Steering Committee 2003).

polymer	*initial properties*	*0*	*3*	*6*	*9*	*12*	*months* / *condition*
C30PA09	very homogenous, absolutely transparent, colorless, both sides smooth	x x	x* x*	x x*	x* x*	x* x*	25 °C / 60 %RH 40 °C / 75 %RH
C2.000PA07	homogenous, but brittle and partially porous, absolutely transparent, colorless, smooth and rough surface	x x	x x*	x* x*	x x*	x x*	25 °C / 60 %RH 40 °C / 75 %RH
C2.000PA09	very homogenous, absolutely transparent, colorless, both sides smooth	x x	≠ x*	x x*	x x*	x x*	25 °C / 60 %RH 40 °C / 75 %RH
HM6PA2910	totally homogenous, absolutely transparent, colorless, both sides smooth	x x	x ≠	≠ ≠	x x	x x	25 °C / 60 %RH 40 °C / 75 %RH
HM50PA2910	totally homogenous, transparent, uncolored, both sides smooth	x x	x x	x ≠	x x	x x	25 °C / 60 %RH 40 °C / 75 %RH
Metolose®	homogenous, absolutely transparent, both sides smooth	x x	x ≠	x x	x ≠	x x	25 °C / 60 %RH 40 °C / 75 %RH
pullulan	homogenous, less transparent, whitely, smooth and rough surface	x x	x ≠	x ≠	x ≠	x ≠	25 °C / 60 %RH 40 °C / 75 %RH
* = films stuck together, not separable		x = corresponded / ≠ = did not correspond					

Table 8: Appearance of drug-free films from formulations A and B by visual inspection.

Drug-free films of basic formulations A and B were manufactured, specified, stored and tested. The oral films were stored under worst-case conditions. Provided that the films were not primary packaged, they had free access to moisture. Every three months samples were assessed against the properties in Table 8 to determine whether the criteria were fulfilled (x) or not (≠). If one criterion did not fit with the specifications, the batch failed. Although the oral films were clamped into slide frames to store them non-contacting, space-saving and separately, polymer films made from different types of CMC coincided with each other and stuck together. This occurrence complicated further investigations. The requirements were not met when homogeneity/consistency changed. C2.000PA09 films became porous, HM6PA2910 films plasticized over time, Metolose® films wrinkled and pullulan films became brittle. In case of HM6PA2910, HM50PA2910, Metolose® and pullulan the color of the films was altered by browning, especially at 40 °C / 75 % RH.

In the same manner drug-loaded wafers of formulation C were assessed (Table 9).

polymer	*initial properties*	*0*	*3*	*6*	*9*	*12*	*months* / *condition*
gelatin	API recrystallized in small crystals, hardly transparent, pearly, smooth and rough surface	x x	x ≠	x ≠	x ≠	x ≠	25 °C / 60 %RH 40 °C / 75 %RH
HM6PA2910	very homogenous, hardly transparent, white, both sides smooth	x x	x ≠	≠ ≠	x ≠	≠ ≠	25 °C / 60 %RH 40 °C / 75 %RH
HM50PA2910	homogenous, slightly transparent, white, both sides smooth, slightly dusty	x x	≠ ≠	≠ ≠	≠ ≠	≠ ≠	25 °C / 60 %RH 40 °C / 75 %RH
Mowiol®	homogenously recrystallized over film, slightly transparent, white, smooth and rough surface, rubbery	x x	x x	≠ ≠	≠ ≠	≠ ≠	25 °C / 60 %RH 40 °C / 75 %RH
pullulan	partially API particles within film, slightly transparent, white, smooth and rough surface	x x	x x	x ≠	x ≠	x ≠	25 °C / 60 %RH 40 °C / 75 %RH
							x = corresponded / ≠ = did not correspond

Table 9: Appearance of drug-loaded films from formulations C by visual inspection.

Most of the polymer films failed the criteria for color and surface texture. Films made of gelatin became yellowish, HM6PA2910 and pullulan films browned. The surface of the gelatin films became fluffy whereas the films of HM6PA2910 and HM50PA2910 became dusty. HM50PA2910 and Mowiol® polymer films appeared opaque. Moreover, films from both Mowiol® and pullulan showed smooth surfaces on the upper and lower side. Visual inspection offers a simple and feasible method for characterization of polymer films. Therewith predictions for usage of polymers in the preparation of fast-dissolving films can be made. Gelatin and pullulan films are stable under the worst-case scenario at 25 °C / 60 % RH.

The rest of the investigated films need to be primary packaged to improve stability.

2.2.2 Microscopic and Image Analysis

Image analysis, light microscope and scanning electron microscope (SEM) served for visual comparison of polymer films made from different film formers, see Figure 3.

2.2.2.1 Comparison of visualization methods

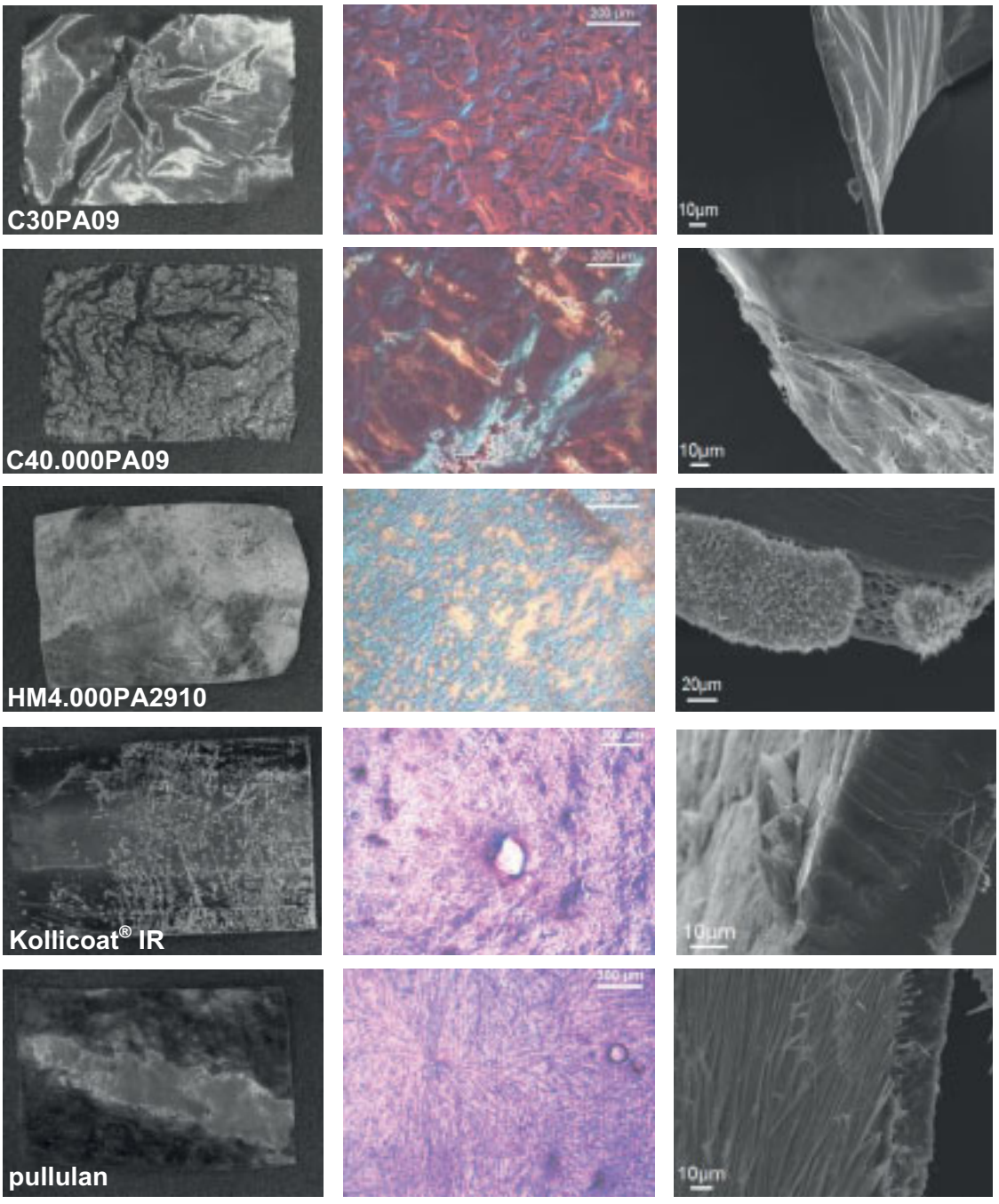


Figure 3: Images of drug-free oral films of basic formulations A (C30PA09, C40.000PA09, HM4.000PA2910 and Kollicoat® IR) and B (pullulan) by different visualization methods.

One film strip displayed in the image analysis is equal to 6 cm² (3 x 2 cm). Already it can be seen that the structure of the polymer films differed. C30PA09 films were relatively smooth in comparison to films of C40.000PA09 which appeared wrinkled. HM4.000PA2910 and Kollicoat® IR films had a white appearance. Films made of pullulan showed a peculiar structure. On closer inspection with light microscopy both CMC grades (C30PA09 and C40.000PA09) appeared to have a similar microstructure. The white appearance at HM4.000PA2910 films was clearly seen and, the polymer film appeared very smooth. Films made from Kollicoat® IR had a wrinkled structure, in comparison to pullulan films which appeared fan-shaped. On the basis of the cross-section the oral wafers using SEM were investigated. The cross-section of the C30PA09 and C40.000PA09 film strips appeared to be smooth, although C40.000PA09 in contrast to C30PA09 showed whitely filaments. The fine structure of HM4.000PA2910 films appeared spongy and a fluff of polymer covered the cross-section as well as the surface of the film strip. The cross-section of Kollicoat® IR films showed a tight structure without any pores, but likewise a slight fluff at the surface of the wafer. Films made from pullulan showed a porous inner structure. Altogether with these three methods differences in the structures of the film formers could be identified, however the differentiation of the inner structure could only be investigated at the cross-section area by SEM. Comparing the visualization methods each method has its advantage and they are complementary. Image analysis offers the depiction of the whole film to get an impression of the film size, light microscopy with polarizer allows the detection of crystals and SEM works with high resolutions to display fine structures.

2.2.2.2 Influence of draw speed

Oral wafers can be cast by different draw speeds, so it should be clarified whether the draw speed influences the structure of the oral wafers, especially with the use of caffeine as API tending to recrystallize. The film applicator used is able to cast films at speeds of 6, 12, 18 and 24 mm per s.

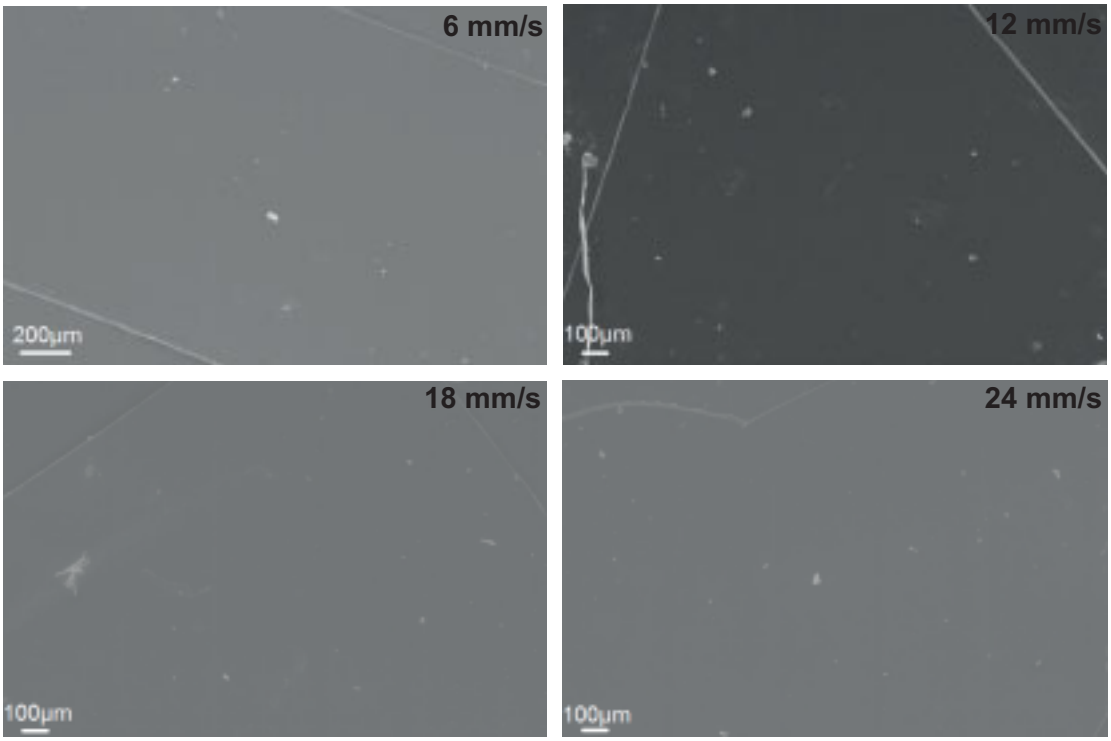


Figure 4: SEM images of drug-free oral wafers (HM50PA2910) cast with four different draw speeds.

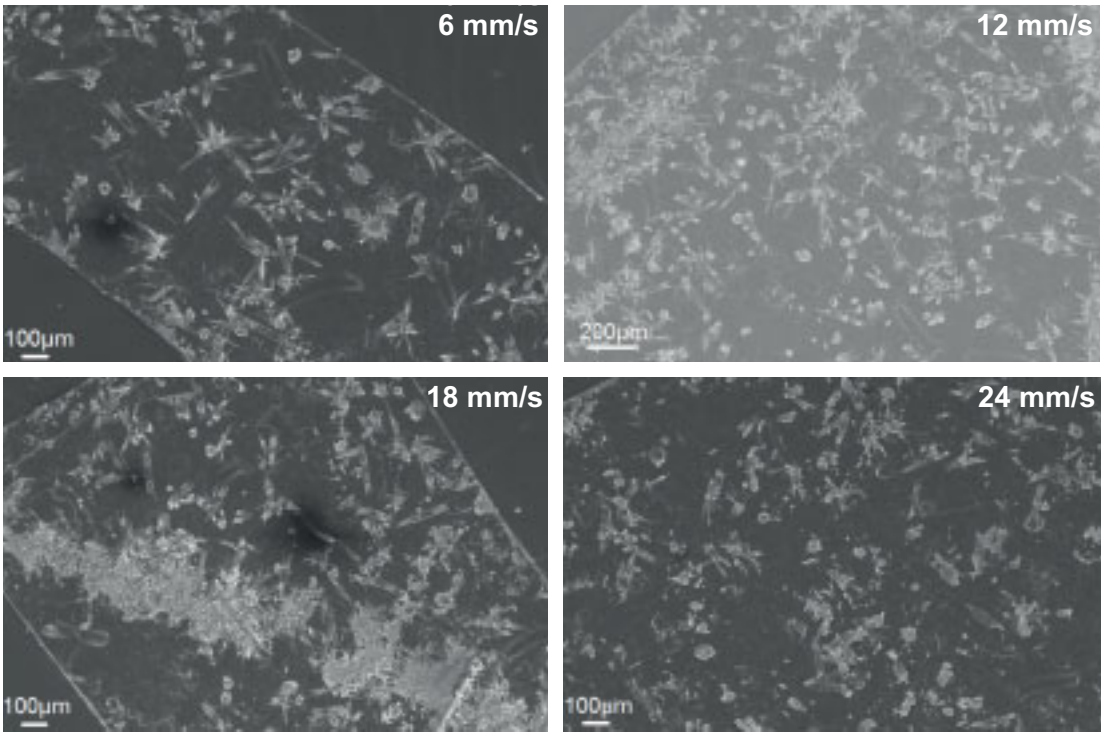


Figure 5: SEM images of drug-loaded oral wafers (HM50PA2910) cast with four different draw speeds.

As can be seen in Figure 4 and 5 neither the drug-free formulations nor the drug-loaded films of formulation C showed differences in the fine structure at different draw speeds. In case of the drug-free oral films of HM50PA2910 all SEM images showed a smooth surface without any traces of the casting process. The oral wafers made from HM50PA2910 with caffeine cast at different speeds showed no differences in recrystallization behavior as well. The caffeine recrystallized in the same manner at each investigated speed. At 18 mm/s a small track with caffeine crystals appeared which can be irregularly found in the cast films.

These observations are important for the manufacturing process of the oral wafers. It can be concluded that the draw speed does not influence the structure of the oral films within the investigated intervals, whether API is incorporated or not. Additionally it should be mentioned that high draw speeds might be helpful for wafers made of gelatin given that the film solutions quickly gelatinize and may hinder the further casting process.

2.2.2.3 Properties of upper and lower surfaces

The film solutions were cast on a release liner on which the films dry after casting. Thus, an upper and a lower side result, in which the upper side is in contact to the air during drying and the lower side is in contact with the release liner. It was assumed that differences between the upper and lower side within the oral wafers occur. Due to the tendency of caffeine to recrystallize and its water vapor volatility, considerable differences between the upper and lower side were expected which may be critical.

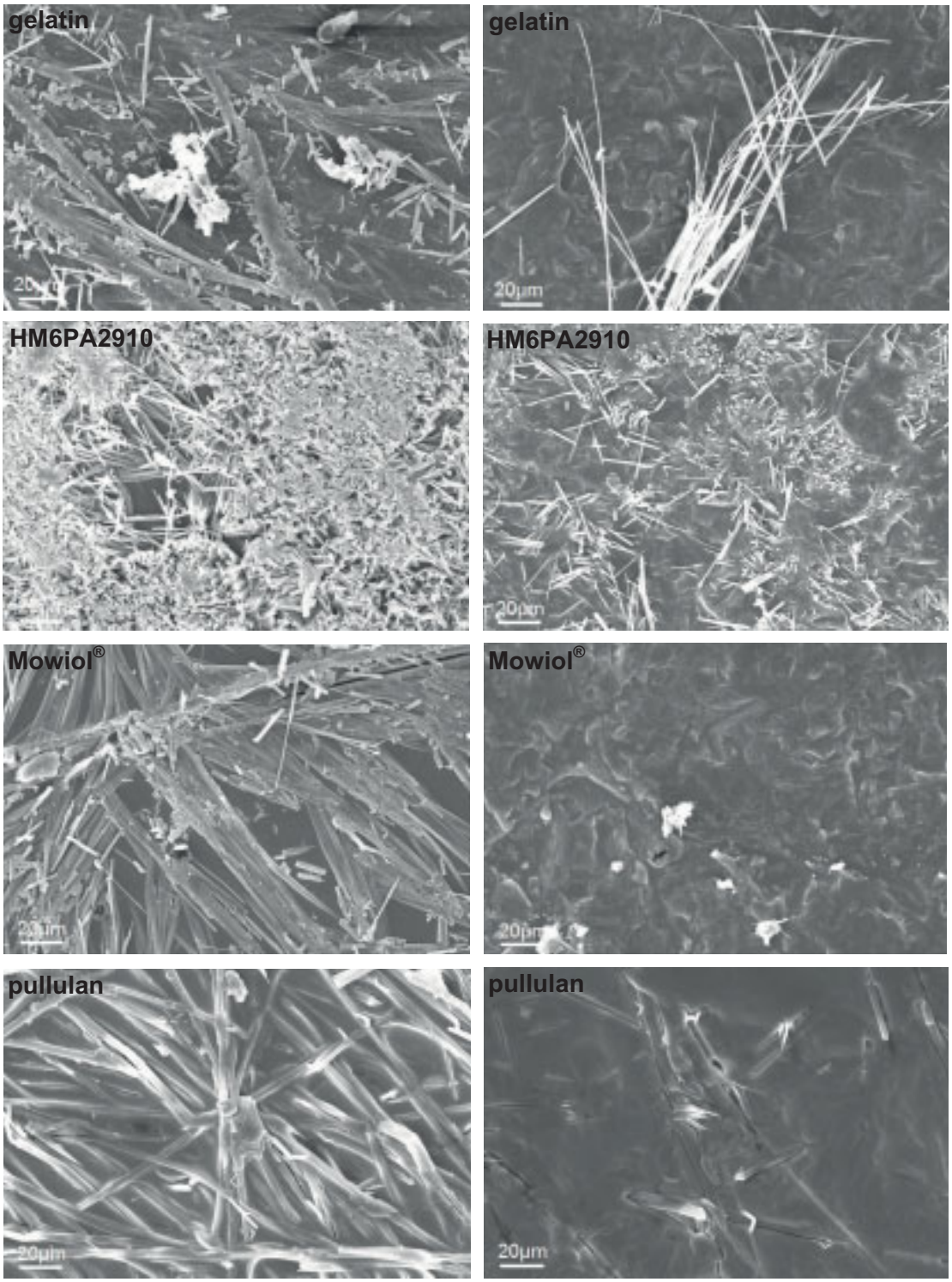


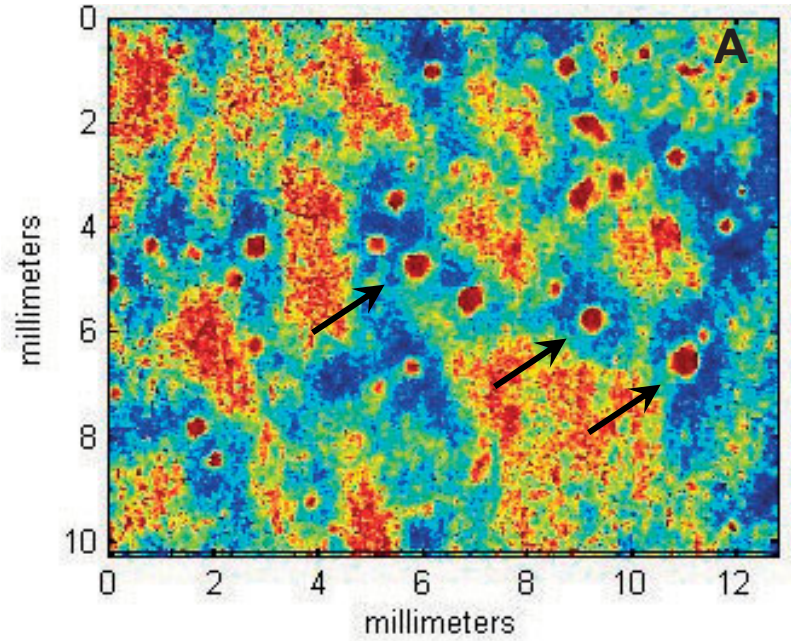
Figure 6: SEM images of polymer films made of different film formers containing API (formulation C, drug-load: 10 mg/film), left: upper side and right: lower side.

As it was assumed that caffeine recrystallized within the oral films the SEM was used again to elucidate this supposition. In the images of Figure 6 differences can be seen between upper and lower surface as well as differences between the films made from gelatin, HM6PA2910, Mowiol® as well as pullulan. Caffeine tends to recrystallize and this was found in all polymer films except the HPMC films. The upper side of the

gelatin wafers showed large frayed needles of caffeine whereas the lower side of the gelatin films exhibited no caffeine crystals, the white filaments in the picture are attributed to the polymer itself. Although the oral wafers made from HM6PA2910 appeared smooth without any recrystallized caffeine by visual inspection, differences between upper and lower side were found by SEM. Small clusters of caffeine were found on both sides, however the frequency of appearance is much higher on the upper side. Mowiol® films showed small thin needles on the upper side compared to its lower side. In the case of pullulan wafers caffeine recrystallized as large needles. As can be expected, the recrystallization tendency is more likely on the upper side than on the lower side of the films which could be shown for all investigated polymers. Recrystallization preferentially occurs on the upper side as there is enough place for nucleation, whereas on the lower side the release liner hinders nucleation. This fact enforces the observed difference in roughness between the upper and lower side and has to be considered for other investigations such as surface pH or mucoadhesion measurements. Furthermore, a correlation between the used polymer and the form of the caffeine crystals in the film is assumed given that differences in the outer appearance in the size of the needles exist. However, these assumptions need further investigations.

2.2.3 Near Infrared Chemical Imaging

Near Infrared Chemical Imaging (NIR-CI) is a non-invasive approach to analyze the distribution of substances within the wafers by the absorption at an NIR band. Initially wavelengths specific for the film former and active substance were identified (see D 2.2.4). In a next step the calibration with the pure substances, polymer powder and anhydrous caffeine, as well as a mixture of both powder substances was conducted. Finally the samples have been measured.



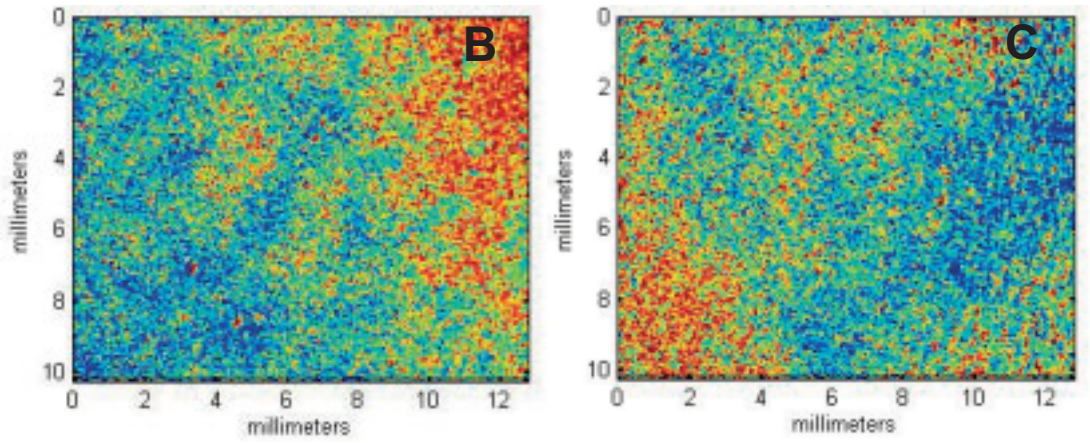


Figure 7: NIR-CI images of drug-loaded films, A: pullulan, B – HM6PA2910 and C – HM50PA2910 (formulation C), red: high caffeine concentration, blue: low caffeine concentration.

As aforementioned, during the manufacturing of the wafers recrystallization processes took place and NIR-CI was used to investigate whether the polymer, the active ingredient or one of the excipients recrystallized. In Figure 7 three images of different polymer wafers are displayed. While polymer and active ingredient are not distributed homogenously in the pullulan wafer (A), the films made of HPMC (B+C) are very homogenous. Within A there are a lot of small agglomerates of caffeine (marked by arrows), in which caffeine is surrounded by a blue area which is mainly attributed to the polymer. During the drying process the caffeine recrystallizes and the area around the crystals deteriorates. In contrast to pullulan (A) no agglomerates of caffeine can be found in HM6PA2910 (B) and HM50PA2910 (C) films.

Comparing the results from NIR-CI to SEM, NIR-CI offers a chemical selective method to investigate the distribution of caffeine within the oral films. The penetration depth of NIR radiation can only be estimated, but anyway with NIR signals in the films are detected. In contrast to SEM which only can visualize surface outlines.

2.2.4 X-ray diffraction

To investigate the crystallographic properties of the observed particles in the drug-loaded oral films X-ray diffraction was used.

Before basic formulation C was developed a preliminary study with some polymers of basic formulation A for drug-loaded films was carried out. Therefore oral wafers from formulation A with the polymers C30PA09, HM6PA2910 and HM50PA2910 as well as caffeine base and caffeine citrate as API were manufactured. The amount of caffeine added to the film solution was 2.0 g (1.5 % of the batch) and 3.98 g (2.94 %) for the free base and citrate respectively.

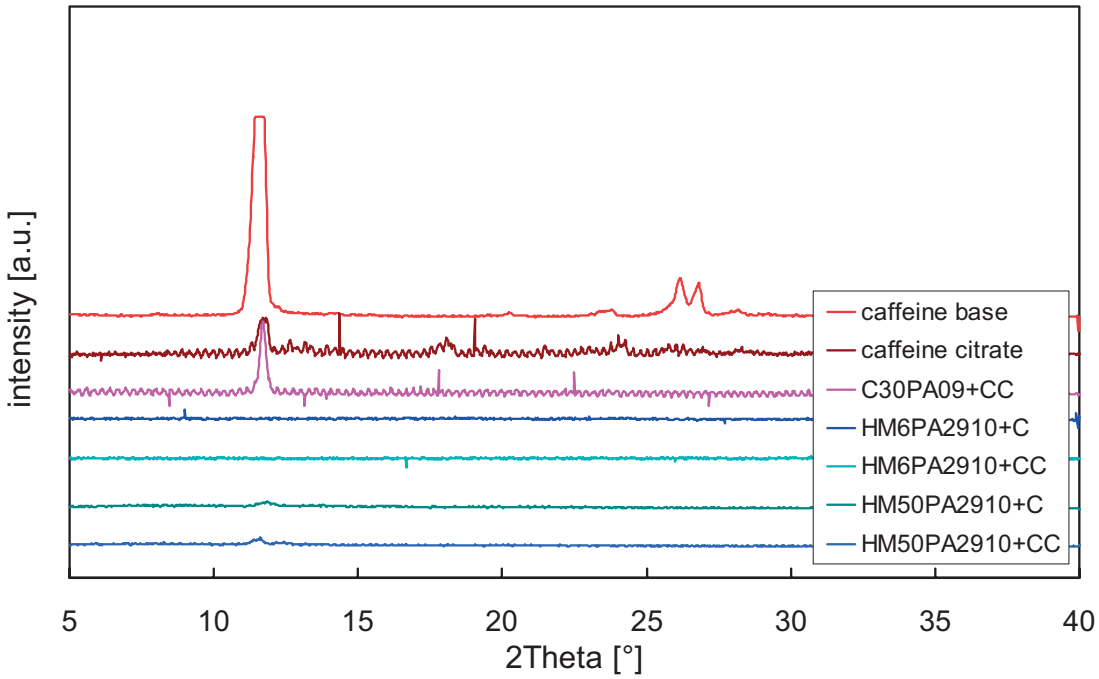


Figure 8: Diffraction patterns of API and drug-loaded oral films of formulation A, (C = caffeine base, CC = caffeine citrate).

The investigations of the drug-loaded films of formulation A showed weak or no signals (Figure 8) of the caffeine peak compared to the signals of the oral wafers from formulation C (Figure 9). One reason is that the amount of incorporated caffeine within one oral wafer was less than 10 mg, determined by HPLC (C 2.8.4), and therefore the signal was less pronounced. The signal of caffeine citrate was weaker than that of caffeine base. For the citrate no signal could be observed. As there was hardly any caffeine signal in HPMC films it is assumed that caffeine is molecularly distributed within the oral film which was already found by NIR imaging (C 2.2.3). Only the oral films made from C30PA09 showed a weak caffeine signal which was caused by crystals forming within the prepared film.

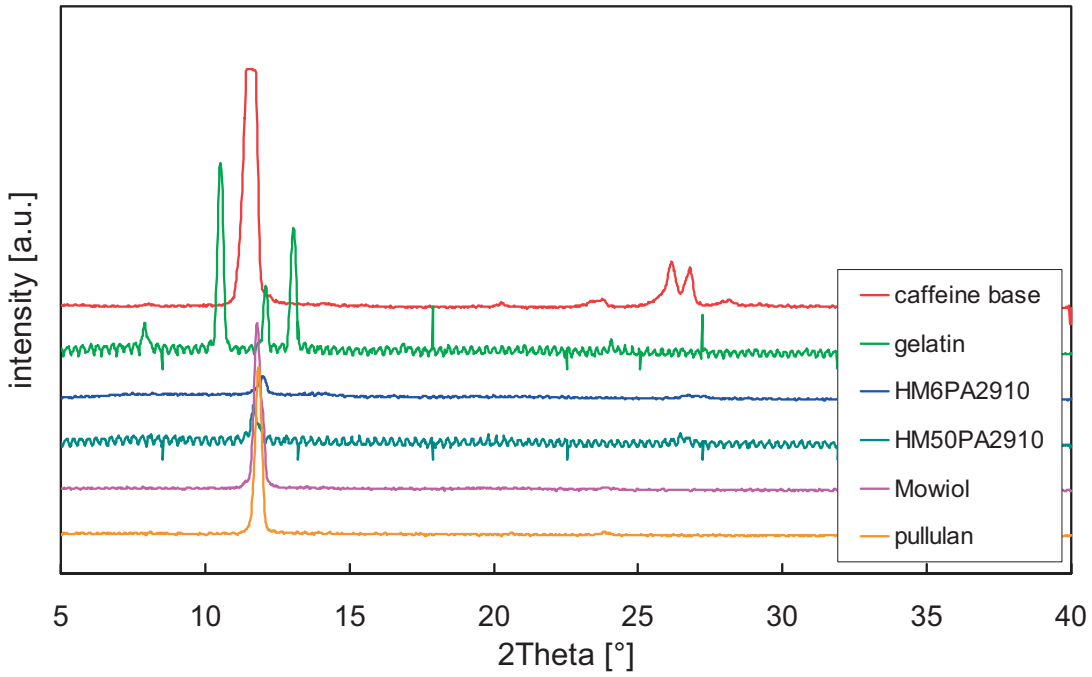


Figure 9: Diffraction patterns from drug-loaded oral wafers of formulation C.

As described above HM6PA2910 and HM50PA2910 showed small caffeine reflections indicating a homogenous distribution of API within the film matrix. Obviously, the anhydrous caffeine recrystallized within Mowiol® and pullulan films in the same modification as present in the raw material. In contrast to these two polymers the oral wafers made of gelatin showed other peaks. Due to the determination of drug content it can be concluded that the API did not decompose, but rather caffeine probably recrystallized in another modification. Further investigations should be performed to elucidate the crystallographic structure of the formed particles.

2.3 Mechanical properties

2.3.1 Variation of mass

When manufacturing the oral films (see D 2.1.2) the film solutions were cast into sheets and then cut into smaller strips of 6 cm² (3 x 2 cm). Oral films were cut from different sheets and the variability between the sheets of the respective polymer as well as the variability between the polymers was investigated. Given that the API recrystallizes within the polymer films to different extents, it was assumed that the individually prepared film strips would differ. Furthermore the masses of oral films on initial analysis showed high standard deviations, thus three polymers (with highest, average and lowest standard deviation) were investigated in more detail for their variation of mass.

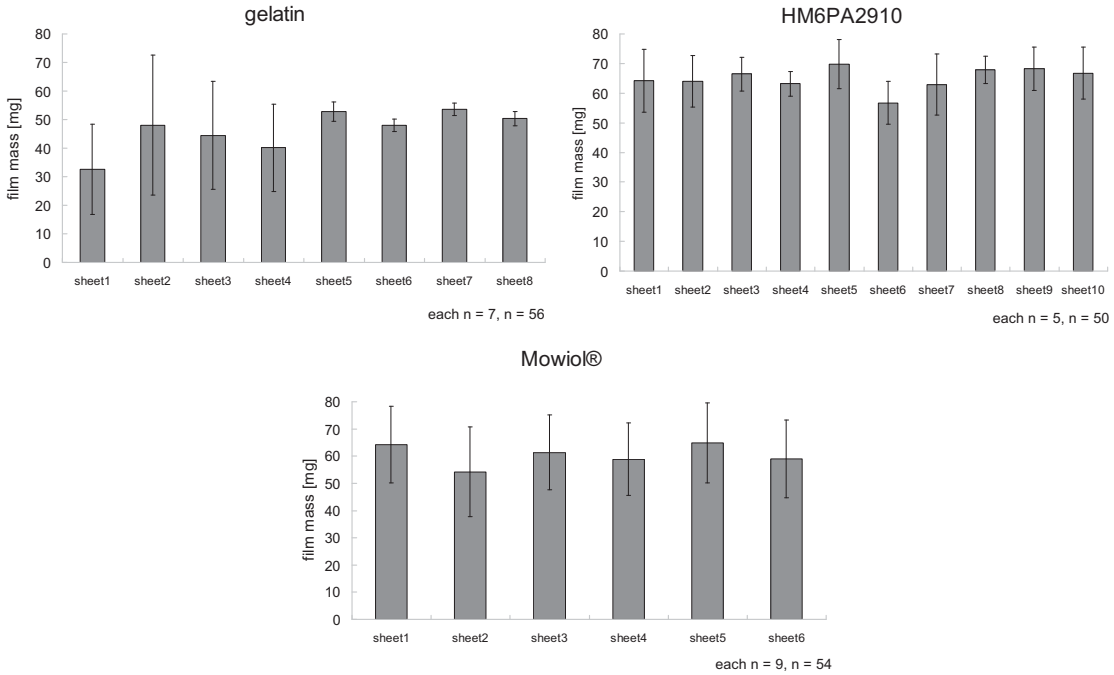


Figure 10: Variation of mass of individually prepared oral films between film sheets made by basic formulation C for the polymers gelatin, HM6PA2910 and Mowiol®, mean ± SD.

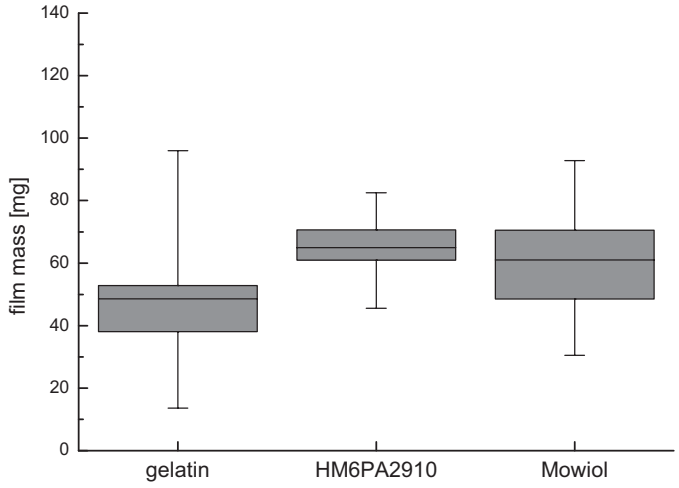


Figure 11: Variation of mass of individual film strips made from different film formers, x_{10}, x_{25}, x_{50}, x_{75}, x_{90}, n = 56, 50, 54.

