

Sugarcane wax as a sustainable excipient for melt extrusion

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Abbreviations

API	Active pharmaceutical ingredient
BCS	Biopharmaceutics classification system
BW	Beeswax
CO ₂	Carbon dioxide
CPA	Change of membrane potential caused by adsorption
CRM	Confocal Raman Microscopy
CV	Coefficient of variation
CW	Carnauba wax
d ₁₀	10% quantile
d ₅₀	50% quantile
d ₉₀	90% quantile
DGF	Deutsche Gesellschaft für Fettwissenschaft
DSC	Differential scanning calorimetry
e-tongue	Electronic tongue
EMA	European Medicines Agency
EU	European Union
FDA	U.S Food and Drug Administration
GFA	Same direction, conveying, comb-out element
GFF	Same direction, conveying, cut-free element
GHG	Greenhouse Gases
GRAS	Generally recognized as safe
HME	Hot-melt extrusion
HPLC	High performance liquid chromatography

Abbreviations

ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IPCC	Intergovernmental Panel on Climate Change
MMT	Million metric tons of CO ₂ equivalent
MT	Mini-tablet
NKH	Non-ketotic Hyperglycinemia
PCA	Principal component analysis
PEG	Polyethylene glycole
Ph. Eur.	European Pharmacopoeia
PVA-PEG	Polyvinylacetate-polyethylen glycole
RPM	Revolutions per minute
SB	Sodium benzoate
SD	Standard deviation
SEM	Scanning electron microscopy
SLE	Solid lipid extrusion
SW	Sugarcane wax
TAG	Triacylglycerides
TSE	Twin-screw Extruder
UNFCCC	United Nations Framework Convention on Climate Change
U.S	United States
USP	United States Pharmacopeia
WHO	World Health Organization
XRPD	x-ray powder diffraction

1 Introduction

Climate change or climate crisis is one if not the biggest challenge societies worldwide are currently dealing with (Intergovernmental Panel on Climate Change, 2022). Industries from all technological sectors are facing the challenge to reduce their emissions of carbon dioxide (CO₂) and switch to sustainable supply chains to reduce their contribution to climate change. The pharmaceutical industry is a great emitter of CO₂ which generates emissions comparable to the emissions of the automotive industry (Belkhir and Elmeligi, 2019). Beside the use of sustainable energy sources (Gielen *et al.*, 2019) and the switch from fossil-based excipients to regrowable excipients can contribute to the reduction of CO₂ emission. A good example of such fossil and regrowable materials which are utilized in the pharmaceutical industry are waxes. Beeswax (BW) and carnauba wax (CW) are well known excipients in the pharmaceutical industry (European Pharmacopoeia, 2023; 2023) and are non-fossil materials. Sugarcane wax (SW) is also a non-fossil material but is not utilized in the pharmaceutical industry but in polishing agents (Krendlinger *et al.*, 2015). SW is obtained from the waste of sugarcane cultivation, the bagasse (Attard *et al.*, 2015). The use of SW in the pharmaceutical industry would contribute to make the pharmaceutical industry more sustainable in two ways. On the one hand, the use of regrowable materials is more sustainable than of fossil material and on the other hand it would be the utilization of a waste material, which contributes to a circular economy. Circular economy is a key aspect of the green transition of the European Union (EU) (European Commission, 2020). Circular economy, enables the reduction of wastes by utilizing them for further processing.

In pharmaceutical applications waxes can be used to modify the release of a drug formulation (Vaassen *et al.*, 2012) or to mask unpleasant taste (Krause, 2008). Taste masking is especially useful for pediatric drug formulations. The pediatric population is sensitive for unpleasant taste (Breitkreutz and Boos, 2007) and taste masking is a useful tool to increase the acceptance of a drug formulation in the pediatric population. Beside taste, the size of the final drug formulation is essential for a good compliance. A lot of research has been done in the last years on the acceptance of mini-tablets (MT) in the pediatric population (Klingmann *et al.*, 2018). Acceptance of multiple units was shown.

Waxes are relatively low melting substances, which makes melting processes such as hot melt extrusion available for drug manufacturing without destroying the active pharmaceutical ingredient (API). Hot melt extrusion has been successfully applied for lipid-based pediatric formulations in the past (Breitkreutz *et al.*, 2003). Drug formulations obtained by melting processes can show changes in their dissolution properties due to changes in the crystalline structure over time (Michalk, 2007). This makes analysis of the crystalline properties as well as dissolution testing over the time of storage necessary.

A solid dosage form made from wax for the pediatric population should have a high drug load to reduce the number of total units applied, and should have no unpleasant taste. The stability of the drug formulation has to be analyzed. Suitable methods are differential calorimetry scanning (DSC) and x-ray powder diffraction (XRPD) to gain information on the crystalline properties, dissolution testing to analyze the drug release and taste analysis via electronic tongue to evaluate the taste properties.

2 General part

2.1 Sustainable use of resources

Significant research has been conducted on climate change and the role of CO₂. One of the earliest research works was done by Tyndall (1861), who demonstrated that CO₂ is able to trap heat, which laid the foundation to the concept of the greenhouse effect. In 1897, Arrhenius and Holden (1897) were the first to quantify the impact of CO₂ on the earth's temperature. Callendar (1938) later collected and analyzed data on global temperatures and linked it to the raising levels of CO₂ in the atmosphere, which represents one of the first studies on global warming. Revelle and Suess (1957) advanced his work and revealed in their research that CO₂ will not only be absorbed by the oceans but also accumulate in the atmosphere and ultimately contribute to the warming of it. Keeling (1960) was able to provide evidence of increasing concentrations of CO₂ in the atmosphere. Based on his research, the "Keeling Curve" was introduced, which tracks the steady increase of the CO₂ concentration in the atmosphere. Awareness for sustainable use of the earth's resources was brought to the world's population by the Club of Rome in 1971, with the publishing of their report "Limits to Growth" in 1971 (Meadows *et al.*, 1972).

However, it was not until 1979 that the first World Climate Conference was held in Geneva (United Nations Environment Programme, 2000). Purpose of the conference was to explore the impact of climate change on human activities and prevent man-made changes in the climate (United Nations Environment Programme, 2000). In 1987, the Brundtland Commission published a report titled "Our Common Future", which helped to popularize the term "sustainable development" (World Commission on Environment and Development, 1987). This report emphasized the need of balancing economic growth, environmental protection, and social equity. In 1988, the Intergovernmental Panel on Climate Change (IPCC) was founded by the United Nations (Intergovernmental Panel on Climate Change, 2024). The periodic reports by the IPCC help defining the international climate policy and summarize the latest knowledge on climate change and its impact (Intergovernmental Panel on Climate Change, 2024).

Following the founding of the IPCC, the Rio Earth Summit was held in 1992 in Rio de Janeiro. A major outcome was the adoption of the United Nations Framework Convention on Climate Change (UNFCCC), which was the first international treaty aiming to address climate change (United Nations, 1993). In 1997, the commitment to reduce the emission of CO₂ was pushed further with the Kyoto Protocol (United Nations, 1997). This protocol defined reduction targets for the emission of greenhouse gases (GHG). Following the Kyoto Protocol, it took almost 20 years until the Paris Agreement was drafted in Paris in 2015 (United Nations, 2015). This agreement contained a major commitment to fighting the climate change by reducing global warming to a maximum of 2.0°C. In comparison to the Kyoto Protocol, the Paris Agreement included commitments from 175 countries ((United Nations, 1997; 2015). With the commitments of all countries in the United Nations, the EU launched its own commitment in 2019 - the European Green Deal (European Commission, 2019). The EU has committed itself to become the first climate-neutral continent by 2050. A milestone on the way to that goal is the reduction of net GHG-emissions by 55% in comparison to the levels emitted in 1990 by 2030. A huge factor in this reduction is the decarbonization of the energy sector, but circular economy and resource efficiency also play a crucial role in the European Green Deal (European Commission, 2019).

2.1 Sustainable use of resources

The ambitions of the EU require efforts from all industries. The pharmaceutical sector emits large amounts of CO₂. It was estimated that the pharmaceutical industry exceeded the emissions of the automotive sector by emitting 52 million metric tons of CO₂ equivalents (MMT) in 2015 in comparison to the 46.4 MMT emitted by the automotive industry (Belkhir and Elmeligi, 2019). The high CO₂ emissions are caused by many relevant factors in the pharmaceutical industry. In order to reduce the carbon footprint, the switch to renewable energy sources is as important a step as it is for any industrial sector (Gielen *et al.*, 2019) due to the high energy consumption of drug manufacture (Malik *et al.*, 2018). Another relevant aspect is the aim of a zero-pollution and toxic-free environment, which is also part of the Green Deal of the EU (European Commission, 2020). This requires the implementation of green chemistry in the pharmaceutical industry (Kar *et al.*, 2022; Park *et al.*, 2021). *“Green chemistry is the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances. Green chemistry applies across the life cycle of a chemical product, including its design, manufacture, use, and ultimate disposal. Green chemistry is also known as sustainable chemistry”* (Anastas and Warner, 1998). Furthermore, pharmaceutical manufacturing is a highly regulated sector. The high regulation results in high quality standards. These may rely on inefficient processes, which cause a high consumption of resources (Roschangar *et al.*, 2015). The EUs Green Deal is also targeting a circular-economy (European Commission, 2020), which helps to lower the emission of GHG by reusing waste material to increase the efficiency of industrial processes. To achieve such a circular economy, the use of bio-waste material from the agricultural sector in the pharmaceutical industry could be implemented. This would also help to lower the GHG-emissions (Park *et al.*, 2021; Xu *et al.*, 2019). The use of SW as a pharmaceutical excipient represents an example of such a circular economy process, as it is a waste product occurring during the massive cultivation of the sugarcane plant for bioethanol manufacture (De Oliveira *et al.*, 2005; Krendlinger *et al.*, 2015).

2.2 Requirements for child-appropriate dosage forms

The pediatric population is a diverse patient group, which are not simply small adults, but a very heterogeneous group whose characteristics depend on the patients' age as well as their state of physical development. According to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline ICH E11, the pediatric population can be divided into the groups shown in Table 2.1.

Table 2.1 Categorization of the pediatric population according to ICH E11 (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2017)

Name	Age
Preterm newborn infants	gestational age in weeks
Term newborn infants	0 to 27 days
Infants and toddlers	28 days to 23 months
Children	2 to 11 years
Adolescents	12 to 16-18 years (dependent on region)

The European Medicines Agency (EMA) uses a slightly modified version of this categorization in their guideline concerning the clinical investigation of medicinal products in the pediatric population (European Medicines Agency, 2018). This modified classification is shown in Table 2.2.

Table 2.2 Categorization of pediatric population according to EMA ICH E11(R1) guideline (European Medicines Agency (2018))

Name	Age
Preterm newborn infants	Gestational age in weeks
Newborn	0 to 28 days
Infants	>28 days to 12 months
Toddlers	>12 month to 23 months
Preschool child	2 to 5 years
School age child	6 to 11 years
Adolescents	12 to 16-18 years

Each of these groups has its own characteristics, for example in regards to their bodies' water-to-fat ratio or their enzymatic activity. These unique characteristics make the development of suitable dosage forms necessary for each of the different pediatric populations.

2.2 Requirements for child-appropriate dosage forms

While the availability of drug formulations suitable for the pediatric population has improved over the last years, there is still a profound gap in availability in comparison to their adult counterparts. Several years ago, the situation was even direr. The lack of evidence-based drug-formulations lead to the application of unsuitable drug-formulations (European Parliament and the Council, 2006). The leading opinion was that liquid formulations were the preferred drug-formulation for pediatric patients, since the oral route is highly favored for treatment and liquid formulations are easy to swallow for every pediatric group. However, this was based on the assumption that neonates or infants generally reject solid dosage forms, because they would find swallowing them very difficult or even impossible (Yeung and Wong, 2005). Based on this assumption, the drawbacks of liquid drug formulations, such as a certain level of error in dosing or the poor patient compliance caused by bad taste, were generally accepted (Breitkreutz *et al.*, 1999).

The World Health Organization (WHO) now considers solid dosage forms suitable for dry administration to the pediatric population and does not limit the selection of suitable dosage forms for the pediatric population to only liquid drug formulations (World Health Organisation, 2008). The EMA however, has a different opinion on this matter and questions the general suitability of solid dosage forms for children. In the opinion of the EMA, the suitability of a solid dosage form is a combination of the age of the patient and the size of the drug dosage form (European Medicines Agency, 2006; 2013). Due to this general concern, a lot of efforts were performed in this field of research. (Oro-)dispersible formulations, which easily disperse into smaller particles with only slight amounts of liquid medium or even saliva, have been developed (Stoltenberg and Breitkreutz, 2011). For example, orodispersible MTs would make it easy for parents to precisely administer the targeted dose, while the drug formulation itself is of small size and rapidly disintegrates once it has been exposed to liquid. This makes swallowing easier, especially for infants and neonates. But not only MTs with orodispersible properties are possible, even orodispersible films can be developed (Klingmann *et al.*, 2020). Additionally, several studies have provided evidence that children are able to swallow solid dosage forms and that these are even preferably accepted by the pediatric population. Klingmann *et al.* (2022) showed that dosage forms with analgesics designed for the adult population (ages 18 and up) are well accepted by school-aged children and could be taken correctly by pediatric patients aging 6 to 17 years. Van de Vijver *et al.* (2011) could show that infants from 6 to 30 months of age accepted enteric coated MTs of 2 mm in diameter very well. These findings were later confirmed by Klingmann *et al.* (2013), who showed that six months to six-year-old children prefer one drug-free 2 mm MT, either coated or uncoated, over a dose of 3 ml of glucose syrup. In another work (Klingmann, 2017), the authors could show that even neonates and infants accepted 2 mm MTs, either coated or uncoated, very well in comparison to 3 ml doses of the glucose syrup. Klingmann *et al.* (2018) also demonstrated that children with 6 to 23 months of age accepted and even preferred a dose of 25 or 100 MTs over 5 ml of syrup. In the age group of two to five years, a non-inferiority was demonstrated (Klingmann *et al.*, 2018). But not only MTs with a diameter of 2 mm have been investigated: MTs with a size of 3 mm in diameter have also been the object of research focusing on acceptance among the pediatric population. Thomson *et al.* (2009) showed in their study that children from two to six years of age accept and can swallow a single 3 mm MT. Further, it was well demonstrated by Kluk *et al.* (2015) that children of age two and three accept swallowing multiple MTs of 2 mm or 3 mm size.

Such publications have shown that the application of solid dosage forms is a valuable alternative in the treatment of children. These findings triggered the development of solid drug dosage forms with their full spectrum of advantages: easier taste-masking, more precise dosing, high stability and non-harmful excipients.

2.3 Waxes

Waxes are a group of substances which have been known to mankind since ancient times. For instance, ancient Egyptians and Romans already knew the substance BW and used it for various purposes, such as the preparation of their mummies or as medicine (Bogdanov, 2016; Deutsche Gesellschaft für Fettwissenschaft, 1974; Fratini *et al.*, 2016; Pinto and Silverio, 2001). Waxes are heterogeneous in their chemical composition, which makes it difficult to formulate a universal definition covering all wax-like materials. A widely accepted definition for waxes has been established by the Deutsche Gesellschaft für Fettwissenschaft (DGF). The members of this society have identified the six properties constituting a wax (Deutsche Gesellschaft für Fettwissenschaft, 1974):

1. Kneadable at 20°C, firm to brittle hard.
2. Coarse to fine crystalline, translucent to opaque, but not glassy.
3. Melting above 40°C without decomposition.
4. Already relatively low viscosity slightly above the melting point.
5. Strongly temperature-dependent in consistency and solubility
6. Polishable under light pressure.

If more than one of these properties does not comply, it is not a wax according to this definition by DGF.

Waxes are materials that are composed of different components like oligomers or polymers of different molecular weights and degrees of branching. These oligomers or polymers may also contain functional groups such as carboxyl, alcohol, ester or ether-functions (Deutsche Gesellschaft für Fettwissenschaft, 1974; Krendlinger *et al.*, 2015).

Figure 2.1 shows a different classification of waxes based on their origin, including synthetic and natural waxes. Synthetic waxes gained a lot of relevance when the Fischer-Tropsch process was invented in 1935, enabling the synthesis of paraffins from carbon or carbon monoxide and hydrogen (Krendlinger *et al.*, 2015). However, this work has focused on the use of natural waxes and therefore synthetic waxes are not further explained here.

2.3 Waxes

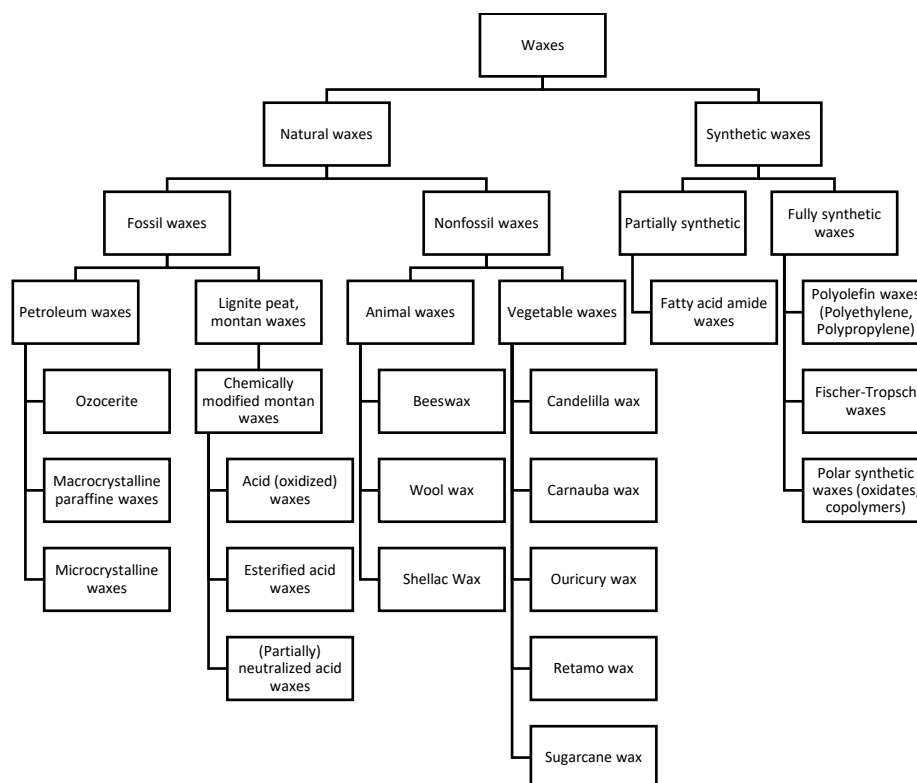


Figure 2.1: Systematic of waxes (adapted from Krendlinger *et al.* (2015))

Natural waxes can be divided into further subgroups according to their origin. Fossil waxes date back to prehistoric times and are usually extracted as components of fossil oil. Representatives of this wax group are petroleum wax and montan wax, both hydrocarbons of different chemical structures, mainly paraffins. The non-fossil waxes come either from animal or plant materials. Well-known examples of waxes from animal origin are wool wax and BW. From the group of vegetable waxes, candelilla wax, CW and SW are widely used as components in polishes (Krendlinger *et al.*, 2015). Additionally CW is used in the food industry as food additive E903 (European Food Safety Authority, 2012) and candelilla wax as food additive E 902 (European Food Safety Authority, 2012). SW was the main focus of this work. BW and CW, which have already been established in the pharmaceutical field, have been used as comparators. In contrast to the fossil waxes, animal and vegetable waxes are from renewable sources. In general, non-fossil waxes have a relatively low melting point (exceptions being Fischer-Tropsch paraffins and CW), which make them energy efficient for hot melting processes. Since energy consumption is an important factor in calculating the ecological footprint of a material, this is a beneficial attribute of these excipients. In addition, as these waxes are obtained from natural sources, they are considered a good substitute for fossil waxes which are not available infinitely. The three waxes used in the present study (BW, SW and CW), are presented in detail in the following paragraphs.

BW (cera alba; lat.: bleached wax, or cera flava, lat.:yellow wax, CAS-No. 8012-89-3 (Chemical Abstract Service)) is the building material of honey bees of the genus *apis*. It is used to build honeycombs in their hives. The BW is formed in the abdomen of the bee, more precisely in their oenocytes (Hepburn *et al.*, 1991; Xu *et al.*, 2024). These specialized cells form the precursors of the wax out of hydrocarbons and fatty acids. These substances are then transported through the epidermis of the honey bee (Cassier and Lensky, 1995; Hepburn *et al.*, 1991) and subsequently processed further by the jaws of the bee to build the honey combs (Krendlinger *et al.*, 2015). The exact composition of the wax varies according to the age of the bee and its species (Hepburn *et al.*, 1991; Piek, 1964). As

is typical for a natural product, this means a certain variability in the composition depending on the source of supply. Freshly secreted BW has a white color. Its yellow color comes from contamination with honey and pollen (Fratini *et al.*, 2016). The yellow color is well shown in Figure 2.2 Chemically, BW is a mixture of different esters, fatty acids and other hydrocarbons (Tulloch, 1972). It melts at approx. 66°C and is kneadable by hand. It is insoluble in water and moderately soluble in organic solvents such as diethyl ether or chloroform (European Pharmacopoeia, 2023; Krendlinger *et al.*, 2015). BW has been used by mankind for a very long time. In ancient Egypt, BW was used in the mummification process or to preserve papyrus (Bogdanov, 2016). In fact, the word “mummy” derives from a Persian word meaning wax. In the EU it is approved as a food additive under the number E901 and is used as a glazing agent as well as a carrier of flavoring agents (European Food Safety Authority, 2007). In the United States (U.S.), it is a material generally recognized as safe (GRAS).



Figure 2.2: Honeybees and their honey combs (Madsen, 2020)

by the U.S Food and Drug Administration (FDA) (Administration, 2019). This GRAS status enables the use of BW for various applications. In the pharmaceutical field; it has been used to prepare microcapsules (Racz *et al.*, 1997), to perform hot-melt fluid bed coating of API containing nonpareils (Kennedy and Niebergall, 1998) or to apply an enteric coating (Habashy *et al.*, 2020). BW is also used as an excipient in semi-solid preparations (Cordeiro *et al.*, 2024; Souza *et al.*, 2017) or ointments (Akombaetwa *et al.*, 2023). It is also a component of many cosmetic and pharmaceutical creams. In addition, since it is a renewable material, unlike the frequently used paraffins, BW is also used to make candles (Bogdanov, 2016).

SW (saccharum officinarum cera, CAS-No. 2496446-24-1 (DEUREX, 2018)) is obtained from the sugarcane plant. This plant is mass-cultivated in Brazil for bioethanol production (De Oliveira *et al.*, 2005). Additionally, South Africa, India and China cultivate large quantities of sugarcane (Krendlinger *et al.*, 2015). Historically, the wax was cultivated in Southeast Asia and Western India around 327 B.C (Chinnadurai, 2017). From there, it spread to Egypt in 647 A.D. and to Spain in 755 A.D. (Chinnadurai, 2017). The wax is obtained from the bagasse, a dry filter cake accruing during bioethanol production, via extraction (Attard *et al.*, 2015). The bagasse contains approximately 1% SW. Based on how much the SW has been processed after extraction, it can vary in color from dark

2.3 Waxes

brown to yellowish-brown (Krendlinger *et al.*, 2015). Depending on the source, it has a melting point of 71°C to 76°C, with the wax from the bagasse melting at around 71°C (Attard *et al.*, 2015; Krendlinger *et al.*, 2015). SW consists of alkanes, methyl and ethyl esters as well as alcohols and fatty acids (Nuissier *et al.*, 2002). For some of these components, potential health benefits are known (Asikin *et al.*, 2012), such as the reduction platelet aggregation (Arruzazabala *et al.*, 2002) and low-density lipoprotein levels in blood (Singh *et al.*, 2006) as well as possible prevention of atherosclerosis development (Noa and Mas, 2005). The wax is generally used in the industrial sector as a replacement for petroleum waxes. A typical application is the use as a polishing agent. As early as 1924, 6000t of SW were produced in South Africa in one year for the candle production of the Russian Orthodox Church (Origen Chemicals, 2024). It is also used as a substitute for CW in cosmetics and food (Nuissier *et al.*, 2002). In addition, it has been proposed as an alternative to candelilla wax in the food technology sector (Rocha *et al.*, 2013). The use in the pharmaceutical field, however, is still limited. While there has been research into producing sugarcane microspheres containing indomethacin (Gowda *et al.*, 2012) or solid lipid nanoparticles based on SW (Shamshad *et al.*, 2025), SW has not been monographed in the United States Pharmacopeia (USP) or European Pharmacopoeia (Ph. Eur.) so far.