The nominal mass of one film strip was calculated (see D 2.1.1) to be 58.75 mg. The variations in mass within the sheets of gelatin were somewhat large, especially within sheet number 2 between 17 and 95 mg (standard deviation = 25.5 mg). Meanwhile the standard deviation within sheet 5 to 8 (standard deviation between 2.2 and 3.4 mg) was much lower than compared to sheet 1 to 4 which occurred random depending on the cut film strip of the sheet. Different sizes of the crystals within a sheet are the main reason. In contrast to gelatin the film strips of the sheets of Mowiol® vary to a lesser

extent. Even though fewer sheets were investigated, presumably the anhydrous caffeine recrystallizes more homogenously within the oral wafers, which can also be observed by visual inspection. The results from HM6PA2910 show that the masses of the films varied the least compared to the other two polymers for which the homogenous distribution of API is responsible.

The variation of mass of oral films between the different polymers is displayed in Figure 11. Gelatin oral wafers showed the highest variation in mass with an average mass of 46.3 mg. The film strips of HM6PA2910 exhibited an average mass of 65.0 mg and Mowiol® 60.4 mg. Thus the mass was either lower or higher than the nominal value which could have consequences on the content uniformity. Although, the film height is adjusted to achieve the nominal mass, the masses of the dried oral wafers vary during manufacturing. In conclusion, a homogenous distribution of API and, if possible, prevention of recrystallization processes of API reduce mass variation and enhance content uniformity.

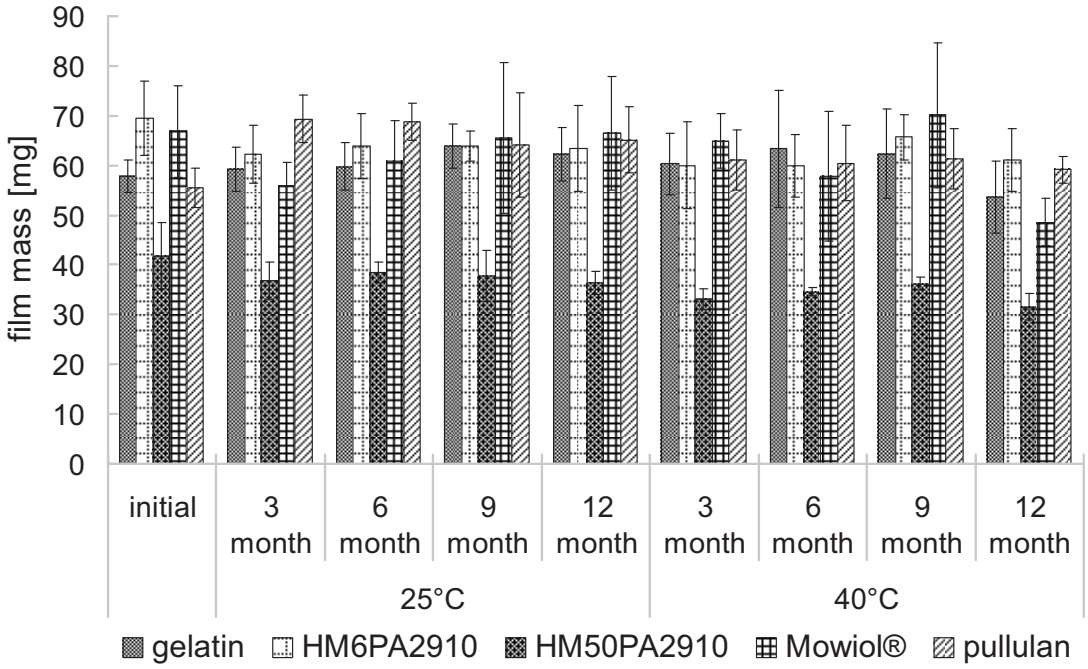


Figure 12: Mass over storage of oral wafers from basic formulation C at different conditions, mean ± SD, n = 10.

Furthermore, the masses during storage of the drug-free (formulation A, data not shown) and drug-loaded films (formulation C) were determined. The nominal weight for each polymer was pre-determined and differs depending on film forming capacity and adherence to release liner. In contrast to the investigations on the variation of mass, the masses during storage differed to a great extent in films of Mowiol® and least in oral wafers of HM50PA2910. For the two conditions investigated, no systematic variations occurred in either polymer which proves that there was no weight loss during storage. The differences result from measuring individual film strips at

different points in time and the recrystallization behavior of the API and are assumed to be coincidence findings.

2.3.2 Film thickness

The determination of film thickness is the most common method to characterize produced oral wafers. The film heights of the basic formulations A and B differ depending on the polymer properties. For the drug-loaded films of formulation C wet thickness heights from 550 to 900 µm were adjusted to an average drug-load of 10 mg per wafer for each polymer.

In the literature the measurement of film thickness was performed by a micrometer screw (Peh and Wong 1999) or light microscopy (Juliano et al. 2007). A coating thickness gauge measures, non-destructively, the thickness of coatings and platings and works with the magnetic-induction principle. In Figure 13 the micrometer screw method is compared to the coating thickness gauge method.

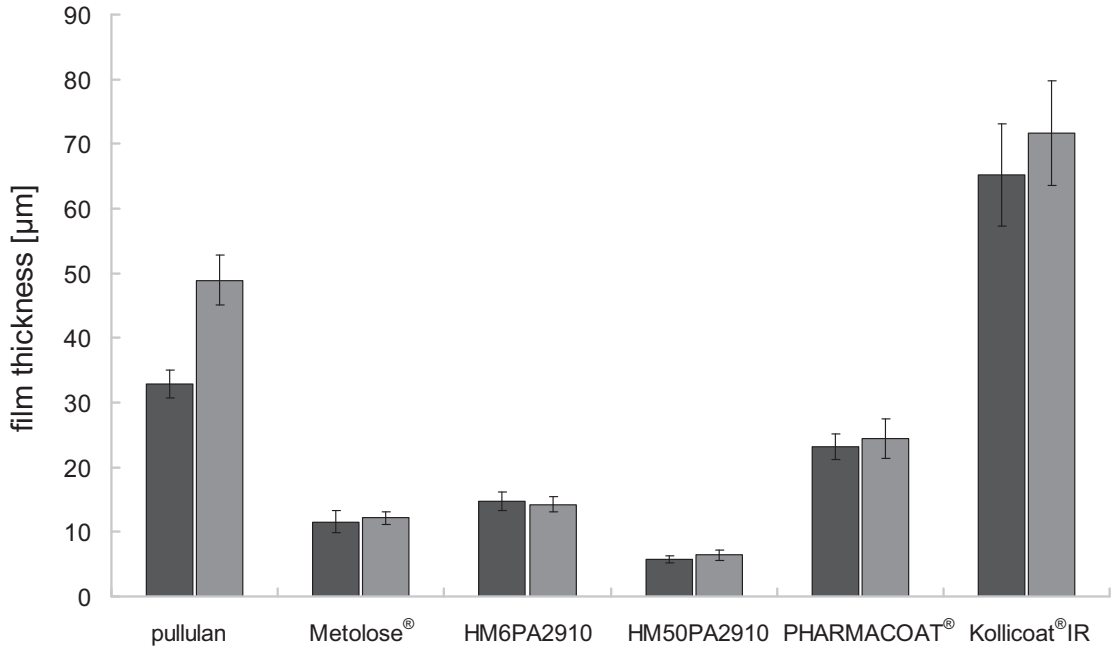


Figure 13: Film thicknesses of drug-free films, left: coating thickness gauge method, right: micrometer screw method, mean ± SD, n = 5.

For these investigations only caffeine-free formulations were used. The usage of two independent measuring methods was chosen to investigate their practicability and accuracy. The introduced reduction of film thickness was determined and calculated according to Equation 6 (D 2.2.7). The thicknesses of dried films (data not shown) decreased about 86 to 97 % compared to the wet films. The thickness of the films made of HM50PA2910, measured by the coating thickness gauge, diminished 97.1 %. The Kollicoat® IR films showed the lowest film thickness reduction, the thickness decreased by about 85.7 %, measured with the micrometer screw. Intermediate values

were found for oral films made of the other polymers. Almost all polymers used in the thickness determination, however, showed no differences between the methods used. Wafers made from pullulan showed an average dry film thickness of 32.9 µm measured by the coating thickness gauge while the determination by micrometer screw revealed a value of 48.9 µm. Using F- and t-test revealed that there is no significant difference ($p > 0.05$) between these two methods. In conclusion, in most cases careful measurement with the micrometer screw offers the same accuracy as the measurement with the coating thickness gauge.

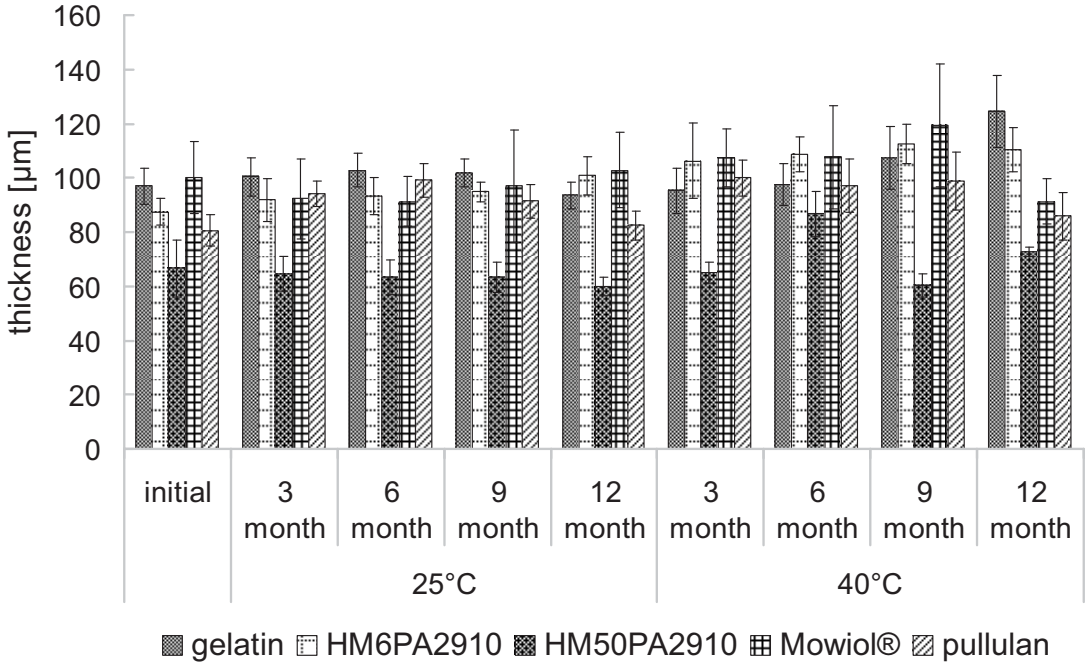


Figure 14: Film thicknesses over storage of drug-loaded oral wafers from formulation C, mean ± CI, n = 10.

The results from 25 °C / 60 % RH compared to 40 °C / 75 % RH did not differ methodically. For gelatin the measured films of the 12 months at 40 °C / 75 % RH differed significantly (CI, $\alpha = 0.05$) from the others. The investigated oral films of HM6PA2910 can be divided into two groups, according to both conditions, which did not differ significantly from each other, but differed from other investigated points of time. The measured film strips of HM50PA2910 did not differ significantly from each other with the exception of 6 and 12 month samples at 40 °C and 75 % RH. In Mowiol® oral wafers no significant differences between all investigated films were found. Finally, for the oral films made of pullulan during storage for the initial investigated films differed not significantly from 12 month at both conditions, but from the rest and 12 month at 25 °C / 60 % RH only differed from 6 month (25 °C / 60 % RH) and 3 month (40 °C / 75 % RH). As seen methodic, significant differences between the measured oral wafers at different points of time were not observed as well.

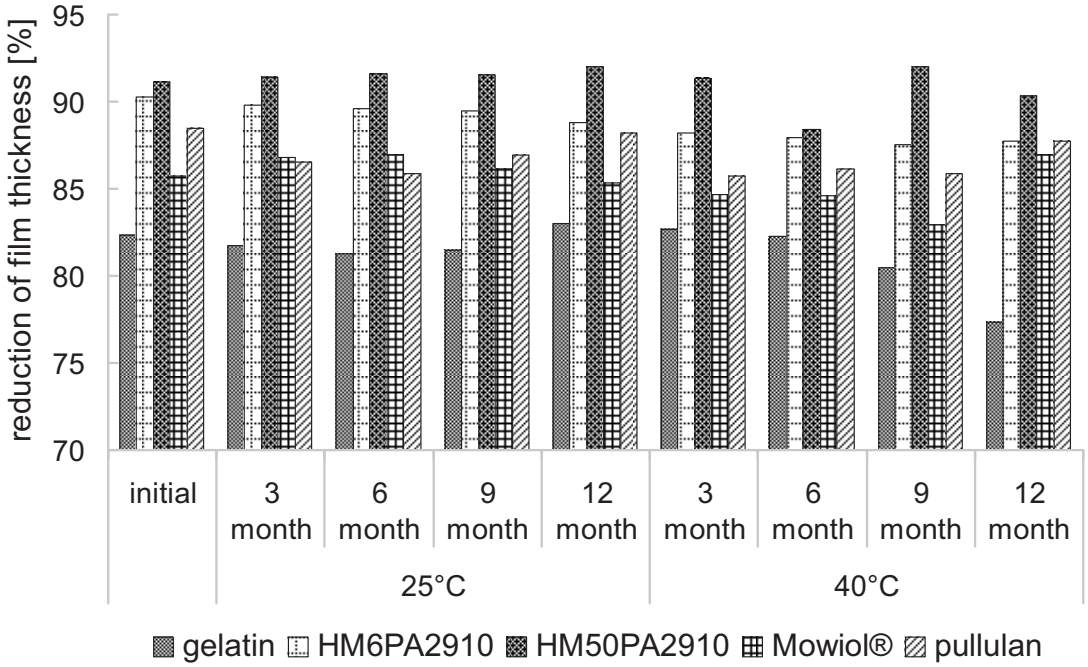


Figure 15: Film thickness reduced about [%] from a wet to a dry film during storage, (respective wet film thickness equates to 100 %).

As the oral wafers made from different film formers had varying wet film heights for achieving 10 mg drug-load, the comparison of dry to wet film thickness as reduction was calculated according to Equation 6 (see D 2.2.7). Given that gelatin films had the lowest film height (550 µm) the reduction in film thickness was also the lowest at 81.4 %. Mowiol® (700 µm, 85.6 %) and pullulan (700 µm, 86.3 %) followed. HM6PA2910 oral wafers showed an 88.8 % reduction of film thickness (900 µm) with lowest standard deviation of 1.01 %. The highest reduction of thickness was shown for oral wafers of HM50PA2910 (750 µm) with 91.1 %. In summary, the calculated ratio for the determination of the film thickness reduction seems to be a robust term due to the low variability of less than 2 % for all investigated polymers during a period of 12 months.

2.3.3 Tensile strength

Mechanical properties of the film formers are important for film casting on release liners, punching and packaging. The aim of this part of the study was to investigate potential differences between the used polymers, without changing the ratio of the excipients, especially the polymers, and to identify variations in the mechanical characteristics. A quality control test was adapted from the plastics industry. Therefore, DIN EN ISO 527-3 was applied using number 5 test specimen (see D 2.2.8). Tensile test was performed to assess strength and elasticity of the prepared films. Depending on the cut film strip used, different results arose. Well-established parameters (DIN Deutsches Institut für Normung e.V. 1996) are tensile strength [σ_M =

maximum tension of test specimen during tensile test] as well as modulus of elasticity in tension [E_t, Equation 1].

Equ. 1:

$$E_t = \frac{\sigma_2 - \sigma_1}{\varepsilon_2 - \varepsilon_1}$$

ε_1	strain, 0.0005
ε_2	strain, 0.0025
σ_1	corresponding stress to ε_1 [MPa]
σ_2	corresponding stress to ε_2 [MPa]

The modulus of elasticity is the proportionality constant of stress and strain. Within the Hooke range the stress is proportional to the strain and thus an elastic deformation occurs which is desirable for oral films. With increasing force the test specimen breaks. In our study the tensile stress at break [σ_B = tension at break of test specimen] and the tensile strain at break [ε_B = elongation of stress at break if break is before achieving an elongation point] were analyzed, at which the tensile stress at break [σ_B] is identical to the tensile strength [σ_M] because the maximum tension of the test specimen during tensile test [σ_M] was identical to the tension at break [σ_B]. Buccal wafers are desirable with a high tensile strength and a low modulus of elasticity (Peh and Wong, 1999).

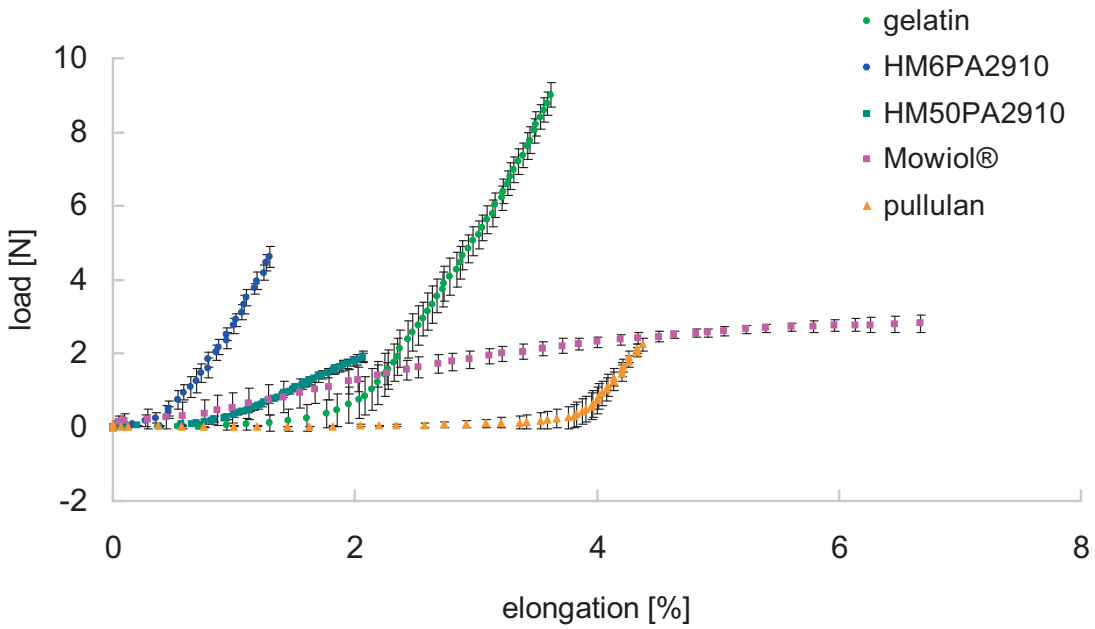


Figure 16: Graph of tensile test measurement of oral wafers made from formulation C, mean ± SD, n = 5.

Exemplarily the graphs of the tensile test of the mean curves are displayed in Figure 16. Films made of Mowiol® tolerate a higher elongation than all other wafers produced. So they can be mechanically stressed more than films prepared from other polymers. The two HPMC types (HM6PA2910 and HM50PA2910) and gelatin are

hardly ductile, therefore, the gelatin films have the highest measured tensile force of 9 N. Pullulan is slightly elastic and breaks at a very low force (2.24 N).

polymer	*ε_B tensile strain at break [%]*	*σ_B tensile stress at break [MPa]*
gelatin	11.77 ± 3.72	20.22 ± 6.40
HM 6 PA 2910	6.46 ± 1.95	12.32 ± 3.71
HM 50 PA 2910	4.43 ± 2.61	11.08 ± 6.52
Mowiol®	3.09 ± 0.57	5.15 ± 0.95
pullulan	3.74 ± 1.29	7.74 ± 2.66

Table 10: Results from tensile test (formulation C), mean ± SD, n = 5.

Further insight into the mechanical properties provides the analysis of the parameters ε_B and σ_B. According to the use of a test specimen all film strips broke in the middle and not at the clamps. Table 10 displays the mechanical properties of caffeine-loaded film preparations. Wafers made of gelatin showed highest values in ε_B (11.8 %) and σ_B (20.2 MPa), which means that these films are hard and tough. Film strips made of Mowiol® are soft and weak with the lowest observed values in ε_B (3.1 %) and σ_B (5.2 MPa). Although the films made of HM50PA2910 showed the smallest film thickness with 66.6 µm, the films did not break below a σ_B of 11.1 MPa. HM6PA2910 and pullulan obtained similar film thicknesses (87.4 µm 81.0 µm respectively), but differed considerably in their obtained ε_B and σ_B. The high standard deviations arose from the fact that the active ingredient recrystallized. Recrystallization occurred throughout the dried film and large crystals grew at the expense of the smaller ones and caused varying structures of the films.

2.4 Thermal properties

2.4.1 Differential Scanning Calorimetry

Polymer films and pure polymer powder were investigated by Differential Scanning Calorimetry (DSC) to characterize the thermal properties of oral wafers. An aim of applying thermoanalysis was to detect emollient properties which may be important for formulation development and film casting.

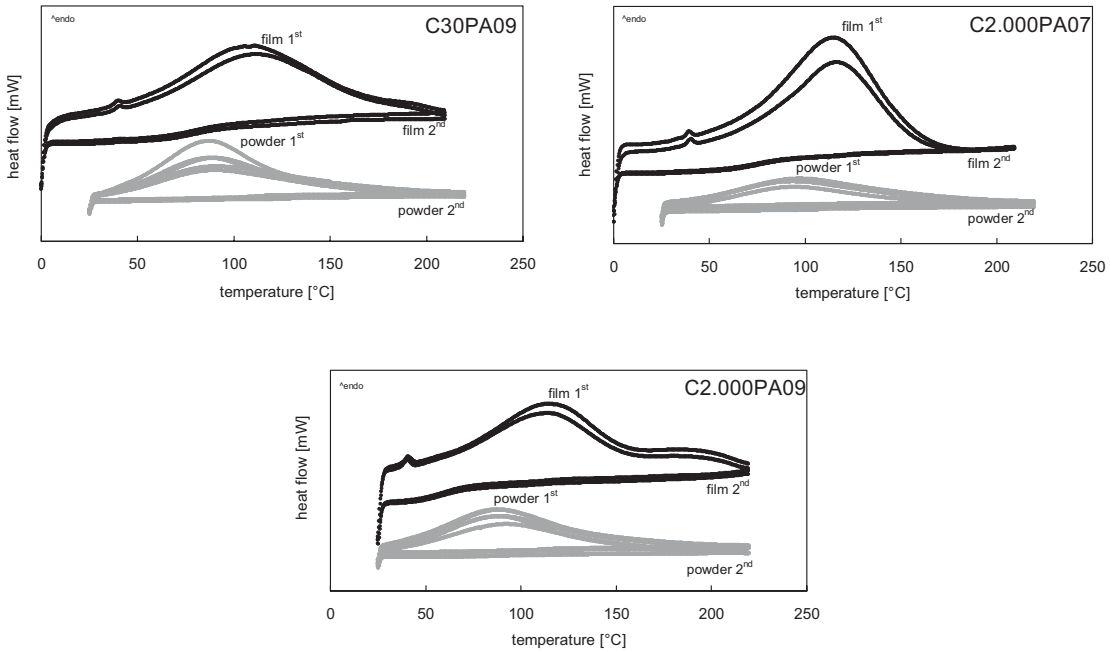


Figure 17: DSC curves of oral films made from different types of CMC (formulation A) and pure polymer powders, 1st indicates first heat scan, 2nd second heat scan, heating rate 10 K/min, 2 replicates per film, 5 per substance.

The DSC measurements (Figure 17) exhibited unsatisfying results. The curves of the polymer powders are clearly compressed compared to the curves of the polymer films. Actually the curves should have shown detectable glass transitions. The glass transition temperatures (Tg) of the polymer powders lay between 85 and 102 °C, showing high standard deviations (Table 11). The Tg of the oral films compared to these results are lower (47 to 63 °C) as assumed. Under these conditions (type and amount of added plasticizer) the Tg cannot be reduced that much. Provided that the glass transitions are not clearly detectable a reason could be that the film matrices overlap the Tg of the polymer. Due to the unconvincing results of Tg determined by DSC, thermomechanical analysis was performed in addition.

Further DSC measurements with drug-loaded oral wafers from formulation C (data not shown) showed no detectable caffeine peak compared to the pure caffeine base at 236 °C. Here the film matrices probably overlap the caffeine peak as well. In former sections (see C 2.2.3, C 2.2.4) it was proved that caffeine recrystallized within the oral films and should have been shown by a melting peak.

2.4.2 Thermomechanical Analysis

Results for the different types of HPMC and pullulan from thermomechanical analysis (TMA) are displayed in Figure 18. It can be seen that the Tg of the oral wafers were hardly determinable. The lowest Tg of the polymer powder was determined for HM6PA2910 at ~ 152 °C, Metolose® ~ 155 °C and HM50PA2910 ~ 241 °C. The films made of HPMC differ in their properties. Presumably the different degrees of

substitution could be a reason for the differing Tg. However, there was not a linear relationship between the Tg of the HMPC films and their degrees of substitution. The determined Tg of pullulan powder was 241 °C which differed significantly from the literature value (Ratto and Schneider 1998) of ~ 180 °C. The Tg of the polymer films were lower than the Tg of their respective polymer powders which is discussed in chapter C 2.4.3.

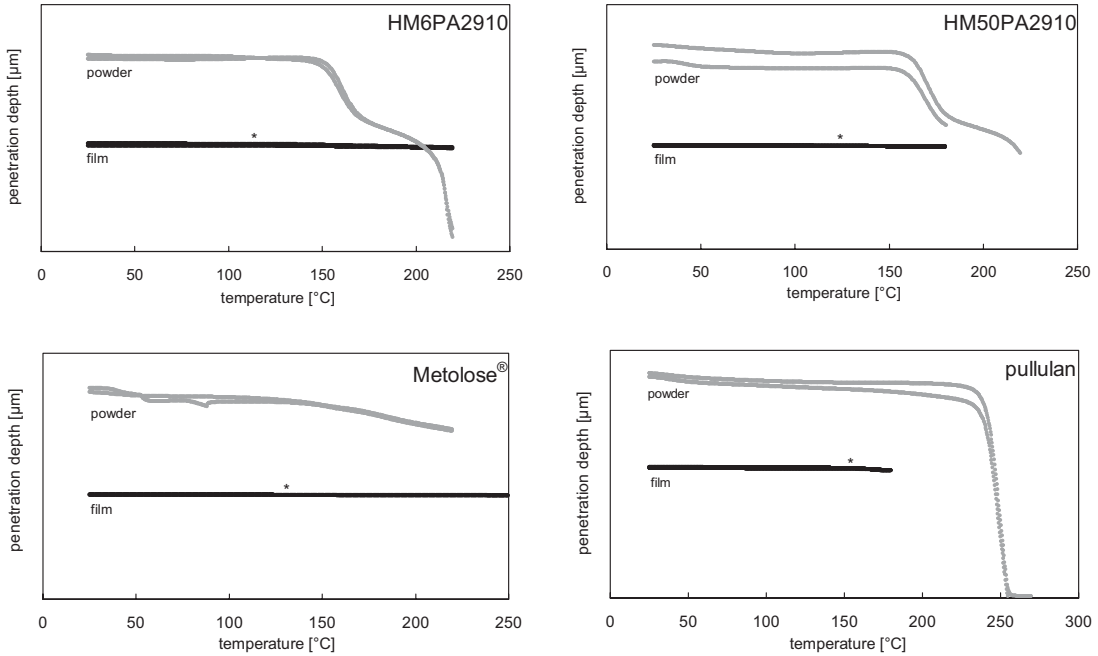


Figure 18: TMA curves of different polymer films (formulation A) and pure polymer powders, n = 2, * = Tg (not apparent due to compressed curve).

2.4.3 Comparison of thermoanalytical methods

	DSC_{powder}		DSC_{film}		TMA_{powder}		TMA_{film}	
	mean [°C]	SD	mean [°C]	SD	mean [°C]	SD	mean [°C]	SD
Walocel C 30 PA 09	102.21	±26.8	56.29	±2.3	19.97	±0.3	32.88	±2.4
Walocel C 2.000 PA 07	85.01	±16.8	62.53	±3.0	n.a.		32.08	±1.1
Walocel C 2.000 PA 09	95.14	±24.1	47.02	±1.1	n.a.		31.35	±2.6
Walocel HM 6 PA 2910	127.82	±4.9	n.d.		151.82	±1.2	113.56	±5.2
Walcoel HM 50 PA 2910	148.91	±4.2	n.d.		160.86	±2.1	126.27	±0.3
Metolose® 65SH-1500	81.08	±4.9	n.d.		155.29	±14.9	131.85	±0.9
Pullulan	116.34	±53.7	n.d.		240.75	±0.3	155.30	±1.8

Table 11: Mean glass transition temperatures [°C] measured by differential scanning calorimetry (DSC) and thermomechanical analysis (TMA), n.d. = not determined, n.a. = not available, mean ± SD, 2 replicates for TMA and DSC_{film}, 5 for DSC_{powder}.

Since precise measurement of the glass transition temperatures of the oral films was not possible with differential scanning calorimetry, the measurements were repeated using thermomechanical analysis. The determined Tg of the polymer powders and oral films by DSC and TMA are shown in Table 11. As can be seen for C30PA09 the

values obtained for the polymer powder and polymer film differed considerably between each other and between the two methods used. The values from DSC_{powder} for most of the polymers, such as C30PA09, C2.000PA07 and C2.000PA09, as well as pullulan, showed high standard deviations and therefore were also measured with TMA. For C2.000PA07 and C2.000PA09 no Tg for the oral wafers could be detected by TMA. The HPMC grades (HM6PA2910, HM50PA2910 and Metolose®) show Tg of the polymer powders close to the literature values (Rowe 2003) of ~ 170 – 180 °C. The TMA results for C30PA09 (Tg ~ 20 °C) and pullulan (Tg ~ 241 °C) did not agree with the values from literature of ~ 220 °C (Rowe 2003) respective ~ 180 °C (Ratto and Schneider 1998). All polymer films were measured by TMA and with the exception of C30PA09, showed lower Tg than the pure powders which was attributed to the incorporated glycerol and sorbitol as plasticizers and the solvent water which acts as a plasticizer as well. In summary, lower Tg, with the exception of C30PA09, were obtained for the oral films than for the pure polymer powders with both methods. Reproducibility of the literature values from CMC was not possible as Rowe (Rowe 2003) presumably investigated other grades of CMC. Furthermore, because oral films consist of a complex matrix, an exact determination of the Tg (especially by DSC) was difficult and resulted in the unmeasurable Tg of HPMC and pullulan films. This fact could be additionally enforced by the measurements of the caffeine-containing oral wafers as described above.

2.4.4 Hot stage microscopy

Hot-stage microscopy is another method used to investigate the thermal behavior of the oral wafers and to understand the scattering results from the determination of the glass transition temperatures. In Figure 19 three of the investigated polymer films are displayed.

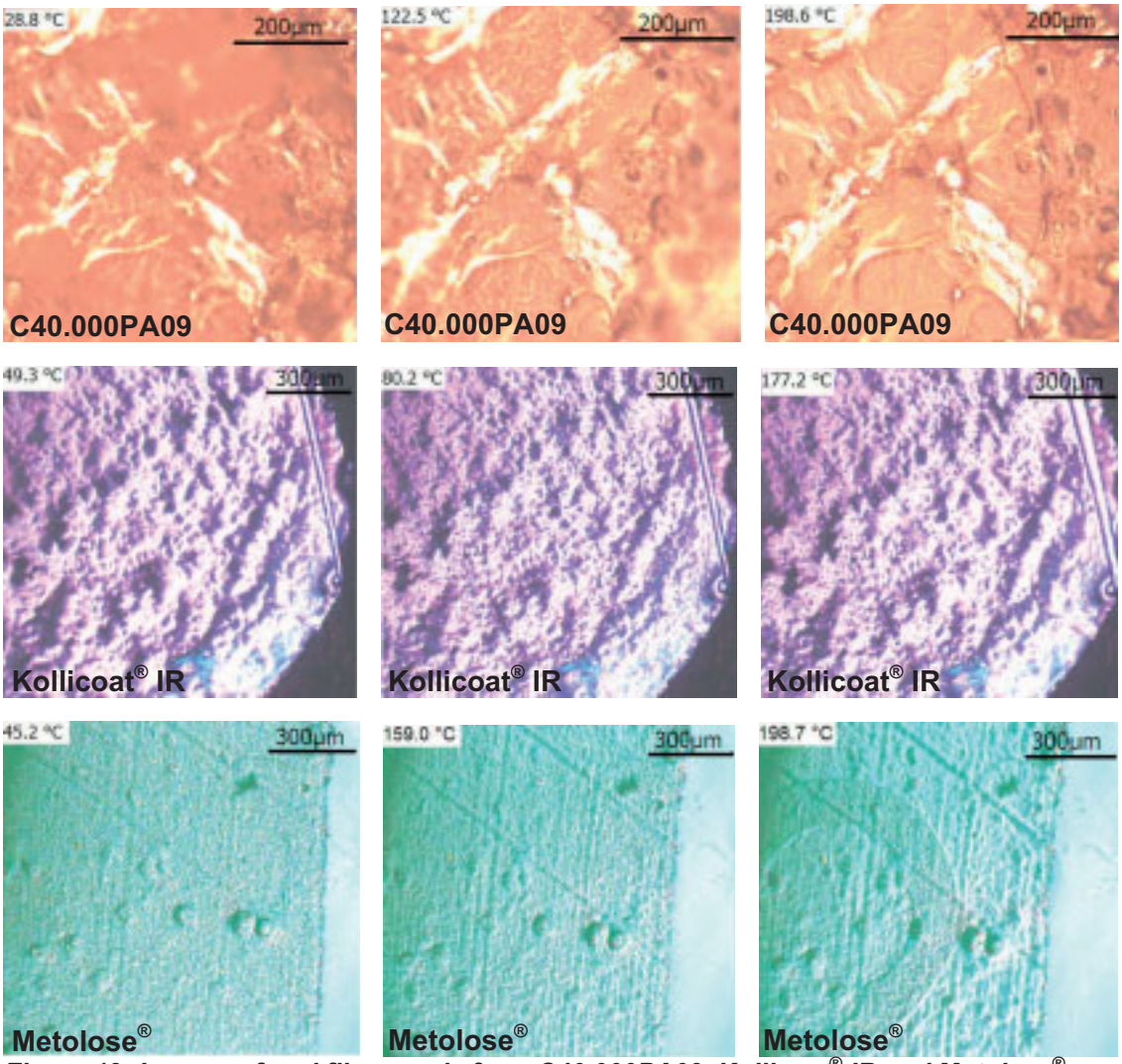


Figure 19: Images of oral films made from C40.000PA09, Kollicoat® IR and Metolose® (formulation A) during heating by light microscopy.

For the CMC, C40.000PA09, and Kollicoat® IR no remarkable thermal events could be observed between 20 and 200 °C. Interestingly, all HPMC grades developed a ring-shaped elevation during heating which can be seen for Metolose®. Even though the oral films of HPMC showed this thermal event, the wafers did not decompose. Overall, the structures of the oral films were not destroyed by increasing the temperature up to 200 °C.

2.5 Residual solvents

2.5.1 Water contents

The determination of residuals from organic solvents is not negligible for the intended application in the young population. A low concentration of organic solvent is important not to exceed acceptable daily intake limits in order to avoid any damage to the human organism. From a technological point of view, the knowledge of the amount of residual solvent within oral films is required for adequate packaging and possible fields of application with extreme climatic conditions. Hence, the determination of all contained residual solvents was performed. The commonly used Karl-Fischer-Titration only detects water. As some polymer films did not completely dissolve in the Karl-Fischer medium comparative investigations by Karl-Fischer-Titration in nitrogen flow were conducted. In contrast to the common titration Karl-Fischer-Titration in nitrogen flow measures all exhausting water during heating. Comparative measurements for detection of all residual solvents were conducted by loss on drying and TGA-MS. However, an unspecified mass loss is detected by loss on drying.

2.5.1.1 Karl-Fischer-Titration

The Karl-Fischer-Titration was used as the standard method to measure the water content of oral films from formulation A, B and C during storage (data not shown). In Figure 20 the initial water contents of the oral films of formulation C are displayed.