Figure 2.3: Sugarcane plantage (Fietz, 2020)

CW (CAS-No. 8015-86-19 (Chemical Abstract Service)) is obtained from the palm *Copernicia cerifera*, which is mainly grown in Brazil. Like other plants, the carnauba palm produces wax to protect itself from water loss. The wax is obtained by harvesting the palm leaves and mechanically removing it from the leaves' surface. It is then further purified (Krendlinger *et al.*, 2015). CW is one of the hardest natural waxes and has one of the highest melting points of all non-fossil waxes at 80-88°C (European Pharmacopoeia, 2023). CW is a mixture of various esters, alcohols and other hydrocarbons (Krendlinger *et al.*, 2015; Tulloch, 1973). It is used as food additive E903 in the EU

(European Food Safety Authority, 2012) and has a GRAS-status by the FDA (U.S. Food and Drug Administration, 2019). Since CW is monographed in the U.S Pharmacopeia (United States Pharmacopeia, 2024) and Pharmacopeia. Europaea. (European Pharmacopoeia, 2023), there has been research using this material for several applications. For instance, microparticles containing Mesalazine (Balducci *et al.*, 2011) or nanoparticles loaded with Bemotrizinol (Medeiros *et al.*, 2020) were made from CW. Tableting has been performed using CW (Arhewoh *et al.*, 2015; Uhumwangho *et al.*, 2009) as well as hot-melt extrusion (HME) to produce granules containing diclofenac sodium (Miyagawa *et al.*, 1996). Melt-coating (Ziegler and Jacobs, 2008), and the manufacture of CW MTs have been investigated as well (Deshpande and Wasule, 2017; Nart *et al.*, 2017).



Figure 2.4: Carnauba palm (Tacarijus, 2007)

Aside from the previously mentioned ecological benefits of vegetable waxes in general, the sugarcane plant specifically has a relatively low consumption of water and a low ecological footprint, making it a perfect candidate for bioethanol and wax production in the present time (Somerville *et al.*, 2010). In the present work, it will be investigated as an extrusion aid for the first time.

2.4 Melt extrusion

The process of extrusion is characterized by pressing or pushing a liquefied or plastic mass through an opening of a defined size. The materials are introduced to the extruder by gravimetric or volumetric feeding and then forced through a die by means of pressure. As a result, extrudates with a constant diameter are obtained. A distinction is made between two basic types of extrusion processes: piston extrusion and screw extrusion (Crowley *et al.*, 2007). In piston extrusion, the material is forced through the die via a piston at high pressure. The disadvantage of this extrusion setup is the lack of further mixing processes within the extruder, which may result in inhomogeneous extrudates.

In screw extrusion, the material is conveyed through the extruder by means of a screw. The screw mixes, conveys and shears the material. Screw extruders come in different varieties. The simplest form is the single-screw extruder. As described in the name, this extruder has one screw that conveys the material through the extruder until reaching the die. In addition to the single-screw extruder, there is also the twin-screw extruder (TSE). This extruder type is built with two screws that rotate in either the same or in opposite directions. A schematic image of a TSE is shown in Figure 2.5. The material is fed from above into the extruder barrel by a suitable dosing tool, either by gravimetric or volumetric means. The material is then transported by the two screws further into the barrel. Screw-elements with different pitches build up a certain amount of pressure, while kneading elements provide a sufficient homogeneity of the mixture inside the barrel. At the end of the barrel, the mixture is pushed through a die, which defines the shape and diameter of the extrudates. In addition, the extruder barrel consists of elements whose temperature can be set individually, which is shown in Figure 2.5. This enables a wide variety of temperature profiles across the extruder housing.

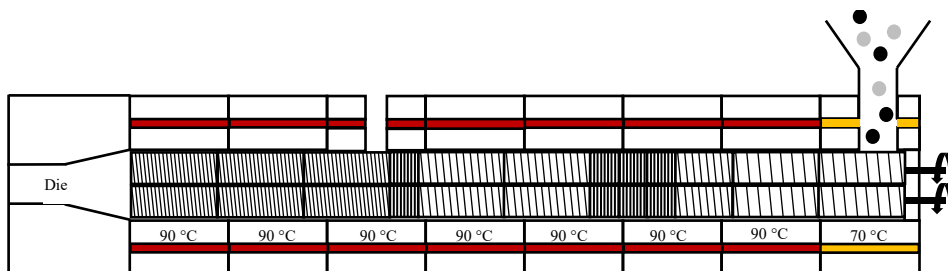


Figure 2.5: Schematic of a TSE. Different conveying and mixing elements are shown. Less distance between the diagonal lines represents a smaller length of the pitch. Vertical lines represent kneading elements.

In wet-granulation, a binder solution can be added, which might subsequently followed by a spheronization process downstream (Kleinebudde and Lindner, 1993). As an alternative to the classical wet-granulation with optional spheronization, HME can be performed. In HME the materials are initially heated up before the material is pushed through the die. In this process, the softening or melting components function as the binder or matrix former (Rosenberg and Breitenbach, 2002). Aside from the binder type and processing temperature, the fill level and the material residence time, which are controlled by the rotation speed of the screws or the metering rate of the feeding hopper, can also be adapted. Furthermore, the material can be processed with differing levels of mechanical shear stress based on the selected screw configuration. A variety of screw configurations is possible. An example of a suitable configuration for a HME process is shown in Figure 2.6.

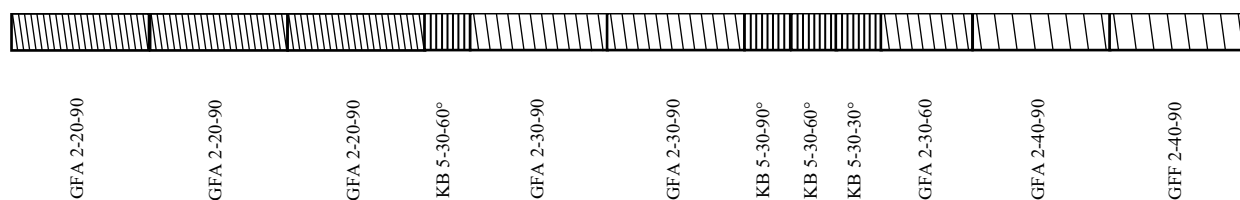


Figure 2.6: Exemplary screw-configuration for hot-melt extrusion process provided in the user manual of MICRO 27GL-28D, Leistritz, Nürnberg, Germany. KB =kneading blocks, GFF and GFA = conveying elements. GFF means same direction, conveying, cut-free element; GFA means same direction, conveying, comb-out element.

The length of a screw is expressed in multiples of the outer screw-diameter. A typical length of an extruder screw is 25 to 40 times the outer diameter of the screw. Extruder screws are composed of the following parts: conveying, kneading, and distributive flow elements (Leister *et al.*, 2012). Conveying elements feed the material forward through the barrel. This type of screw component is generally found at the entry point of the extruder barrel where the material has to be quickly conveyed further into the extruder barrel to avoid a pile-up of material at the entry zone. The purpose of kneading elements is to provide a homogeneous mixture by disturbing the material flow and to improve the blending process. Distributive flow elements are also used to improve the mixing process inside the extruder barrel. However, due to their non-self-wiping design, they typically are used only when liquids are mixed into a solid blend (Leister *et al.*, 2012).

Melt extrusion has become increasingly important in the food and plastics industries since the 1930s. In the 1970s, melt extrusion technology entered the pharmaceutical field. At that time, the use of poly (vinyl acetate-co-methacrylic acid) as a polymer matrix was being investigated.

Nowadays, HME has been used to produce a wide spectrum of different drug formulations (Alzahrani *et al.*, 2022). Follonier *et al.* (1995) were able to produce granules based on ethylcellulose, cellulose acetate butyrate, poly (ethylene-co-vinyl acetate) and polymethacrylate as well as pellets via HME (Follonier *et al.*, 1994). Crowley *et al.* (2002), Zhang and McGinity (1999) and (Sawant *et al.*, 2018) utilized the HME-process to obtain sustained release systems. Trans-dermal systems (Aitken-Nichol *et al.*, 1996; Marreto *et al.*, 2020; Mendonsa *et al.*, 2018; Repka *et al.*, 1999) as well as implants (Bhardwaj and Blanchard, 1997; Sam, 1992) have been developed as well. Nanostructured lipids have been developed by Bhagurkar *et al.* (2017) and solid lipid particles by Bagde *et al.* (2019). HME-technology has become more and more important based on its advantages. It offers the possibility to process an active pharmaceutical ingredient (API) solvent free, which eliminates the requirement of an additional drying process (Crowley *et al.*, 2007). The bioavailability of an API can be improved by using the HME-process (Forster *et al.*, 2001) by processing amorphous solid dispersions (Ashour *et al.*, 2016) and poor taste can also be masked (Malaquias *et al.*, 2018; Sugao *et al.*, 1998; Walsh *et al.*, 2014; Wang *et al.*, 2020).

A sub-discipline of HME is the processing of lipids. The term “lipids” is applicable for various materials. A lot of research has been conducted on the processing of these substances via HME. This type of component showed a promising potential in improving the bioavailability of many Class II substances of the biopharmaceutics classification system (BCS) (Prabhu *et al.*, 2005). The BCS classifies drugs based on their solubility and permeability (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2019). There are four different classes of substances according to the BCS to be distinguished: class I are substances with high solubility and high permeability, class II are substances with low solubility and high permeability, class III are substances with high solubility and low permeability and class IV are substances with low solubility and low permeability (International Council for Harmonisation of Technical

2.5 Solid Lipid Extrusion

Requirements for Pharmaceuticals for Human Use, 2019). Mono, di- and triacylglycerides (TAGs) such as glycerol mono-, di- and tristearate, glycerol tripalmitate, and glycerol trimyristate have been used to develop solid dosage forms (Rosenberg and Breitenbach, 2002). However, changes in the crystalline modification of the lipids (Bertoni *et al.*, 2021; Sato, 2001; Sutananta *et al.*, 1994; Windbergs *et al.*, 2009) have been observed after the HME-process, with some materials forming metastable crystalline structures when solidifying from the molten state. Since these changes altered the dissolution profiles of the drug products over time, another type of extrusion process was developed for such materials: the solid lipid extrusion (SLE) process.

2.5 Solid Lipid Extrusion

SLE processes lipids below their melting point. It was developed due to the aforementioned observed changes in crystalline modifications of lipids after complete melting during HME, which can drastically impact the dissolution profile (Choy *et al.*, 2005). Pinto and Silverio (2001) proved that extrusion of lipid components below their melting point is feasible via ram-extrusion. Such processing below the lipids' melting point prevents the material from changing into metastable crystalline structures during re-solidification. Ultimately, this avoids the transition from metastable crystalline structures to stable structures and thus the observed changes in the drug product quality after HME-processes. SLE can be performed using formulations with a high drug loads of 80% (Krause *et al.*, 2009; Petrovick *et al.*, 2015). The manufacturing of implants loaded with monoclonal antibodies was shown by (Vollrath *et al.*, 2018). Michalk (2007) developed a lipid-based formulation with glycerol dibehenate for the two APIs enrofloxacin and praziquantel using a twin screw extruder. Both formulations improved the desired taste properties but showed insufficient stability over the time of storage. Krause (2008) investigated drug formulations of spheronized lipid-based extrudates containing sodium benzoate (SB). As lipid binders, hard fat as well as combinations of hard fat with either glycerol dibehenate, glycerol trimyristate, or glycerol distearate were used. The process of SLE + spheronization was successfully applied and taste masking properties based on a self-coating mechanism were found. Witzleb *et al.* (2011) investigated the impact of the addition of pore-forming agents and the reduction in diameter of the extrusion die on extrudates made from the SLE process. Vaassen *et al.* (2012) designed a targeted dissolution profile by combining different proportions of hard fat, crospovidone and Polyvinyl acetate-Polyethylene glycole(PVA-PEG)-craft polymer in a SLE process. Petrovick and Breitskreutz (2018) performed SLE with a combination of metformin hydrochloride and either hard fat, glyceryl distearate or Glycerol trimyristate followed by spheronization into pellets. Petrovick *et al.* (2018) processed pellets made from SLE into orodispersible tablets.

SLE combines the advantages of HME, such as being solvent-free and providing taste masking, with additional advantages based on the lower applied temperature. These SLE-specific benefits include drug formulations with stable a crystalline modification as well as reduced energy consumption.

2.6 Taste evaluation

A sufficient taste and thus the palatability of a drug formulation plays an important role for patient compliance to a therapy. Especially younger patients struggle with drug formulations which come with a bad taste sensation because they have not yet adopted the cultural consent that a bad taste is not an unusual characteristic of healthy medicine. As a bad taste will negatively influence the compliance of younger patients and a sweet taste is highly favored by this group (Liem and Mennella, 2002), it makes the evaluation of taste during drug development an important tool for the successful development of a well-accepted therapy for the pediatric population.

The impression of “taste” is a combination of olfaction and gustatoric signals. The gustatoric signals are categorized into the five qualities sweet, umami, bitter, sour and salt. Spiciness and astringency are not taste qualities but somatosensory impressions which can influence the taste (Diepeveen *et al.*, 2022). Each of the five taste qualities provides information on specific nutrition. Sweetness is a signal for a meal rich with carbohydrates, which are a valuable source of energy (Yoshida and Ninomiya, 2024). Umami is a signal for a meal rich with amino acids, which are important to keep vital processes running (Chaudhari *et al.*, 2009; Zhao *et al.*, 2019). The salty taste is important for the body to absorb sufficient minerals like sodium (Sood *et al.*, 2025). Sourness is an indicator of valuable acids (Diepeveen *et al.*, 2022) and bitterness protects the body from absorbing harmful substances such as toxins (Wilson *et al.*, 2019). The different taste sensations are enabled by taste receptors, which are located in the taste buds of the tongue. Within each taste bud, three different types of receptor cells can be found (Baumer-Harrison *et al.*, 2020; van der Werf *et al.*, 2017). Type 1 cells make up 50% of all the cells in the taste buds. Type 2 cells 20 – 30% and type 3 cells make up to 10% (Baumer-Harrison *et al.*, 2020; Miura *et al.*, 2001). Each of these types of cells contributes in a different way to the processing of taste impressions. Type 1 cells mainly regulate the uptake and degradation of neurotransmitters. Type 2 cells are sensitive for substances which trigger a sweet, bitter or umami stimulus (Baumer-Harrison *et al.*, 2020; Zhang *et al.*, 2003). Type 3 cells are sensitive for sour and salty stimuli (Wilson *et al.*, 2022). All the above mentioned information emphasizes that the taste sensory system of the human body is a highly complex system with specialized cells for individual taste impressions and regulation.

An analytical instrument which can partially mimic these sensory capabilities is the electronic tongue (e-tongue). Different variants of this device have been developed in the past (Legin *et al.*, 2004; Toko, 1998). Currently, such devices are being commercialized by the companies AlphaMOS (Toulouse, France), which sells the α Astree e-tongue, and by Intelligent Sensor Technology (Atsugi, Japan), which sells the Taste Sensing System TS-5000Z. Both systems operate by measuring a potentiometric signal against an Ag/AgCl reference electrode (AlphaMOS, 2004; Kobayashi *et al.*, 2010). The α Astree e-tongue uses cross-selective sensors, which are not specialized on a single taste sensation (AlphaMOS, 2004). The Sensing System TS-5000Z uses several sensors with lipid membranes which are each sensitive to different types of substances. Thus, the sensors mimic the specialization of individual taste buds for sweet, sour, bitter, salty and umami taste. If a membrane is sensitive to a substance, the substance will interact with the membrane and alter its electrical potential. On the inside of the membrane, an Ag/AgCl reference electrode is in place in order to record the change in the electronic potential (Insent, 2024; Toko, 1998). It was shown by Ishizaka *et al.* (2004) and Sadrieh *et al.* (2005) that these instruments provide valuable insight into the palatability of a drug-formulation during the development stage. Since there are pronounced differences in taste sensation between children and adults, conducting palatability studies on adult people for pediatric formulations is not sufficient to ensure product acceptance. Furthermore, palatability studies with

2.7 Model drug sodium benzoate

infants are challenging to realize, rendering the use of an objective analytical tool is an invaluable contribution in the development of a drug formulation.

2.7 Model drug sodium benzoate

SB is usually used as a preservative in pharmaceutical drug formulations. As such, it is often part of liquid and semi-solid drug formulations. Apart from functioning as a preservative agent, SB is also applied as an API in urea cycle disorders, which cause hyperammonemia (Nassogne *et al.*, 2005; Snehavardhan *et al.*, 2020). Urea cycle disorders result in elevated ammonium levels, which can be reduced by the administration of SB (Green *et al.*, 1983). A combination with sodium phenylbutyrate is often used in that case (Batshaw *et al.*, 2001). Furthermore, SB is also used in the treatment of hyperarginemia (Baranello *et al.*, 2014; Qureshi *et al.*, 1984) and glycine encephalopathy (Haddad *et al.*, 2025).

Glycine encephalopathy, alternatively known as non-ketotic hyperglycinemia (NKH), is an autosomal recessive disease of the metabolism. In the human body, four proteins are responsible for the elimination of glycine. The P-protein (glycine decarboxylase), T-protein (aminomethyltransferase), H-protein (hydrogen carrier protein) and the L-protein (dihydrolipoamide dehydrogenase) (Coughlin *et al.*, 2017). If there is a malfunction in any of the four proteins due to a gene defect, this will manifest itself in NKH. There are reports in the literature specifically on defects in the P-protein (Coughlin *et al.*, 2017; Kanno *et al.*, 2007; Tada and Hayasaka, 1987). This appears to be the most commonly affected protein. However, there have also been reports of defects at the T-protein (Coughlin *et al.*, 2017; Schutgens *et al.*, 1986; Toone *et al.*, 2003) and at the H-protein (Coughlin *et al.*, 2017; Koyata and Hiraga, 1991).

There are different forms known of NKH, comprising a neonatal form (Hennermann *et al.*, 2002; Parviz and Hooshyar, 2025), an atypical form (Flusser *et al.*, 2005), a transient form (Applegarth and Toone, 2001) and a late onset form. Most affected patients show symptoms of lethargy, coma, respiratory distress, and seizures in neonatal life. As a result, most patients die of respiratory distress within the first two weeks after birth. If the patients survive the initial two weeks after birth, respiratory distress usually improves, but patients show mental abnormalities (van Hove *et al.*, 2005). Defects in one of the 4 proteins that control the Glycine cleavage system cause glycine to accumulate in blood plasma, urine, and cerebrospinal fluid. Elevated glycine levels are problematic because glycine is a substrate at both inhibitory and excitatory amino acid receptors (Stark *et al.*, 2000). Due to excessive levels of glycine, NMDA-receptors are permanently excited, resulting in atypically high calcium levels, which lead to long-term neuronal damage. Even with a correct diagnosis during pregnancy and immediate treatment after delivery, the already caused damage can no longer be compensated, which means a varying degree of mental retardation depending on the severity of the disease.

Since too high levels of glycine cause permanent excitation of NMDA-receptors, therapy is directed at reducing the patients' glycine levels. Glycine can be bound and removed from the body by sodium benzoate (Nowak *et al.*, 2022). Sodium benzoate and glycine are converted to hippuric acid in the mitochondria under participation of coenzyme A. Hippuric acid can subsequently be renally eliminated (Kubota and Ishizaki, 1991). Both sodium benzoate and benzoic acid are suitable for intestinal absorption. Since sodium benzoate is even more water soluble than benzoic acid, the former is preferred. Common doses for treatment of NKH are 250 mg - 750 mg/kg of body weight (Hoover-Fong *et al.*, 2004; Nowak *et al.*, 2022; van Hove *et al.*, 2005). Toxic symptoms may occur if the

dosage of 750 mg/kg body weight is exceeded (Van Hove *et al.*, 1995). Complementary to sodium benzoate, NMDA-receptor antagonists such as dextromethorphan (Hamosh *et al.*, 1998; Randak *et al.*, 2000) or ketamine (Boneh *et al.*, 1996; Ohya *et al.*, 1991) are also used for treatment.

In 2002, sodium benzoate received the orphan drug status for the treatment of NKH by the Committee for Orphan Medicinal Products at EMA. NKH is an orphan disease with a prevalence estimated as 0.02 of 10,000 inhabitants. Breitzkreutz *et al.* (2003) developed formulations containing sodium benzoate via different technologies such as dry roller compaction, cold extrusion and HME with either a hydrophilic or lipophilic binder. The obtained granules were further processed by applying an additional coating with Eudragit®E to mask the unpleasant taste. Further, Krause (2008) developed a sodium benzoate formulation containing combinations of sodium benzoate with either Witocan 42/44, Compritol 888ATO, Dynasan 114 or Precirol ATO 5 using solid lipid cold extrusion.

3 Aim of this work

SW has not yet been used as an excipient in the pharmaceutical field. The aim of this work is to show that this material can potentially be used as a lipophilic-wax like binder when processed via twin screw extrusion. HME as well as SLE should be applied and the properties of the obtained extrudates analyzed. Since molten substances can transition into different crystalline modifications depending on the applied process, the properties of the obtained product and raw material should be investigated via DSC and XRPD. Furthermore, the extrudates will be analyzed in dissolution studies to evaluate the dissolution of extrudates with different drug loads. The results of the SW products should be compared to the results of products made from BW and CW, which have been processed the same way. As these two waxes have previously been monographed in the Ph. Eur, they can be used as pharmaceutical reference materials representing the current state of the art for pharmaceutical lipid-based extrusion.

Once a manufacturing process has been established, the extrudates require further processing into a suitable pharmaceutical dosage form. Since there is a pre-existing knowledge base of MTs made from lipophilic binders, a MT drug formulation based on SW is chosen as the desired drug formulation. SB is chosen for this work as a model substance based on existing processing experience as well as prior market authorization for drug products using lipophilic binders. However, it is known from that development and from literature, that this API has poor taste qualities. Thus, it requires additional processing after undergoing lipid-based granulation and tableting to prevent a bad taste experience during the intake of the drug. In conclusion, a suitable functional coating should be applied to the potential MTs to minimize the risk of taste-based noncompliance. In order to reliably determine if the applied coating successfully prevents a bad taste experience, uncoated and coated product samples should be analyzed using an electronic tongue.

4 Results and discussion

4.1 Characterization of starting materials

4.1.1 Introduction

A thorough understanding of the morphological and physicochemical properties of the materials employed is of great importance for drug development. Knowledge of attributes such as flowability or particle size are important for designing a process. Further morphological information is crucial for establishing a sufficient quality for the final product. For instance, a change in crystal morphology of lipids over storage time may adversely affect the release profile of a drug formulation (Choy *et al.*, 2005; Windbergs *et al.*, 2009), which results in an immense loss of quality and safety.

SW does not have a pharmacopeial specification yet. It has not been used in pharmaceutical preparations so far. However, some research has been done characterizing SW. The chemical industry has characterized SW via determining the saponification number (Krendlinger *et al.*, 2015), which is an established method in the Ph. Eur for the characterization of fats (European Pharmacopoeia, 2023). The food industry has conducted research on organogels formed by SW (Rocha *et al.*, 2013). Other research characterized the thermal properties of SW via DSC (Attard *et al.*, 2015).

For the physicochemical characterization of SB, BW, SW and CW DSC and XRPD were used to investigate potential polymorphism of waxes (Basson and Reynhardt, 1988; 1988; Kameda and Tamada, 2009) as well as to investigate the crystalline properties of SB. Helium pycnometry was also conducted to determine the density of the materials. For an insight into the morphological attributes of the raw materials, both the Carr index and particle size distribution were determined and scanning electron microscopic images were taken.

4.1.2 Particle size distribution and particle shape

SB was purchased as a fine powder, which did not require additional processing before it could be used in experiments. Thus, no pre-treatment of the material was needed prior to determining the particle size distribution shown in Table 4.1. BW, SW and CW were obtained as pellets or flakes (Figure 4.1) and were milled before further processing to harmonize all excipient particle sizes. The particle size distribution was determined using laser diffraction spectrometry. In this technique, the sample is dispersed either in air (dry dispersion) or in a liquid medium (wet dispersion) to ensure an even distribution of particles. The dispersed sample is introduced into a measurement chamber, where it interacts with a laser beam. As the laser light encounters the particles, it is scattered at angles dependent on particle size. Detectors positioned around the chamber collect the scattered light, generating an intensity distribution pattern. The particle size distribution is then calculated by applying either Mie scattering theory or Fraunhofer diffraction theory, depending on the optical properties and size of the particles (de Boer *et al.*, 1987).



Figure 4.1: Waxes before milling; (left) beeswax pellets, (middle) carnauba wax flakes, (right) raw powder sugarcane wax

Due to the low melting point of natural waxes, the material and the mill were cooled with liquid nitrogen, counteracting the otherwise observed temperature increase by energy dissipation, during the milling process to avoid melting or sticking.



Figure 4.2: Waxes after milling; (left) beeswax, (middle) carnauba wax, (right) sugarcane wax

After grinding, agglomerates were still present in both BW and SW (Figure 4.2). These agglomerates however, were very fragile and easily disintegrated by light force. The particle size, span (**BaHav19-004b**) and true density (**BaHav18-008**) of the milled waxes as well as of the unmilled SB were determined and are shown in Table 4.1.