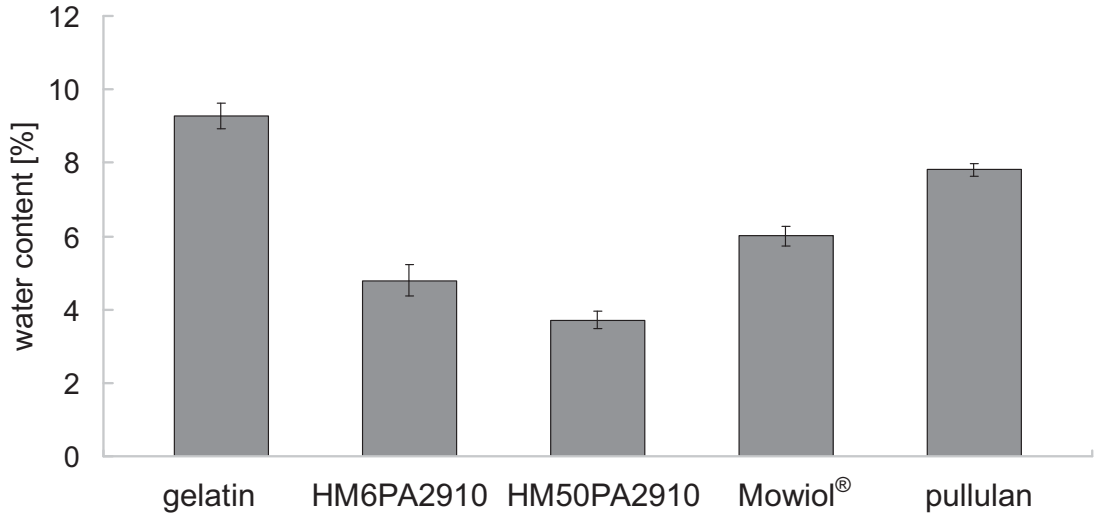


Figure 20: Initial water content [%] of oral films from formulation C determined by Karl-Fischer-Titration, mean ± SD, n = 3.

Within the pharmacopeias no recommendations for water contents of oral films are available due to the lack of monographs as discussed in chapter C 2.1. Manufacturers

of oral films recommend 4 to 12 % (Labtec, personal communication, 2006) which satisfied all investigated polymer films. The water content of all investigated films ranged between 3 and 10 %. The lowest average water content was detected in HM50PA2910 (3.7 %). Wafers made of gelatin exhibited the highest average water content (9.3 %). Finally, on the one hand water content must not be too low, otherwise oral wafers become brittle, but on the other hand the films must not contain too much water, otherwise they become sticky. In both cases the oral wafers become difficult to administer and thus patient-unfriendly.

2.5.1.2 Loss on drying

Loss on drying was used as a comparative method to Karl-Fischer-Titration. The drying was carried out in an oven with a temperature of 105 °C at atmospheric pressure. The loss on drying is the loss of mass expressed as per cent (mass/mass). The weight of the oral wafers was measured until constant mass was achieved. The results are displayed in Figure 21.

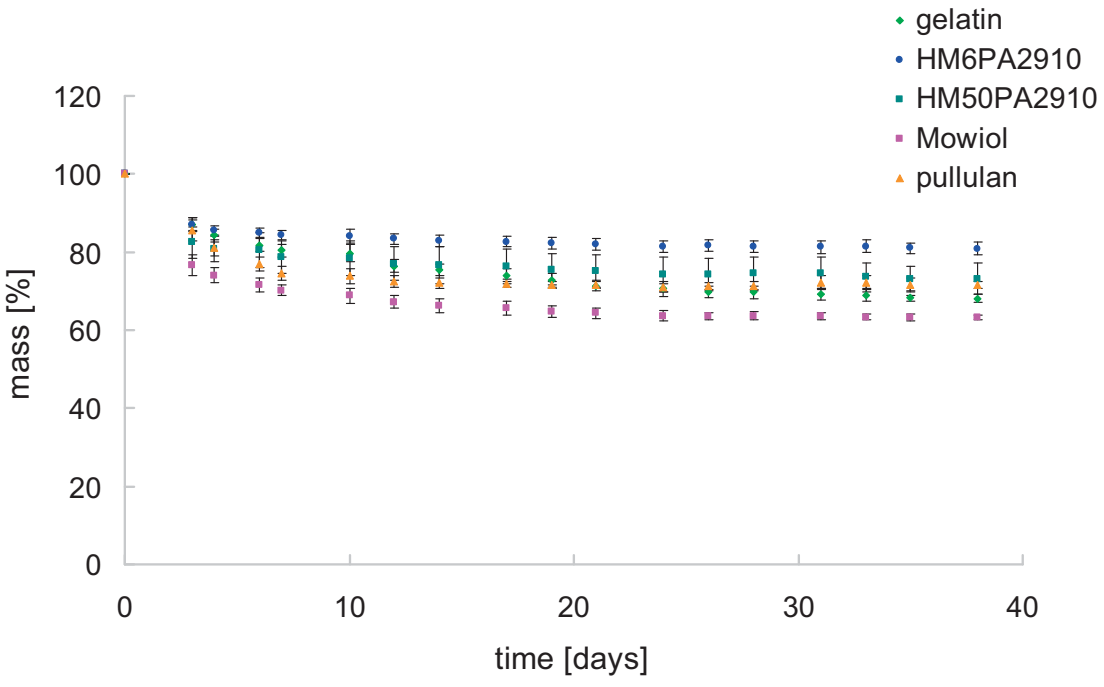


Figure 21: Mass loss during loss on drying determination of oral wafers from formulation C, mean ± SD, n = 5.

The determination of residual solvents by loss on drying was unsuitable for all the oral wafers produced. The films decomposed under the experimental conditions which was observed by a yellowish-brown discoloration of the films and a significant mass loss. The oral films lost in ascending order, ~ 19 % for HM6PA2910, ~ 27 % for HM50PA2910, ~ 28 % for pullulan and ~ 32 % for gelatin, of weight. The highest mass loss occurred with Mowiol® ~ 37 %. Since the films were dry and could be handled without any difficulties, the oral wafers must have lost other ingredients of the film matrix during the measurement procedure.

2.5.1.3 TGA-MS

The simultaneous determination of water in addition to ethanol content was performed by thermogravimetric chromatography which is coupled to a mass spectrometer (TGA-MS). The curves of the diagram (Figure 22) are only shown until 120 °C although the samples were heated up to 650 °C.

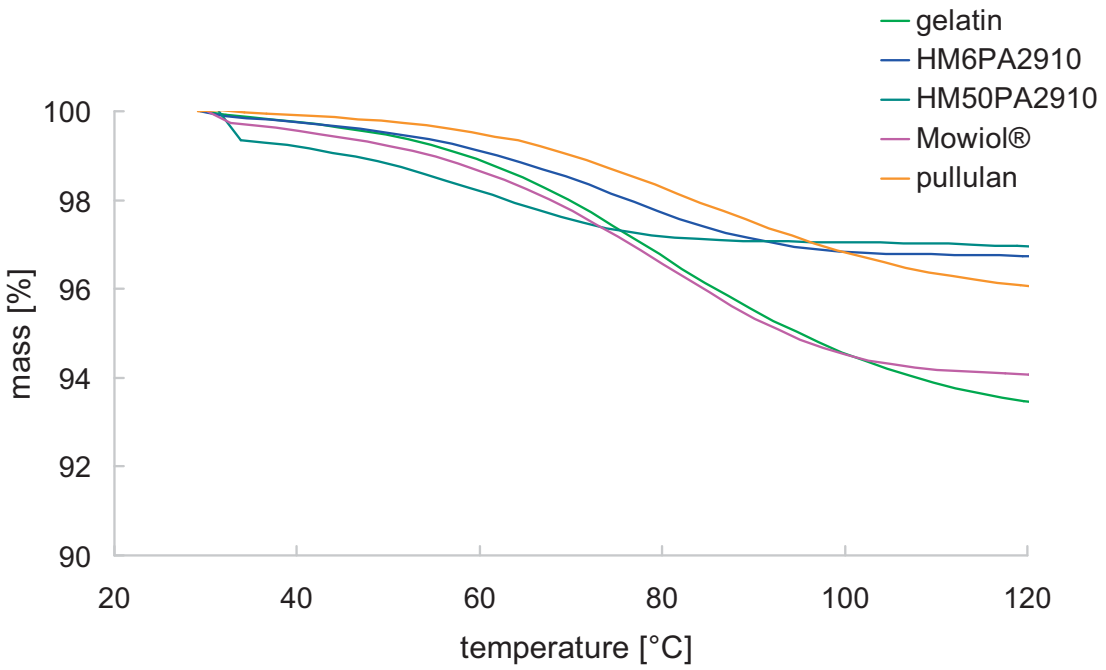


Figure 22: Mass loss by temperature increase measured by TGA-MS, n = 1.

For all investigated films heating up of the samples up to 650 °C led to final masses of less than 25 %, which can be ascribed to the decomposition of the oral wafers.

It could be seen that films made of gelatin and Mowiol® rapidly lost water compared to the other three polymer films investigated. The TGA-MS results showed that the films lost varying amounts of water at 105 °C. Gelatin films contained the largest amount of water with 6.0 %. Mowiol® lost approximately an equal amount of residual solvent with 5.8 %. The two HPMC qualities (HM6PA2910 and HM50PA2910) and pullulan lost similar amounts of 3.0, 3.2 and 3.5 %.

The results from the ethanol determination are thoroughly discussed in chapter C 2.5.2.

2.5.1.4 Methodological comparison

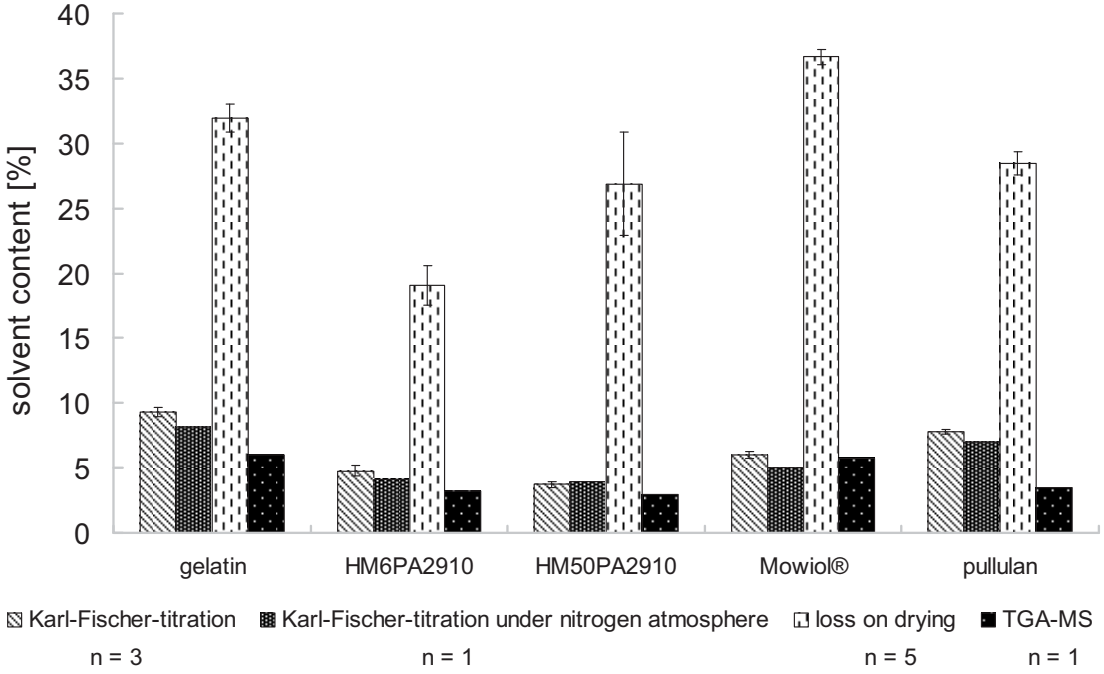


Figure 23: Comparison of various methods for determination of residual solvent, oral wafers from formulation C, mean ± SD.

The comparison of the different methods used show, especially for loss on drying, varying results. As described above, presumably, the decomposition of other ingredients was responsible for this occurrence.

The remaining three methods showed comparable results. Both types of Karl-Fischer-Titration demonstrated similar values. The results from TGA-MS differed slightly which was because at 105 °C bound water was still present within the film matrix. In these three methods gelatin wafers showed highest water content with 9.3 % for Karl-Fischer-Titration, 8.2 % for Karl-Fischer-Titration in nitrogen flow and 6.0 % for TGA-MS. The lowest water content was shown by HM50PA2910 with 3.7 % (Karl-Fischer-Titration), 3.9 % (Karl-Fischer-Titration in nitrogen flow) and 3.0 % (TGA-MS). The ranking order of water content between Karl-Fischer-Titration and Karl-Fischer-Titration in nitrogen flow was in agreement. The ranking order of TGA-MS agreed as far as possible, only Mowiol® and pullulan were not concurrent compared to the both Karl-Fischer methods. In Karl-Fischer-Titration Mowiol® contained 6.0 % water, 5.0 % in Karl-Fischer-Titration in nitrogen flow and 5.8 % at TGA-MS. Pullulan showed second highest values for Karl-Fischer-Titration (7.8 %, Karl-Fischer-Titration and 7.0 %, Karl-Fischer-Titration in nitrogen flow), but third highest values determined by TGA-MS (3.5 %).

In conclusion, the Karl-Fischer-Titration under standard conditions is a valid method which leads to reliable results although some film matrices did not completely dissolve within the medium methanol. The differences between the tested methods could be

caused by the insolubility of film compounds, but also from the differing points of time at which the samples were measured.

2.5.2 Ethanol content

Ethanol was used as a solvent in some formulations such as HPMC which have a good solubility in binary ethanol/water mixtures. However, Mowiol® has a low solubility in ethanol, but in both cases the ethanol volatilizes during drying more easily and faster than water does. Gelatin and pullulan precipitate when in contact with alcohol in any concentration. The question arose whether any and how much ethanol remained within the film matrices after the drying process because the oral wafers are intended for application in pediatrics and may be hazardous for them if containing ethanol in high amounts.

The Pharmacopoeia classifies ethanol within the monograph “5.4 Residual solvents” (European Pharmacopoeia 2009) as a solvent with low toxic potential (class 3), but the intake has to be limited. Therefore the term “permitted daily exposure” (PDE) for residual solvents has been created. The PDE is to avoid confusion of values for ADI of the same substance. For ethanol as a class 3 solvent the PDE has been fixed at 50 mg per day. The data of the Pharmacopoeia refer to a guideline from the International Conference on Harmonization (ICH Expert Working Group 2005a) which based a risk assessment for residual solvent for an adult weighing 50 kg. This relatively low body weight was chosen to provide an additional safety factor compared to standard weights of 60 kg or 70 kg which are often used for such calculations. On assumption that the permitted daily maximum amount of ethanol can be converted by using body weight, a maximum dose of 2.5 mg for a preterm and 3.5 mg for a term infant would arise as PDE. This value compared to the natural occurrence of ethanol in some fruits and fruit juices (Morad et al. 1979), for example bananas, apple or grape juice, is much lower. Indeed, it is debatable whether the conversion via body weight is applicable when giving chemical substances to very young children. It is a matter of common knowledge that the immature organism differs considerably from that of adults in absorption, distribution, metabolism and elimination of substances.

For exact determination of the residual amount of ethanol within the manufactured oral wafers, investigations by the use of a headspace gaschromatograph and TGA-MS were performed. As the TGA-MS detected no ethanol a quantitative statement is not possible. The gas chromatography enforces these results. For all investigated polymer films the residual amount of ethanol was determined as less than 1 ppm. This obtained amount within the oral wafers is certainly fewer than stated within the Ph. Eur. and contained in some fruits and fruit juices. Thus, the application of the prepared oral wafers should be absolutely safe.

2.6 Disintegration testing

2.6.1 Slide frame and Petri dish methods

Disintegration is defined as the deaggregation of a solid dosage form (for example a tablet or capsule) into its primary particles. The disintegration of a solid dosage form is thereby distinguished from drug dissolution. The dissolution of a drug substance may depend on the drug dosage form, on the rate of disintegration and on the properties of the drug itself, such as high or low solubility, which determines the dissolution rate. Although oral lyophilisates or orodispersible tablets show a fast disintegration, the dissolution depends on the properties of the active substance. In comparison, the disintegration of a mucoadhesive dosage form is either very slow or negligible, but the dissolution can be fast or slow. Thus, on the one hand the excipients influence the disintegration and dissolution behavior, and on the other hand, the properties of the API can also have an influence, especially on the dissolution behavior. In the case of oral films the disintegration and dissolution is hardly distinguishable. If the oral film disintegrates it concurrently dissolves in a small amount of saliva which makes it difficult to mimic these natural conditions and measure with an adequate method. Different methods have been described in the literature, but a standard method does not exist. Previous disintegration and dissolution tests were performed with large amounts of media (Ali et al. 2002, Cilurzo et al. 2008, Desai and Kumar 2004, Dinge and Nagarsenker 2008, Peh and Wong 1999) which are physiologically not present in the oral cavity. Furthermore, a disintegration measurement setup for fast-dissolving oral dosage forms, in this case ODTs, has been described using a texture analyzer as well (Abdelbary et al. 2005, Bohnacker et al. 2005, Dor and Fix 2000), but this setup cannot be transferred to oral wafers. In the present work consideration was given to developing a simple test with a few milliliters of medium.

For the assessment of disintegration and dissolution behavior two independent methods will be introduced in this chapter. For both methods only a small amount of medium was needed, so natural conditions could be simulated. Due to the use of the small amount of medium the dissolved drug substance could not be measured by spectral analysis. In the first method one drop of distilled water was dropped by a pipette onto the oral films. Therefore the films were clamped into slide frames and were placed planar on a Petri dish. The time until the film dissolved and caused a hole within the film was measured. For the second method 2 mL of distilled water was placed in a Petri dish and one film was added on the surface of the water and the time measured until the oral film was dissolved completely. Drug-free and drug-loaded films were investigated under both methods.

The results from the drug-free films (Figure 24) show that the complete dissolution of all polymer films took less than 35 seconds on average.

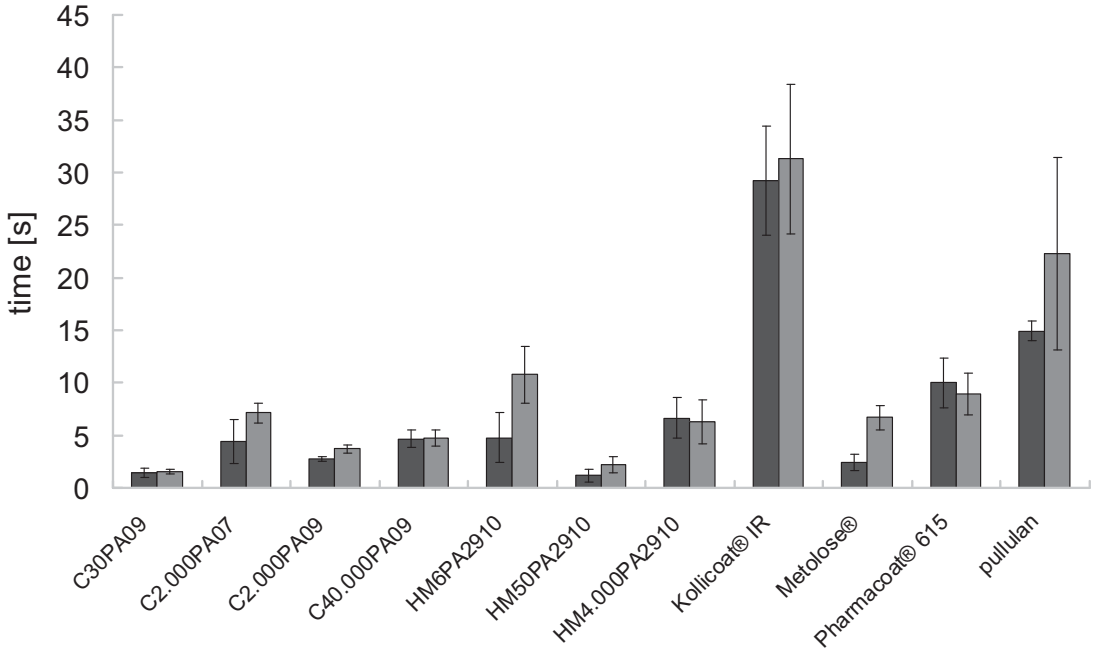


Figure 24: Dissolution times of different drug-free polymer films (formulation A and B) measured by two methods, left: slide frame, right: Petri dish, mean ± CI, n = 5.

Most of the polymers, namely C30PA09, C2.000PA07, C2.000PA09, C40.000PA09, HM50PA2910, HM4.000PA2910 and Metolose® needed less than 10 seconds, fulfilling the criterion of a fast dissolution. The other HPMC grades, HM6PA2910 and Pharmacoat® 615, dissolved in less than 15 seconds. Only the oral films made from Kollicoat® IR and pullulan dissolved in more than 15 seconds, but still in less than one minute. It can be seen in Figure 24 that higher dissolution times resulted with the Petri dish method in any case (right bar) due to the dissolution of a complete polymer film. Furthermore, the comparison of the results from both methods for each polymer showed no significant differences, except in the case of films made from HM6PA2910 and Metolose® (CI, $\alpha = 0.05$). It can be concluded that the polymer Kollicoat® IR seemed to be inappropriate for use in fast-dissolving films.

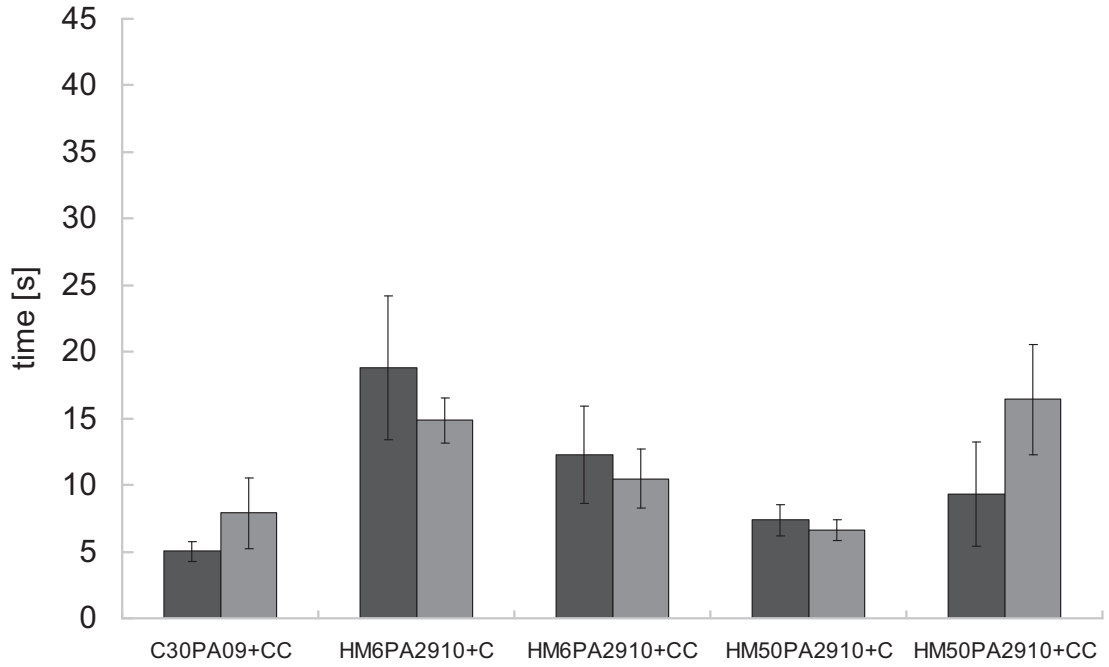


Figure 25: Dissolution times of drug-loaded films (formulation A, C = caffeine, CC = caffeine citrate) measured by two methods. Films made from C30PA09 with anhydrous caffeine could not be prepared. Left: slide frame, right: Petri dish, mean ± CI, n = 5.

The drug-loaded polymer films made from C30PA09, HM6PA2910 and HM50PA2910 of formulation A showed higher dissolution times (Figure 25) from 5 to 25 seconds than their respective drug-free formulation. Although all drug-loaded films had a wet film thickness of 500 µm, the dissolution times between the different film formers differed significantly ($\alpha = 0.05$). However, a significant difference ($\alpha = 0.05$) between the slide frame method (left bar) and Petri dish method (right bar) for each polymer could not be shown. In comparison to the drug-free formulations the Petri dish method did not always show longer dissolution times than the slide frame method. The fastest dissolution times of the drug-loaded formulations showed oral films made from C30PA09 (CMC) with caffeine citrate (CC) with less than 5 seconds for the slide frame method and films made from HM50PA2910 with caffeine (C) and caffeine citrate (CC) with less than 10 seconds for both measured methods. Films made from HM50PA2910 with caffeine citrate (CC) measured by the slide frame method, HM6PA2910 with caffeine (C) by the Petri dish method and HM6PA2910 with caffeine citrate (CC) by both methods showed dissolution times of less than 20 seconds. Only the films made from HM6PA2910 with caffeine (C) for the slide frame method and HM50PA2910 with caffeine citrate (CC) for the Petri dish method showed dissolution times longer than 20 seconds. Actually it was anticipated that the films made with caffeine citrate would dissolve faster than films made with anhydrous caffeine, due to the better solubility of the salt, but this assumption could not be proved in these investigations. The assumption could only be found for HM6PA2910 in contrast to HM50PA2910. Films made from C30PA09 with anhydrous caffeine could not be

prepared, so a prediction for this formulation based on the results from HM6PA2910 and HM50PA2910 was not possible.

Both methods allow an evaluation of drug-free and drug-loaded oral films regarding their disintegration/dissolution behavior. A ranking can be established which may be useful for further pharmaceutical developments. Furthermore, it must be considered that API loading in larger quantities hinders drug release from the film matrix which can be seen in the disintegration/dissolution test for drug-loaded films. Incorporated API and hence resulting changes in dissolution rate influences the disintegration and dissolution behavior. Nevertheless, the obtained short dissolution times comply with the criteria of fast-dissolving oral dosage forms. The Petri dish method (right bar) may simulate the natural conditions better than the first one.

2.6.2 Swelling

Swelling is defined as expansion in aqueous media. In the case of oral wafers, the films swell and subsequently dissolve, so it is not a swelling process in the usual sense. The developed setup (Knop and Matthée 1998, Renner 2003) was applied to predict the disintegration behavior of the prepared films. Metal discs were film-coated and placed into the measuring apparatus. The measuring sensor dipped onto the surface of the film. A defined volume (250 µL) of purified water was added and the sensor movements recorded. In our study the swelling process is considered to be complete when the sensor dips onto the surface of the metal disc. The results for the polymers gelatin, HPMC in two different degrees of polymerization and Mowiol® are shown in Figure 26.

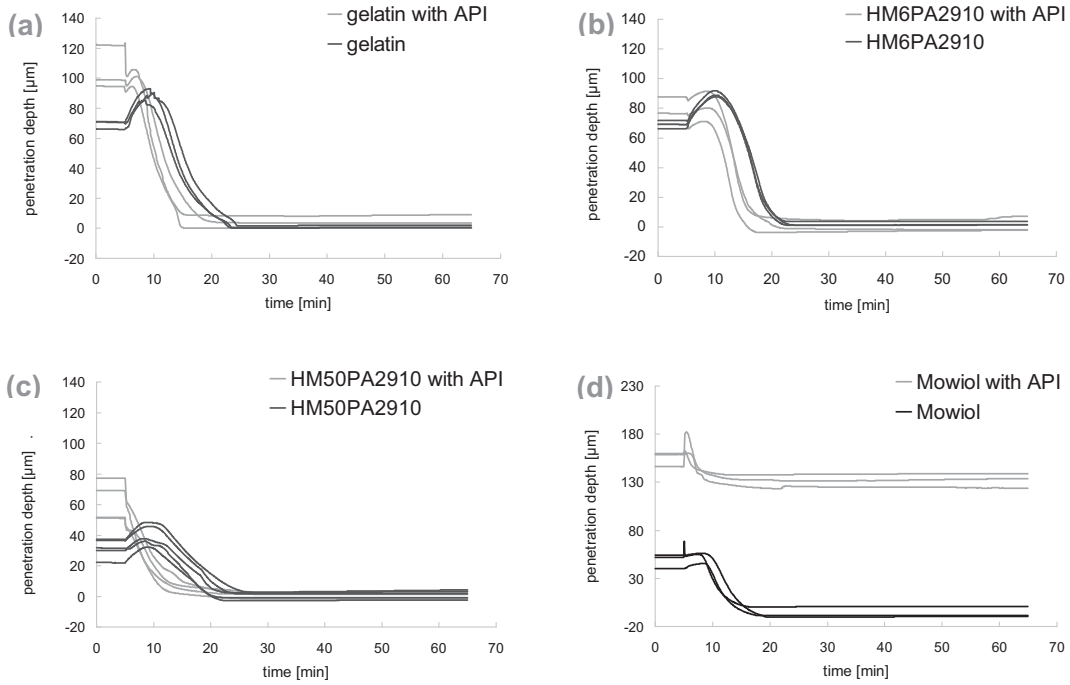


Figure 26: Swelling behavior of films made from gelatin (a), HM6PA2910 (b), HM50PA2910 (c) and Mowiol® (d). Testing fluid was 250 µL purified water (37 °C).

Both, (a) and (b), API-free formulations show initial swelling and dissolve afterwards. Similar behavior shows (c) in contrast to (d) in which swelling does not occur. While the addition of caffeine hardly influenced the curves of gelatin and HM6PA2910, HM50PA2910 with API showed a completely different behavior. The film-coated discs with the drug-loaded formulation did not swell, but dissolved instantly. The curves of Mowiol® with API showed a short swelling phase on the one hand and on the other hand curves at a complete different y-axis intercept. Although the discs of the polymers were filmed with the same height of 900 µm wet film thickness, differences between the dried discs resulted. Dry film thicknesses were determined by measuring the thickness of the filmed disc by micrometer screw and subtracting the thickness of the pure disc. Discs with dry gelatin films (a) differed between 54 µm (drug-free) and 69 µm (drug-loaded) and HM6PA2910 films (b) had dry thicknesses between 65 µm (drug-free discs) and 85 µm (drug-loaded discs), while discs of HM50PA2910 (c) lay between 28 µm (drug-free) and 65 µm (drug-loaded). Mowiol® showed highest thicknesses with 78 µm for the drug-free discs to 93 µm for the drug-loaded ones. Furthermore, the negative values after complete dissolution result from the fact that the discs did not have exactly the same thicknesses, so the zero balance did not fit exactly with every disc. These differing thicknesses of the discs are presumably the reason for the varying y-axis intercepts at the curves of Mowiol®. In conclusion, in less than 25 minutes all formulations, independent of drug content, were completely dissolved although only a small amount (250 µL) of medium was utilized. In dependence of the polymer, the incorporated API influenced swelling and subsequently disintegration as well as dissolution behavior.

2.6.3 Contact angle measurement

One drop (7.5 µL) of testing fluid was added onto the planar surface of the oral film. The contact angle as the ratio of cohesion versus adhesion was determined. The drop shape analysis apparatus records visual data for the measurement of contact angles. The recording starts automatically if the drop passes a predetermined mark. Afterwards, images at different time points, time 0 and after 30 seconds (Figure 27), were evaluated.

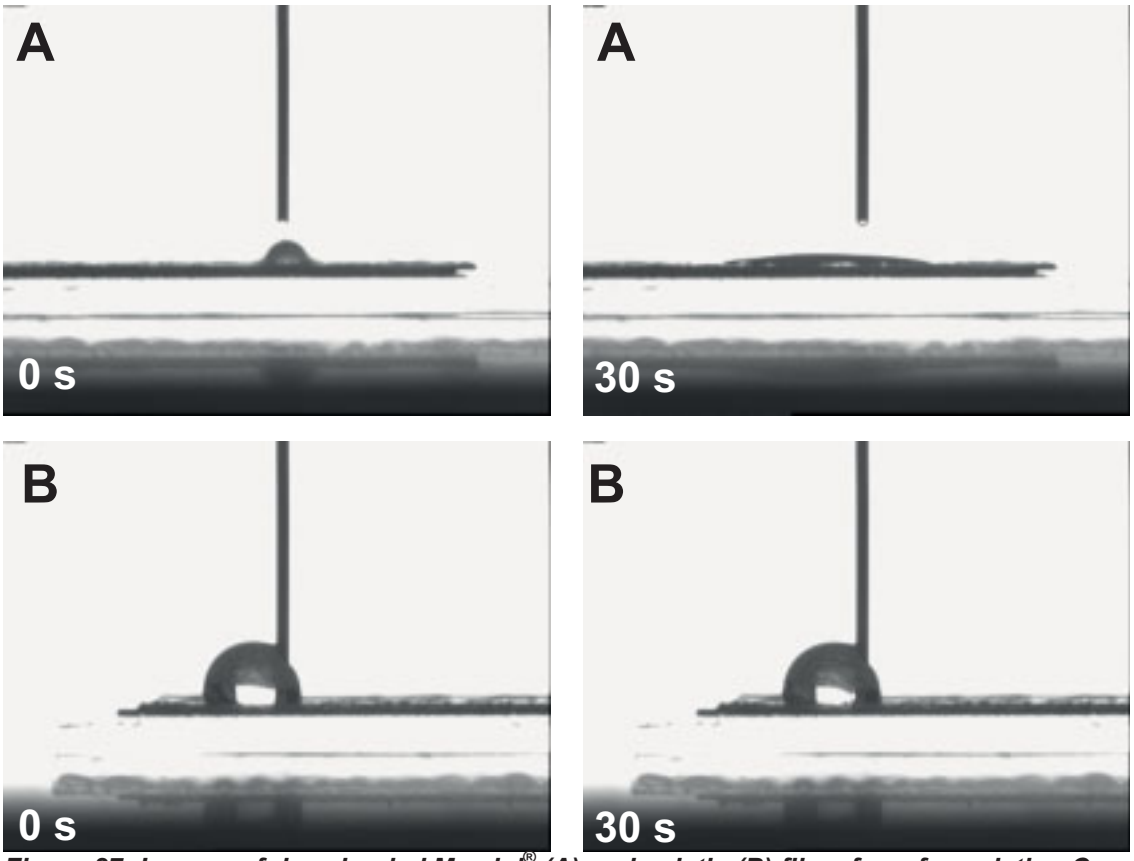


Figure 27: Images of drug-loaded Mowiol® (A) and gelatin (B) films from formulation C during contact angle measurement at different points of time.

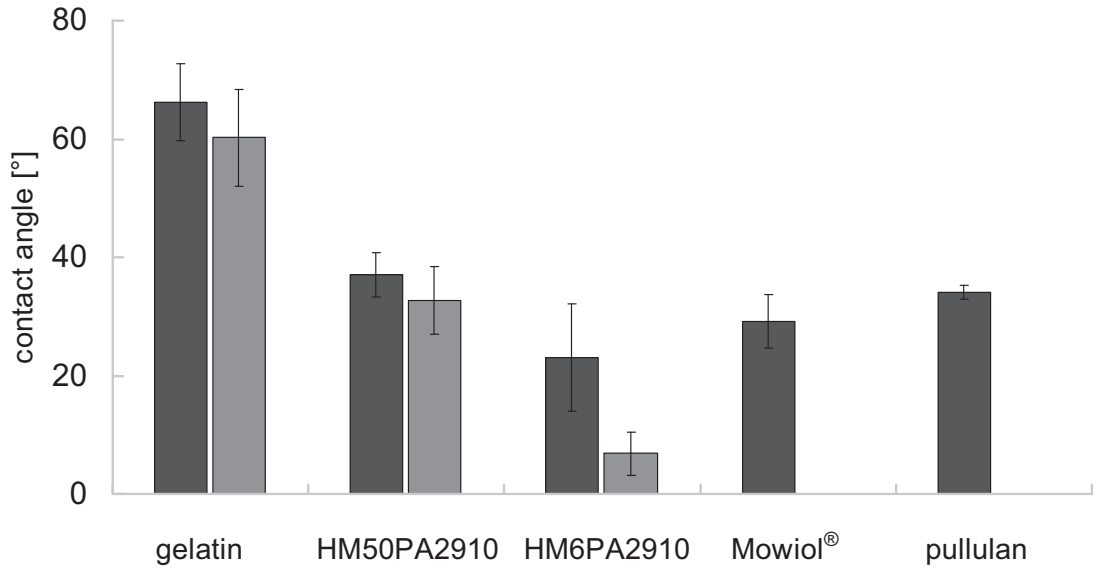


Figure 28: Contact angles measured of drug-loaded formulations (C) with phosphate buffer pH 6.0 as wetting fluid, left: measurement after 0 seconds, right: measurement after 30 seconds, mean ± SD, n = 5.

In all investigated polymers the contact angle changed during measurement due to penetration of the drops into the surface of the films (Figure 28). Gelatin films are hardly wetted by phosphate buffer within 30 seconds (see also Figure 27 B). The contact angle only decreased from 66.0° to 60.3°. HM50PA2910 showed the same behavior decreasing from 37.0° to 32.8°. In contrast the contact angles of Mowiol® (29.2°) and pullulan (34.1°) decreased considerably within 30 seconds such that a contact angle could not be measured. A centered position with a decrease from 23.1° to 6.9° was measured for HM6PA2910. The obtained results allow possible predictions for the wetting behavior of the oral films, their drug disintegration and dissolution within the oral cavity.

2.7 Drug release

2.7.1 Introduced methods

As discussed in chapter C 2.1 no adequate method for dissolution testing of dosage forms administered into or within the oral cavity can be found within the pharmacopoeias. In some papers dissolution testing of novel dosage forms (Azarmi et al. 2007, Siewert et al. 2003) has been reviewed, however, those include orally disintegrating tablets, chewable tablets as well as chewing gums, but not oral films. To address this lack, different methods have been developed in the present work. Approaches with USP type 1 (basket method) or dissolution media of less than 50 mL (Charde et al. 2008, Dinge and Nagarsenker 2008) were developed, but proposed measuring conditions were not transferable to fast-dissolving oral films. Other published approaches (see C 2.6.1) have the major problem of the large amount of dissolution medium which exceeds the natural conditions by far. Therefore, the present study tried to achieve the best agreement between the use of the common dissolution apparatus of the pharmacopoeia and a modification of this to better approach the natural conditions. Furthermore, achieving a measurement procedure which is practicable without any difficulties was essential. Another problem which arose during the studies was the lack of an adequate artificial dissolution medium for the oral cavity. A biorelevant medium for human saliva is not available. The NRF lists a formulation “Künstlicher Speichel” (artificial saliva, Neues Rezeptur-Formularium 2006), but it is used as a saliva substitute in the treatment of xerostomia and differs in viscosity and taste. Therefore it is not comparable to human saliva and may not serve as an appropriate dissolution fluid. In the literature (Morjaria et al. 2004) formulations for artificial saliva are proposed, but no uniform composition exists. To overcome this problem the dissolution medium of the dissolution test for medicated chewing gums of the Ph. Eur. was used. In the performance, it is quite important, among other things, to standardize an artificial saliva as a uniform release medium, analogous to the gastric juice, and thus to enable comparative studies at different labs. The specified gastric juice of the Ph. Eur. and the USP contains sodium chloride, pepsin and hydrochloric acid and is adjusted to a pH of 1.2.

2.7.2 Dissolution apparatus with manual sampling

Due to the missing monograph for oral films in the pharmacopoeia, a definition of fast dissolution does not exist. One oral film of each used polymer type was pre-tested for dissolution time. 250 seconds was the longest time which some of the oral films needed for complete dissolution, thus the criterion of measuring 250 seconds was estimated. Given that in some investigations a number of oral films were not completely dissolved within 250 seconds, the criterion was upgraded to 300 seconds.

The common dissolution apparatus with manual sampling only allows samples to be withdrawn at 30 seconds intervals, because it is not feasible to obtain samples within

shorter time intervals. The samples were withdrawn by a bulb pipette and diluted prior to the measurement with a spectrometer. The missing dissolution volume has to be considered and calculated within the values.

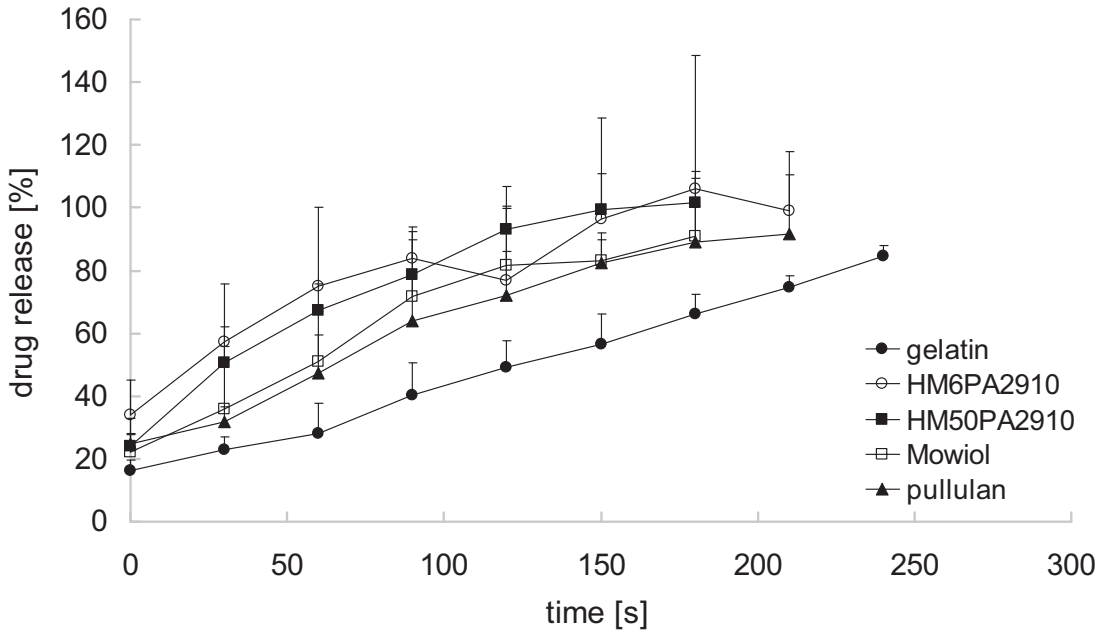


Figure 29: Drug release of oral films (formulation C) by manual withdrawing of samples, dissolution medium: phosphate buffer pH 6.0, 37 °C, 50 rpm, 272 nm (conventional UV/VIS spectrometer), mean + SD, n = 5.

The initial burst (Figure 29) is caused by the experimental design due to the samples were manually withdrawn. Within 250 seconds gelatin films released 84.5 %, Mowiol® wafers 90.9 %, pullulan wafers 91.6 %, HM6PA2910 99.0 %, and HM50PA2910 101.7 %, although all the wafers were completely dissolved. The slowest and lowest drug release was seen with gelatin films, while HM6PA2910 showed the fastest and highest drug release at the 180 second time point. The release rates are inferior compared to the sensor method due to variations between actual and nominal content of the wafers. A main disadvantage of the presented method was the low number of samples that could be withdrawn - a maximum of 9 times within the 250 seconds and thus an exact observation of the drug release profile was impossible.

2.7.3 Fiber-optic sensor system

The fiber-optic sensor system allows an on-line measurement in the dissolution vessel without sample withdrawing. Due to the required volume of 250 mL dissolution medium the positioning of the sensor is difficult. On the one hand a correct sample withdrawing according to the Ph. Eur. should be ensured and on the other hand the sensor should not touch the rotating paddle. The dissolution test was performed according to the Ph. Eur. paddle method except from the described modifications.

2.7.3.1 Differences in polymer properties

All polymer films were investigated regarding their drug release in two dissolution media, purified water and phosphate buffer pH 6.0.

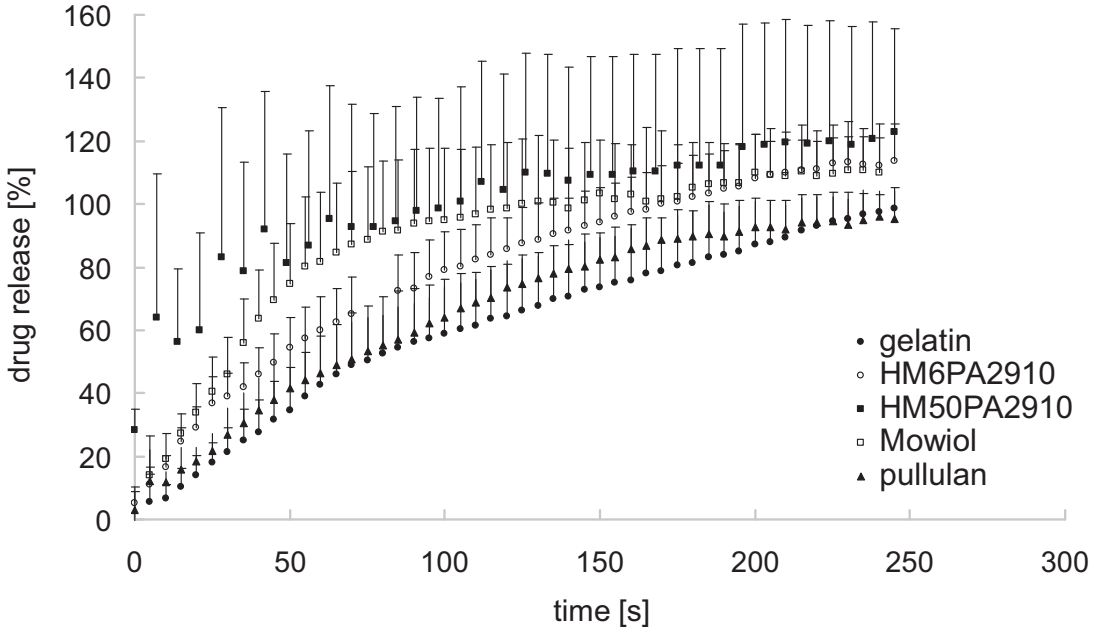


Figure 30: Drug release of polymer films of formulation C, dissolution medium: purified water, 37 °C, 50 rpm, 272 nm (fiber-optic sensor system), mean + SD, n = 5.

Given that the fiber-optic sensor allows in-line measurement without sample withdrawing, the burst decreased. The total drug release in purified water varied between 95.3 % (pullulan) and 123.0 % (HM50PA2910) which resulted from the deviation from actual to nominal content. Furthermore, as shown before, caffeine tends to recrystallization, thus varying contents arise and high deviations could arise for the same reason. HM6PA2910 showed a total drug release of 113.8 %, Mowiol® 109.9 % and gelatin 98.7 %. The ranking slightly changed compared to the method by manual withdrawing. Total drug release was observed after 250 seconds although not all films obtained 100 % drug release which is caused by a minor content. The results for drug release of oral films made of gelatin are in accordance with the determination of content uniformity during storage (C 2.9.2) in which gelatin films showed minor content.

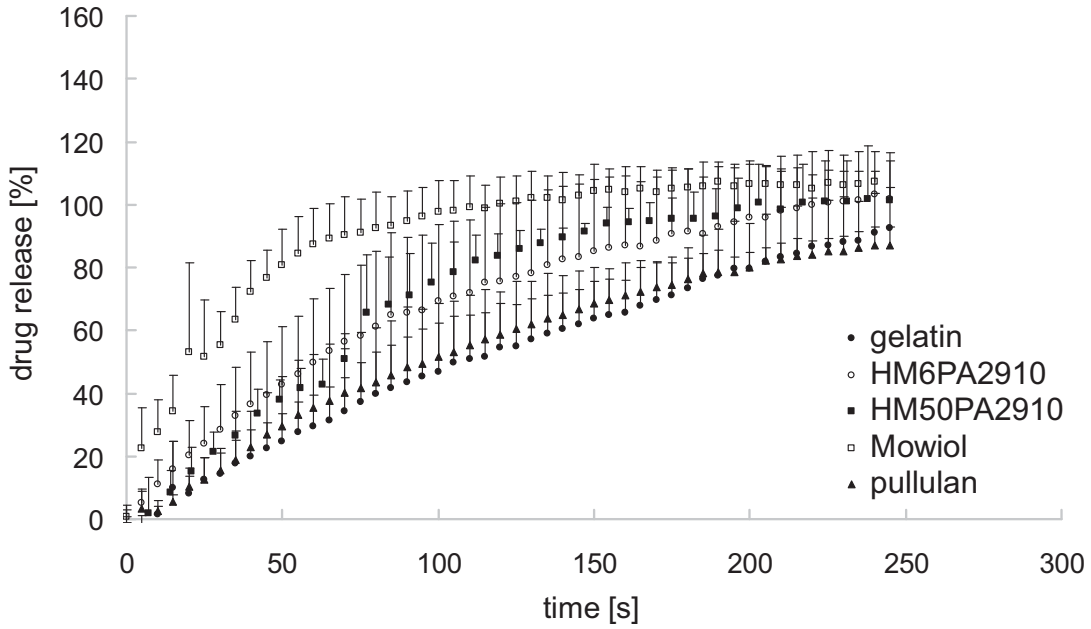


Figure 31: Drug release of polymer films of formulation C, dissolution medium: phosphate buffer pH 6.0, 37 °C, 50 rpm, 272 nm (fiber-optic sensor system), mean + SD, n = 5.

In phosphate buffer pullulan films achieved a total release of 87.1 % and gelatin films 92.5 %. The other three polymers achieved in descending order the following total drug release: Mowiol® 107.3 %, HM50PA2910 105.6 % and HM6PA2910 with 101.9 % of the nominal content.

As seen in Figure 30 and 31 the oral wafers dissolved and released drug immediately after placing an oral film into the vessel. Furthermore, it can be seen that high standard deviations arose which could be have been caused by the sensor touching the rotating paddle. The highest standard deviations had HM50PA2910. In conclusion the total drug release in purified water for all investigated polymers was higher than in phosphate buffer. The deviation from actual to nominal content was not considered in both cases. A major reason is probably the coincidental higher drug content within the oral wafers measured in purified water.

2.7.3.2 Influence of dissolution fluid

Oral wafers of each polymer were investigated in the two dissolution media. Furthermore, oral wafers were prepared with surfactant to investigate changes in dissolution behavior. Surfactant-containing films were only investigated within phosphate buffer pH 6.0. Gelatin, HM6PA2910 and HM50PA2910 films were compared respectively (Figure 32-34).

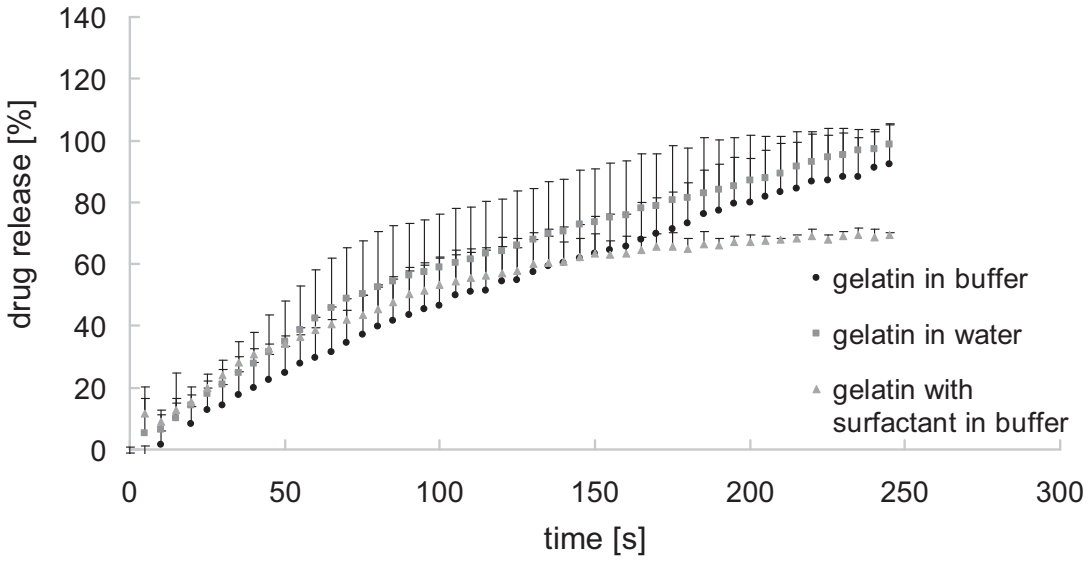


Figure 32: Drug release of gelatin oral wafers of formulation C, 37 °C, 50 rpm, 272 nm (fiber-optic sensor system), mean + SD, n = 5.

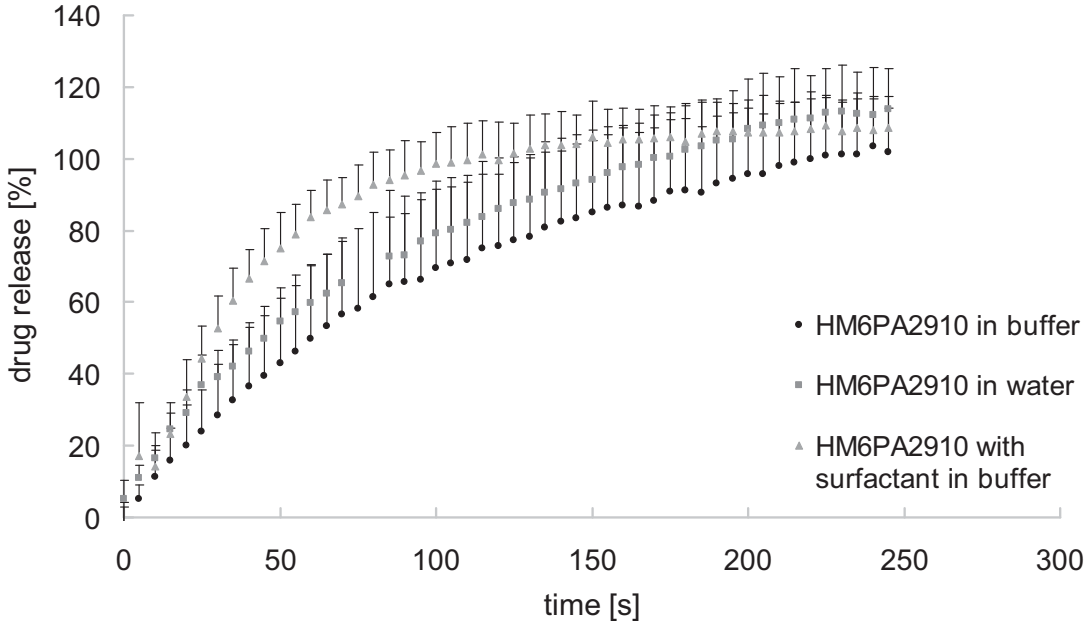


Figure 33: Drug release of HM6PA2910 oral wafers of formulation C, 37 °C, 50 rpm, 272 nm (fiber-optic sensor system), mean + SD, n = 5.

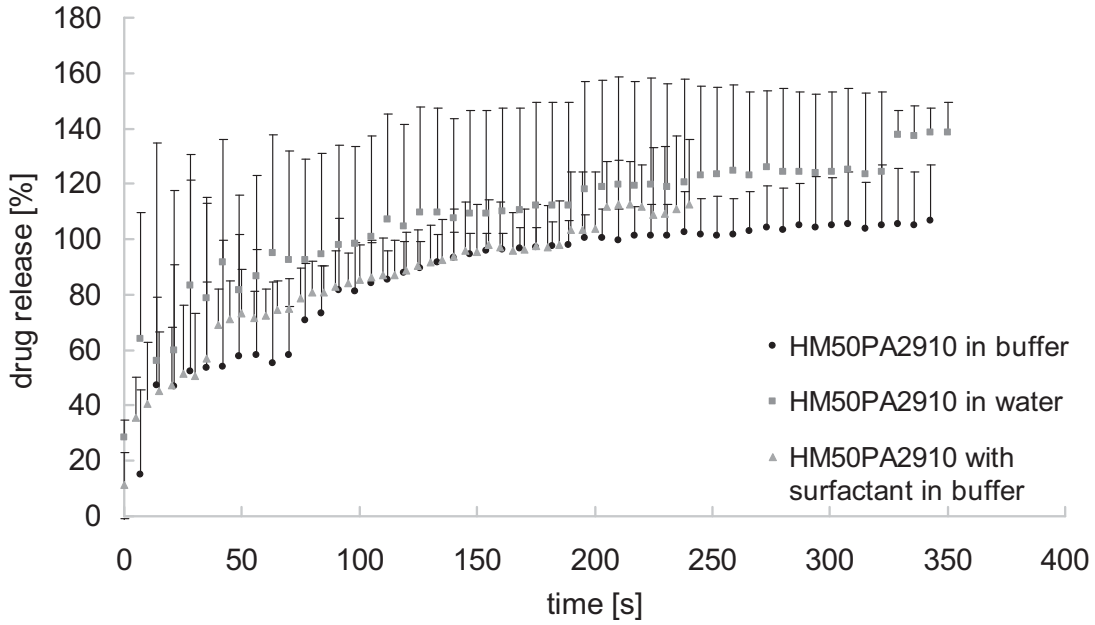


Figure 34: Drug release of HM50PA2910 oral wafers of formulation C, 37 °C, 50 rpm, 272 nm (fiber-optic sensor system), mean + SD, n = 5.

In the case of gelatin the wafers with surfactant showed fastest onset, but lowest total drug release. HM6PA2910 without surfactant in phosphate buffer pH 6.0 showed results similar to the HM6PA2910 wafers with surfactant. The latter also showed faster onset. However, the addition of surfactant to the oral films led to higher total drug release compared to the surfactant-free films. This fact enforces the assumption that drug content varies accidentally.

As mentioned above the oral wafers of HM50PA2910 showed high standard deviations. Thus, trends could not be seen compared to the other two polymer types (gelatin, HM6PA2910). However, it was evident that the oral wafers of HM50PA2910 with surfactant dissolved completely within 250 seconds whereas the films without surfactant needed 350 seconds to dissolve in each of the dissolution media.

In conclusion, the addition of surfactant to the wafers leads to faster dissolution of gelatin and HM6PA2910 films. As can be seen and mentioned above the total drug release of the polymer films was always higher in purified water than in phosphate buffer which presumably occurred by accident.

2.7.3.3 Determination of dissolution kinetics

By using the fiber-optic measurement setup, it became possible to investigate the drug release profiles. The prepared oral wafers were investigated for their drug release profiles in different dissolution media. After the dissolution tests their release kinetics were evaluated.

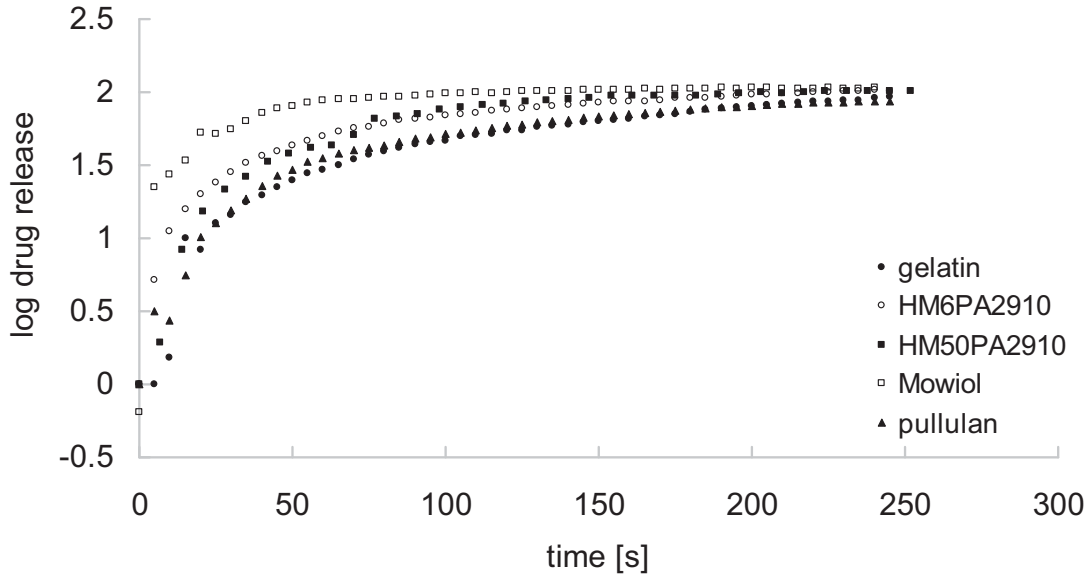


Figure 35: Drug release profiles of oral wafers modeled for 1st order kinetics (formulation C), dissolution medium: phosphate buffer pH 6.0, 37 °C, 50 rpm, 272 nm (fiber-optic sensor system), mean, n = 5.

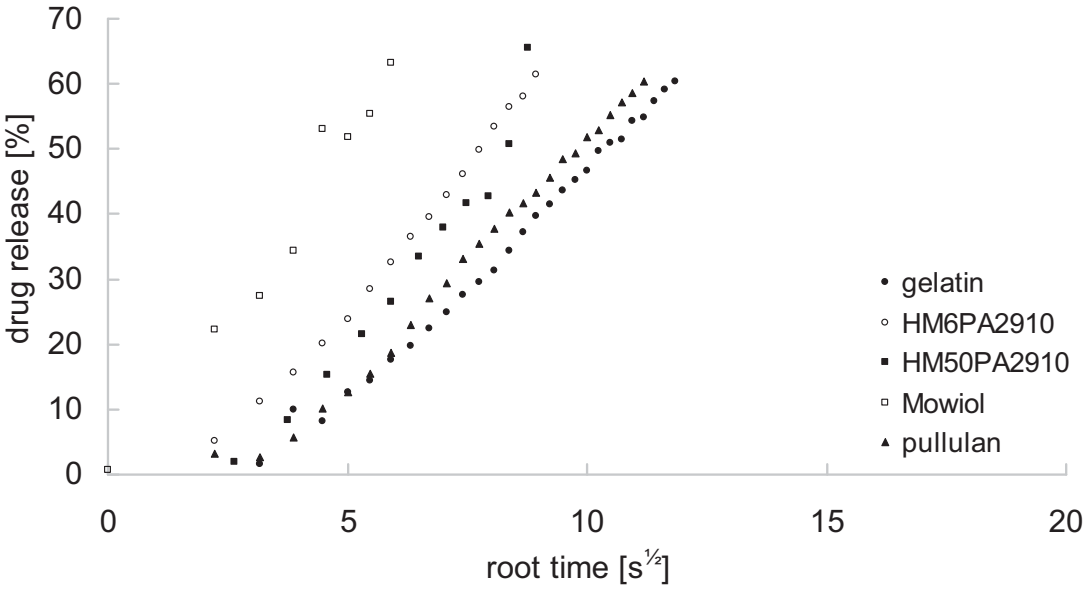


Figure 36: Drug release profiles of oral wafers modeled for root-time kinetics (formulation C), dissolution medium: phosphate buffer pH 6.0, 37 °C, 50 rpm, 272 nm (fiber-optic sensor system), mean, n = 5.

As seen in Figure 30 and 31 the drug release of oral wafers obviously does not follow zero order kinetics. Therefore, the data were analyzed for first order and root-time kinetics. A logarithmic ordinate (Figure 35) offered no linear correlation indicating first order kinetics. The coefficient of determination for pullulan in the root-time kinetics

graph ($R^2_{pullulan}$ = 0.966) was much better, so the root-time kinetics was found to describe the drug release kinetics at least for pullulan. For Mowiol® and HM50PA2910 the curves fit less than all other polymers which resulted from the observed high standard deviations during measurement. The oral wafers showed a similar correlation when water was used as the dissolution fluid and corresponded to the root-time kinetics ($R^2_{pullulan}$ = 0.963), too. In the literature (Perumal et al. 2008b) Higuchi's square-root model has been mainly proposed as the drug release kinetics occurring, which can be confirmed by the present studies.

2.7.4 Dissolution apparatus with modified sampling

2.7.4.1 Drug release profile

The method of withdrawing samples for dissolution testing is not specified in the pharmacopoeia. Common dissolution testing lasts over time periods from hours to days. Thus, sample withdrawal each minute using glass capillaries with filters is sufficient to simulate dissolution processes and generally used. In fast dissolution sample withdrawal and analysis is required more often. Since sample withdrawal is not required for the fiber-optic sensor system, sampling limitations were overcome. In the present work samples were withdrawn each second and analyzed by a spectrometer in situ. However, this modified setup exhibited several problems. The wafers were often stuck on the filters and gave incorrect results. These curves showed rapidly increasing values and decreased afterwards and therefore were not included in the analysis. Furthermore, it must be considered that it is not an on-line measurement, which is actually important for a fast-dissolving dosage form. The dissolution medium has to pass through apparatus tubing and as such the time taken for the sample to reach the spectrometer varies. In this study short tubes were used so the delay time was only 15 seconds.

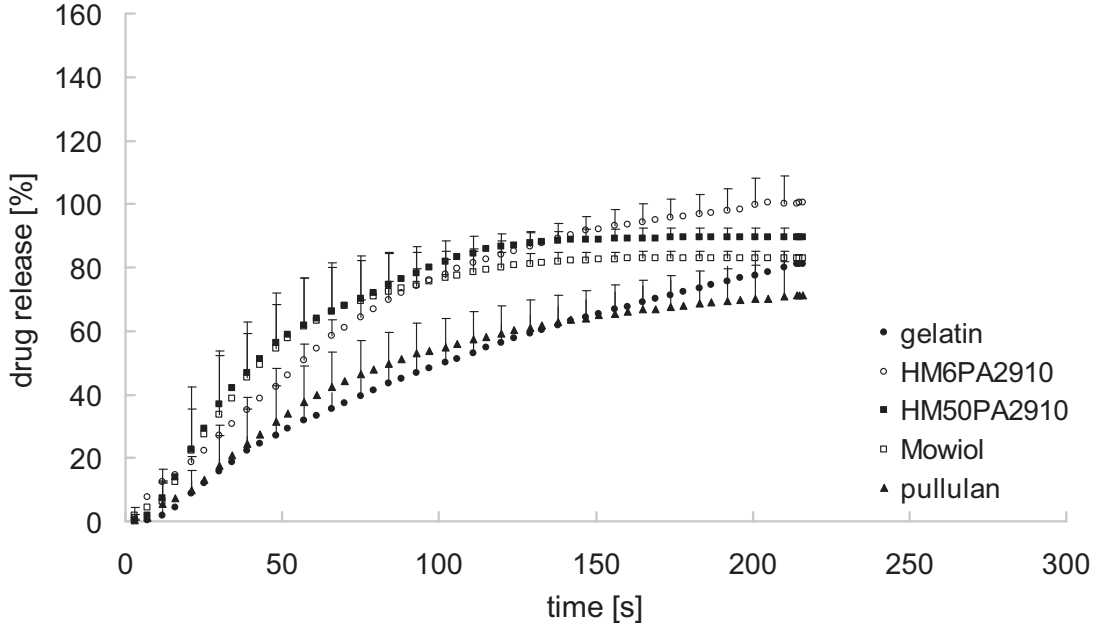


Figure 37: Drug release of oral films (formulation C) by modified withdrawing of samples, dissolution medium: phosphate buffer pH 6.0, 37 °C, 50 rpm, 272 nm (conventional spectrometer), mean + SD, n = 5.

In this measurement setup the actual API content was considered, but the actual to nominal film weight was not considered, and thus the release rates were still too low. The lowest value was shown with pullulan with 71.3 % total drug release, followed by gelatin films (81.2 %) with the slowest drug release from Mowiol® with 83.1 %. 100 % release was only achieved by HM6PA2910 (100.4 %). HM50PA2910 showed 89.4 % total release after 250 seconds.

The oral wafers from formulation C were also investigated by this method during storage. Since the curves did not differ from the depicted graph, the results are not shown. All investigated films dissolved in less than 300 seconds.

This method of measurement allowed observation of fast dissolution instances. However, samples are withdrawn and analyzed after a 15 second delay compared to the fiber-optic sensor. In conclusion, both methods are suitable for dissolution testing of fast dissolution.

Further dissolution tests were performed with varying amount of API to investigate the effect of drug concentration on dissolution behavior. Furthermore, the hydrodynamics were modified by using a sinker and varying the rotational speed.

2.7.4.2 Varying content

Polymer films of gelatin (Figure 38) and HM6PA2910 (Figure 39) were manufactured with varying amounts of anhydrous caffeine. Prepared wafers had nominal drug

contents of 2.5 mg, 5.0 mg, 7.5 mg and 10.0 mg caffeine base. The effect of varying the drug concentration on the drug release profiles was investigated.

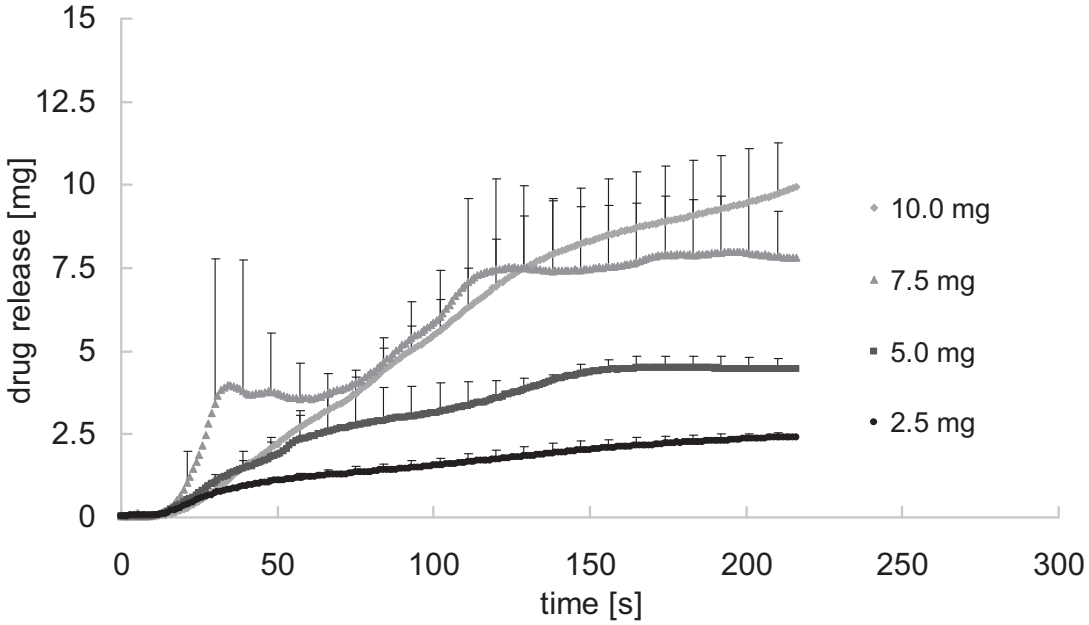


Figure 38: Drug release of gelatin films (formulation C) with varying amount of API, dissolution medium: phosphate buffer pH 6.0, 37 °C, 50 rpm, 272 nm (conventional spectrometer), mean + SD, n = 5.

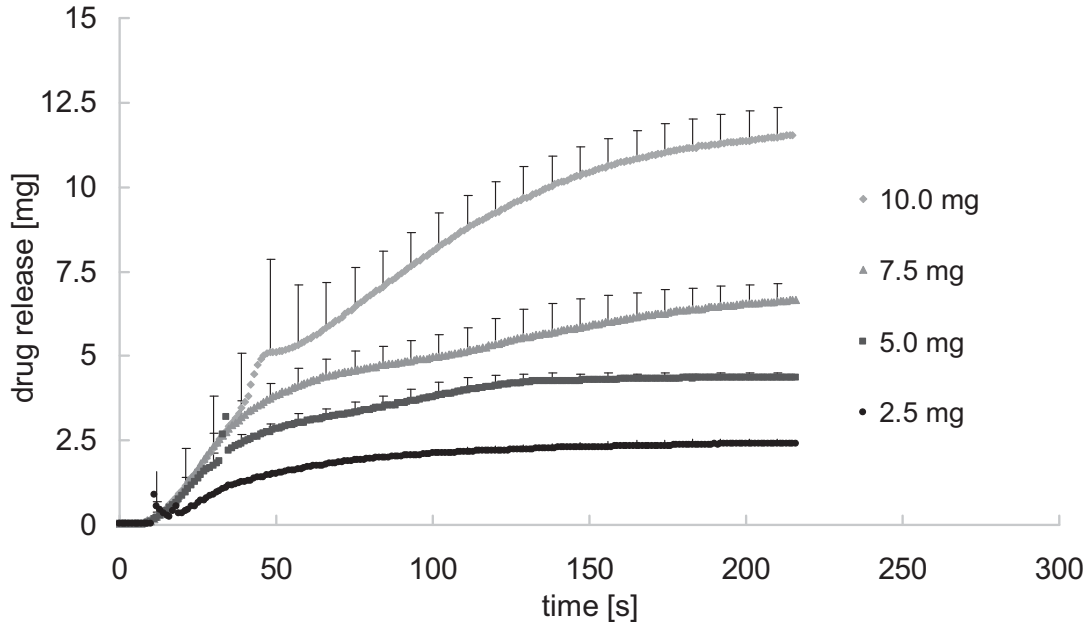


Figure 39: Drug release of HM6PA2910 films (formulation C) with varying amount of API, dissolution medium: phosphate buffer pH 6.0, 37 °C, 50 rpm, 272 nm (conventional spectrometer), mean + SD, n = 5.

It can be seen that the actual content highly deviates from the nominal content, especially for HM6PA2910 films. All investigated oral films dissolved completely in less than 250 seconds. Furthermore, with increasing drug content an increase in the standard deviations was observed. The dissolution curves for various drug-loaded films were comparable and thus, the drug load does not influence the dissolution profile of the oral films.

2.7.4.3 Sinker

A small stainless steel mesh (0.71 x 0.71 mm mesh, 0.315 mm wire diameter, 1.3 x 1.3 x 2.7 cm size) was used as a sinker. One oral wafer was set into the sinker then placed into the dissolution medium and the measurement started. The sinker was used to investigate whether the standard deviation could be reduced by preventing films adhering to a surface and ensuring they are positioned at the bottom of the dissolution vessel. Furthermore, dissolution behavior/drug release profiles were analyzed additionally to ascertain the influence of the sinker.

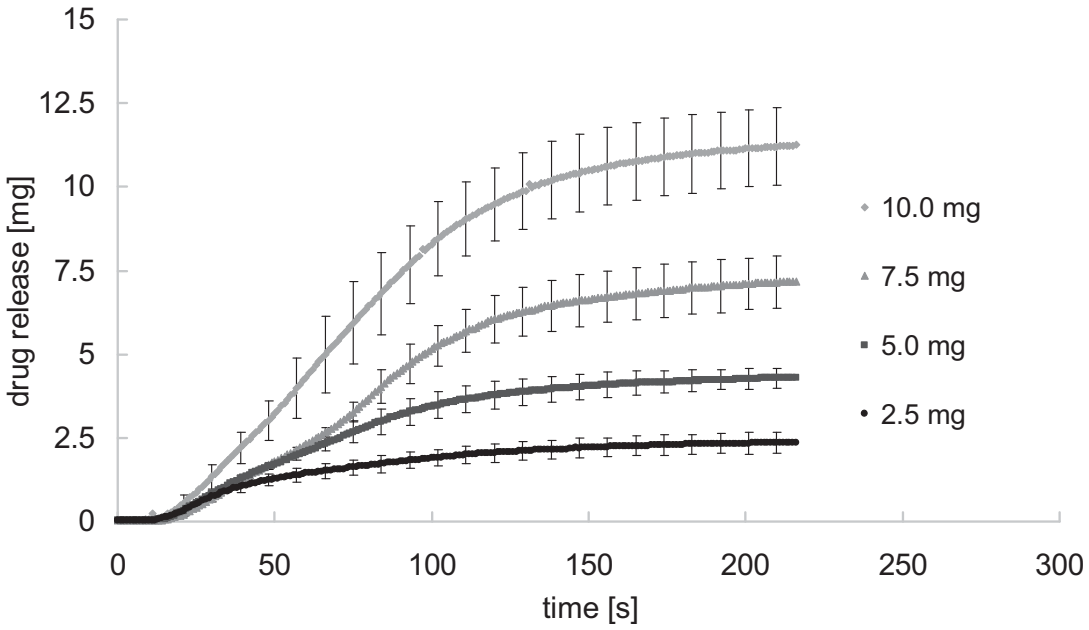


Figure 40: Drug release of gelatin films (formulation C) by use of a sinker, dissolution medium: phosphate buffer pH 6.0, 37 °C, 50 rpm, 272 nm (conventional spectrometer), mean ± SD, n = 5.