Table 4.1: Particle size distribution values and true density of raw materials

Material	Mean $\pm$ SD (n = 3)				True density [g*cm ⁻¹]
	d ₁₀ [μm]	d ₅₀ [μm]	d ₉₀ [μm]	Span	
Sodium benzoate	16.5 $\pm$ 0.1	67.2 $\pm$ 0.2	129.0 $\pm$ 0.5	1.673 $\pm$ 0.002	1.511 $\pm$ 0.006
Beeswax	160.2 $\pm$ 4.2	708.3 $\pm$ 9.5	1702.8 $\pm$ 29.8	2.180 $\pm$ 0.012	0.964 $\pm$ 0.001
Sugarcane wax	3.6 $\pm$ 0.1	8.2 $\pm$ 0.2	606.9 $\pm$ 130.9	73.683 $\pm$ 15.128	1.030 $\pm$ 0.002
Carnauba wax	122.2 $\pm$ 4.7	431.7 $\pm$ 5.8	836.4 $\pm$ 7.4	1.655 $\pm$ 0.016	1.001 $\pm$ 0.003

4.1 Characterization of starting materials

Even after cryomilling of the other excipients, SB showed the smallest particle size with respect to the 90% quantile (d_{90}) at 129.0 μm . The particle size of SB was in total narrow with 16.5 μm at the 10% quantile (d_{10}), 67.2 μm at the 50% quantile (d_{50}) and 129.0 μm at the d_{90} (Figure 4.3a). This narrow particle size distribution is a result of the production process of spray drying, which allows producing particles in a narrow range and was used to produce SB (Fahr, 2018). In contrast to SB,

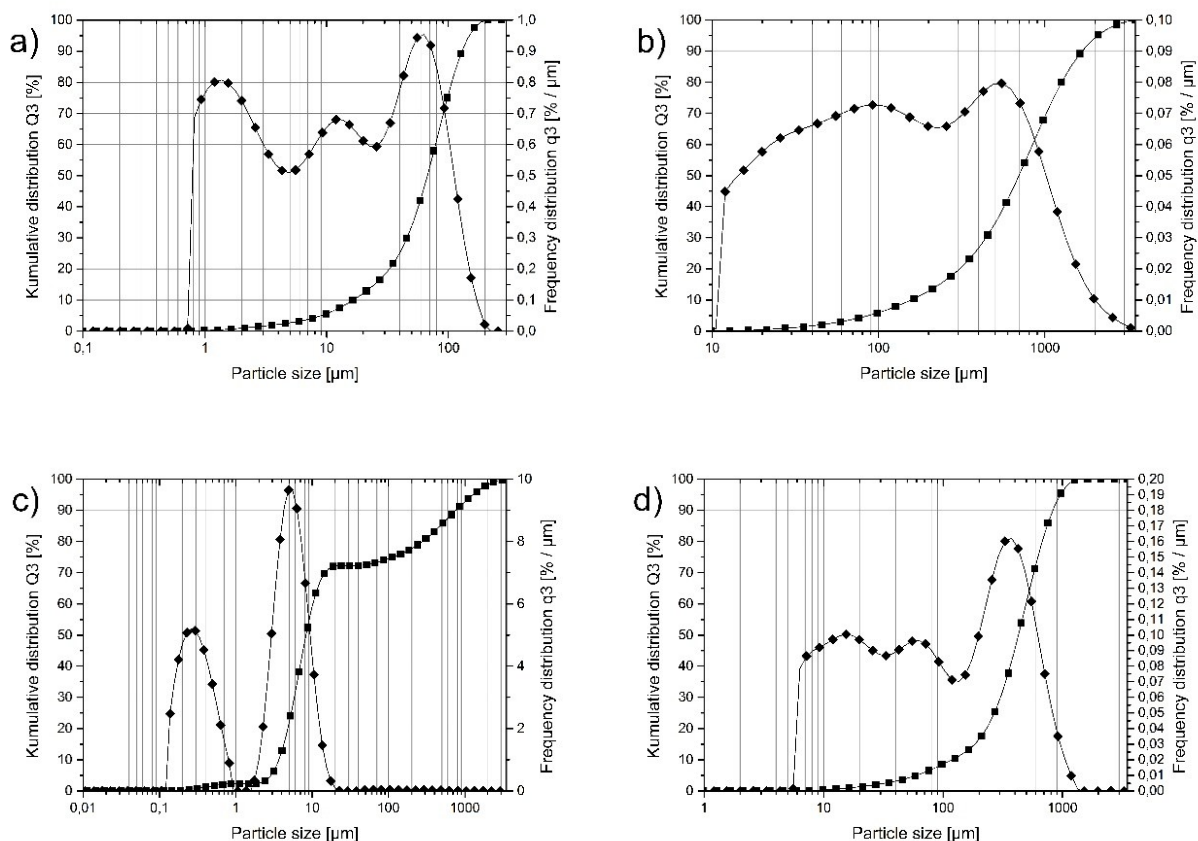


Figure 4.3: Cumulative and frequency distribution of a) sodium benzoate, b) beeswax, c) sugarcane wax and d) carnauba wax

the waxes showed a broad particle distribution. This is caused by the milling process which typically generates bimodal particle size distributions: large particles are broken into smaller particles during milling, resulting in a mode in the range of smaller particle sizes while a significant amount of raw material does not get milled, which results in a second mode at larger particle sizes (Staniforth and Taylor, 2018). This bimodal distribution of the milled waxes prevented an accurate representation of the particles' size based solely on a single value. For example, while the d_{90} of all three wax samples was larger than that of SB, the d_{10} of the milled SW smaller than that of SB at 3.6 μm . Therefore, the span was calculated to quantify the width of the particle size distribution. As previously mentioned, SB showed a narrow particle size distribution with a span of 1.673 ± 0.002 , as did CW and BW at span values of 1.655 ± 0.016 (CW) and 2.180 ± 0.012 (BW), respectively. SW on the other hand showed a significantly larger span of 73.683 ± 15.128 . Reproducibility of the measurement of d_{10} , d_{50}

and d_{90} was checked to ensure a correct measurement (International Organization for Standardization, 2009) with the following equation:

$$CV \text{ in}\% = \frac{SD}{\bar{x}} \times 100 \quad \text{Equation 4.1}$$

SD = standard deviation

$\bar{x}$ = mean value

All samples were measured three times. The quantiles of all materials except for the d_{90} of SW showed a coefficient of variation (CV) within the acceptance criteria of less than 3% for d_{50} and less than 5% for d_{10} and d_{90} . The d_{90} of SW showed a CV of 21.57% and thus exceeded the limits of ISO 13320:2009. BW and SW, although milled, showed the formation of agglomerates over storage time. While the BW agglomerates were completely destroyed by the dispersion pressure of the laser diffraction spectrometry apparatus, those of the SW were not and resulted in non-reproducible d_{90} values for the SW. This formation of agglomerates might negatively influence the processing capabilities by impacting blend homogeneity. On the other hand, an addition of a certain amount of non-sticking excipients might neutralize or reduce the formation of BW or SW agglomerates. From the images taken via scanning electron microscope (SEM) (**BaHav17-008**) it was concluded that SB particles were produced via spray drying. They showed a spherical shape with a hole (Figure 4.4A), which is typical for products from spray drying processes (Fahr, 2018). A closer look at the spheres revealed that they were comprised of needle-shaped crystals. Some spheres were not fully intact but are broken into smaller fragments. The impact of the fragmentation on the particle size distribution is shown in Figure 4.3a. The frequency distribution shows three peaks, indicating three major particle size groups. BW particles showed fragments of the former pellets with irregular shape and size (Figure 4.4B). SW particles were mainly small, since the raw material had a powder like appearance before milling, with irregular shape (Figure 4.4C). This picture is in good accordance with the findings of the frequency distribution of SW (Figure 4.3c) showing a particle size population for 0.3 μm and 5 μm . CW, like BW, showed primary particles of various shapes and sizes derived from the milled flakes (Figure 4.4D). The measured densities of the raw materials corresponded to the values found in literature except for SW. SB has a true density of 1.5 $\text{g}\cdot\text{cm}^{-3}$ (Merck Millipore, 2006), BW of 0.96 $\text{g}\cdot\text{cm}^{-3}$ (European Pharmacopoeia, 2023) and CW of 1.00 $\text{g}\cdot\text{cm}^{-3}$ (European Pharmacopoeia, 2023). SW showed a noticeable higher density than described by the supplier with a value of 1.03 $\text{g}\cdot\text{cm}^{-3}$ instead of 0.80 – 0.85 $\text{g}\cdot\text{cm}^{-3}$ (DEUREX, 2018).

4.1 Characterization of starting materials

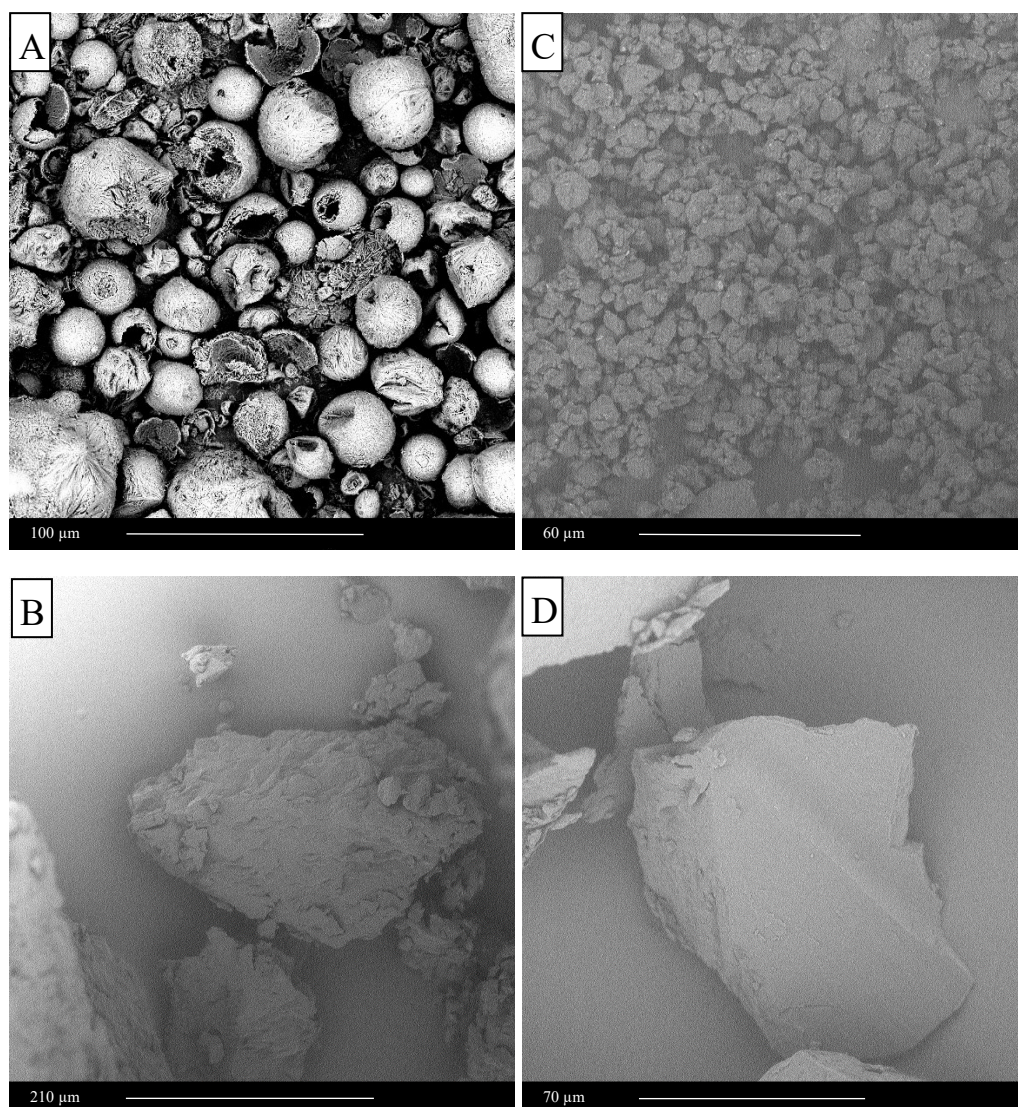


Figure 4.4: SEM images of the raw material: A) sodium benzoate, B) beeswax, C) sugarcane wax and D) carnauba wax

4.1.3 Flowing properties

BW and SW are materials which show sticky properties if compressed under slight pressure. During the extrusion process, the material is conveyed via screws by a gravimetric feeder. The conveying process applies small amounts of pressure on the material, which therefore could cause sticking and thus impeding the precise feeding of material into the extruder barrel. According to the Ph. Eur., by calculating the Hausner Ratio or the compressibility index from the bulk and tap densities of a material, its flow properties can be quantified (European Pharmacopoeia, 2023). A large difference between these two densities expresses an initially insufficient flow of material, resulting in more intraparticle space and thus a low bulk density. By applying vibration or tapping the vessel, the material starts to densify by filling the intraparticle space until finally reaching its tap density. In Table 4.2, the Hausner Ratios and compressibility indices of the raw materials are shown (**BaHav19-017b**). All materials showed sufficient flow properties. However, SW only fulfilled the criteria for a “Passable” flow character according to Ph. Eur. classification. SB had a “Fair”, BW a “Good” and CW an “Excellent” flow character. The excellent flow properties of CW can be explained with its

brittle properties. The particles of CW do not show plastic deformation, which would increase the contact surface and ultimately adhesion to other CW particles or the surface of the funnel. The good flow properties of SB can be explained by its combination of flow-enhancing and flow-reducing particle morphologies. As previously described, the SB consisted of particles with a spherical shape, which provides good flow properties, and the fragments of needle-shaped crystals, which decrease its flowability. The BW particles showed plastic deformation during measurement of the tap density, resulting in a compact powder and in an increase in density compared to the bulk density. The “Passable” flowing character of SW is caused by a very poor bulk density. The material is not able to initially form a compact powder bed. This could be caused by the ability to form agglomerates very easily, resulting in larger particle groupings and thus preventing the small gaps in between agglomerates from being filled. After determining the tap density, BW and SW had formed agglomerates, which stuck to the cylinder. The subsequently performed extrusion tests clarified whether this could lead to a problem during processing.