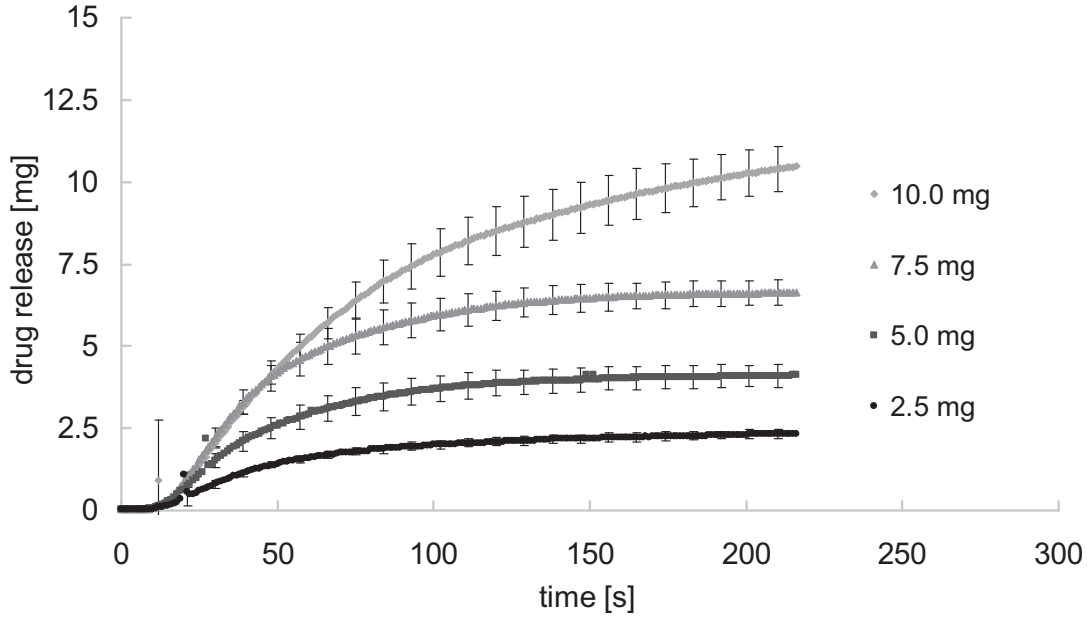


Figure 41: Drug release of HM6PA2910 films (formulation C) by use of a sinker, dissolution medium: phosphate buffer pH 6.0, 37 °C, 50 rpm, 272 nm (conventional spectrometer), mean ± SD, n = 5.

The dissolution data obtained using a sinker were analyzed for zero-order, first-order and root-time kinetics (data not shown). The results were the same as described above. The root-time kinetics was found to be the best for describing kinetics of drug release of oral wafers, although the fitting is still rather poor.

Gelatin films showed with increasing drug content a decrease in the y-axis intercept in which the coefficient of determination hardly altered. The curve progression was not significantly influenced by changing the drug content.

The coefficient of determination of HM6PA2910 films was worse than those of gelatin films, but still better than those of zero and first order kinetics. The slope and y-axis intercept showed no systematic effect.

In comparison to the conventional proceeding, use of a sinker improved the standard deviation and ensured the same position of all wafers during measurement. The sinker does not influence the drug release kinetics and is a mandatory and meaningful tool to obtain comparable data.

2.7.4.4 Changing rotational speed

The hydrodynamic properties were varied to evaluate possible changes in dissolution behavior.

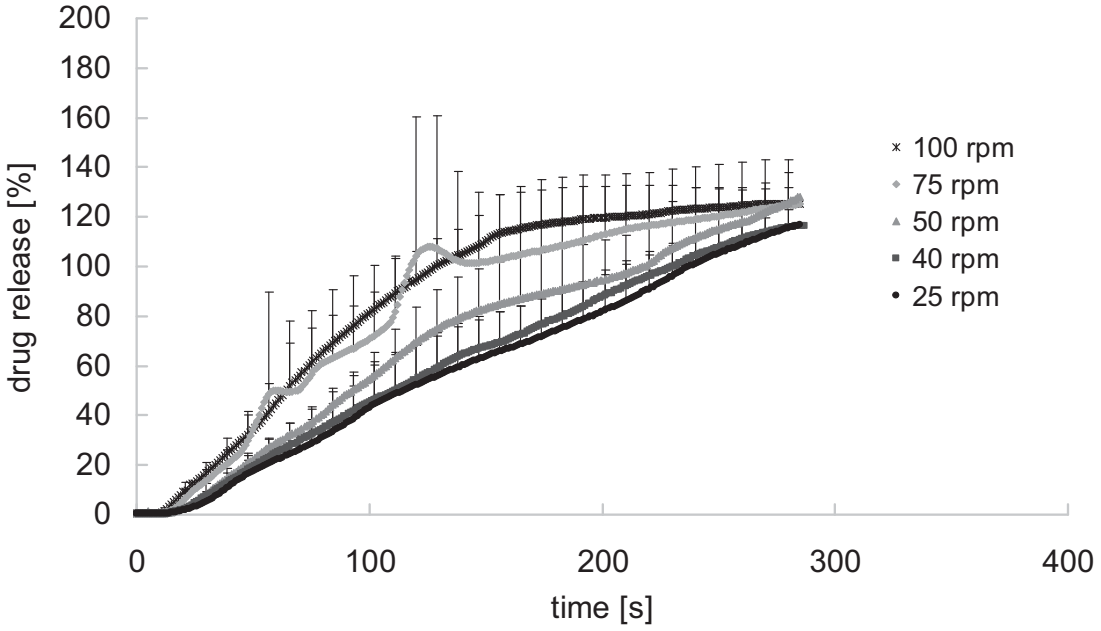


Figure 42: Drug release profiles for gelatin wafers by changing rotational speed, dissolution medium: phosphate buffer pH 6.0, 37 °C, 272 nm (conventional spectrometer), mean + SD, n = 5.

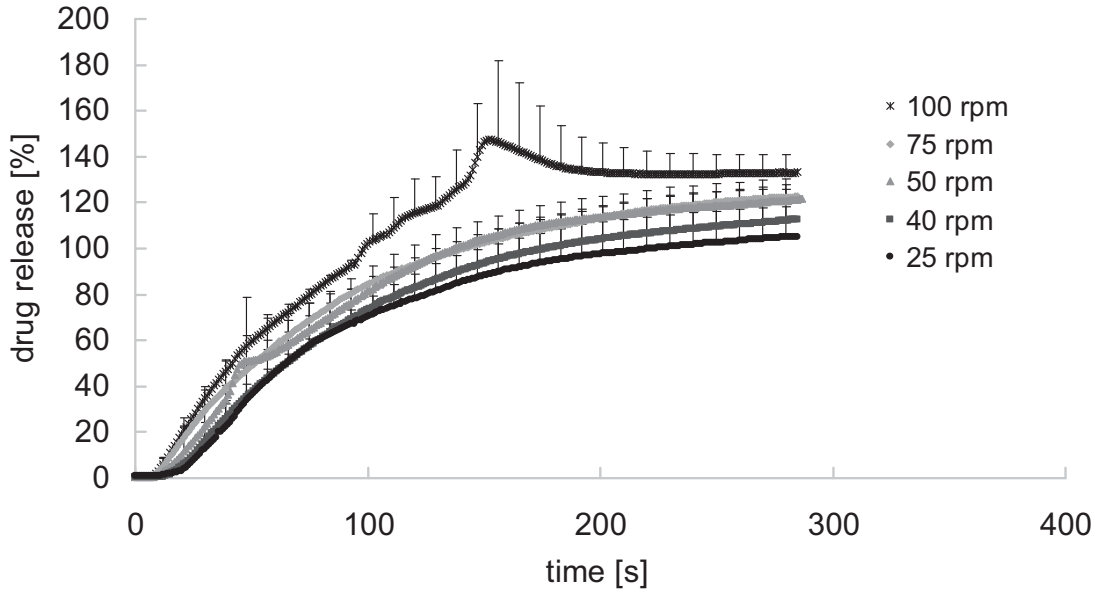


Figure 43: Drug release profiles for HM6PA2910 wafers by changing rotational speed, dissolution medium: phosphate buffer pH 6.0, 37 °C, 50 rpm, 272 nm (conventional spectrometer), mean + SD, n = 5.

It was found that with increasing rotational speed the oral wafers showed faster onset of drug release. Varying content within the films and therefore varying total drug release is caused by the recrystallization of the API. Gelatin wafers showed ~ 116 % drug release at 25 and 40 rpm whereas at 50, 75 and 100 rpm ~ 125 % was released. The oral wafers made of HM6PA2910 showed increasing drug release with increasing rotational speed. These data exhibit that the test conditions affect the results, but nevertheless the drug release kinetics remains the same and complete dissolution of the oral wafers was achieved before the criterion of 300 seconds.

2.7.4.5 Conclusion

As a criterion for fast dissolution is not prescribed, it was tested and defined. In all the tested method the wafers dissolved completely within 300 seconds. Depending on the polymer type and the film height the dissolution profiles differed. The measurements with the fiber-optic sensor and the common dissolution apparatus with manual sampling did not consider the deviations from actual to nominal API content. Furthermore, the nominal to actual film weight was not considered in all methods as well. The different positions of the wafer within the vessel are also problematic. After placing the wafer in the dissolution vessel, the film moves to different locations: at the paddle, at the vessel, at the surface, at the sampling probe, at the sensor or – preferably – at the bottom of the vessel.

A method which regards the drug release in time was only achieved with the fiber-optic sensor system. The common dissolution apparatus with a modified procedure for sampling showed advantages compared to the conventional setup. Samples were withdrawn every second and a precise diagram of the dissolution behavior can be presented. The curves of both methods are similar. Calculations of the obtained data revealed that the drug release from oral wafers best follow the Higuchi's square-root model, although the fitting is still rather poor.

The use of a sinker avoids sticking of the oral wafers at the paddle, vessel walls, sampling probe or sensor and thus reduces the standard deviation of the measurements. The change of rotational speed leads to changes in the onset of drug release, but complete dissolution is not influenced. Summing up, both modifications of dissolution testing did not influence the drug release kinetics.

2.7.5 Disintegration in human oral cavity

Given that dissolution testing is not comparable to natural conditions, the oral wafers were assessed by adult volunteers. As with the disintegration of the oral film the API caffeine dissolves within saliva and the terms disintegration and dissolution were used synonymously.

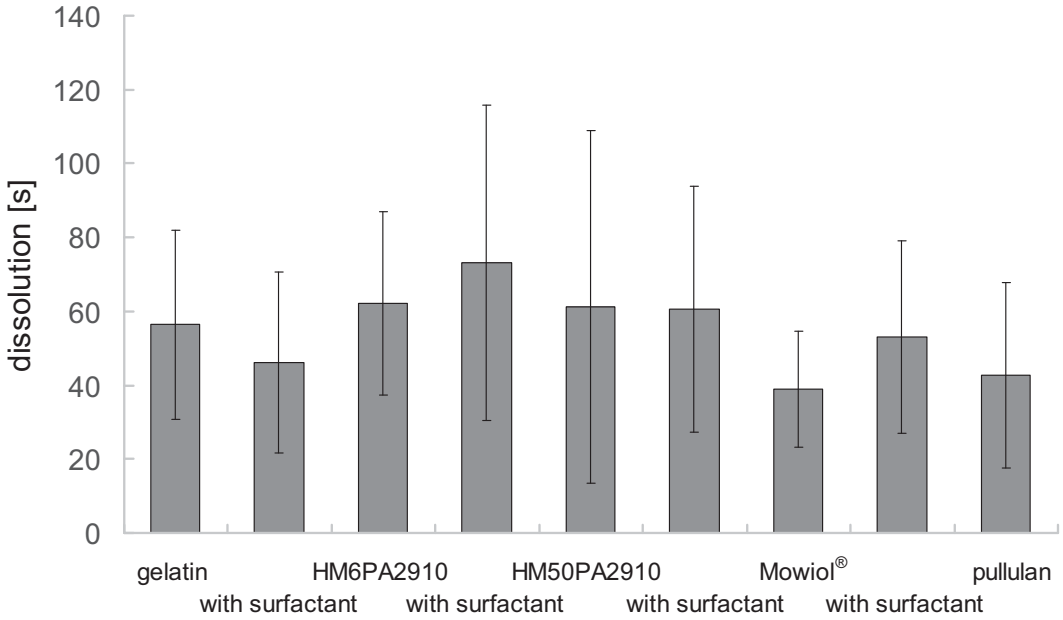


Figure 44: Dissolution times of oral films (formulation C) from human taste panel with adult volunteers, mean ± SD, n = 15.

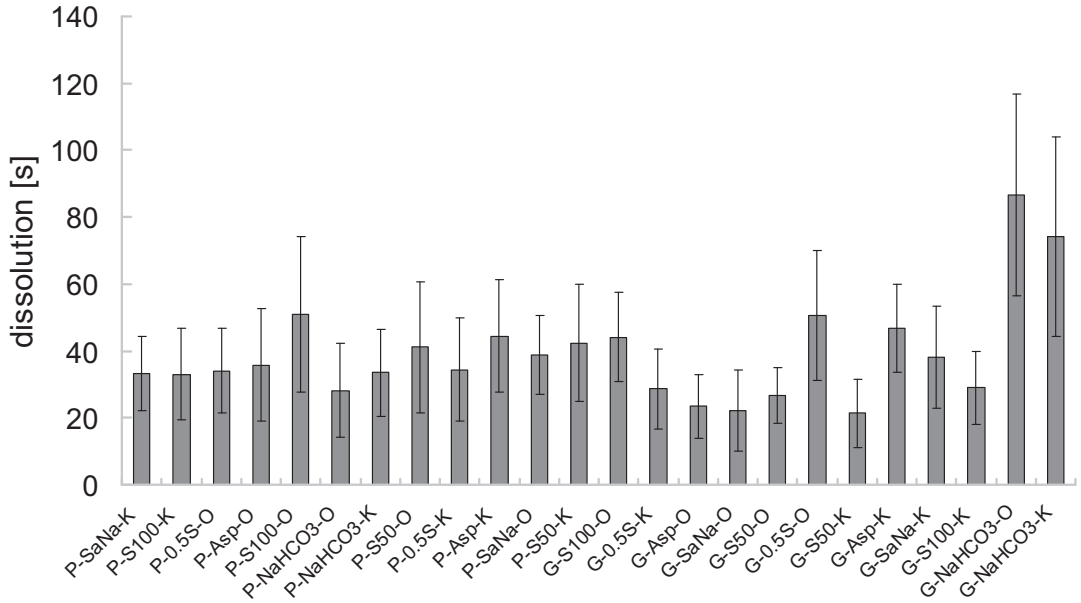


Figure 45: Dissolution times of oral films (formulation C') from human taste panel with adult volunteers, mean ± SD, n = 15.

The results from Figure 44 and 45 show that the oral wafers dissolved in less than 90 seconds on average and thus dissolution was much faster than in the *in vitro* dissolution testing above. The overall mean of the dissolution time was about 55 seconds for formulation C and about 39 seconds for formulation C'. It can be concluded that a transfer of the analytical data of the applied systems to *in vivo* disintegration/dissolution is not possible. Furthermore, the advancement of formulation C did not only lead to better taste, but also to faster dissolution in formulation C'.

2.8 Other properties

2.8.1 Rheological behavior

The rheological properties of drug-free and drug-containing film solutions of formulation C were investigated for possible consequences on film casting using a rotational viscometer. The solutions studied were those that were later used for casting films (see D 2.1.2).

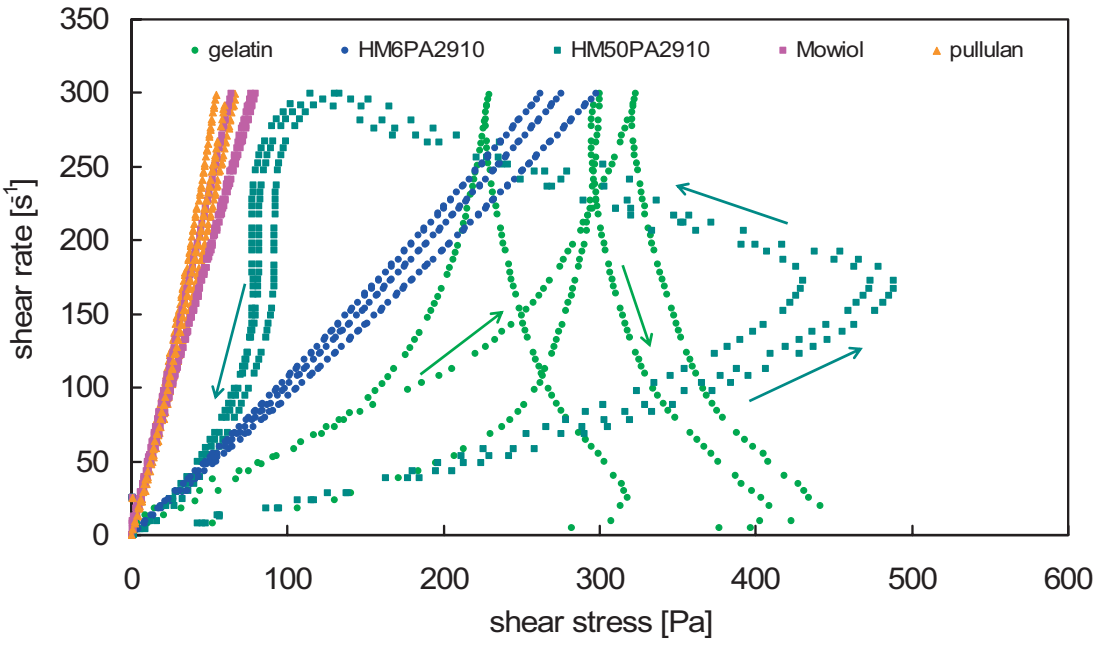


Figure 46: Rheogram of film solutions without API made from formulation C, n = 3.

The rheograms of the API-free formulations, displayed in Figure 46, show that HM6PA2910, Mowiol® and pullulan film solutions had ideal rheological behavior. For each of these three film solutions the rheogram is a straight line passing the point of origin. In comparison, film solutions of gelatin and HM50PA2910 differ in their up- and downwards curve. When considering the viscosity curves (data not shown, analog shear rate D = 0 – 300 s^{-1}) the viscosity of the gelatin film solution decreases by

increasing shear rate and remains at this value after decreased shear rate. The observed phenomenon is called rheodestruction. The viscosity of the HM50PA2910 film solution decreases during increasing shear rate and increases during decreasing shear rate pointing to thixotropic behavior. Comparing the viscosities (data not shown, D = 0 – 300 s^{-1}) of the API-free film formulations, Mowiol® and pullulan solutions showed lowest viscosities (~ 0.25 respective ~ 0.2 Pa·s, D = 0 – 300 s^{-1}), followed by HM6PA2910 (~ 1 Pa·s, D = 0 – 300 s^{-1}). Film solutions with HM50PA2910 showed an initial viscosity of about 6 Pa·s (D = 0 – 300 s-1) and gelatin film solution initially showed the highest viscosity of about 80 Pa·s (D = 0 – 300 s^{-1}).

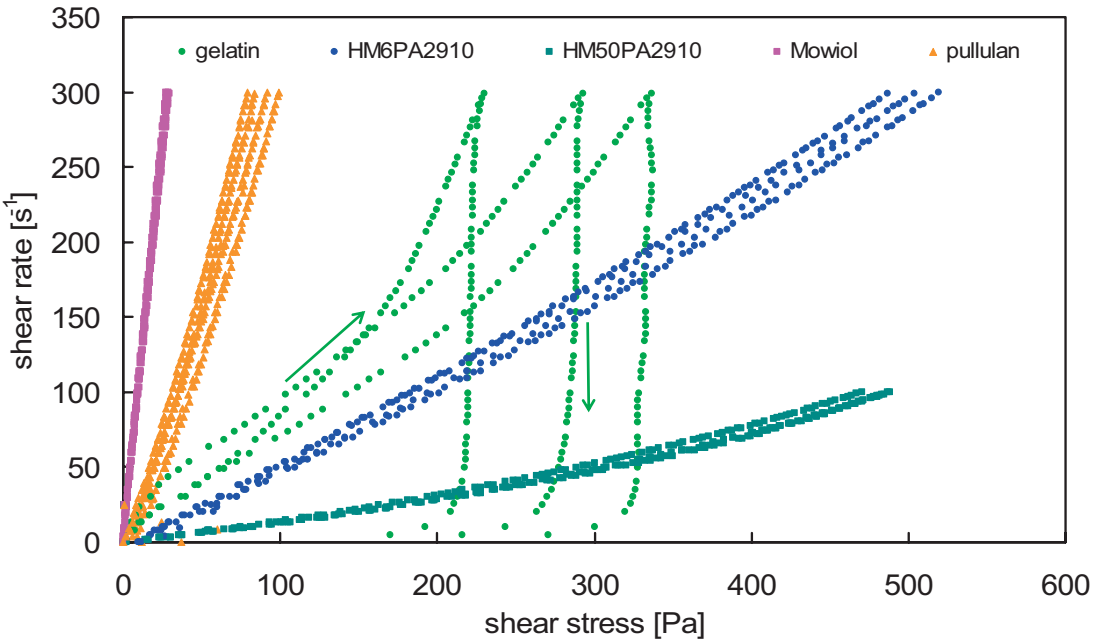


Figure 47: Rheogram of film solutions with API incorporated into formulation C, n = 3.

It can be seen in Figure 47 that the film solution of Mowiol® with API shows ideal viscous behavior again due to the straight line through the point of origin. However, drug-loaded Mowiol® film solution had a lower viscosity (data not shown, D = 0 – 300 s^{-1}) of ~ 0.1 Pa·s compared to the API-free formulation. Drug-loaded HM6PA2910 and pullulan solutions showed slightly thixotropic behavior in contrast to the API-free film solutions. Furthermore, slightly higher initial viscosities (HM6PA2910: 6.5 Pa·s, pullulan 4 Pa·s, D = 0 – 300 s^{-1}) in contrast to the drug-free solutions were observed for both. The viscosity of the film solution of HM50PA2910 is increased by the API (~ 10 Pa·s, D = 0 – 100 s^{-1}), thus a shear rate above 100 s^{-1} could not be measured. Gelatin film solution with API showed similar curves compared to the API-free solution. During shearing the viscosity decreased from ~ 56 Pa·s (D = 0 – 300 s^{-1}) by increasing shear rate and remained at the lower values after decreased shear rate.

Adding of API led to lower as well as higher viscosities of the film solutions depending on the respective polymer. Rheological behavior differs between the different film

solutions and it can be concluded that no standard solution can be used for manufacturing of oral wafers.

2.8.2 pH value

The pH-value was determined by dissolving one oral film in 2 mL distilled water and measuring the pH of the obtained solution. Differences were expected because various polymers were used as well as the addition of API.

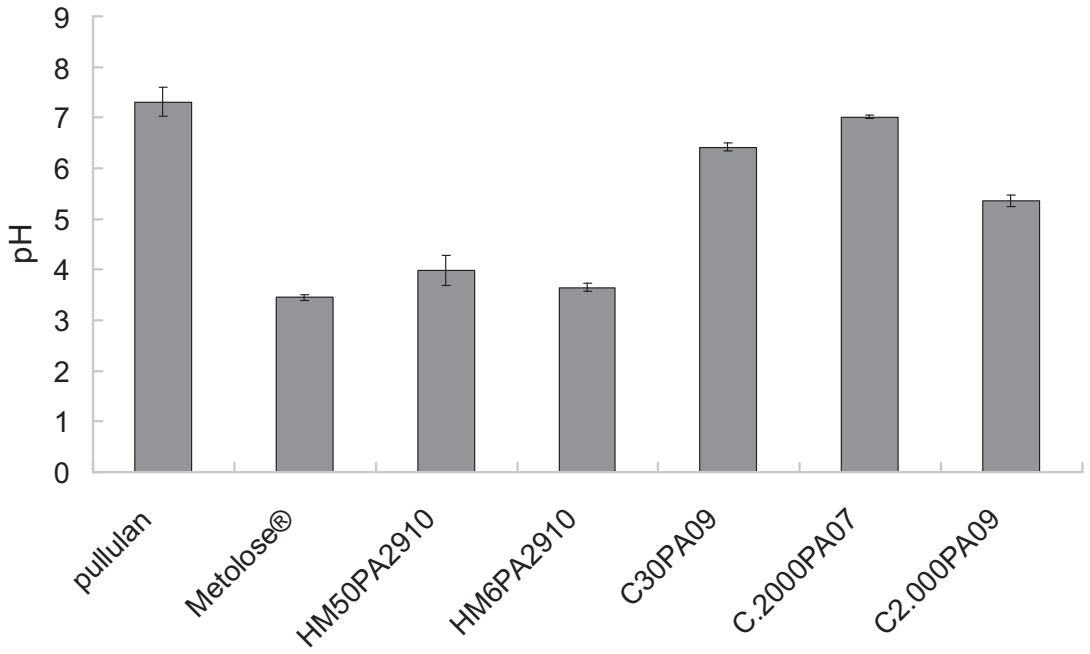


Figure 48: pH of solutions from drug-free polymer films (formulation A and B), mean ± SD, n = 5.

As formulation B (for pullulan oral films only) does not contain citric acid, the pH of this solution was the highest with an average pH of 7.3. From formulation A the CMC solutions show pH-values in neutral range between 5.4 (C2.000PA09) and 7.0 (C2.000PA07). Although, HPMC is a non-ionic polymer, surprisingly, the HPMC grades showed lower values of the solutions with 3.5 (Metolose®), 3.7 (HM6PA2910) and 4.0 (HM50PA2910) compared to the CMC.

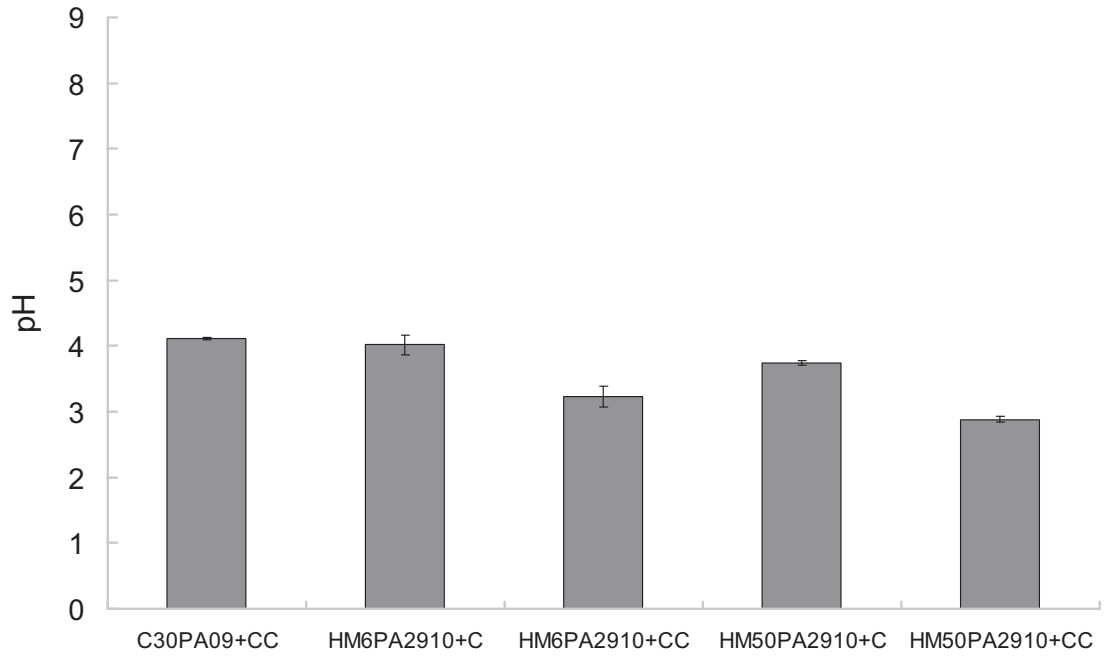


Figure 49: pH of solutions from drug-loaded polymer films (formulation A), (C = caffeine base, CC = caffeine citrate), mean ± SD, n = 5.

In contrast to the solutions of drug-free films, the pH of the solution with drug-loaded films of CMC (C30PA09) was considerably influenced by the incorporated caffeine citrate. The pH decreased from 6.4 to 4.1. The HPMC grades, HM6PA2910 and HM50PA2910, showed similar values compared to the solutions with drug-free oral films (Figure 48). However, incorporated caffeine citrate decreased the pH-value for both HPMC solutions in contrast to caffeine base.

2.8.3 Osmolality

Given that a high osmotic pressure has been described in the literature as causing the development of necrotizing enterocolitis in premature infants, neonates and toddlers, the osmolality of a liquid drug formulation has to be considered. It is the most frequent case of emergencies in neonatology and leads to intestinal necrosis culminating in sepsis. A threshold of 1000 mosmol/kg has been proposed for cell necrosis (Bruns 2006), the optimal value is assumed to be 350 mosmol/kg.

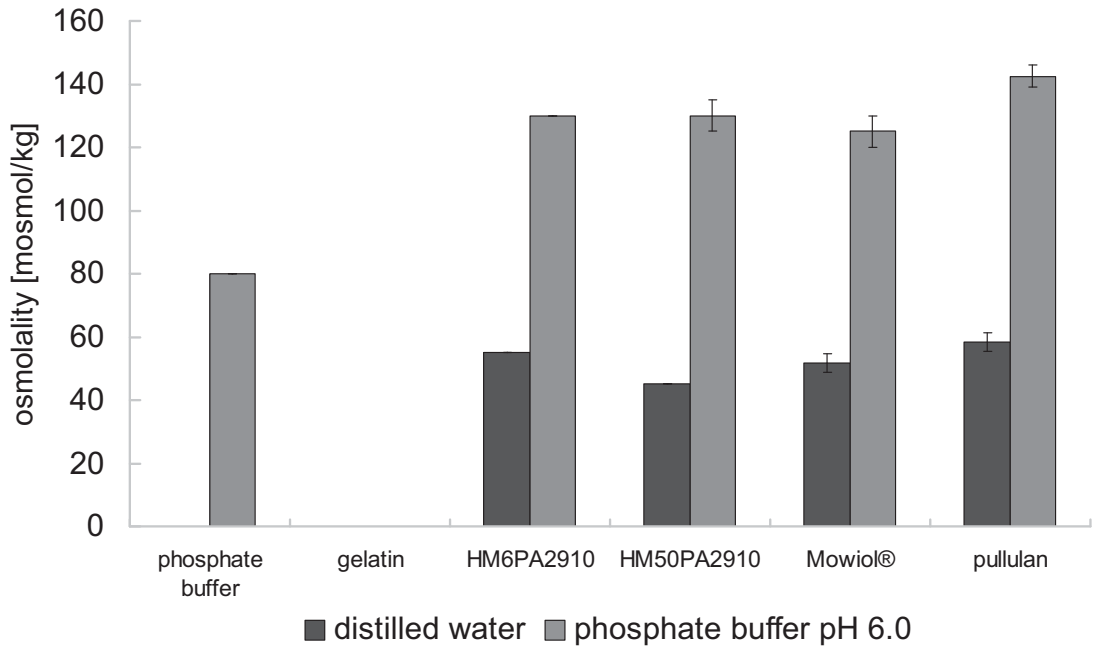


Figure 50: Osmolalities of solutions prepared from drug-loaded oral wafers of formulation C dissolved within 2 mL testing media, mean ± SD, n = 3.

The results from the measurement of osmolalities showed (Figure 50) that the solution with the highest value was still far below the threshold of 1000 mosmol/kg. Films made of gelatin could not be measured due to gelification of the solution by super cooling and thus the samples could not be frozen. As the phosphate buffer pH 6.0 itself has an osmolality of 80 mosmol/kg the osmolalities of solutions from oral wafers within phosphate buffer are higher than those dissolved in distilled water. The osmolalities ranged from 45 to 145 mosmol/kg. HM50PA2910 in distilled water showed the lowest value (45 mosmol/kg) and pullulan in phosphate buffer pH 6.0 showed the highest value (145 mosmol/kg).

Osmolalities of solutions from drug-free oral films of formulation A and B (data not shown) ranged between 0 and 10 mosmol/kg. Thus the higher values of the solutions from drug-loaded films were mainly due to addition of API.

The osmolalities of the investigated solutions are clinically negligible. The threshold value of 1000 mosmol/kg which is supposed to be responsible for necrotizing enterocolitis in children was not reached by far. It can be concluded that no risk of developing necrotizing enterocolitis is associated with the use of oral wafers in pediatrics.

2.8.4 Content

Preliminary studies of drug-loaded oral films were performed with three polymers namely C30PA09, HM6PA2910 and HM50PA210 from the basic formulation A. The free base of caffeine as well as the more soluble salt caffeine citrate were chosen as API. It was only possible to manufacture drug loaded films using the two HPMC

grades. Attempts to manufacture CMC films with anhydrous caffeine failed because the films converged during drying.

A drug load of 10 mg per oral film (6 cm²), which corresponds to 1.67 mg/cm², should be achieved. The ratio of molar masses of 1:2 for caffeine base (194.19 g/mol) to caffeine citrate (386.62 g/mol) was considered, thus double the amount of caffeine citrate was weighed in. All film solutions were cast with a width of 500 µm. The oral films were only initially investigated for their drug content. The content of the oral wafers was determined by high performance liquid chromatography (HPLC).

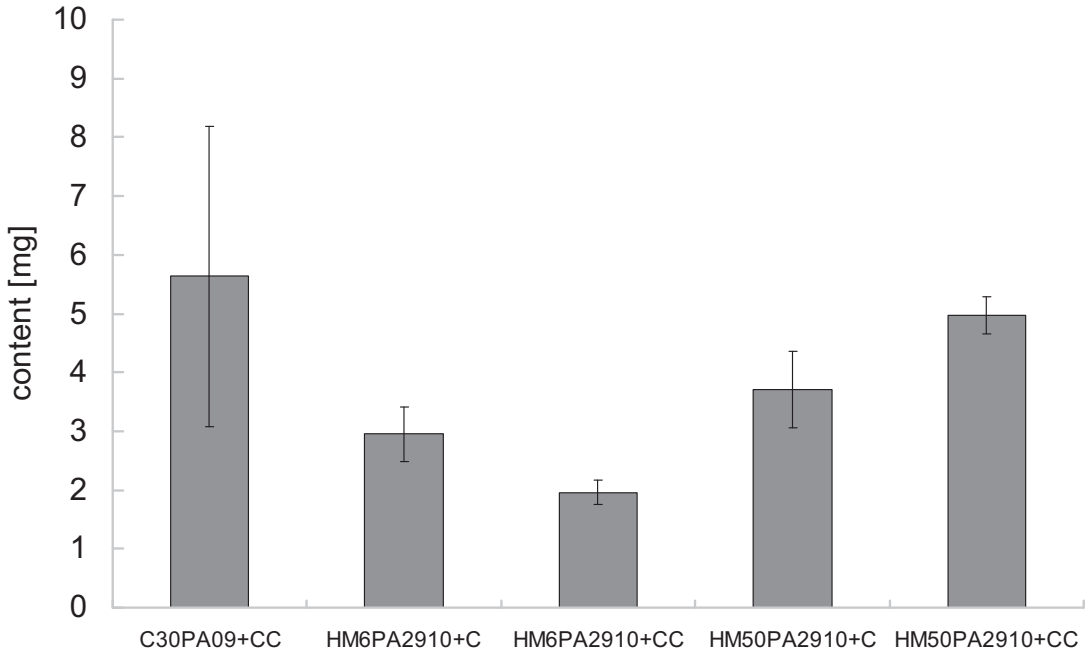


Figure 51: Content of drug-loaded oral wafers of formulation A, (C = caffeine base, CC = caffeine citrate), mean ± SD, n = 5.

As can be seen in Figure 51 no polymer film achieved the nominal content of 10 mg per film (6 cm²). The lowest content showed HM6PA2910+CC films with 0.17 mg/cm², followed by wafers made from HM6PA2910+C with 0.5 mg/cm². C30PA09+CC films showed highest values (0.93 mg/cm²), but the highest standard deviation as well. Presumably the reason was recrystallized caffeine citrate within the polymer films that resulted in an inhomogeneous distribution of API. The approach to determine drug content of 10 mg per 6 cm² once, considering the weight of films per area unit, and transfer the data to the other polymers seems inappropriate. The film height, and thus weight of film per area, has to be adjusted to achieve the nominal drug content individually for each polymer. The results from that preliminary study led to exclusion of CMC from further studies and were used for the development of formulation C as well as for drug-loading of prepared oral wafers.

2.9 Stability testing

For stability testing the oral wafers were stored under controlled conditions of 25 °C / 60 % RH as well as 40 °C / 75 % over a period of 12 months according to the ICH guideline (ICH Steering Committee 2003).

During storage the oral wafers were checked for their morphological properties (C 2.2.1), mass (C 2.3.1), thickness and reduction of film thickness (C 2.3.2), tensile properties, water content and dissolution behavior. Consecutively, pH and content during storage are displayed.

Furthermore, a stress test was performed during which the oral wafers were exposed to a relative humidity of 98 % at 25 °C to investigate whether they were stable.

2.9.1 pH during storage

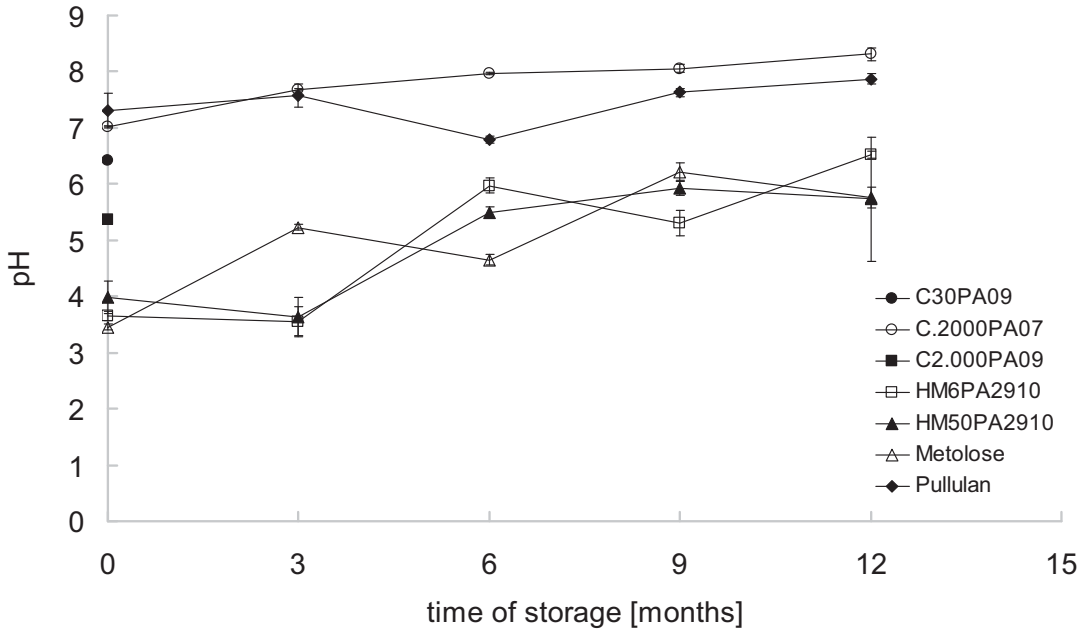


Figure 52: pH of solutions from drug-free polymer films (formulation A and B) during storage over 12 months at 25 °C / 60 % RH, mean ± SD, n = 5.

All investigated polymer solutions (one oral wafer dissolved in 2 mL distilled water) showed an increase in the pH-value of about 1 to 3 units (Figure 52) during storage at 25 °C and 60 % RH over 12 months. However, this phenomenon cannot be explained with the different film widths of the respective polymer during storage and the real cause is still dubious. Presumably, the oral wafers absorbed water during storage, increased in weight with absolute lower polymer proportion and thus, the pH increased.

The formulation with pullulan did not contain citric acid which explains the pH in the neutral pH-range (6.8 to 7.9). C2.000PA07 is an anionic polymer which showed neutral pH-values as well (7.0 to 8.3). All investigated HPMC films (Metolose®,

HM6PA2910 and HM50PA2910) showed pH-values of lower range, although HPMC is a non-ionic polymer. Probably the citric acid used decreased the pH of these polymers in contrast to the CMC films.

The films made from C30PA09 and C2.000PA09 stuck together and therefore no values for these polymers over the time of storage are available.

2.9.2 Content uniformity

Caffeine liquid formulations (an extemporaneous injection, two oral solutions and an enteral product) have been described (Trissel 2000) as being stable during storage of 12 months at different climatic conditions. To compare the proposed liquid formulations with the newly developed wafer formulation, the content of the oral wafers was determined over the storage time by high performance liquid chromatography (HPLC). All investigated polymers showed no degradation in 12 months at both climatic conditions. This indicates that the caffeine-loaded oral wafers are stable for at least one year at moderate and accelerated conditions.

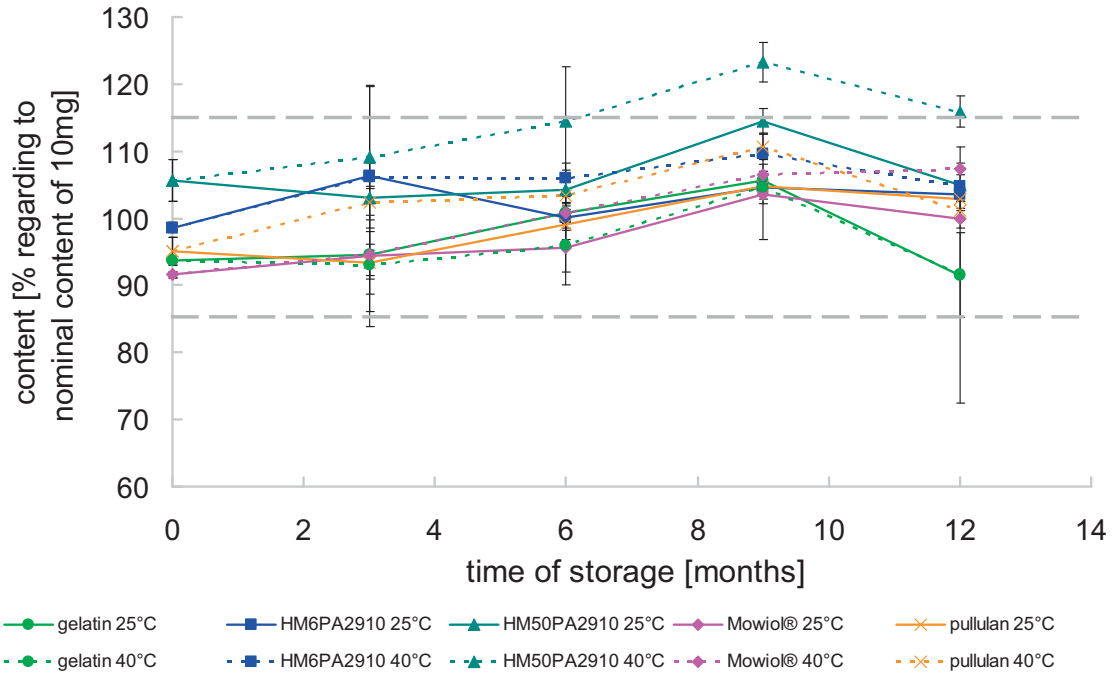


Figure 53: Content of oral wafers of formulation C during storage of 12 months, mean ± SD, n = 5.

It is commonly known, but sparsely described in the literature (Perumal et al. 2008a, Perumal et al. 2008b) that cast films show poor content uniformity. This study investigated the extent to which oral wafers were stable and how content uniformity was affected. Due to the prescribed recrystallization process of API within the oral films occurring, over- and underdosing and thus poor content uniformity was expected. As described above no degradation peak could be detected by HPLC and no systemic decrease of API content could be observed which leads to the assumption that oral

wafers containing caffeine are stable. However, content uniformity could not be proven. In accordance with the monograph 2.9.6. “uniformity of content of single-dose preparations” of the Ph. Eur. the mean nominal content (10 mg) of the oral wafers almost corresponds to the requirements of a content between 85 and 115 %. The newer severe monograph 2.9.40. “uniformity of dosage units” was adopted for the oral wafers. The required Acceptance Value (AV) is calculated according to Equation 2.

Equ. 2:

$$AV = \left|M - \overline{X}\right| + k \cdot s$$

$\overline{X}$ is the mean of the individual contents, expressed as percentage of the label claim and M is the reference value taking the value of 98.5 % for $\overline{X}$ < 98.5 %. The standard deviation of the sample is s, k is the acceptability constant with a value of 2.4 (n = 10, first test condition) or 2.0 (n = 30, second test condition). The Ph. Eur. only lists acceptability constants for sample quantities of 10 and 30; thus, the acceptability constant was extrapolated with 2.8 due to 5 samples were measured in the present investigations. The test is successful if the AV value is ≤ 15.

polymer \ month		0	3	6	9	12
gelatin	25°C / 60% RH	5.5	**20.3**	3.7	8.0	**60.4**
	40°C / 75% RH		**31.2**	**19.0**	**25.3**	**24.6**
HM6PA2910	25°C / 60% RH	2.1	10.2	9.0	7.5	5.0
	40°C / 75% RH		9.6	6.2	11.2	6.0
HM50PA2910	25°C / 60% RH	12.7	**48.5**	10.9	**18.1**	9.9
	40°C / 75% RH		**37.1**	**35.8**	**30.0**	**20.8**
Mowiol®	25°C / 60% RH	8.0	9.0	12.9	6.3	4.1
	40°C / 75% RH		14.0	7.1	9.5	8.4
pullulan	25°C / 60% RH	9.3	10.5	9.1	6.4	6.7
	40°C / 75% RH		8.0	**15.4**	14.9	9.1

Table 12: AV values for polymer films during storage according to Ph. Eur. monograph 2.9.40, AV > 15 highlighted, k = 2.8, n = 5.

In Table 12 it can be seen all oral wafers made from different polymers initially showed content uniformity with AV values of less than 15. Considering the values from storage, in particular films made from gelatin and HM50PA2910 did not fulfill the requirement of an AV of ≤ 15. Oral films stored at 40 °C / 75 % RH of both polymers throughout failed the criterion. The other films made from HM6PA2910, Mowiol® and pullulan (with the exception of the 6th month, 40 °C / 75 % RH) always had AV values of less than 15 exhibiting content uniformity within the whole storage time at both climatic conditions. In conclusion, recrystallization processes which occur with the API caffeine and lead to an over- (HM50PA2910, 40 °C / 75 % RH) and underdose (gelatin, 40 °C / 75 % RH) may cause poor content uniformity.

2.9.3 Dynamic vapor sorption

All investigated films showed an increase in weight by water sorption (Figure 54). While the oral films made from HPMC and pullulan showed an increase at the beginning of the measuring cycle and slowly equilibrated to constant masses, the films made of CMC showed a decrease after a rapid initial mass increase. Furthermore, the CMC films equilibrated very slowly and still maintained after 24 hours. After completion of the measurement all films possessed their original size. No film was dissolved at 25 °C and 98 % RH. Nevertheless, the films made from CMC seemed to be inappropriate for the use at high relative humidity due to the high initial water sorption which made the films sticky and difficult for application.

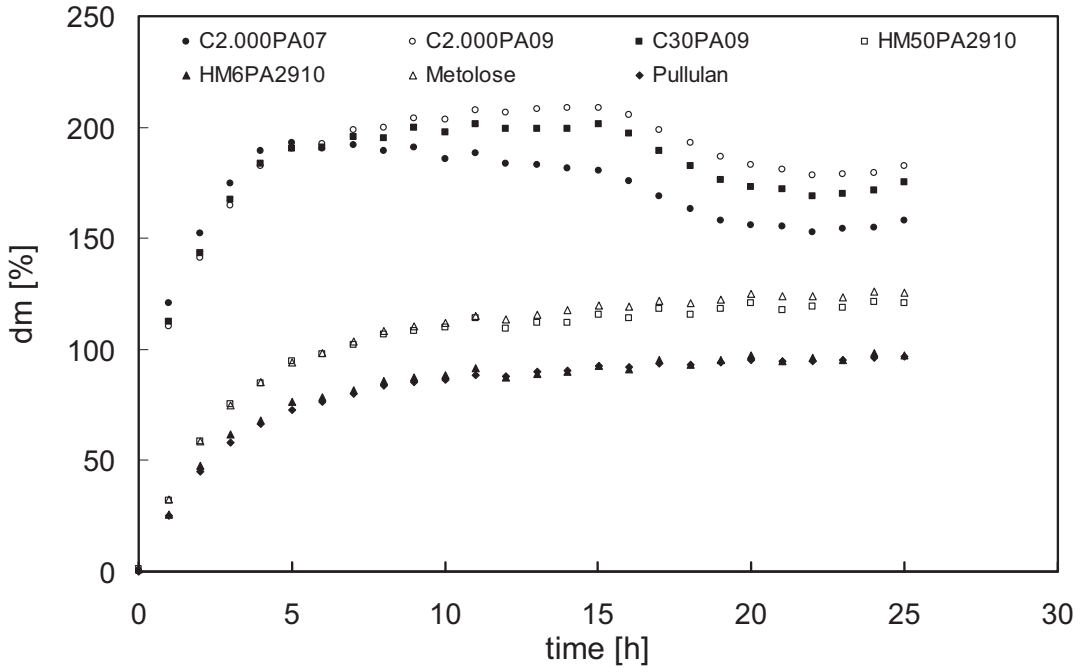


Figure 54: Water uptake capacity of drug-free oral films, 25 °C, 98 % RH, measurement until equilibrium was achieved, n = 1.

2.10 Taste assessment

2.10.1 General aspects of taste testing

Taste is recognized by humans by different gustatoric receptors and divided into five categories: bitter, salty, sour, sweet and umami. Umami is the Japanese word for palatable and is mediated by amino acids such as arginine or glutamate. Each of the mediation of the taste signal is mediated by pathways.

Usually taste testing is performed with the target group the formulation is developed for. In the case of child-appropriate formulations taste testing is rather problematic due to ethical concerns and difficulties of interpreting the response of the pediatric subpopulations (Cram et al. 2009). In contrast to the UK, in Germany a palatability study is categorized as a clinical study, with all resulting necessities. Approximately more than 90 % of guardians would reject participation of their child in a clinical study (Kleist 2001). This assumption would make it impossible to perform taste tests within the pediatric population.

Furthermore, there is the question of which children should participate in a palatability study. A lot of aspects have to be considered such as age, ethnic origins, practical aspects, duration and location of a study, whether sick or healthy children are included as well as measurement scales. In the EU healthy children are not allowed to be included in clinical studies. As the pediatric population is a very inhomogeneous group, some are not able to express themselves verbally. Therefore, so-called hedonic scales are very often used tools which mainly describe the mood or the feeling of the child, but hardly the formulation properties. It is difficult to obtain results for distinguished taste sensations, e.g. how bitter or sweet the taste is for the children.

In the past many practical approaches of taste masking have been developed to improve the palatability of medication. For liquid formulations various flavors and sweeteners are beneficial, but particularly for children, the cariogenic potential of certain sweeteners should be considered. Furthermore, the complexation of the API with β-cyclodextrins or ion exchange resins (Bess et al. 2006) has been established. However, β-cyclodextrins are nephrotoxic due to the impossible metabolism which often leads to an accumulation in the smaller blood vessels like renal arteries. As aforementioned carbonates are able to reduce bitterness (C 1.4), sodium salts act similarly (Mennella et al. 2003), but it must be evaluated separately for each drug of interest whether the bitterness sensation can be inhibited. Another approach is the inactivation of G-protein coupled receptors by nucleotides (McGregor et al. 2004). These receptors convey sensory perception of bitter taste. All methods mentioned are applied to solid dosage forms as well, but other technologies are are used in addition. Solid dispersions, manufactured by melt granulation, melt extrusion or precipitation, chemical modification in the form of reducing solubility or derivatizations as well as introducing physical barriers such as microencapsulation are commonly used techniques (Douroumis 2007).

An approach without human palatability studies is offered by the electronic tongues. Two systems are already commercially available: the αAstree E-tongue (Alpha-MOS, Toulouse, France) and the Insent Taste Testing System SA402B (Intelligent Sensor Technology, Atsugi, Japan). The electronic tongues measure potentiometrically with Ag/AgCl reference electrodes. The Insent Taste Testing System consists of sensors made of lipid-polymer membranes, whereas the αAstree system uses ChemFET (Chemical field effect transistor) technology. However, these electronic systems still need to be developed further (Cram et al. 2009). In addition other electronic tongues are under development as well (Anand et al. 2007).

2.10.2 Human taste panel

After pre-evaluation of different film formers which led to formulation C (see D 2.1.1), a palatability study with oral films of that basic composition including caffeine as API was performed. Oral wafers were prepared from different polymers. Gelatin, HM6PA2910, HM50PA2910 and Mowiol® were prepared without and with surfactant (Tween® 80) to investigate the influence on dissolution behavior (see C 2.7.3.2). Films made of pullulan were generally handled with surfactant; otherwise they could not be cast. The incorporated API, caffeine, is a bitter tasting substance and hence, the taste of the oral wafers was investigated. The results from the human taste panel are displayed in Figure 55.

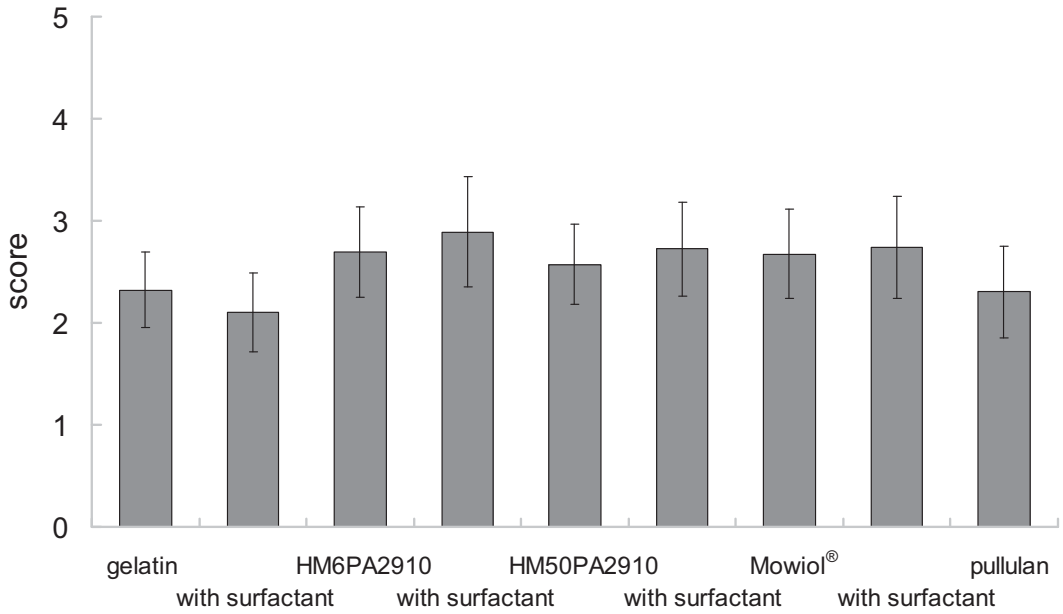


Figure 55: Results from human taste panel with adult volunteers (formulation C), assessment of taste in general of oral films on a scale from 1 to 5 according to school grades, mean ± CI, n = 15, sample labeling according to column 1 table 14.

Since taste is an individual sensation, there were large differences in assessment of taste between volunteers. Taste, especially bitter taste, is a complex taste impression mediated by many different receptors, so it is difficult to assess bitter taste above an individual threshold. Therefore different bitterness strengths are hardly distinguishable from each other for humans.

In the present human taste panel the adult volunteers were asked to assess the taste in general (Figure 55) as well as the taste in particular subdivided into bitterness, saltiness, sourness and sweetness. A tendency was distinguishable. The most pleasant taste was shown by gelatin films with surfactant, followed by gelatin without surfactant. Pullulan and the two HPMC qualities as well as Mowiol® followed. The formulations with surfactant, besides gelatin, showed worse taste throughout, compared to the respective oral films without surfactant. The volunteers described the films made from HM6PA2910 and HM50PA2910 as slimy when dissolving in the mouth and hence unacceptable. For further investigations the best compromise between a pleasant taste and fast dissolution was crucial and led to further use of the two polymers gelatin and pullulan.

On the basis of formulation C, the gelatin and pullulan oral films were further developed by varying plasticizer, adding flavors and dyes and using other sweeteners resulting in films of formulation C' (see D 2.2.28.1). The prepared oral wafers from formulation C' were tested by a palatability study as well (Figure 56) in order to ascertain whether taste masking was successful.

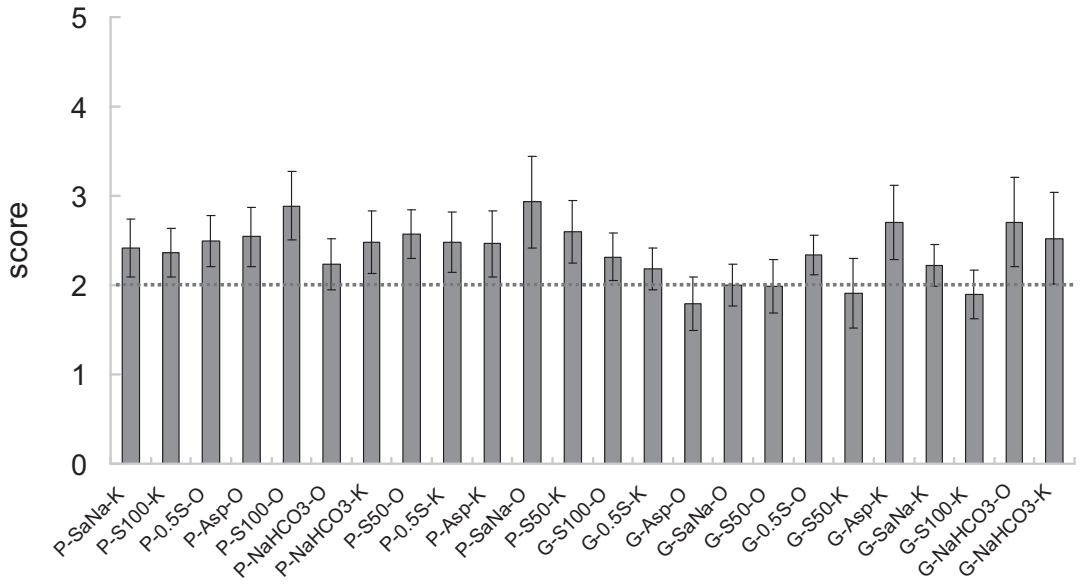


Figure 56: Results from human taste panel with adult volunteers of the improved formulations (formulation C'), assessment of taste in general of oral films on a scale from 1 to 5 according to school grades, mean ± CI, n = 15, sample labeling according to column table 16.

It can be seen (Figure 55 and 56) that the grading is not remarkably better than for the initial formulations, but some mean values were better than grade 2. Furthermore, two formulations, which were G-Asp-O and G-S_{50}-K, differ significantly (CI, $\alpha = 0.05$) from most of the other formulations which was not the case in the first taste series. Generally, the oral films made from gelatin had a more pleasant taste than the pullulan films. Although the volunteers found that oral wafers made of pullulan were better distinguished from each other, this could not be represented in the diagram. Most of the volunteers did not detect the difference between the cherry and orange flavor, although they could have been influenced by the different colors of the oral films. Therefore no preference between cherry and orange flavor could be observed. Furthermore, the volunteers did not evaluate the corresponding partner with similar scores, for example gelatin with aspartame flavored with cherry or gelatin with aspartame flavored with orange. Surprisingly, the taste panel results show that taste masking with sodium bicarbonate was not successful for caffeine as indicated in the literature (Ludwig and Krumme 2001). In conclusion, the manufacture of oral wafers with a drug-load of 10 mg anhydrous caffeine per film strip with pleasant taste was successful. Oral wafers made of gelatin with aspartame as sweetener orange flavored (G-Asp-O) and gelatin cherry flavored with glycerol and sorbitol as plasticizers (G-S_{50}-K) were the most pleasant oral wafers and accepted by all volunteers.

2.10.3 Electronic tongue

Before measuring the samples with the electronic system calibrations with anhydrous caffeine (Figure 57) and caffeine citrate (data not shown) solutions were performed. The obtained raw data contain direct sensor responses [mV] which were used for the calibration. The results of caffeine citrate offered a more distinctive sensor response due to its ionic character compared to the data of caffeine base. In particular, the sensor for sourness showed a linear behavior for caffeine citrate solutions from concentrations of 0.01 to 30 mg/mL. Due to a drug-load of 10 mg of caffeine per oral wafer the drug-loading capacity of the films was exhausted. As twice the amount of caffeine citrate had to be added to the films due to the ratio of molar mass to caffeine base, it could not be used as API. To get a better sensor response for caffeine base 10 mmol/L of potassium chloride (KCl) as ionic substance was added to the solutions (0.01 to 15 mg/mL caffeine base) to improve conductivity at the membranes of the sensors. The results from the calibration for all five sensors (astringency: SB2AE1, bitterness: SB2C00, saltiness: SB2CT0, sourness: SB2CA0 and umami: SB2AAE) are displayed in Figure 57. The filled symbols represent the results from the first measurement cycle whereas the open symbols show mV values obtained from a subsequent measurement series with another sensor set. As seen, the reproducibility is excellent except for the sour sensor.

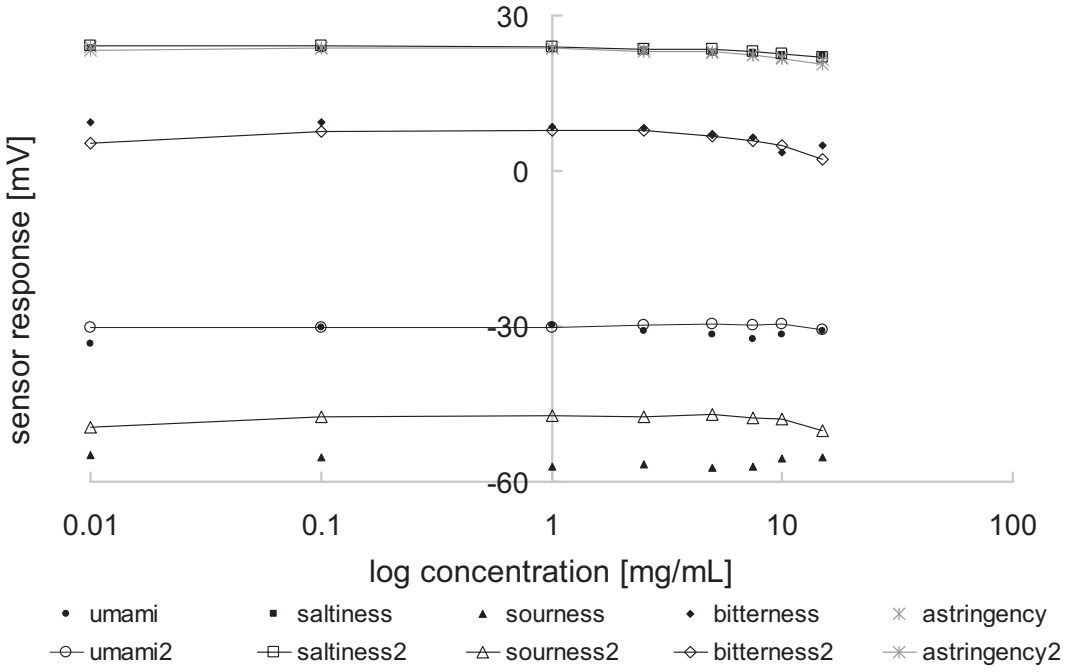


Figure 57: Calibration curve sets for caffeine base, (0.01 to 15 mg/mL) with added 10 mmol/L KCl, two different series: first measurement cycle: filled symbols, second measurement: open symbols, mean, n = 3.

For all sensors a concentration dependent sensor response from the raw data, however not in Figure 57, can be observed. With increasing concentration the sensor response decreased, except for the sourness and umami sensor. There was no correlation between concentration and sensor response for sourness and umami. Linearity for astringency, bitterness and saltiness sensor were found for concentrations from 1 to 10 mg/mL. The observed relationship is relevant for interpretation of the results of the oral wafers.

However, the differences in sensor response within the concentration series are low. A multivariate data analysis with the tool “projection to latent structures by means of partial least squares” (PLS) was performed additionally. In Figure 58 the PLS-plot with linear regression for caffeine base of 9 different concentrations with 10 mmol/L KCl is displayed. The PLS-plot includes all 5 sensors and shows that a quantification of unknown samples is possible (R^2 = 0.973). It can be seen that the RMSEE is 0.912 which means that the concentration can be predicted with an error rate of 0.912 mg/mL.

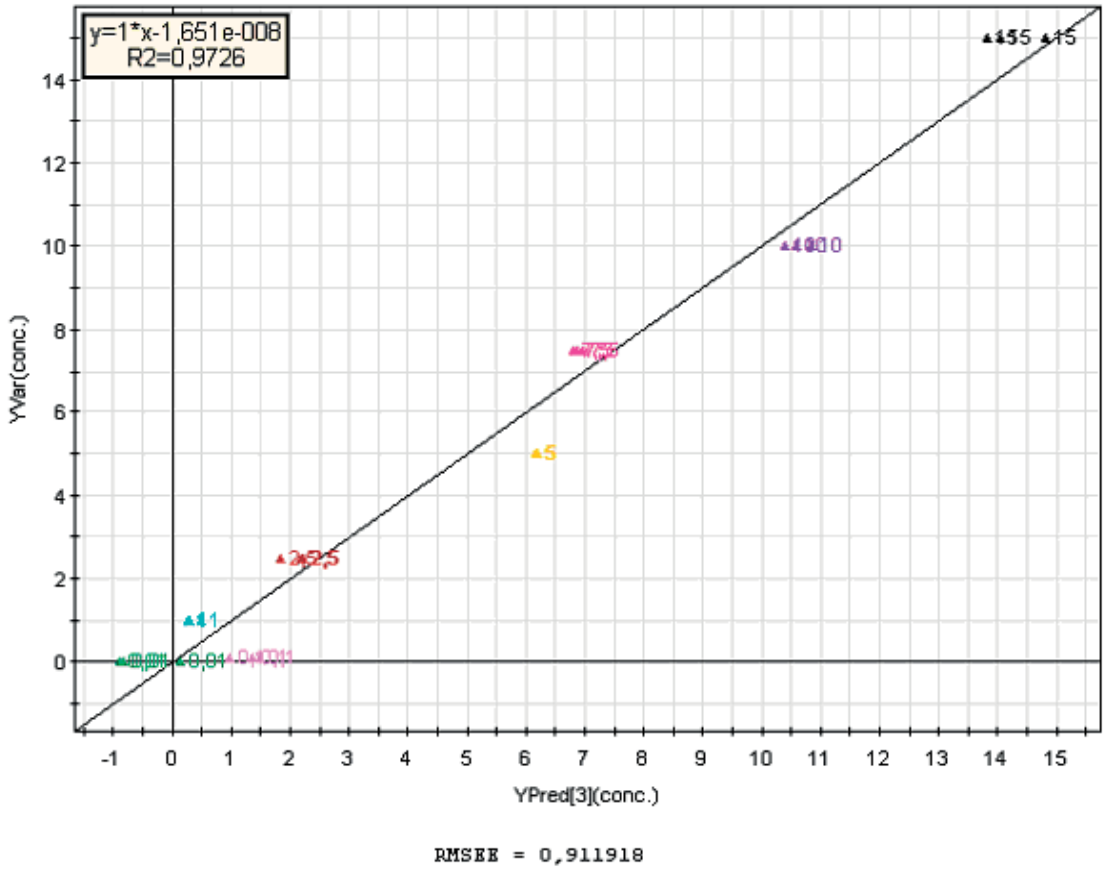


Figure 58: PLS-Plot with linear regression of caffeine base solutions in different concentrations with added 10 mmol/L KCl, 3 replicates displayed.

Initially solutions of oral wafers made from formulation C, except for gelatin, were investigated by the electronic tongue. As a result of the calibration of each investigated polymer, 10 and 15 oral wafers were dissolved within 100 mL distilled water (equal to 1 and 1.5 mg/mL caffeine). 10 mmol/L KCl was added to all solutions. Actually more oral wafers could have been dissolved in 100 mL distilled water, but it was intended to avoid polymers interacting or at the worst destroying the lipid membranes of the sensors. A pure 10 mmol/L KCl solution served as a blank. Given that there were no anomalies with the blank results, the raw data were used as obtained.

In Figure 59 sensor responses from the solutions of oral wafers of formulation C are displayed. The umami sensor (SB2AAE) is missing as it was not working during the measurements. In the diagram the raw data are depicted due to the transformation into taste information (see D 2.2.28.4) resulted in the same predictions. The transformation into the taste information is a recommendation of the manufacturer of the Insent electronic tongue, but is not mandatory. The bitterness (SB2C00) and astringency (SB2AE1) sensor responses are transformed into taste information with the saltiness (SB2CT0) sensor response and can lead to mistakes if the raw data do not show strict dependence on the concentration at the SB2CT0 sensor. As seen in Figure 59 the sensor responses for the 1.5 mg/mL caffeine concentration were more distinctive than for the concentration of 1.0 mg/mL. The most bitter solutions contained

polymer films made of pullulan followed by Mowiol®, HM50PA2910 and HM6PA2910. However, the polymers overlapped the bitterness for the sensors in comparison to the pure caffeine solution of 1.0 mg/mL. An increase in astringency correlates with decreasing values, thus the results of the electronic tongue showed that the polymers provided an astringent taste. With reference to the pure caffeine solution of the same concentration, the polymers intensify the salty taste and simultaneously weaken the sourness.

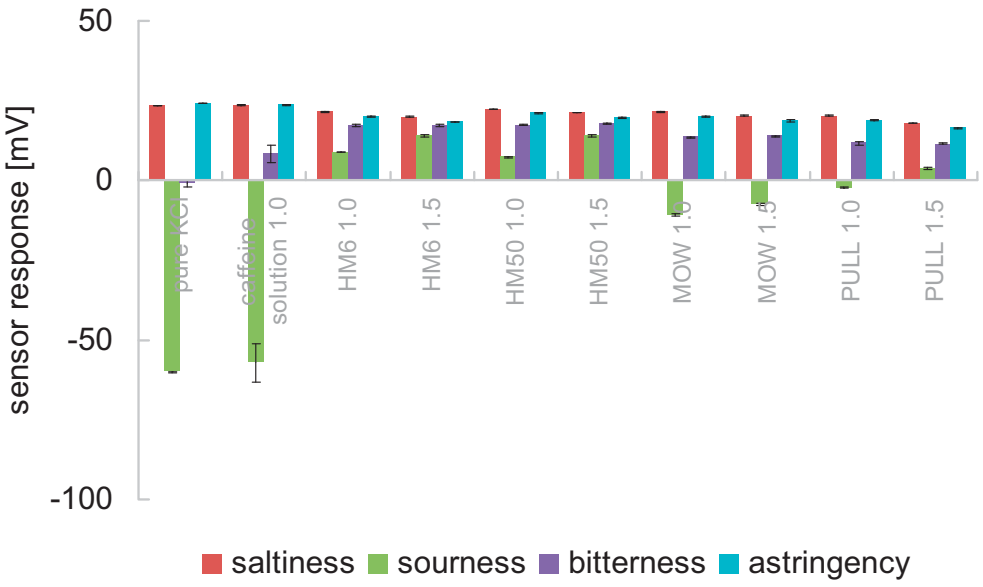


Figure 59: Sensor responses from four sensors of solutions of oral wafers from formulation C, (concentration: 1 and 1.5 mg/mL caffeine), mean ± CI, n = 3.

In a second trial the improved formulations (C', see D 2.2.28.1) were measured by the electronic tongue as well. Provided that the umami sensor was working again, it could be used for the analysis which is especially important for the wafers made of gelatin.

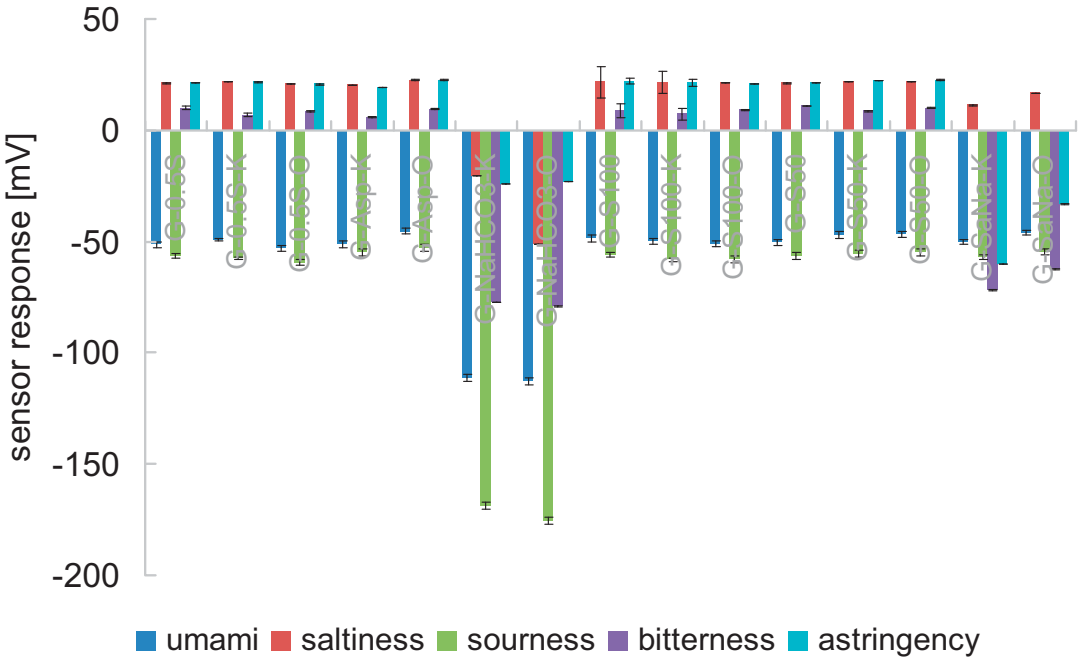


Figure 60: Sensor responses from all sensors of solutions of gelatin oral wafers from formulation C', mean ± CI, n = 3.