Table 4.2: Bulk and tap densities of raw materials and their calculated Hausner Ratio and compressibility index.

Material	Mean $\pm$ SD (n = 3)				
	Bulk density [g * ml ⁻¹]	Tap density [g * ml ⁻¹]	Hausner Ratio	Compressibility Index	Flow character
Sodium benzoate	0.351 $\pm$ 0.005	0.420 $\pm$ 0.001	1.197 $\pm$ 0.018	16.44 $\pm$ 1.29	Fair
Beeswax	0.440 $\pm$ 0.006	0.510 $\pm$ 0.004	1.155 $\pm$ 0.007	13.43 $\pm$ 0.51	Good
Sugarcane wax	0.175 $\pm$ 0.003	0.230 $\pm$ 0.001	1.306 $\pm$ 0.017	23.42 $\pm$ 0.98	Passable
Carnauba wax	0.514 $\pm$ 0.005	0.566 $\pm$ 0.003	1.102 $\pm$ 0.012	9.23 $\pm$ 0.96	Excellent

4.1.4 Thermal properties

DSC enables insight into the solid-state properties of a material. Heat is applied to a pan containing the substance of interest alongside an empty reference pan. Changes in the crystalline structure can be identified by measuring the increase or decrease in heat flow of the first pan in comparison to the reference pan, indicating endothermic or exothermic events caused by the material under investigation (Fahr, 2018; Ghanbari *et al.*, 2023). The melting point of a material is visible in DSC-measurements as an endothermic heat flow, because the applied energy is needed to overcome the strong molecular binding in the crystal lattice (Buckton, 2018). Figure 4.5 depicts the thermograms of the waxes (**BaHav19-040**) heated with 1°C/min. In Figure 4.5a, the first heating cycle of the three waxes is shown combined in one graph. It was observed that CW melted at the highest temperature, followed by SW at the second highest temperature and BW at the lowest temperature. This conforms to the values found in literature (European Pharmacopoeia, 2023; 2023; Krendlinger *et al.*, 2015). The thermogram of BW (Figure 4.5b) reveals four characteristic peaks at 46, 52, 58 and 62°C, which are in agreement with previously published findings (Flaherty, 1971; Gaillard *et al.*, 2011). The value of melting enthalpy of 192.39 J*g⁻¹ also align with values from literature (Attama and Muller-Goymann, 2008), which were found to be around 190 J*g⁻¹. In the first heating phase, the three

4.1 Characterization of starting materials

different peaks at 46, 52 and 62 °C were detected very easily, while the fourth peak at 58°C is better detected in the second heating cycle and far better in the cooling cycle, as known from literature (Gaillard *et al.*, 2011). The three peaks at 46, 52 and 62°C represent transitions within the BW mixture from an orthorhombic crystalline state over a low-temperature rotary crystalline state to high-temperature hexagonal crystalline rotation state (Basson and Reynhardt, 1988; Kameda and Tamada, 2009; Kotel'nikova *et al.*, 2007). At 68°C, BW was completely melted. During cooling, recrystallization of each polymorph took place at the same temperatures. A second heating cycle revealed small changes in the heat flow intensity for the solid-solid transition at 52°C. Additionally, the peak at 46°C was less clearly detectable while the peak at 58°C was revealed. Due to some similarities in composition of the waxes, it is not surprising that SW showed polymorphic transitions as well (Attard *et al.*, 2015; Flaherty, 1971). During the first heating cycle, transitions occurred at 44.0, 52.6 and 66.5°C. In contrast to the thermogram of BW, the recrystallization peaks of SW showed noticeable differences in temperature regarding the transition peaks (Figure 4.5c). SW had a first peak at 52°C and a second peak at 66°C which was followed by a broad melting event with less heat flow until the wax was completely molten at 79°C. Recrystallization during cooling started at 67 °C, followed by the second transition which occurred at 58.8°C instead of the formerly observed 52.6°C. In the second heating cycle, the first

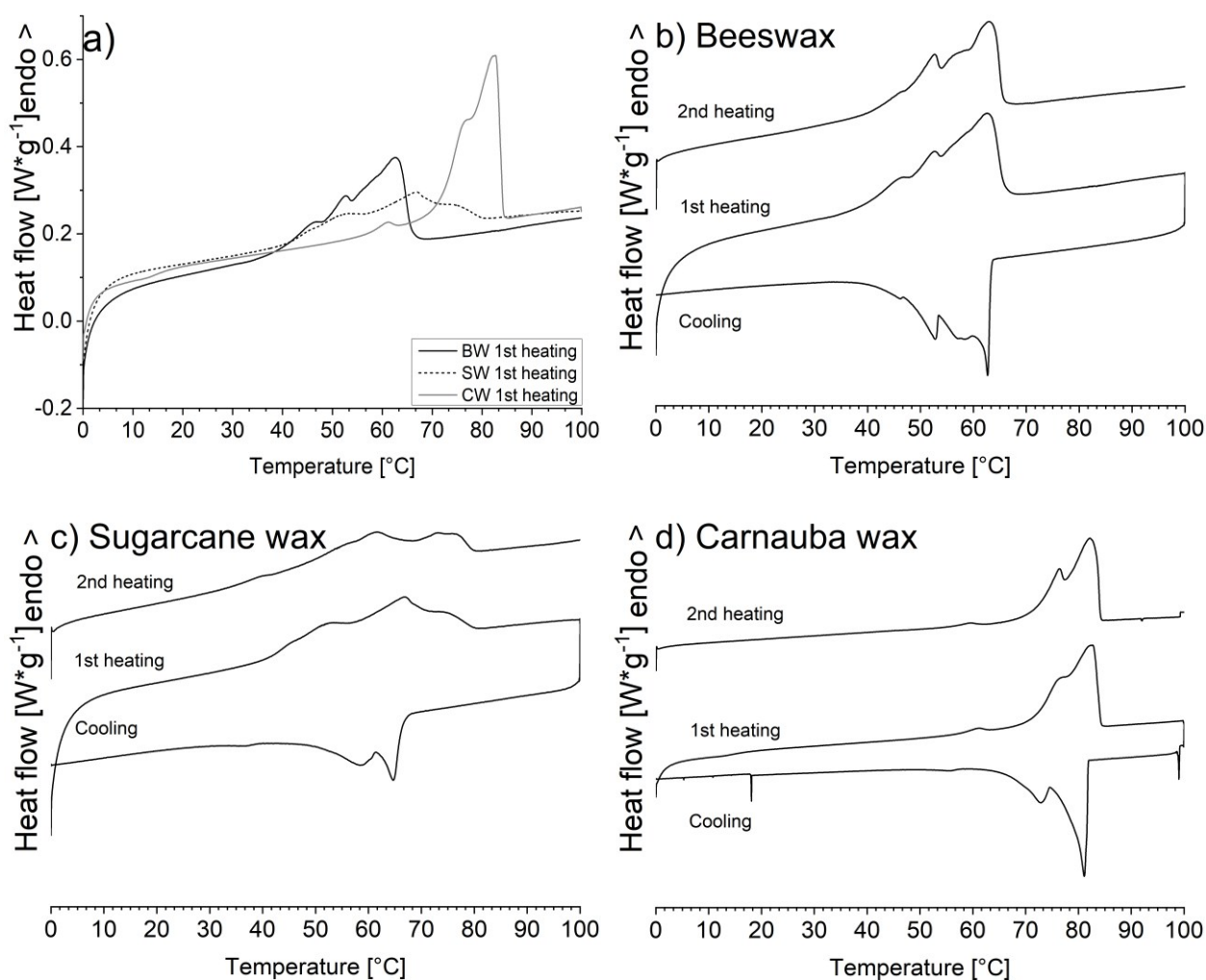


Figure 4.5: Thermograms of a) the used waxes plotted in one graph, b) beeswax, c) sugarcane wax and d) carnauba wax; heating rate 1°C/min.

solid-solid transition occurred at 61.3 and the second at 72.9°C. This indicates a change in the crystalline structure in comparison to the first heating cycle. The solid-solid transition at 66.5°C was no longer present. CW, like bees wax, showed a solid-solid transition at 61.2°C (Figure 4.5d) (Basson and Reynhardt, 1988). Unlike BW, this transition occurred at a temperature below the melting range. During the first heating, a second transition occurred at 77.2°C before melting took place at 82.6°C. During cooling, the liquid-solid transition at 81.2°C and solid-solid transition at 72.9°C occurred as mentioned in literature (Flaherty, 1971). By heating the sample a second time, small changes in the solid-solid transition at 77°C could be observed. The peak was located at 76.3°C and was followed by a new minimum at 77.3°C. The melting enthalpy of CW was $201.8 \text{ J} \cdot \text{g}^{-1}$, which is in the same magnitude as found in literature (Ritter *et al.*, 2001).

Measurements with a heating rate of $1^\circ\text{C}/\text{min}$ are very time-consuming and slow down the analysis of many samples. For that reason, a second DSC analysis was performed with a heating rate of $10^\circ\text{C}/\text{min}$. In Figure 4.6 the results of DSC-analysis of pure waxes with a heating rate of $10^\circ\text{C}/\text{min}$ and a cooling rate of $20^\circ\text{C}/\text{min}$ are shown (BaHav17-017). With an increased heating rate, the transitions described above for BW could not be detected. The melting peak shifted towards higher temperatures from formerly 62.6°C to 64.5°C for pure BW. In the second heating cycle, a transition at 52.9°C was detected. This transition occurred at temperatures similar to the previously performed DSC measurements with a slower heating rate.

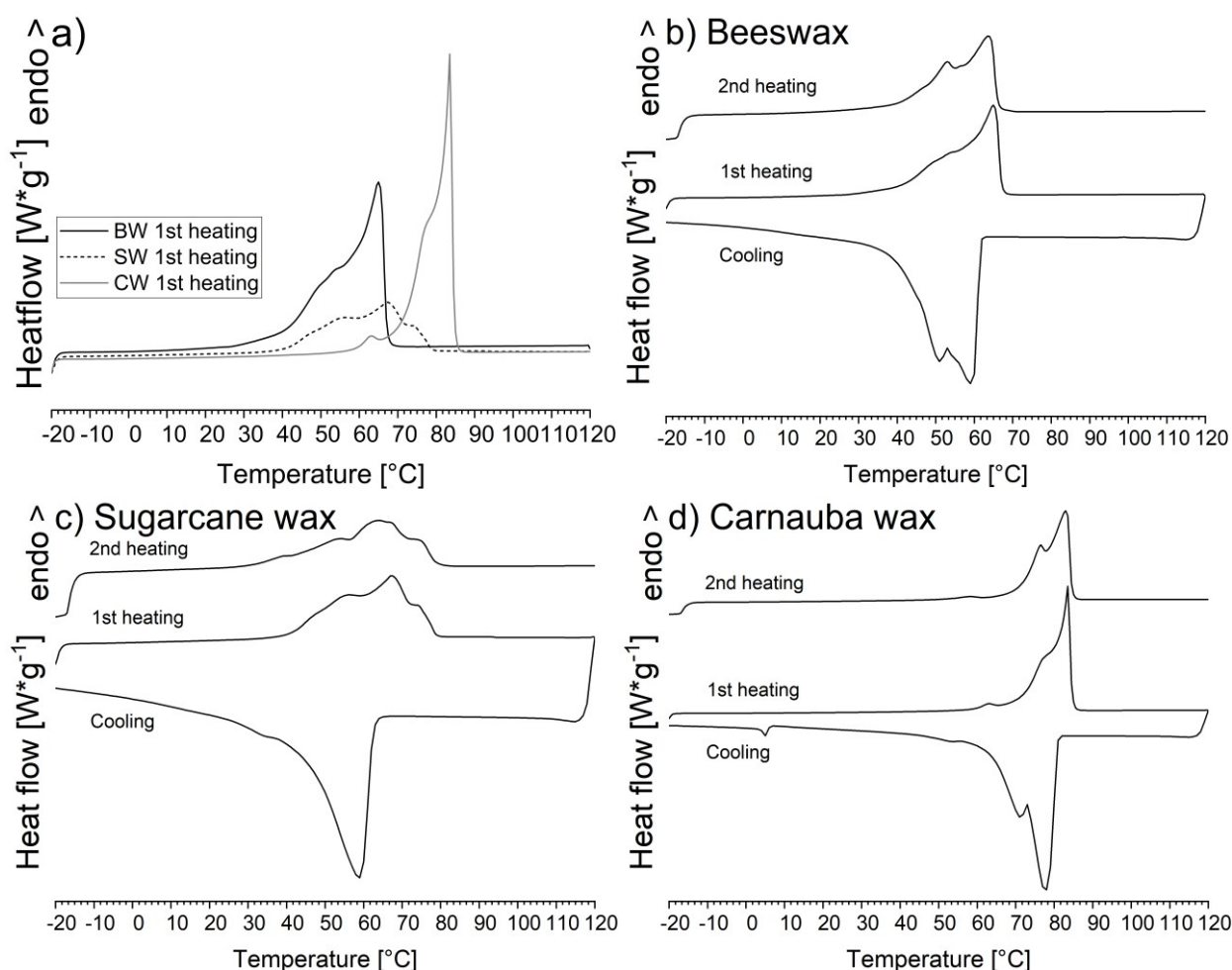


Figure 4.6: Thermograms of a) the used waxes plotted in one graph, b) beeswax, c) sugarcane wax and d) carnauba wax; heating rate $10^\circ\text{C}/\text{min}$, cooling rate $20^\circ\text{C}/\text{min}$.

4.1 Characterization of starting materials

However, the other transition at 46.7°C could no longer be detected. Such behavior is typical for increased heat rates because of the thermal lag of a sample (Sandu and Singh, 1988). The maximum peak of SW was slightly shifted to 67.2°C by the increased heating rate. Its solid-solid transition, which was previously 52.6°C, was similarly shifted to 56.4°C. The increased heating rate did not influence the melting peak of CW, which was detected at 82.9°C, but the solid-solid transition formerly detected at 61.2°C was then found at 63.1°C. However, in the second heating cycle all three transitions previously detected at a heating rate of 1°C/min were found with a slight alteration but with the first solid-solid transition now being detected at 58.0°C. All of the measurements shown above were done in duplicate. The measurement of the second sample confirmed the measurements shown above.

In summary, the waxes showed thermal properties in accordance with the properties described in literature. In a second heating cycle, slight alterations were observed. Some solid-solid transitions could not be observed anymore when increasing the heating rate, as is often the case with DSC-measurements. When increasing the heating rate, single thermal events are not separated as well as before due to the thermal lag of the sample (Hutchinson *et al.*, 1988). In consequence, some peaks are overlaid by others.

The obtained data provides valuable insight for the selection of the process temperature. It could be shown that the waxes melt in a broad temperature range, which could in turn enable a broad process parameter range. Processes below the melting point could also be feasible due to the partially molten state at a chosen temperature. Additionally, potential changes in the crystalline state must be kept in mind in regards to product stability.

4.1.5 Crystalline properties

XRPD analysis is a valuable tool to gain insight into the crystalline character of a material. With XRPD, the crystalline structure can be analyzed by measuring the crystalline lattice via X-rays. Depending on the structure of the crystal lattice, the x-rays are reflected in varying angles. From the reflection angle, measured as angle of detector 2Θ , conclusions regarding the structure of the crystalline lattice can be drawn. (Chauhan and Chauhan, 2014). It is possible to supplement information gained by DSC measurements, for instance. The results of the performed XRPD analyses of SB, BW, SW, and CW (**BaHav17-017**) are shown in Figure 4.7. The diffractograms of BW, SW, and CW show significant peaks at the same angles. The two characteristic peaks at 20.9 2Θ and 23.2 2Θ for BW are in accordance with values known from literature (Basson and Reynhardt, 1988; Gaillard *et al.*, 2011). They are caused by the orthorhombic morphology of BW crystals at room temperature. Basson and Reynhardt (1988) recognized an orthorhombic morphology for CW as well, which explains the two characteristic peaks observed in the diffractogram of CW at 21.0 2Θ and 23.2 2Θ . Furthermore, BW and CW show peaks of small intensity at 29.2 2Θ , 35.3 2Θ and 39.6 2Θ , which are connected to the orthorhombic arrangement of hydrocarbons and monoesters (Gaillard *et al.*, 2011).

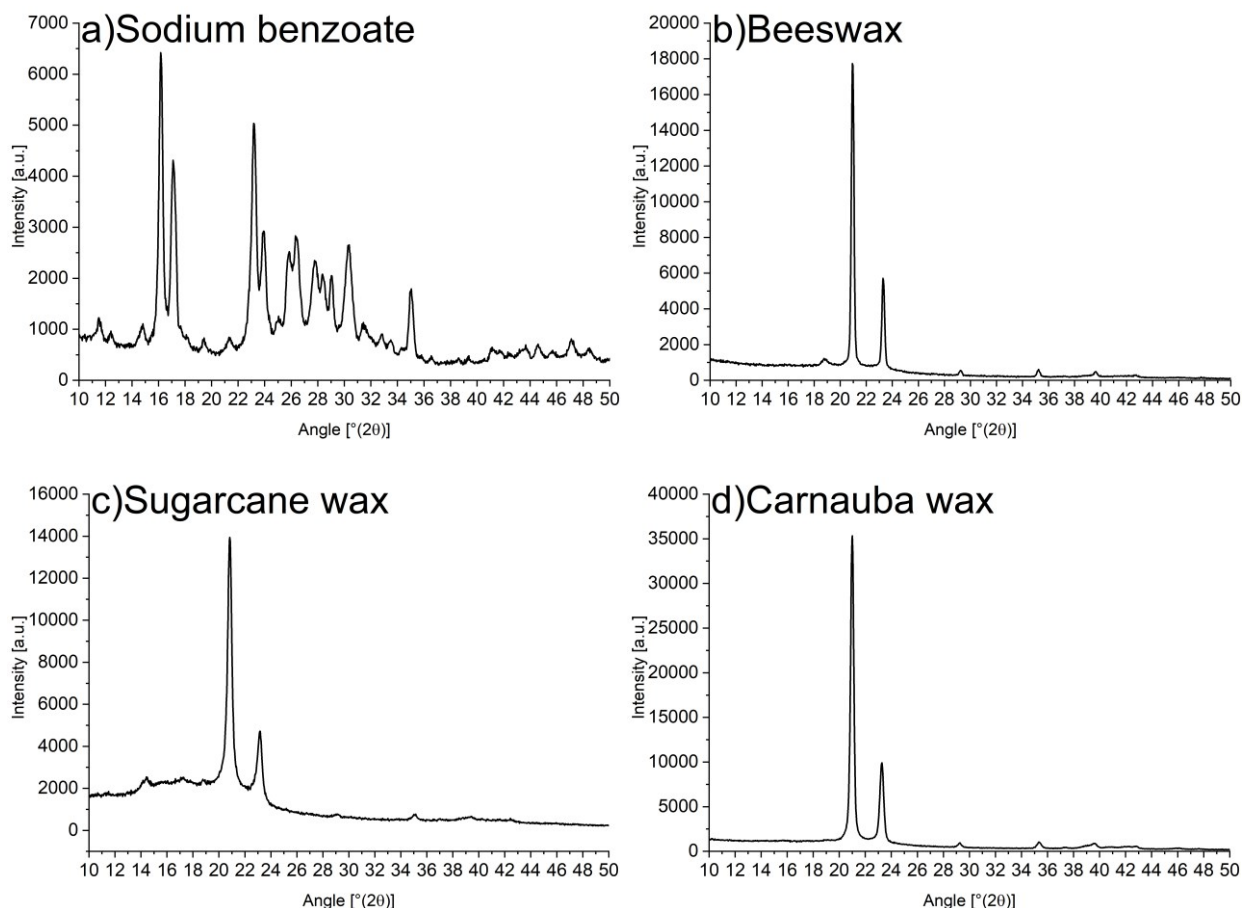


Figure 4.7: X-ray powder diffractograms of the raw excipients measured in transmission mode: a) sodium benzoate, b) beeswax, c) sugarcane wax and d) carnauba wax. 2θ interval was used from 10 to 50 degree.

SW is similar in composition to BW and CW, since it contains a significant amount of hydrocarbons and monoesters (Nuissier *et al.*, 2002), and therefore shows two characteristic peaks at $20.8\ 2\theta$ and $23.2\ 2\theta$ as well as at $35.0\ 2\theta$ and $39.4\ 2\theta$. SB shows multiple characteristic peaks, which comply with the values from literature (Butterhof *et al.*, 2012).

4.1.6 Conclusion

The morphological and physicochemical properties of SB and the natural waxes were characterized. The milled natural waxes had a d_{90} larger than that of the SB. BW exhibited the largest d_{90} due to the formation of agglomerates which were not destroyed by the air pressure of the measurement process. CW and SW had similar d_{90} similar to each other, although the SW also showed some agglomerate formation. However, these agglomerates were easily destroyed during the measurement, which is indicated by the small d_{10} and d_{50} of the SW (Table 4.1). SB showed a d_{90} of $129.0\ \mu\text{m}$, which is significantly smaller than those of the milled waxes. Good flowing properties according to the classification of the Ph. Eur. were measured for all substances except for SW. Sticking was observed after the measurement of SW and BW in the tap volumeter, which was caused by the forces applied during tapping. This sticking tendency could impact further processing of the materials. The analysis of the crystalline properties via DSC revealed a broad melting range for BW and SW with multiple peaks, which indicate soli-solid transitions according to literature (Flaherty, 1971; Gaillard *et al.*,

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2011). The thermogram recorded for CW was also in alignment with the values found in literature (Basson and Reynhardt, 1988; Flaherty, 1971; Ritter *et al.*, 2001). The XRPD analysis revealed peaks for BW, SW, and CW well known in literature (Basson and Reynhardt, 1988; 1988; Gaillard *et al.*, 2011). The three waxes showed peaks around $20\ 2\theta$ and $23\ 2\theta$ as well as at $35\ 2\theta$ and $39\ 2\theta$. The waxes were easily distinguishable from SB, which showed several additional peaks at for instance $26\ 2\theta$ and $30\ 2\theta$, which were not detected for any of the waxes. The peaks measured for SB comply with the values found in literature (Butterhof *et al.*, 2012). In further studies, the DSC-measurement should be applied to analyze the impact on the crystalline structure after the processing of the wax materials via HME.

4.2 Characterization of physical mixtures

4.2.1 Introduction

Morphological and physicochemical properties of the used excipient have been discussed in chapter 4.1. Furthermore, knowledge about the interactions of these excipients, caused by creating a mixture of these, is essential for further processing. Components may interact on a molecular level and alter their properties in a significant way, ultimately changing the attributes of the final product. In the previous chapter, it was already observed for BW and SW that these two materials tend to form adhesive agglomerates when force is applied to them.

4.2.2 Flow properties

During the flow character analysis of the raw materials, it was observed that BW and SW tend to stick in the tap cylinder after applying tap force. This sticking tendency might impede their processing into a drug formulation. Physical mixtures with ratios of 75:25, 50:50 and 25:75 (w/w) (wax/SB) were prepared and their flow characteristics were evaluated (**BaHav19-027**) (Table 4.3).