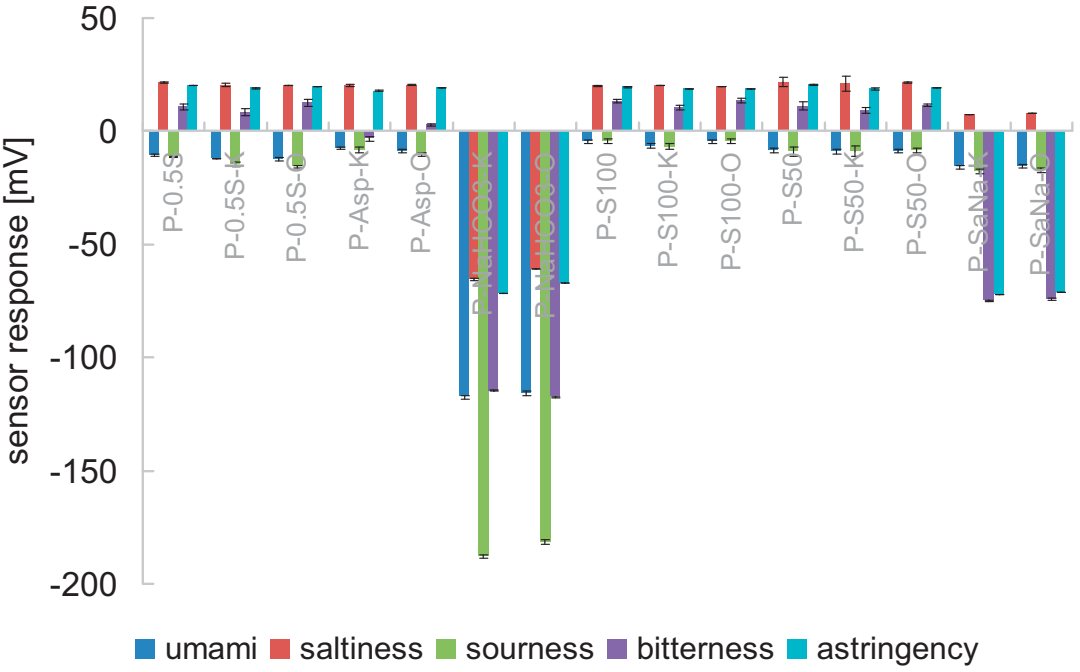


Figure 61: Sensor responses from all sensors of solutions of pullulan oral wafers from formulation C', mean ± CI, n = 3.

The results of the electronic tongue measurements of formulation C' are displayed in Figure 60 and 61. Generally, it can be seen that the electronic tongue detected the corresponding partner of each formulation. More precisely, the samples were measured randomly and the electronic tongue was able to detect similar responses for cherry flavored gelatin with saccharin compared to orange flavored gelatin with

saccharin. Furthermore, the sensor response of sourness and umami was more characteristic for gelatin oral wafers (Figure 60) compared to the pullulan films (Figure 61). As umami is mediated by amino acids, the polypeptide gelatin showed a stronger sensor response. In terms of the sensor response, especially for the bitterness and astringency sensors, polymers prepared with both saccharin as sweetener or sodium bicarbonate as masking agent had stronger responses compared to all other formulations. Saccharin is a sweetener with slightly bitter bland taste which can be retrieved in these results. Sodium bicarbonate is an ionic substance which explains the stronger sensor response for bitterness, saltiness, sourness and umami. However, the possibility of masking bitter taste with bicarbonate can be disproved, which was observed in the taste panel as well. Although the electronic tongue can detect corresponding formulations, no significant differences between the unflavored and flavored formulations (CI, $\alpha = 0.05$) could be observed.

In conclusion, the electronic systems provide a suitable and cost-effective tool for screening of formulations excluding safety concerns and subjective bias, but they are still not able to replace human taste panels.

D EXPERIMENTAL PART

1 Materials

substance	*trade name*	*quality*	*Lot. No.*	*source*
API				
caffeine anhydrous		Ph. Eur.	31247243	Caesar & Loretz, Hilden, Germany
caffeine citrate		DAC	53695256	Caesar & Loretz, Hilden, Germany
chemical reference standard				
caffeine		CRS	3a	EDQM, Strasbourg, France
polymers				
carboxymethyl cellulose	Walocel® C30PA09	Ph.Eur.		Dow Wolff Cellulosics, Bomlitz, Germany
	Walocel® C2.000PA07	Ph.Eur.		Dow Wolff Cellulosics, Bomlitz, Germany
	Walocel® C2.000PA09	Ph.Eur.		Dow Wolff Cellulosics, Bomlitz, Germany
	Walocel® C40.000PA09	Ph.Eur.		Dow Wolff Cellulosics, Bomlitz, Germany
gelatin	GELITA®, Type B, 260 Bloom	Ph.Eur.	L625456	GELITA Europe, Eberbach, Germany
hydroxypropyl cellulose	Klucel® GF	Ph.Eur.	2038	Hercules Inc., Aqualon, Wilmington, USA
hydroxypropyl methyl cellulose	Metolose® 65SH-1500	Ph.Eur.	109650	Syntapharm, Mülheim/Ruhr, Germany
	PHARMACOAT® 615	USP	506286	Shin Etsu Chemical Co., Ltd., Tokyo, Japan
	Walocel® HM6PA2910	Ph. Eur.		Dow Wolff Cellulosics, Bomlitz, Germany
	Walocel® HM50PA2910	Ph. Eur.		Dow Wolff Cellulosics, Bomlitz, Germany
	Walocel® HM4.000PA2910	Ph. Eur.		Dow Wolff Cellulosics, Bomlitz, Germany
sodium alginate, talc, titanium dioxide, triacetin	Instacoat® P-4		RD-8889	Syntapharm, Mülheim/Ruhr, Germany
polyethylene glycol-polyvinyl alcohol copolymer	Kollicoat® IR		72580716K0	BASF, Ludwigshafen, Germany
polyvinyl alcohol	Mowiol® 4-88		15786637	Carl Roth, Karlsruhe, Germany
pullulan			TCP0978	ABCR, Karlsruhe, Germany
other excipients				
aspartame		Ph.Eur.	06I07-N03	Fagron, Barsbüttel, Germany
κ-carrageenan	Gelcarin® GP-911 NF		40701120	FMC, Philadelphia, PA, USA
citric acid anhydrous		Ph. Eur., USP	621774	Lohmann, Emmerthal, Germany
dodecyl-poly(ethylene oxide-23) ether	Brij® 35		N5003	Uniqema, Bromborough, UK
flavoring cherry 0 VP			9022837	Dragoco, Holzminden, Germany
flavoring orange (juice)			9012884	Dragoco, Holzminden, Germany
food colorant red	E124		72759487	Caesar & Loretz, Hilden, Germany
food colorant yellow	E102, E124		63288427	Caesar & Loretz, Hilden, Germany
glycerol		Ph. Eur.	502974-0	Caesar & Loretz, Hilden, Germany
locust bean gum				C.E.Roeper, Hamburg, Germany
mannitol	Mannitol 60	Ph.Eur.	706961	Roquette, Lestrem, France
polysorbate 80	Tween® 80	Ph. Eur.	307E0185	Uniqema, Bromborough, UK
polyvinyl pyrrolidone	Kollidon® 30		49-0064	BASF , Ludwigshafen, Germany
potassium sorbate			278837888	Fluka Chemie, Buchs, Schweiz
sodium bicarbonate			623322	Welding, Hamburg, Germany
sodium saccharin		Ph.Eur.	70562028	Caesar & Loretz, Hilden, Germany
sorbitol	Sorbidex® P5	Ph. Eur.		Cerestar, Krefeld, Germany
sorbitol solution 70 %, not crystallizing	Sorbitol LGK	Ph.Eur.	140805	DHW, Rodleben, Germany
sucralose	InnTense™ SL 6210		20051101	InnoSweet, Braunschweig, Germany
xanthan gum	Xantural® 11K		3C7561K	CP Kelco, Atlanta, USA
lab chemicals				
acetonitrile	Chromanorm®	HPLC grade	various	VWR, Leuven, Belgium
alcohol 99 %	Normapur®	p.a.	various	VWR, Leuven, Belgium
alcohol 96 %		Ph.Eur.		Bundesmonopolverwaltung für Branntwein
demineralized water		Ph.Eur.		reverse osmosis
distilled water		Ph.Eur.		lab destillation
hydrochloric acid 1mol/L		p.a.	various	Merck, Darmstadt, Germany
methanol	HiPerSolvChromanorm®	HPLC grade	various	VWR, Leuven, Belgium
potassium chloride		p.a.	Z12008	Grüssing, Filsum, Germany
potassium phosphate monobasic puriss.		Ph.Eur.	18786828	Riedel-de Haën, Seelze, Germany
silver chloride		purum	50770	Riedel-de Haën, Seelze, Germany
sodium acetate trihydrate		p.a.	A013053401	Acros Organics, Geel, Belgium
sodium hydroxide 1mol/L		p.a.	various	J. T. Baker, Deventer, The Netherlands
tartaric acid		Ph.Eur.	50610	Riedel-de Haën, Seelze, Germany
tetrahydrofurane		p.a.		Merck, Darmstadt, Germany
theophylline anhydrous		Ph.Eur.	114521AX1D	BASF, Ludwigshafen, Germany

Table 13: Table of all substances used within the present studies.

2 Methods

2.1 Preparation of oral wafers

2.1.1 Film formulations

The following table shows the detailed compositions of film formulations which were used in the present study. Formulation C' is displayed in chapter D 2.2.28.1 because the manufactured oral wafers were exclusively prepared for taste assessment.

The loading of the oral wafers with API requires the calculation of mass per film strip to achieve the desired drug-load of 10 mg caffeine. Initially the mass of API per batch was calculated.

Equ. 3:

$$m_{API\ in\ b} = \left(\left(\frac{m_{API\ in\ f}}{con}\right) \cdot \left(\frac{A_b}{1000 \cdot A_f}\right)\right)$$

A_b	batch size [cm²]
A_f	size of one film [cm²]
con	content of API [% / 100]
$m_{API\ in\ b}$	mass of API in the batch [g]
$m_{API\ in\ f}$	mass of API in one film [mg]

A drug-load of 10 mg caffeine per oral wafer should be achieved. The content of the caffeine base was determined by HPLC analysis and amounted 98.85 % related to the reference, caffeine CRS Ph. Eur.. The batch size was 1500 cm² and each film strip had an area of 6 cm², with a rectangular shape measuring 3 x 2 cm. The factor 1000 is the conversion factor from [g] into [mg].

In a next step the amount of each ingredient that should be contained in a single film strip was calculated.

Equ. 4:

$$m_{ing\ in\ f} = \left(\frac{m_{ing\ in\ b}}{\left(\frac{A_b}{A_f}\right)}\right) \cdot 1000$$

A_b	batch size [cm²]
A_f	size of one film [cm²]
$m_{ing\ in\ b}$	mass of ingredient in the batch [g]
$m_{ing\ in\ f}$	mass of ingredient in one film [mg]

Finally, the mass of one film strip can be calculated from the amount of each ingredient per film strip excluding the solvent, given that the solvent is completely evaporated.

Equ. 5:

$$m_f = \sum_1^n m_{ing\ in\ f} - m_{sol\ in\ f}$$

m_f mass of one film [mg]
$m_{ing\ in\ f}$ mass of ingredient in one film [mg]
$m_{sol\ in\ f}$ mass of solvent in one film [mg]

A	poly-mer	caffeine anh.	sucra-lose	sorbi-tol	glycerol 85 %	citric acid	Tween® 80	Brij® 35	ethanol 96 %	water	Total
Walocel® C 30 PA 09	4.57			2.28	0.91	0.46	0.08	0.30	45.70	45.70	100
Walocel® C 2.000 PA 07	4.57			2.28	0.91	0.46	0.08	0.30	45.70	45.70	100
Walocel® C 2.000 PA09	2.72			2.33	0.93	0.47	0.08	0.31	46.58	46.58	100
Walocel® C 40.000 PA 09	1.96			2.35	0.94	0.47	0.08	0.31	46.95	46.95	100
Walocel® HM 6 PA 2910	10.67			2.14	0.86	0.43	0.07	0.29	42.77	42.77	100
Walocel® HM 50 PA 2910	4.91			2.28	0.91	0.46	0.08	0.30	45.53	45.53	100
Walocel® HM 4.000 PA 2910	1.96			2.35	0.94	0.47	0.08	0.31	46.95	46.95	100
Instacoat® P-4	34.50			1.57	0.63	0.32	0.05	0.21	31.36	31.36	100
Kollicoat® IR	19.31			1.93	0.77	0.39	0.06	0.26	38.64	38.64	100
Metolose® 65SH-1500	4.59			2.28	0.91	0.46	0.08	0.30	45.69	45.69	100
Pharmacoat® 615	6.00			2.25	0.90	0.45	0.08	0.30	45.01	45.01	100

B	poly-mer	pot-assium sorbate	sucra-lose	mann-itol	glycerol 85 %	xanth-an gum	locust bean gum	carra-geenan	Tween® 80	water	Total
Pullulan	13.16	0.05	1.15	1.64	1.23	0.04	0.06	0.25	0.58	81.85	100

B'	poly-mer				glycerol 85 %		Kollidon® 30		ethanol abs.		Total
Klucel® GF	8.16				2.25		16.28		73.31		100

C	poly-mer	caffeine anh.	sucra-lose	sorbi-tol	glycerol 85 %	citric acid	Tween® 80	Brij® 35	ethanol 96 %	water	Total
Gelatin	13.24	3.65	0.51		3.57	0.45				78.58	100
Walocel® HM 6 PA 2910	13.24	3.65	0.51		3.57	0.45			39.29	39.29	100
Walocel® HM 50 PA 2910	7.08	3.90	0.55		3.83	0.48			42.08	42.08	100
Metolose® 65SH-400	2.46	4.10	0.57		4.02	0.51			44.17	44.17	100
Mowiol® 4-88	13.24	3.65	0.51		3.57	0.45			39.29	39.29	100
Pullulan	13.17	3.63	0.51		3.55	0.45	0.49			78.20	100

Table 14: Detailed film formulations, labeling according to column 1: numbers from Walocel® products, otherwise short names of trade marks or polymers were used, content in [%].

Formulation B' was already excluded from the study during pre-formulation. Therefore it was not displayed in chapter C 1.4.

2.1.2 Manufacturing

The oral films were prepared according to a standard procedure as displayed in Figure 62.

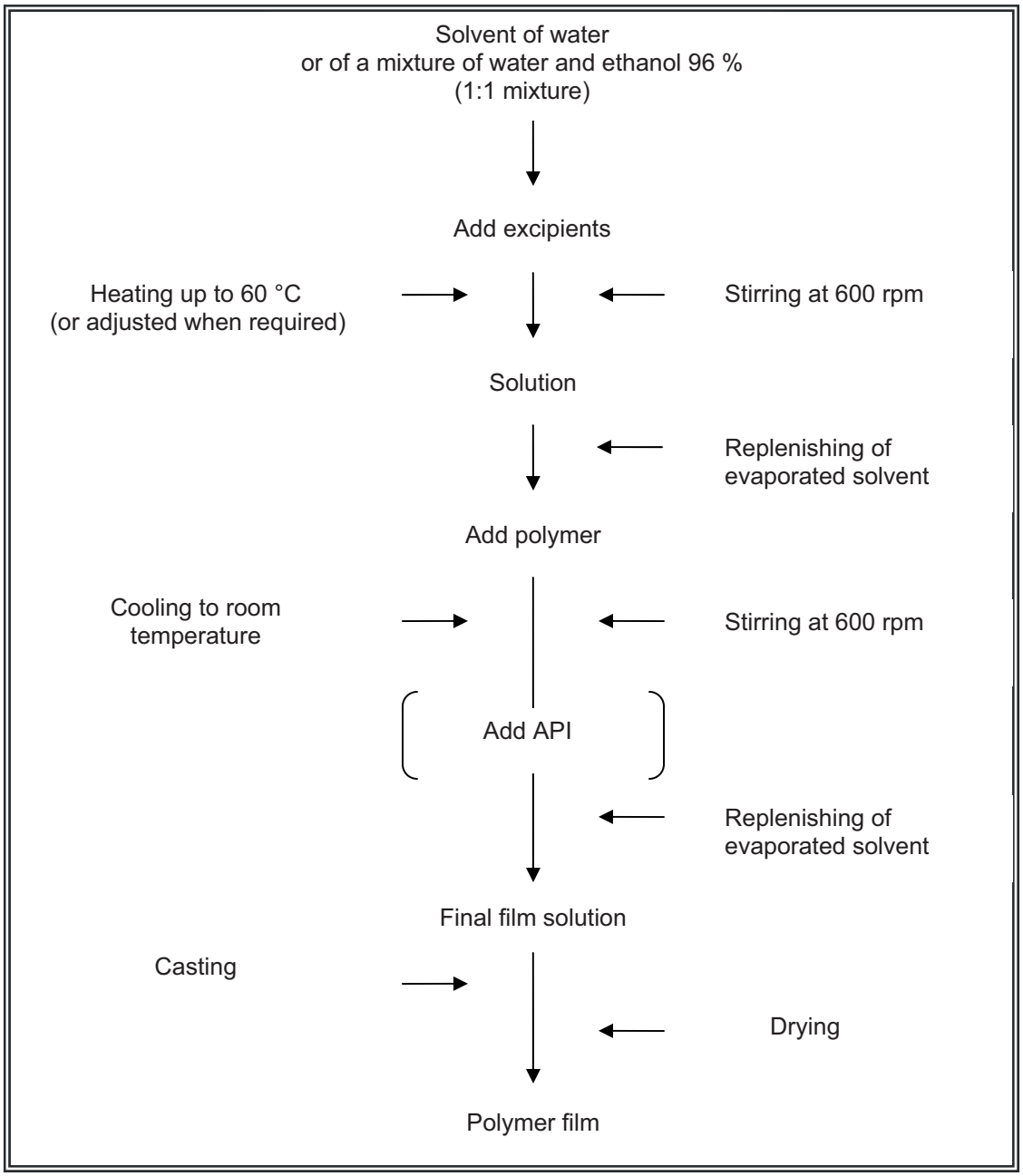


Figure 62: Preparation scheme of oral wafers, (modifications have been described in C 1.4).

The film solutions were prepared in closed brown wide-mouth glass containers and evaporated solvent was replenished by weighing of the glass containers. The film solutions were cast on two types of release liners (Polyslik JU 460 and FL 2000 PET

75 µm 1S, Loparex, Apeldoorn, the Netherlands) with an Erichsen film applicator (Coatmaster 509/1, Erichsen, Hemer, Germany). To adjust different heights a vertically adjustable doctor knife was used and then the film solutions with speeds of 6, 12, 18 or 24 mm/s were cast. The process of film formation has been thoroughly described (Alanazi et al. 2007) and is divided into three stages: (a) evaporation of solvent casting and subsequent concentration of polymer particles, (b) deformation and coalescence of polymer particles and (c) further fusion by interdiffusion of polymeric molecules of adjacent polymer particles. The cast films were dried in a drying oven (Memmert U50, Memmert, Schwabach, Germany) at 40 °C or at room temperature until dryness. The duration of drying depended on the properties of each polymer. The films containing caffeine developed long needles, especially the wafers made from gelatin, Mowiol® and pullulan. Individual wafers were prepared by cutting the films into strips of regular dimension of 2 x 3 cm with a paper cutter (Flofto), cutting die and a surgical scalpel (B. Braun Melsungen AG, Tuttlingen, Germany).

2.1.3 Storage

After cutting the wafers were stored under controlled conditions at 25 °C / 60 % RH in a hygrostat with saturated sodium bromide solution and at 40 °C / 75 % RH in a conditioning cabinet over a period of 12 months according to the ICH guideline Q1A (ICH Steering Committee 2003). The films were clamped into slide frames to store them non-contacting, space-saving and separately (see Figure 63).

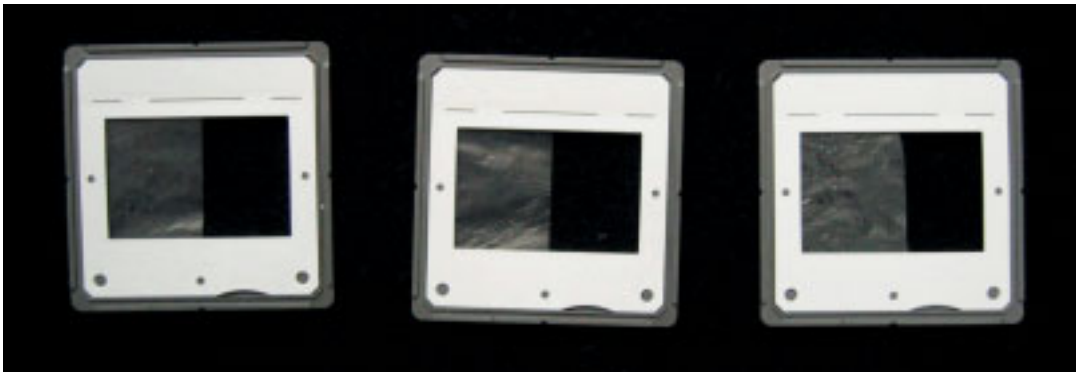


Figure 63: Storage of the films clamped into slide frames.

2.2 Characterization

2.2.1 Light microscopy

Light microscopic investigations were conducted by a polarization microscope (Leica DM LB, Leica, Cambridge, UK) with transmitted light modus. Images were taken by software (Leica QWin, Leica, Cambridge, UK).

2.2.2 Image analysis

For imaging the complete film strips a system containing a stereomicroscope (Leica MZ 75, Leica, Cambridge, UK), a cold light source (Leica KL 1500, Leica, Cambridge,

UK) and a digital camera (Leica CS 300 F, Leica, Cambridge, UK) was used. Images were taken and evaluated by software (Leica QWin, Leica, Cambridge, UK) with a magnification of 1 pixel = 8.75 µm.

2.2.3 Scanning Electron Microscopy

Upper and lower sides as well as the cross-section of the obtained films were gold sputtered for 180 s (Agar Manual Sputter Coater B7340, Agar Scientific Ltd., Stansted, Essex, UK). Thereafter, the surface and the distribution of caffeine as well as the differences between upper- and lower side of the films were examined by scanning electron microscopy (Leo 1430 VP, Leo Elektron Microscopy, Cambridge, UK) at a working voltage of 20 kV.

2.2.4 Near Infrared Chemical Imaging

The film strip samples and powder substances were analyzed with the SyNIRgi® (Malvern, Worcestershire, United Kingdom), a near infrared chemical imaging digital analyzer, which builds on the Spectral Dimensions Sapphire®/NIRCI-2450 platform. 81920 NIR spectra can be generated from a 13 x 10 mm sample area in two minutes. The spectral range was 1200 to 2450 nm and the detector size was 320 x 256 pixels. The drug-loaded films were imaged with the 40 µm/pixel object lens. The images were taken at a band of 1670 nm.

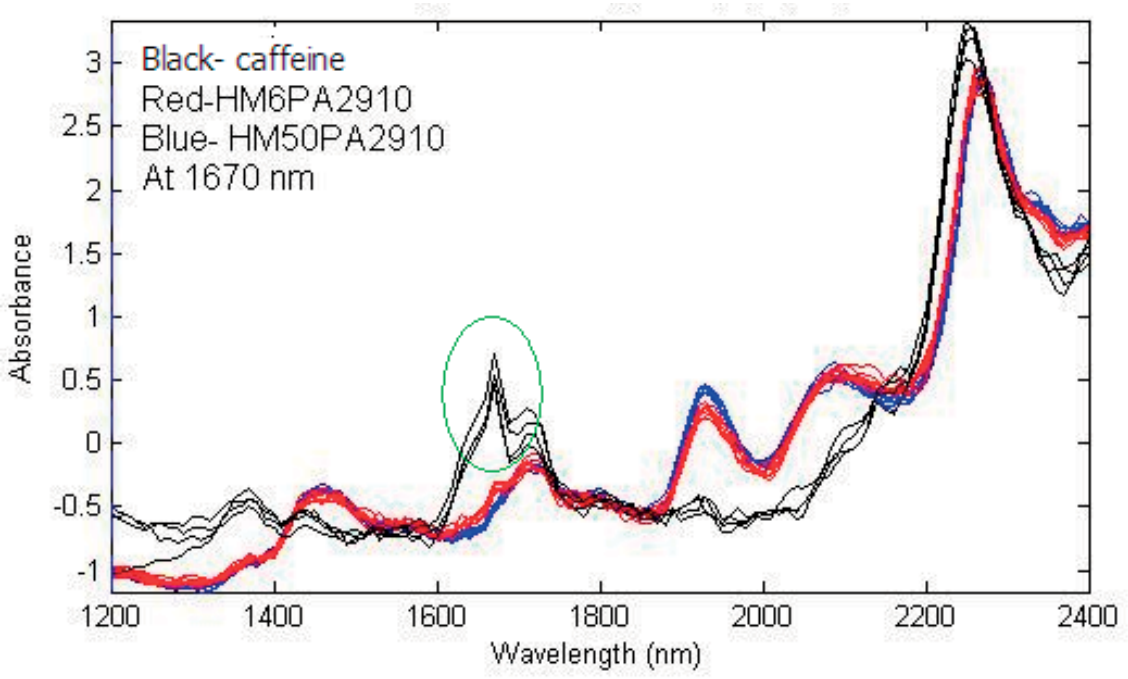


Figure 64: Spectra of pure substances from anhydrous caffeine, HM6PA2910 and HM50PA2910 with a characteristic wavelength peak at 1670 nm.

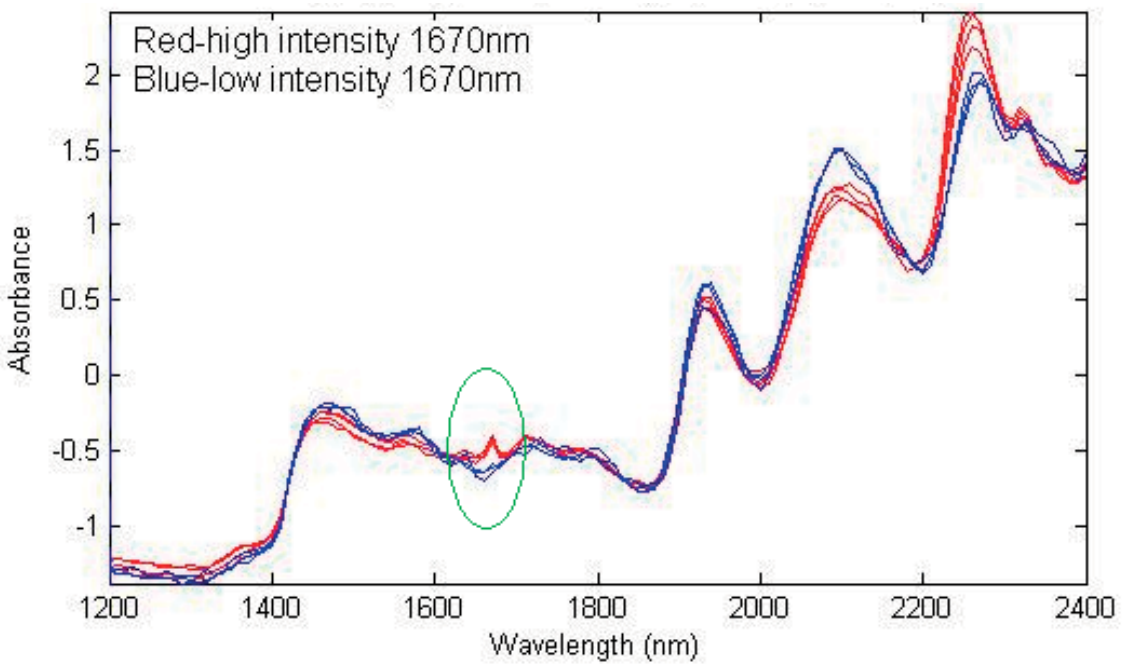


Figure 65: Spectrum of pure pullulan powder

Statistical and image analysis were conducted by ISys®, the Malvern's data analysis software. Differences between the drug-loaded films referring to the distribution of active substance and possible recrystallizations were visualized.

2.2.5 X-ray diffraction

The crystallinity of the powders of caffeine base, caffeine citrate and of the polymer films were investigated using a Miniflex apparatus (Rigaku, Tokyo, Japan) with CuKα radiation. To get a smooth surface for the measurement procedure the powder samples were compressed into aluminum frames which were mounted on an aluminum plate. Otherwise the oral films were mounted with adhesive tape on the aluminum frames. This procedure is required to avoid preferential orientation of the particles, especially of the powders. Diffraction patterns were obtained at a working voltage of 20 kV and a current of 10 mA. The samples were scanned in a 2Theta range from 5 to 40°. The used intensity was 1000 cps, the scanning speed was 2 °/min by a distance between two data points of 0.02°.

2.2.6 Film mass

The mass of films was determined by an analytical balance with five decimal places (Sartorius MC 210 P, Sartorius, Göttingen, Germany).

2.2.7 Film thickness

Film thicknesses were determined using two different methods. The measurement with the micrometer screw (Mitutoyo, Neuss, Germany) is product-contacting in comparison

to the coating thickness gauge (Minitest 600, Erichsen, Hemer, Germany), which is a contact-free method.

The coating thickness gauge works with the magnetic-induction principle. Before measuring a two-point calibration, one point on a metal (steel) plate and one point on the respective foil (for non-magnetic material), was performed. The metal plate serves as transverse anchor. The films show no ferromagnetic properties and behave like a head gap. The inductance depends on the thickness, the strength of the measuring signal diminishes with increasing coating thickness.

Each wafer was measured at five positions (central and the four corners) and the mean thickness was calculated.

The following equation was used to calculate the reduction of film thickness.

Equ. 6:

$$red = 100\,\% - \left(\frac{thi_d \cdot 100\,\%}{thi_w} \right)$$

red	reduction of film thickness [%]
thi_d	film thickness of the dried film [µm]
thi_w	film thickness of the wet film [µm]

2.2.8 Tensile strength

The load [N] and elongation [%] were measured during tensile test by a universal testing apparatus (H10KM, Hess, Sonsbeck, Germany) using a load cell of 1000 N (Hounsfield, Surrey, England). Each strip of film was cut and prepared according to the standard DIN EN ISO 527-3 (DIN Deutsches Institut für Normung e.V. 2003). The test specimen No. 5 (see Figure 66) was clamped between the tensioning tools.

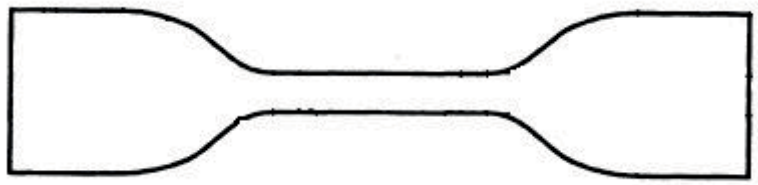


Figure 66: Test specimen type 5 according to DIN EN ISO 527-3.

The draw speed was 50 mm/min and no preload was used. Tensile strain at break [%] and tensile stress at break [MPa] were subsequently calculated (DIN Deutsches Institut für Normung e.V. 1996). The stress is defined as force per area [Pa]. The area is the width in which the film breaks which is the smaller segment as pretermined breaking point multiplied by the respective film thickness.

2.2.9 Differential Scanning Calorimetry

Glass transition temperatures (Tg) were determined by differential scanning calorimetry (DSC) as well as thermomechanical analysis (TMA).

DSC measurements were performed by a DSC 821e apparatus (Mettler Toledo, Gießen, Germany). The heating rate was 10 K/min and the temperature ranged individually depending on the properties (i.e. Tg) of the polymer respective polymer film. The samples (2 – 3 mg) were sealed in pierced aluminium pans of 40 µL. The measurement was repeated after annealing to eliminate effects of moisture. The reference was an empty aluminium pan. The Tg was determined in the 2nd measurement as onset temperature.

2.2.10 Thermomechanical Analysis

For thermomechanical analysis a Mettler TA 3000 Apparatus (Mettler Toledo, Gießen, Germany) with TC 10A Processor was used. The TMA sensor was overlaid with a load of 0.5 N. The subsidence of the sensor within the softening films was measured (penetration method). The heating rate was 10 K/min, the temperature ranged individually as well and the nitrogen flow was 50 mL per minute. To measure the polymeric powders, they were pressed into molds of 20 mg.

The pure polymer powders as well as the polymer films were measured with both methods. The oral films were folded to fit into the small pans. The determination of the Tg was carried out by Mettler Star^{e} software (version 6.01).

2.2.11 Hot stage microscopy

A polarization microscope (Leica DM LB, Leica, Cambridge, UK) with transmitted light modus was used to investigate polymer behavior during heating. Polymer films were prepared on a temperature controlled microscope stage (THMS 600, Linkam Scientific Instruments, Surrey, UK) and were heated with the temperature control system TMS 94 (Linkam Scientific Instruments, Surrey, UK) at a heating rate of 10 K/min. Images were taken by software (Leica QWin, Leica, Cambridge, UK).

2.2.12 Karl-Fischer-Titration

The oral films were dissolved in equal amounts of a Hydranal®-methanol dry (Lot. No. 6235A) and Hydranal®-formamide dry (Lot. No. 6025A) medium and titrated using Karl-Fischer-Method. An automatic titrator (Karl-Fischer-Titrator DL 18, Mettler Toledo, Gießen, Germany) was used at room temperature (20 °C). The system was calibrated with the Hydranal®-water standard 10.0 (Lot. No. 61160) and the one-component-reagent Hydranal®-composite 5 (Lot. No. 7022D). The calibrating solution contained 10.05 mg water. The required mass of a film was between 20 and 30 mg. The determination of water content was performed in triplicate.

2.2.13 Karl-Fischer-Titration in nitrogen flow

The water content of the oral wafers was determined using the Moisture Meter® CA05 with dry heat oven VA05 (Mitsubishi Chemical Industries Limited, Tokyo, Japan). For weighing the samples in an aluminum pan an analytical balance (Mettler AT261®,

Mettler Toledo, Gießen, Germany) was used. After weighing the moisture was expelled through heating at 170 °C and led over by nitrogen flow to the measuring cell in which the determination of water was performed.

2.2.14 Loss on drying

Loss on drying was carried with five samples of each polymer. The mass loss was calculated (Equ. 7) as the ratio from initial mass to final mass after drying.

Equ. 7:

$$LOD = 100\,\% - \left(\frac{m_i \cdot 100\,\%}{m_f} \right)$$

LOD	loss on drying [%]
m_i	initial mass of one film [mg]
m_f	final mass of one film when constant weight was achieved [mg]

An air circulating drying oven (Heraeus ET 6130, Kendo, Hanau, Germany) with 105 °C was used for drying process until the samples achieved constant weight.

2.2.15 Thermogravimetric chromatography coupled with mass spectrometer

The thermogravimetric analysis was performed by a thermobalance (STA 499C, Netzsch, Selb, Germany) coupled with mass spectrometer (Thermo Star, Pfeiffer Vakuum, Asslar, Germany). Samples of 50 mg were heated up from 30 to 650 °C during measurement. The rate of heating was 2.5 K/min and the emerging gases over the sample were conveyed to the mass spectrometer by nitrogen.

2.2.16 Gas chromatography

The gas chromatographic determination of residual ethanol within the oral wafers was carried out by a headspace (TurboMatrix 40, Perkin-Elmer, Juegesheim, Germany) coupled with gas chromatograph (Clarus 500, Perkin-Elmer, Juegesheim, Germany). Approximately 50 mg of sample, exactly weighed, was dissolved in inner standard solution consisting of 20 µL methyl isobutyl ketone in 1000 mL water. 2 mL of this solution were pipetted in a headspace vial and heated up to 90 °C. The equilibrated headspace gas was injected into the gas chromatograph with flame ionization detector. The external standard contained 20 µL ethanol within 500 mL inner standard solution. The headspace and gaschromatograph settings are displayed in Table 15.

headspace			gas chromatograph	
carrier gas	helium		carrier gas	helium
pressure setting	105 kPa		initial primary pressure	80 kPa
temperature setting	oven	90 °C	capillary column	6 % cyanopropyl-phenyl- 94 % dimethylpolysiloxane; DB-1301-DF1.0 µm; 30 m * 0.32 mm; Agilent Technologies
	needle	100 °C	split flow	10 mL/min
	transfer conduction	120 °C	initial oven temperature	40 °C
time setting	thermostatting	50 min	initial time	4.5 min
	pressurizing	2 min	ramp 1 ramp 2	10 °C/min / 120 °C / 5 min 40 °C/min / 200 °C / 3 min
	withdraw	0.2 min	total time	22.5 min
	cycle	35 min	equilibration time	2 min
	injection	0.04 min	temperature	detector 300 °C injector 200 °C

Table 15: Gas chromatographic settings for quantitative analysis of residual ethanol

The ethanol peak showed a retention time of 2.25 minutes under these conditions. For calculation of residual solvent (ethanol) the ratio of standard solution (Equ. 8) and sample (Equ. 9) were calculated:

Equ. 8:
$$ratio_{sta} = \left(\frac{peak\ area_{analyte\ (ethanol)}}{peak\ area_{inner\ sta}} \right)$$

Equ. 9:
$$ratio_{sample} = \left(\frac{peak\ area_{analyte\ (ethanol)}}{peak\ area_{inner\ sta}} \right)$$

Then the determination of content was calculated according to Equ.10:

Equ. 10:
$$con = \left(\frac{ratio_{sample} \cdot V_{analyte\ within\ sta} \cdot D_{analyte} \cdot F_{sta} \cdot V_{sample} \cdot 1000\ g}{ratio_{sta} \cdot V_{sta} \cdot W_{sample} \cdot 1\ kg} \right)$$

con	content [mg/kg]
$D_{analyte}$	density of the analyte (ethanol: 0.7937 g/mL)
F_{sta}	content factor of the alcohol reference standard
$V_{analyte\ within\ sta}$	volume of the analyte (ethanol) within standard solution [µL]
V_{sample}	volume of sample solution [mL]
V_{sta}	volume of standard solution [mL]
W_{sample}	weighted sample [g]

The samples were measured once in which the relative standard deviation of the method was determined with 0.52 % by six repeat measurements of the standard.

2.2.17 Petri dish method

Due to the films being stored in slide frames and therein positioned planar, the development of a disintegration/dissolution test was considered. The slide frames with the oral films were laid on a Petri dish and one drop of distilled water was added by a pipette. The time taken for the drop to dissolve the film and form a hole in the film was recorded.

2.2.18 Slide frame method

To compare the results a second method was acquired. 2 mL of distilled water were placed in a Petri dish. One film was added at the surface of the water and the time measured until the oral film was completely dissolved.

2.2.19 Swelling determination

Swelling was measured by thermomechanical analysis using a Mettler TA 3000 Apparatus (Mettler Toledo, Gießen, Germany) with TC 10A Processor and a TMA 40 load cell. Discs were film-coated either with drug-free or drug-loaded film solutions, each with the same height of 900 µm. A film-coated disc was placed into a crucible and was annealed. The measuring sensor was placed onto the surface with a constant force of 0.02 N at a constant temperature of 37 °C. 250 µL of purified water were added by a syringe with a canula.

2.2.20 Contact angle measurement

Drop shape analysis was used to determine contact angles. Time-dependent contact angles were measured by an optical contact angle meter (Drop Shape Analysis System DSA100, Krüss, Hamburg, Germany) at room temperature. 7.5 µL of distilled water (500 µL/min) were dropped by a syringe (Hamilton 1750 TLL without Stop, 500 µL, Hamilton, Bonaduz, Switzerland) onto the film lying planar on the surface by fixing with double-faced adhesive tape. The contact angle was determined after 30 seconds by using the supplied software (Drop Shape Analysis DSA1 v 1.90, Krüss, Hamburg, Germany).

2.2.21 Drug release

The *in vitro* drug release of the wafers was determined using the paddle method (USP 24 apparatus type 2). The dissolution medium was either 250 mL of purified water or phosphate buffer pH 6.0 R2 annealed to 37 °C according to the Ph. Eur. 6.3 dissolution test for medicated chewing gums. The rotational speed was set at 50 rpm. The drug release was analyzed spectrophotometrically at 272 nm by using a calibration. To exclude overlapping between absorption peak of API and film matrix a chromatogram at 272 nm by HPLC was recorded. The spectrum showed no interference. One film was placed into each vessel. All tests were performed until the wafers were completely dissolved (judged by visual inspection). The measurement was replicated five times with the standard deviation as a measure of variation.

2.2.21.1 Dissolution apparatus with manual sampling

The dissolution test was performed with Sotax AT6 apparatus (Sotax GmbH, Lörrach, Germany). Every 30 seconds 0.5 mL samples were manually withdrawn and measured by UV-VIS-spectroscopy (Spekol 1200, Analytik Jena, Jena, Germany). The withdrawn amount of dissolution medium was calculated. Since measurements were

only carried out in phosphate buffer pH 6.0, calibration was only performed for this medium.

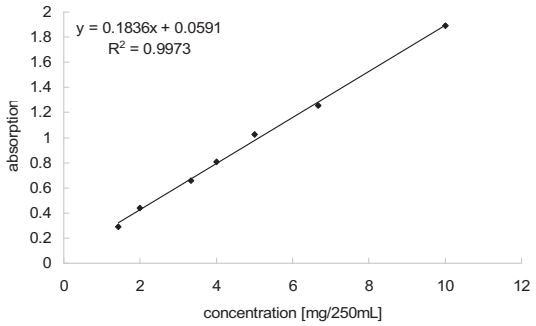


Figure 67: UV absorption of caffeine base in phosphate buffer pH 6.0 by UV/VIS spectrometer (Spekol 1200) at 272 nm, 1 cm cuvette.

2.2.21.2 Fiber-optic sensor system

For dissolution testing a Sotax AT6 apparatus (Sotax GmbH, Lörrach, Germany) was used. A fiber-optic submersible sensor (T300-RT-UV-VIS, Mikropack, Ostfildern, Germany) was dipped into the medium. The obtained absorptions during drug release were measured on-line via spectrometer (USB4000-UV-VIS, Mikropack, Ostfildern, Germany). Spectra between 200 and 800 nm were recorded and analysed. The calibration was performed for purified water as well as phosphate buffer pH 6.0 with 5 concentrations.

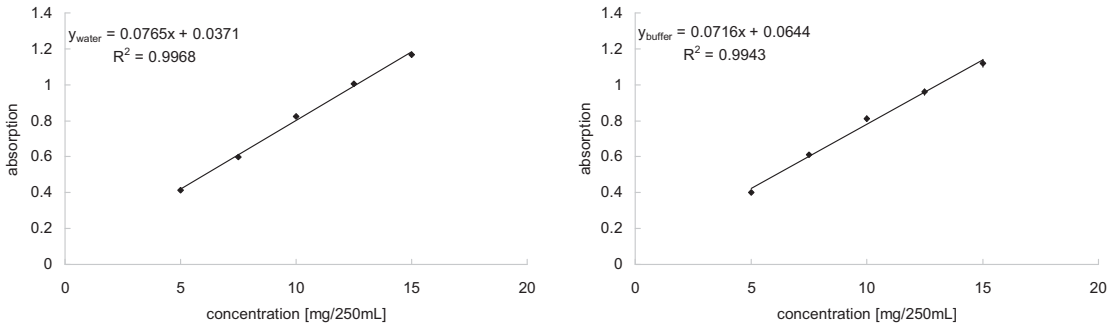


Figure 68: UV absorption of caffeine base in purified water and phosphate buffer pH 6.0 measured by fiber-optic sensor at 272 nm.

2.2.21.3 Dissolution apparatus with modified sampling

During dissolution tests (PTWS 600, Pharmatest, Hainburg, Germany) the dissolution medium was permanently pumped (IPC-8, Ismatech, Zurich, Switzerland) through a flow cell in a photometer (Lambda 2S, Perkin-Elmer, Juegesheim, Germany) and returned. The time delay was determined to be 15 seconds.

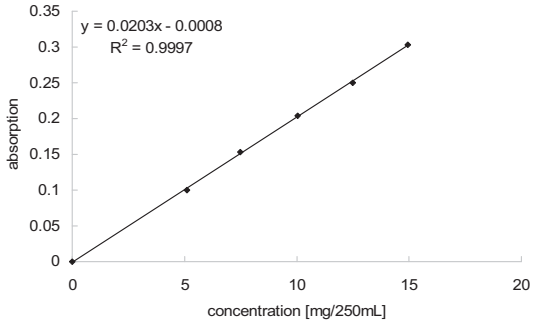


Figure 69: UV absorption of caffeine base in phosphate buffer pH 6.0 by UV/VIS spectrometer (Lambda 2S) at 272 nm, 1 mm cuvette.

2.2.22 Disintegration in human oral cavity

For a detailed description see section D 2.2.28.2 human taste panel. The layout of the questionnaire was based on Desai and Kumar (Desai and Kumar 2004).

2.2.23 Rheological measurements

Rheological measurements of the film solutions were performed by a rotating viscosimeter (CV20, Haake, Karlsruhe, Germany) with plate-cone-system (PK45/4, Haake, Karlsruhe, Germany) at 25 °C. All samples were measured at least in triplicate. During measurement the shear rate was raised from 0 to 300 s^{-1} within one minute and directly in turn decelerated within one minute from 300 to 0 s^{-1}. The film solution of HM50PA2910 with API could only be sheared below a shear rate of 100 s^{-1}.

2.2.24 pH value

One oral film was dissolved in 2 mL of distilled water in a test tube and the pH was measured by a pH meter (Knick Type 507, Knick, Berlin, Germany). The measurement was repeated five times.

2.2.25 Osmometry

The osmolalities of the dissolved oral wafers in 2 mL distilled water and phosphate buffer pH 6.0 were measured by a semi micro osmometer (Knauer, Berlin, Deutschland). Calibration was performed before measurement by two calibration solutions with an osmolality of 0 and 400 mosmol/kg. 150 µL of the film solution were inserted into a measuring cap and super cooled below the melting point. A stirring needle (vibrator) initiated crystallization. The measurement could be directly read off the display.

2.2.26 High performance liquid chromatography

Solutions with dissolved oral wafers were analyzed by high performance liquid chromatography (HPLC). The method used was adopted from Baroth (Baroth 1998). Chromatograms were obtained by an automated HPLC apparatus (Hewlett-Packard 1090L Series, Agilent, Böblingen, Germany) with an external UV-diode array detector (HP 1040M Series II, Agilent, Böblingen, Germany). Samples of 15 µL were injected

by an auto-injector with a draw speed of 42 µL/min. Data analysis was performed using the associated software (Hewlett-Packard ChemStation Rev. A.06.03, Agilent, Böblingen, Germany). The method was validated according to the ICH-Guideline “Validation of Analytical Procedures” (ICH Expert Working Group 2005b). Identification of the peaks was based on the relative retention time referring to the chemical reference standard (CRS) as external standard.

Samples of decomposition were prepared by adding an overage of API to 1 N hydrochloric acid (acid decomposition), 1 N sodium hydroxide (basic decomposition) or 3 % hydrogen peroxide solution (oxidative decomposition) by heating until boiling over half an hour. To avoid desiccation and thus charring of the sample distilled water was added. The obtained solution was inserted into diluent and subsequently chromtagraphed. Chromatograms of the samples of decomposition showed no overlaying with the caffeine peak.

For the mobile phase, a mixture of methanol and water (volume ratio 25:75) with an isocratic flow of 0.9 mL/min was used. The stationary phase was a reversed phase column (Hypersil™ ODS, Thermo Fisher Scientific, Waltham, USA) with the following dimensions: 125 x 4 mm, 5 µm. The oven temperature was 40 °C. Peaks were evaluated at a wavelength of 272 nm.

Validation of the HPLC method added up to the following characteristics. Linearity was proved (Figure 70) with 10 aqueous solutions of concentrations from 0.0005 to 0.5 mg/mL caffeine (0.5 – 500 % content of the labeled claim) measured in ascending and randomized order (n = 3).

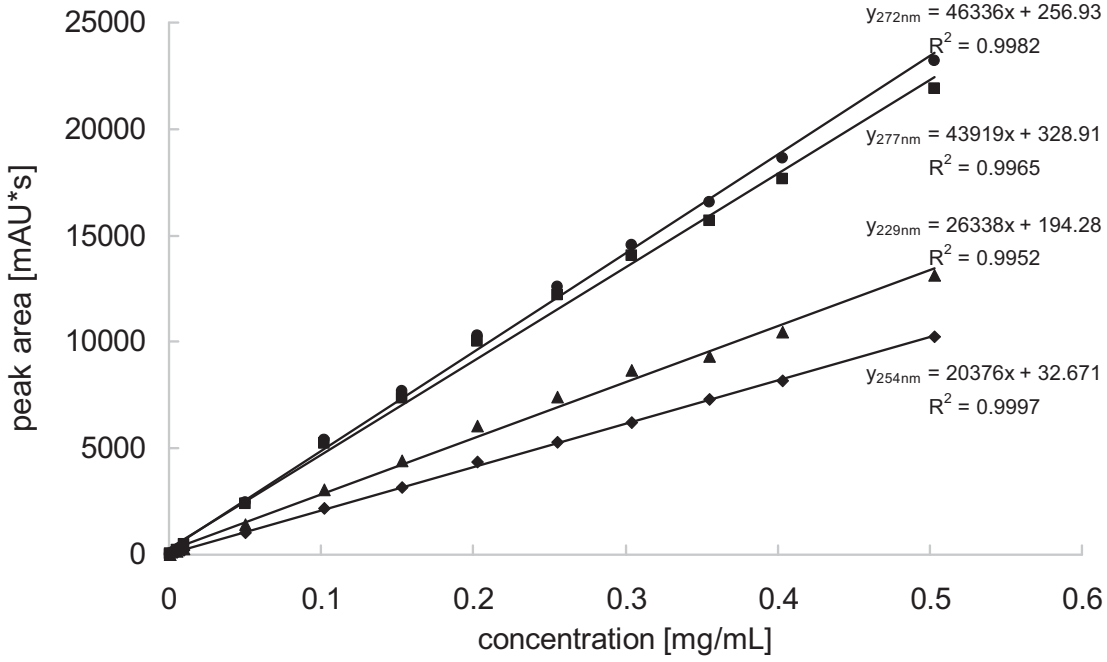


Figure 70: UV-DAD absorption of 10 solutions containing caffeine base in distilled water.

The limit of quantification was found to be less than 0.5 µg/mL and the limit of detection was 15.625 ng/mL. Precision was determined by quintuplicate injection of the same sample (0.06 mg/mL caffeine CRS) and amounts 1.21 % for the absorption maximum of 272 nm. The sensitivity is highest at 272 nm with a slope of 46336 mAU·s·mg/mL. After complete dissolution of the oral wafers in solvent and subsequent determination of content by HPLC, the excipients present in the wafers could also absorb UV. Consideration had to be given to the potential of interference with the caffeine peak. As seen in Figure 71 the matrix of excipients did not overlay the caffeine peak.

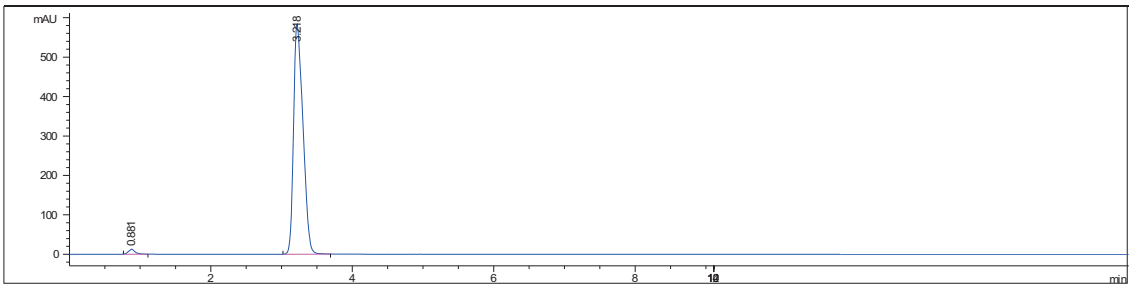


Figure 71: Chromatogram of a dissolved pullulan film within 100 mL mobile phase at 272 nm.

Samples were prepared by dilution of one oral wafer in a 100 mL volumetric flask with mobile phase as solvent which is equal to a concentration of 0.1 mg/mL. This concentration is within the linear range of the method (Figure 70). For oral wafers made of gelatin the mobile phase was warmed up until the wafers dissolved. Afterwards the solutions were cooled down and lost solvent replaced. Before injection these solutions were filtered through 0.45 µm polypropylene filters to separate suspended sediment. Besides sample measurement a lab standard solution was used as reference or rather external standard. 10 mg of caffeine base was weighed and dissolved in distilled water in a 100 mL volumetric flask. The calculated contents were referred to the lab standard.

The USP describes in the monograph of caffeine a method (United States Pharmacopoeia 2007) for determination by HPLC. The mobile phase is a buffer containing anhydrous sodium acetate, water, acetonitrile as well as tetrahydrofuran which is adjusted with glacial acetic acid to pH 4.5. The flow is specified with 1.0 mL/min. The peaks were evaluated at 275 nm wavelength at 22 °C. System suitability (containing theophylline), standard (containing theophylline and caffeine) and assay preparation (containing caffeine) were injected (20 µL instead of recommended 10 µL) and chromatographed by a Hypersil™ ODS, 150 x 4.6 mm, 5 µm column according to the requirements of the USP. With regard to the impurities, the relative retention times of theophylline and caffeine, the resolution between both peaks, the tailing factors and the standard deviations, all complied with the requirements.

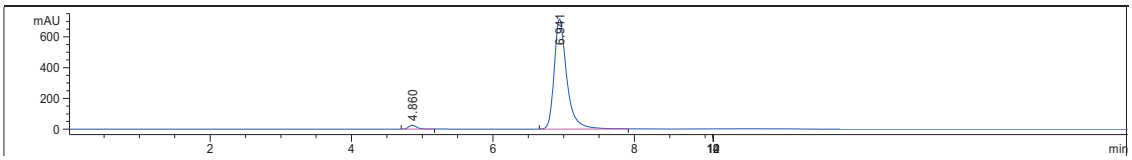


Figure 72: Chromatogram of standard preparation of the USP method at 272 nm, left peak: theophylline, right peak: caffeine.

In summary, the USP method is also suitable to distinguish caffeine from other substances qualitatively and quantify unknown amounts. However, it could not be proved that a better separation occurred and accordingly products of decomposition could be better separated as compared to the previously acquired method. The USP method is not advantageous compared to the used method. A major disadvantage is the use of acetonitrile as well as tetrahydrofuran which are polluting substances and their disposal is difficult and expensive compared to the conventional methanol-water-mixture of the used method. Furthermore, the mobile phase has to be prepared and no automatic merging by the HPLC is feasible. Due to the longer column the relative retention times are higher which causes longer run times and thus more wastage of solvent. Hence, costs for supply and disposal increase again. Given that the method of Baroth has been developed for the analysis of black tea as a multicomponent mixture, it is usable for film matrices which are complex multicomponent mixtures as well.

2.2.27 Dynamic vapor sorption

The films were exposed to a humidity of 98 % RH at 25 °C until constant weight was achieved. This increase in mass [%] was measured by a moisture sorption test system (SPS11, Projekt Messtechnik, Ulm, Germany). The measurement was complete when the weight did not change about 0.1 % within 30 minutes. One film was given in each weighing unit. This method was tested to determine whether a prediction is possible, on how the oral films made from different polymers behave in the presence of a high RH.

2.2.28 Taste assessment

2.2.28.1 Formulations for assessment

For the first trial of human taste panel as well as electronic tongue measurements oral films of formulation C (C 2.1.1) were investigated. The results from these investigations led to the compositions of formulation C' which are displayed in Table 16. The oral films were dyed according to the used flavor (red color for cherry flavor and orange color for orange flavor) to enhance acceptance by the volunteers as the color of the film is associated with an expected taste impression.

C'	polymer	caffeine	sucra-lose	aspar-tame	saccharin	sodium bicar-bonate	sorbitol	glycerol 85 %	citric acid	Tween®80	water	Total	dye	flavor
	gelatin / pullulan												yellow / red [gtt.]	orange / cherry [gtt.]
G-0.5S	13.27	3.65	0.26					3.58	0.45		78.79	100		
P-0.5S	13.21	3.64	0.25					3.56	0.45	0.49	78.40	100		
G-0.5S-K	13.27	3.65	0.26					3.58	0.45		78.79	100	30	3
P-0.5S-K	13.21	3.64	0.25					3.56	0.45	0.49	78.40	100	30	3
G-0.5S-O	13.27	3.65	0.26					3.58	0.45		78.79	100	20 2	3
P-0.5S-O	13.21	3.64	0.25					3.56	0.45	0.49	78.40	100	20 2	3
G-Asp	13.20	3.64		0.76				3.56	0.45		78.39	100		
G-Asp-K	13.20	3.64		0.76				3.56	0.45		78.39	100	30	3
P-Asp-K	13.14	3.62		0.76				3.55	0.45	0.49	77.99	100	30	3
G-Asp-O	13.20	3.64		0.76				3.56	0.45		78.39	100	20 2	3
P-Asp-O	13.14	3.62		0.76				3.55	0.45	0.49	77.99	100	20 2	3
G-$NaHCO_3$	13.04	3.59	0.25			1.80	1.72	1.72	0.44		77.44	100		
G-$NaHCO_3$-K	13.04	3.59	0.25			1.80	1.72	1.72	0.44		77.44	100	30	3
P-$NaHCO_3$-K	12.98	3.57	0.25			1.79	1.72	1.72	0.44	0.49	77.04	100	30	3
G-$NaHCO_3$-O	13.04	3.59	0.25			1.80	1.72	1.72	0.44		77.44	100	20 2	3
P-$NaHCO_3$-O	12.98	3.57	0.25			1.79	1.72	1.72	0.44	0.49	77.04	100	20 2	3
G-S_{100}	13.27	3.65	0.26				3.58		0.45		78.79	100		
P-S_{100}	13.21	3.64	0.25				3.56		0.45	0.49	78.40	100		
G-S_{100}-K	13.27	3.65	0.26				3.58		0.45		78.79	100	30	3
P-S_{100}-K	13.21	3.64	0.25				3.56		0.45	0.49	78.40	100	30	3
G-S_{100}-O	13.27	3.65	0.26				3.58		0.45		78.79	100	20 2	3
P-S_{100}-O	13.21	3.64	0.25				3.56		0.45	0.49	78.40	100	20 2	3
G-S_{50}	13.28	3.66	0.26				1.76	1.76	0.45		78.83	100		
P-S_{50}	13.22	3.64	0.25				1.75	1.75	0.45	0.49	78.45	100		
G-S_{50}-K	13.28	3.66	0.26				1.76	1.76	0.45		78.83	100	30	3
P-S_{50}-K	13.22	3.64	0.25				1.75	1.75	0.45	0.49	78.45	100	30	3
G-S_{50}-O	13.28	3.66	0.26				1.76	1.76	0.45		78.83	100	20 2	3
P-S_{50}-O	13.22	3.64	0.25				1.75	1.75	0.45	0.49	78.45	100	20 2	3
G-SaNa	13.26	3.65			0.31			3.58	0.45		78.75	100		
G-SaNa-K	13.26	3.65			0.31			3.58	0.45		78.75	100	30	3
P-SaNa-K	13.20	3.63			0.31			3.56	0.45	0.49	78.36	100	30	3
G-SaNa-O	13.26	3.65			0.31			3.58	0.45		78.75	100	20 2	3
P-SaNa-O	13.20	3.63			0.31			3.56	0.45	0.49	78.36	100	20 2	3

Table 16: Detailed film formulation and labeling of oral wafers (column 1) from human taste panel and electronic tongue measurements, content in [%].

2.2.28.2 Human taste panel

15 adult volunteers participated in the palatability study and were asked to assess the disintegration/dissolution time as well as the taste of oral wafers. With the disintegration of the oral film caffeine dissolves within saliva and thus, the terms disintegration and dissolution were used synonymously. The sample, one oral wafer of 6 cm² with 10 mg caffeine, had to be laid on the tongue and the volunteers had the option whether the film disintegrated/dissolved on the tongue, was moved around in the mouth or adhered to the palate. However, due to the small amount of saliva present, the complete dissolution probably took more time. Initially, the adult volunteers had to stop the time when no solid particles were distinguishable in the mouth anymore. Then they had to assess the oral wafers in general, which meant its comfort or possible irritation, whether saliva production was increased or dryness in mouth occurred. In addition the consistency/texture of the wafers was estimated. Furthermore, the volunteers had to assess the taste in general and in particular the bitterness, saltiness, sourness and sweetness, only umami (and astringency) was not be evaluated. The taste of the oral wafers was rated on a scale from 1 to 5 for the general taste according to school grades and for the specific taste from 1 to 5 as well, but 1 was equal to no taste and 5 was equivalent to too much of the respective taste. Between the samples the volunteers had unlimited time to rinse their mouth with water. Moreover, the volunteers had the opportunity to add other notes or remark further on taste impressions like metallic, astringent and so on.

Within the second palatability study the adult volunteers were asked only to test the flavored oral wafers (-K, -O) to avoid an irritation of the taste buds contrary to the measurements using the electronic tongue where all formulations were investigated.

2.2.28.3 Electronic tongue

The Insent electronic tongue is distributed with 7 sensors revealing 8 taste qualities (including 3 aftertastes) and 2 reference electrodes. Within the presented measurements 5 taste (astringency, bitterness, saltiness, sourness and umami) and 3 aftertaste qualities were evaluated. The 5 tastes are expressed as relative values. The aftertaste (CPA) is only detectable for astringency, bitterness and umami and results from the change of membrane potential caused by adsorption of the sample. The electronic system measurements were based on the principle of potentiometry. The sensors and reference electrodes were filled with an inner solution (3.33 mol/L KCl/saturated AgCl) and soaked for one day. They either can be stored filled for a short time (not more than one week) or dry. The sensors are closed by a lipid membrane. The reference electrodes are small glass tubes with a porous ceramic field at the bottom. The sensors and electrodes were attached to the sensor heads according to their numbering. A standard solution serving as cleaning and reference solution (30 mmol/L KCl/0.3 mmol/L tartaric acid) was prepared, filled into beakers (80 mL) and arranged. Up to 10 samples can be measured by a measurement cycle.

Before measuring the samples calibrations with anhydrous caffeine as well as caffeine citrate were performed. Aqueous solutions of anhydrous caffeine (concentrations: 0.01 to 15 mg/mL) and caffeine citrate (concentrations: 0.01 to 30 mg/mL), close to the solubility limit of the API, were measured. The measurement cycle encompasses 4 standard cycles, but the first run is disregarded in taste prediction as recommended by the supplier, so the calibration contains 3 runs. The calibration was repeated with a second sensor set, at different days, to check reproducibility of sensor response. The calibration solutions were measured in ascending order. Before starting the measurement cycle a sensor check to assure intact sensors has to be performed. The sensors should be in the specified voltage range and not differ more than 0.5 mV from the set value otherwise the sensor check has to be repeated or finally the sensors are not used. In addition, a maintenance measurement with solutions representing the taste stimuli is done monthly. The measurement cycle encompasses 4 runs in which the first run is omitted. The samples can be referred to the reference (= standard) solution or any other solution, as here used for example a 10 mmol/L KCl solution. After starting the cycle the measurement follows automatically the program. A reference value (V_r) is firstly measured within the standard solution. Afterwards the sample value (V_s) is measured in the sample solution, and then the sensors are shortly washed with standard solution. To determine the reference value V_r' the sensors dip into another standard solution representing the aftertaste. Relative value and CPA are calculated by subtracting V_r from V_s or V_r' respectively. After this procedure the sensors are fully washed with alcohol solution. This procedure is repeated for every active sample and lasts about 10 minutes including washing time. Every sample is measured in rotating order and measured again after one full round until the fourth run is completed.

2.2.28.4 Data analysis of the electronic system

For data analysis relative values (raw data) were used, however the taste information can be used as well. The transformation into taste information is performed by the Insent software by calculating the relative values with respective equations (Table 17) from mV data into dimensionless taste values.

Taste information	Equation of taste information
Sourness	= 0.332×sensor SB2CA0 + 12.0
Bitterness	=－0.140×sensor SB2C00 + 0.084 ×sensor SB2CT0
Astringency	=－0.1575×sensor SB2AE1 + 0.1575 ×sensor SB2CT0
Umami	=－0.1575×sensor SB2AAE
Saltiness	=－0.252×sensor SB2CT0
Aftertaste from Bitterness	=－0.210×CPA(SB2C00)
Aftertaste from Astringency	=－0.252×CPA(SB2AE1)
Substance	=－0.420×CPA(SB2AAE)

Table 17: Transformation of raw data into taste information (Intelligent Sensor Technology, Ltd.).

A multivariate analysis was performed for the concentration series by SIMCA software (SIMCA-P+, version 11.5, Umetrics AB, Umeå, Sweden). Therefore the multivariate data analysis tool “projection to latent structures by means of partial least squares” (= PLS) by SIMCA was used. All variables were mean centered and scaled to unit variance. For estimation of the predictability of the models R^2 and RMSEE (Root Mean Square Error of Estimation) were calculated. The Q^2-leave-one-out cross validation test within the PLS models was generated. Therefore components and variables were chosen to decrease complexity of the model and ease interpretation. The more components were included the higher the Q^2 was. If the Q^2 did not further improve, the calculation stopped. The aim of the selection of variables and components was achieving high predictivity of the model by removing information not directly related to variables and components to increase the robustness of the model. However, the amount of components should never exceed the number of variables.

E SUMMARY

Oral films, also called oral wafers, are intended for the application in the oral cavity and they are an innovative and promising dosage form especially for use in pediatrics and geriatrics. On the European market no licensed drug product is available yet. In one part of this project the studies focused on the development of such a dosage form for pediatric use with an appropriate active substance. In the other part, the aim was to develop adequate analytical methods for their characterization as well as improving already existing approaches.

Caffeine was chosen as the API according to a prospective field study on prescribed drug formulations in German pediatric wards as it plays an important role in neonatology and a licensed medicinal product is still missing. Hence, the masking of the bitter taste of caffeine arose as a further requirement in drug development to avoid the refusal of the new dosage form. In the study it was found that a single dose of 10 mg caffeine may cover several age groups within the pediatric population. The amount, 10 mg of API, represents a high drug-load for oral wafers.

Drug-free films were prepared according to the patent literature starting with a pre-evaluation of different film formers such as cellulose ethers, polyethylene glycol-polyvinyl alcohol copolymer (Kollicoat® IR), pullulan and sodium alginate. Gelatin, hypromellose, polyvinyl alcohol and pullulan were evaluated for further use in drug-loaded oral films. The best compromise between fast dissolution and pleasant taste was shown for oral films made of gelatin and pullulan. Improving their palatability by using different sweeteners, flavors and dyes led to two formulations with pleasant taste without any bitterness.

The oral films, based on different formulations, were evaluated with regard to their morphology, mechanical and thermal properties. Recrystallization of caffeine occurred within the drug-loaded oral wafers, which led to non-uniform distribution of API and caused limited content uniformity for oral wafers made of gelatin and one hypromellose type (HM50PA2910). Furthermore, residual solvent was determined by different methods. In the formulations that contained ethanol as solvent, this alcohol could not be quantified in the finished products making the oral wafers safe for pediatric use. The results from the investigations of osmolalities of dissolved films in appropriate medium showed values far below the critical threshold for cell necrosis which additionally approves the applicability of oral wafers to pediatrics.

An attempt to simulate the disintegration and dissolution behavior in the human oral cavity was made by developing methods using a fiber-optic sensor, contact angle meter or determination of swelling. Since only a small amount of saliva is present in the oral cavity, the development of an adequate method proved to be difficult. It was

revealed that oral wafers showed fast-dissolving behavior, both *in vitro* and *in vivo*, although they had a drug-load of 10 mg caffeine.

Child-appropriate dosage forms have been described for liquids, but recently the WHO has recommended solid dosage forms, including oral films, for global use due to their better stability and lower costs. The stability testing over 12 months at two climatic conditions showed sufficient stability of all investigated oral wafers.

In conclusion, in the present work, the development of oral drug-loaded wafers was successful. Although the wafers contain 10 mg caffeine, which is a bitter tasting substance, the taste was assessed as comfortable and pleasant. The manufactured oral wafers were characterized by several methods and found out to be stable even without primary packaging. An evaluation of appropriate film formers for oral use could be undertaken.

However, the present study revealed that recrystallization of API may be problematic. Further studies should be aimed at preventing the recrystallization which occurred in the case of caffeine. The developed approaches, especially for dissolution testing, should be improved to better mimic the natural conditions. Adequate methods to determine mucoadhesion are another possibility for prediction of the suitability of film formers for use in the oral cavity. Ultimately, the packaging of those oral wafers will play a considerable role in ascertaining and increasing their stability.

Finally, as the development of caffeine-containing oral wafers was successful and have been thoroughly characterized, some of the analytical approaches developed may attract interest in the scientific community and may be further developed.

F ZUSAMMENFASSUNG

Orale Filme, auch oral Wafers genannt, sind für die Anwendung in der Mundhöhle bestimmt und stellen eine innovative und viel versprechende Darreichungsform insbesondere für den Einsatz in der Pädiatrie und Geriatrie dar. Bisher ist auf dem europäischen Markt kein solches lizenziertes Arzneimittel erhältlich. In der vorliegenden Arbeit wurde einerseits auf die Entwicklung dieser Darreichungsform mit einem geeigneten Wirkstoff für die Pädiatrie fokussiert. Andererseits sollten neue, adäquate analytische Methoden für deren Charakterisierung entwickelt werden. Bereits vorhandene Ansätze sollten dabei implementiert und adaptiert werden.

Koffein wurde als Arzneistoff in Anlehnung zu einer prospektiven Studie an deutschen Krankenhäusern zur Verschreibung von Rezepturarzneimitteln in pädiatrischen Fachabteilungen ausgewählt, weil es eine wichtige Rolle in der Neonatologie spielt und ein geeignetes Fertigarzneimittel bisher fehlt. Mit der Maskierung des bitteren Geschmackes von Koffein ergab sich eine weitere Fragestellung, um eine Ablehnung der neuen Darreichungsform zu vermeiden. Der Studie zufolge wird für diverse pädiatrische Altersgruppen eine Einzeldosis von 10 mg benötigt. Letzteres erreicht damit die Beladungsgrenze von oralen Filmen.

Zunächst wurden wirkstofffreie Filme auf der Basis einer Patentrecherche entwickelt. Eine Evaluierung unterschiedlicher Filmbildner, die von Celluloseethern, Polyethylenglykol-Polyvinylalkoholcopolymer (Kollicoat® IR), über Pullulan bis zu Natriumalginat reichte, wurde durchgeführt. Gelatine, Hypromellose, Polyvinylalkohol und Pullulan wurden zur weiteren Herstellung von wirkstoffhaltigen oralen Filmen verwendet. Schließlich wurden Filme auf der Basis von Gelatine und Pullulan durch die Verwendung unterschiedlicher Süßungsmittel, Aromen und Farbstoffe geschmacklich weiter verbessert. Zwei unterschiedliche Formulierungen konnten entwickelt werden, die einen angenehmen Geschmack ohne jegliche bittere Eigenschaften zeigten.

Die oralen Filme aus unterschiedlichen Formulierungen wurden im Hinblick auf ihre Morphologie sowie die mechanischen und thermischen Eigenschaften evaluiert. In den arzneistoffbeladenen oralen Filmen wurde eine Rekristallisation von Koffein nachgewiesen, was zu einer ungleichmäßigen Verteilung des Arzneistoffs und damit zu unbefriedigender Gleichförmigkeit des Gehalts vorwiegend bei Gelatine- und Hypromellosefilmen führte. Darüber hinaus wurde der Restlösemittelgehalt durch verschiedene Methoden bestimmt. Es konnte kein Ethanol, welcher in den Lösungen zur Filmherstellung enthalten war, nachgewiesen werden. Somit kann die Verwendung von oralen Wafern in der Pädiatrie als unbedenklich angesehen werden. Die Ergebnisse der Untersuchungen zur Osmolalität von aufgelösten oralen Filmen zeigten Werte weit unterhalb des problematischen Grenzwertes, was die Verabreichung von oralen Wafern in der Pädiatrie ebenfalls unbedenklich erscheinen lässt.

Der Zerfall der oralen Filme und das Auflöseverhalten des Wirkstoffes in der Mundhöhle wurden mit verschiedenen Methoden wie faseroptischer Tauchsonde, Kontaktwinkelbestimmung sowie Quellungsmessungen nachgeahmt. Angesichts der Tatsache, dass in der Mundhöhle nur geringe Mengen Speichel vorhanden sind, erwies sich die Entwicklung einer angemessenen Methode als schwierig. Es konnte sowohl *in vitro* als auch *in vivo* gezeigt werden, dass orale Filme schnell zerfallen und sich auflösen obwohl sie 10 mg Koffein Arzneistoffbeladung enthielten.

Als kindgerechte Arzneiformen zur peroralen Anwendung wurden bisher Flüssigkeiten beschrieben. Aufgrund ihrer besseren Stabilität und geringeren Kosten empfiehlt die WHO neuerdings feste Darreichungsformen, zu denen die oralen Filme gehören. Die Prüfung der Stabilität über 12 Monate bei zwei klimatischen Bedingungen zeigte, dass alle untersuchten oralen Filme stabil waren. Schließlich spielt die Verpackung eine nicht zu vernachlässigende Rolle, um eine ausreichende Stabilität sicher zu stellen oder diese gar zu erhöhen.

Die vorliegende Arbeit hat gezeigt, dass das Rekristallisieren von Koffein in vielen Fragestellungen problematisch ist. In weiteren Untersuchungen sollte die Rekristallisation soweit wie möglich verhindert werden. Die entwickelten Ansätze, vor allem zur Untersuchung des Auflösungsverhaltens, sollten weiterentwickelt werden, um den natürlichen Gegebenheiten möglichst nahe zu kommen. Die Bestimmung der mukoadhäsiven Eigenschaften wäre eine weitere Möglichkeit zur Vorhersage der Eignung von Polymeren zur Anwendung in der Mundhöhle.

Zusammenfassend lässt sich sagen, dass die Entwicklung von oralen Wafern mit 10 mg Koffeinbeladung mit einem angenehmen Geschmack gelungen ist. Die hergestellten oralen Wafer wurden mittels verschiedener Methoden charakterisiert und zeigten eine ausreichende Stabilität über einen Zeitraum von mindestens 12 Monaten. Eine Bewertung von geeigneten Filmbildern konnte vorgenommen werden.

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