Table 4.3: Flow properties of physical mixtures of wax and sodium benzoate; BW = beeswax, SW= sugarcane wax, CW = carnauba wax

Material	Mean $\pm$ SD (n = 3)				
Ratio (Wax : SB)	Bulk density [g * ml ⁻¹]	Tap density [g * ml ⁻¹]	Hausner Ratio	Compressibility Index	Flow character
BW 75:25	0.471 $\pm$ 0.003	0.550 $\pm$ 0.000	1.167 $\pm$ 0.008	14.28 $\pm$ 0.62	Good
BW 50:50	0.437 $\pm$ 0.002	0.512 $\pm$ 0.003	1.171 $\pm$ 0.006	14.58 $\pm$ 0.47	Good
BW 25:75	0.381 $\pm$ 0.002	0.460 $\pm$ 0.000	1.205 $\pm$ 0.006	17.04 $\pm$ 0.4	Fair
SW 75:25	0.242 $\pm$ 0.001	0.288 $\pm$ 0.001	1.191 $\pm$ 0.003	16.02 $\pm$ 0.21	Fair
SW 50:50	0.280 $\pm$ 0.002	0.334 $\pm$ 0.003	1.192 $\pm$ 0.005	16.11 $\pm$ 0.37	Fair
SW 25:75	0.308 $\pm$ 0.007	0.373 $\pm$ 0.000	1.210 $\pm$ 1.767	17.30 $\pm$ 1.77	Fair
CW 75:25	0.445 $\pm$ 0.006	0.560 $\pm$ 0.006	1.260 $\pm$ 0.029	20.58 $\pm$ 1.87	Passable
CW 50:50	0.434 $\pm$ 0.008	0.515 $\pm$ 0.003	1.189 $\pm$ 0.024	15.88 $\pm$ 1.68	Fair
CW 25:75	0.393 $\pm$ 0.003	0.467 $\pm$ 0.000	1.188 $\pm$ 0.010	15.83 $\pm$ 0.70	Fair

Both BW/SB-mixtures of 75:25 (w/w) and 50:50 (w/w) showed a flow character of “Good”, which is the same as for pure BW. A mixture of 25% BW and 75% SB was rated as “Fair” according to the specifications of Ph. Eur.. Nevertheless, the pharmacopeial method is not suitable to assess the stickiness of a material. All physical mixtures of BW and SW showed a reduced sticky character and

4.2 Characterization of physical mixtures

were easier to remove from the tap cylinder. Physical mixtures of SW/SB showed an improved flow character according to the classification system of the Ph. Eur. The physical mixture of SW and SB showed a “Fair” flow character while pure SW showed a “Passable” flow character. All ratios of SW/SB showed a flow behavior corresponding to “Fair” according to Ph. Eur. specifications and showed reductions in their stickiness as well as their agglomeration tendency. Physical mixtures of CW/SB showed a reduction in flow character in comparison to pure CW, resulting in a rating of “Passable” and “Fair” for the two mixtures. The reduction in stickiness by combining SB and BW or SW leads to the conclusion that SB is functioning as a flow-regulating agent in this mixture. A glidant reduces the adhesion forces of a mixture (Fahr, 2018) and thus increases flowability.

4.2.3 Thermal characteristics of natural waxes

By creating a physical mixture of two or more components, the thermal properties of the single components within the mixture may change. For instance, the formation of a eutectic mixture is not rare, which results in a decrease of the melting temperature (Fahr, 2018). For processes like HME, this can have a significant impact on the minimum process temperature.

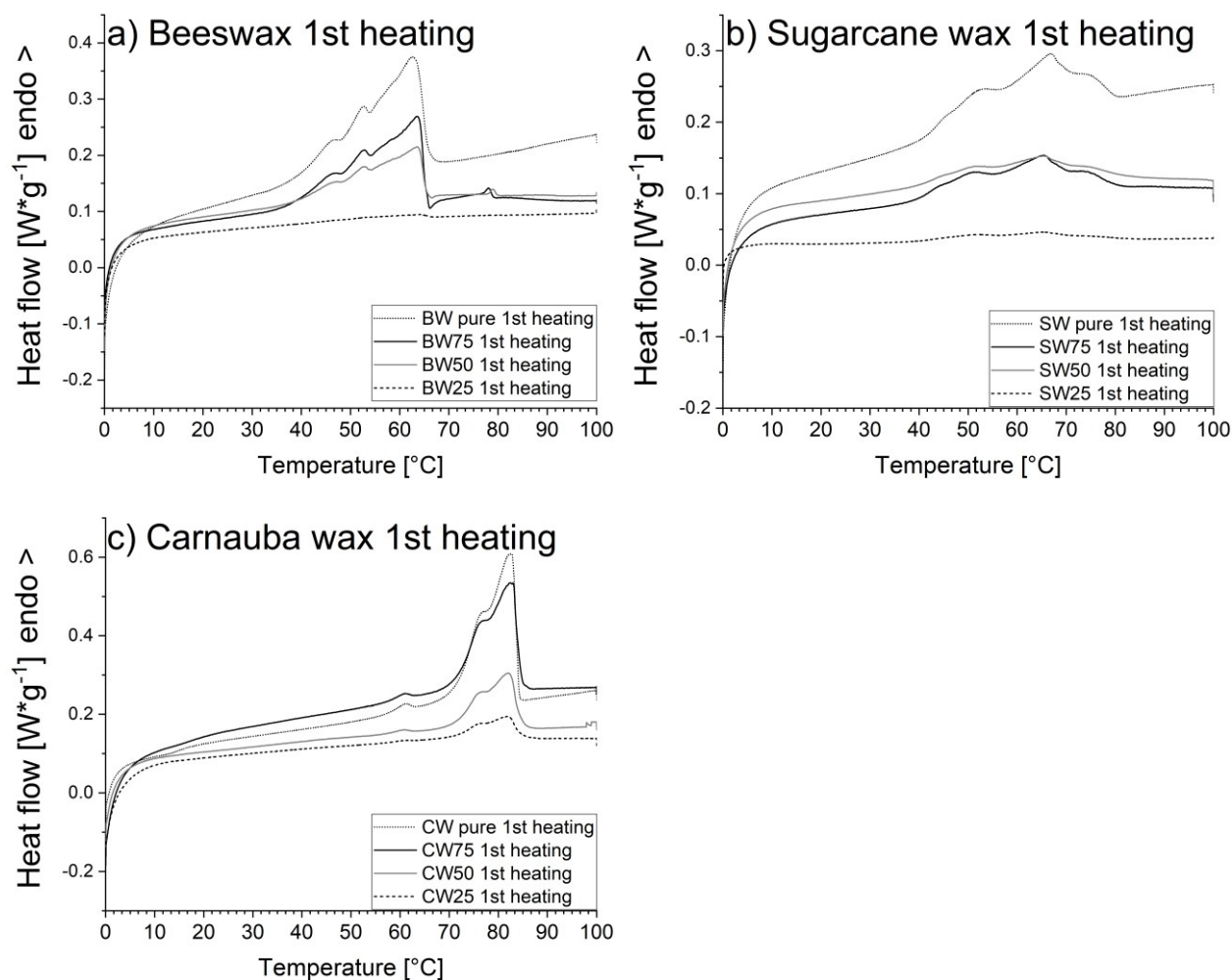


Figure 4.8: Thermogram of the 1st heating cycle of pure wax and physical mixtures of 25:75, 50:50 and 75:50 (w/w) (SB/wax); a) beeswax, b) sugarcane wax and c) carnauba wax; heat rate 1°C/min.

The thermograms of physical mixtures with ratios of 25:75, 50:50 and 75:25 (w/w) (SB/wax) (**BaHav19-040**) are depicted in Figure 4.8. The main part of the thermogram of BW mixtures remained unchanged in comparison to pure BW (Figure 4.8a). The solid-solid transitions at 46.9°C and 52.8°C of BW75 as well as of BW50 at 47.2°C and 52.5°C were the same as in the thermogram of pure BW. However, in the thermogram of the physical mixture, a new endothermic thermal event was recorded after the wax was melted at 78°C. Possible sources of that thermal event could be, the formation of a eutectic mixture. The observed peak would then correspond to the eutectic of beeswax and sodium benzoate.

Contrarily, when analyzing the cooling curve of the respective mixture (Figure 4.9a), a corresponding exothermic event could be observed during cooling of the sample. This finding rules out a chemical reaction or degradation as the cause of the aforementioned thermal event, instead supporting the idea of a eutectic mixture occurring after melting of the BW.

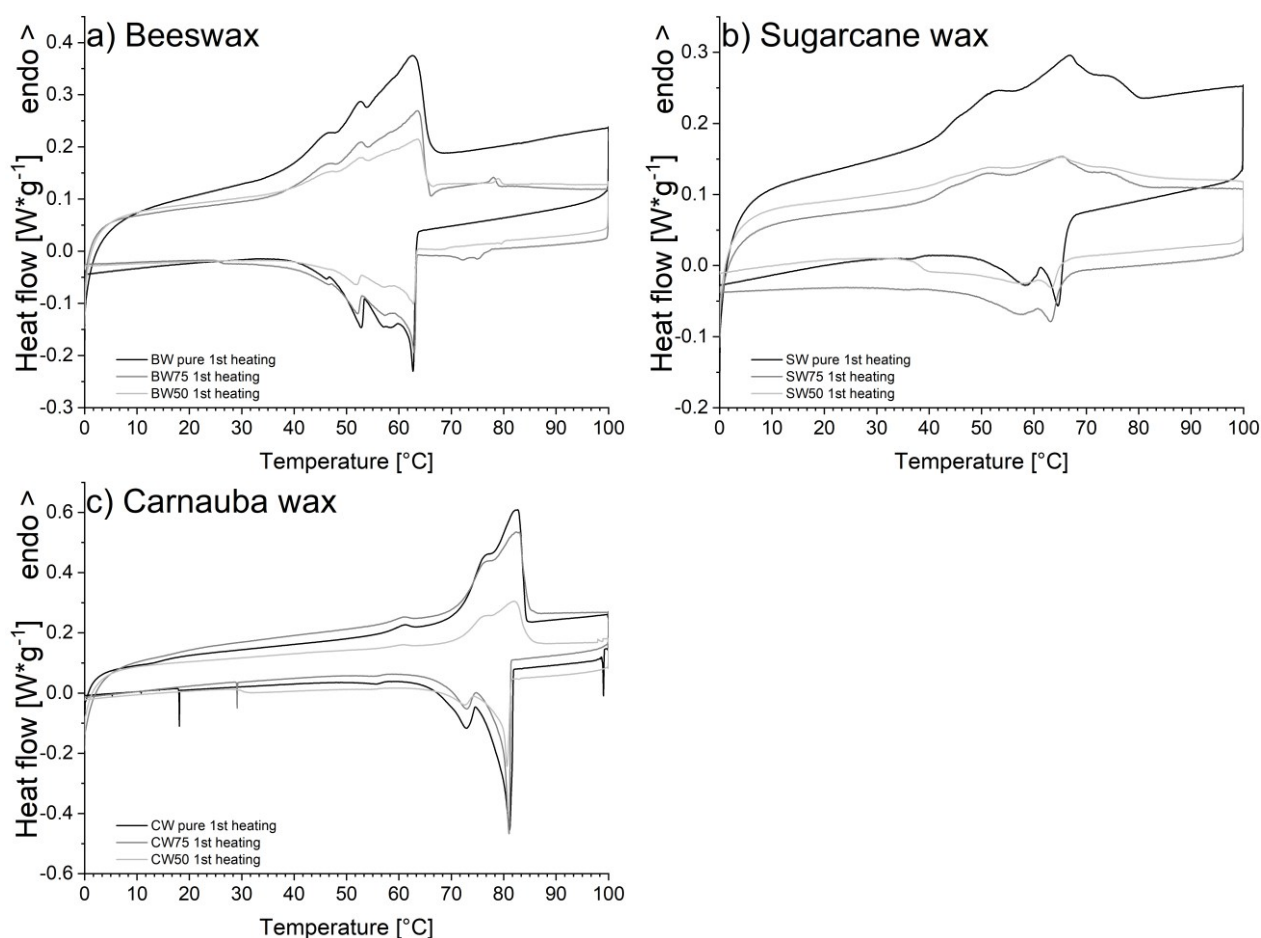


Figure 4.9: Thermogram of pure wax and physical mixtures of ratios of SB:wax 25:75 and 50:50 (w/w); a) beeswax, b) sugarcane wax and c) carnauba wax; heat rate and cooling rate 1 C/min.

When the sample was cooled down, recrystallization took place as represented by the corresponding exothermic event from 77.5 to 63.7°C. However, physical mixtures of SW or CW with SB showed neither an additional endothermic event after melting (Figure 4.8b + c) nor an additional crystallization peak during cooling (Figure 4.9b + c). Physical mixtures of SW and SB showed peaks at 51.9°C, 65.5°C and 71.5°C for SW75 and at 51.2°C, 65.2°C and 71.2°C for SW50. These peaks were in alignment with the findings from the analysis of pure SW. CW75 and CW50 showed characteristic peaks at 60.8°C / 61.0°C, 77.4°C / 77.0°C and 82.2°C / 81.9°C, indicating that the thermal properties of CW were also not influenced by SB. A possible explanation for physical

4.2 Characterization of physical mixtures

mixtures of BW showing a eutectic mixture while SW and CW did not is the temperature of the observed peak. SW and CW exhibited endothermic events at 78°C, which overlapped the peak of the eutectic mixture. This could explain the absence of a similar observation in the thermograms of SW and CW. However, this hypothesis could not be proven. The physical mixtures were heated up a second time right after the cooling phase had been completed. The thermograms are shown in Figure 4.10.

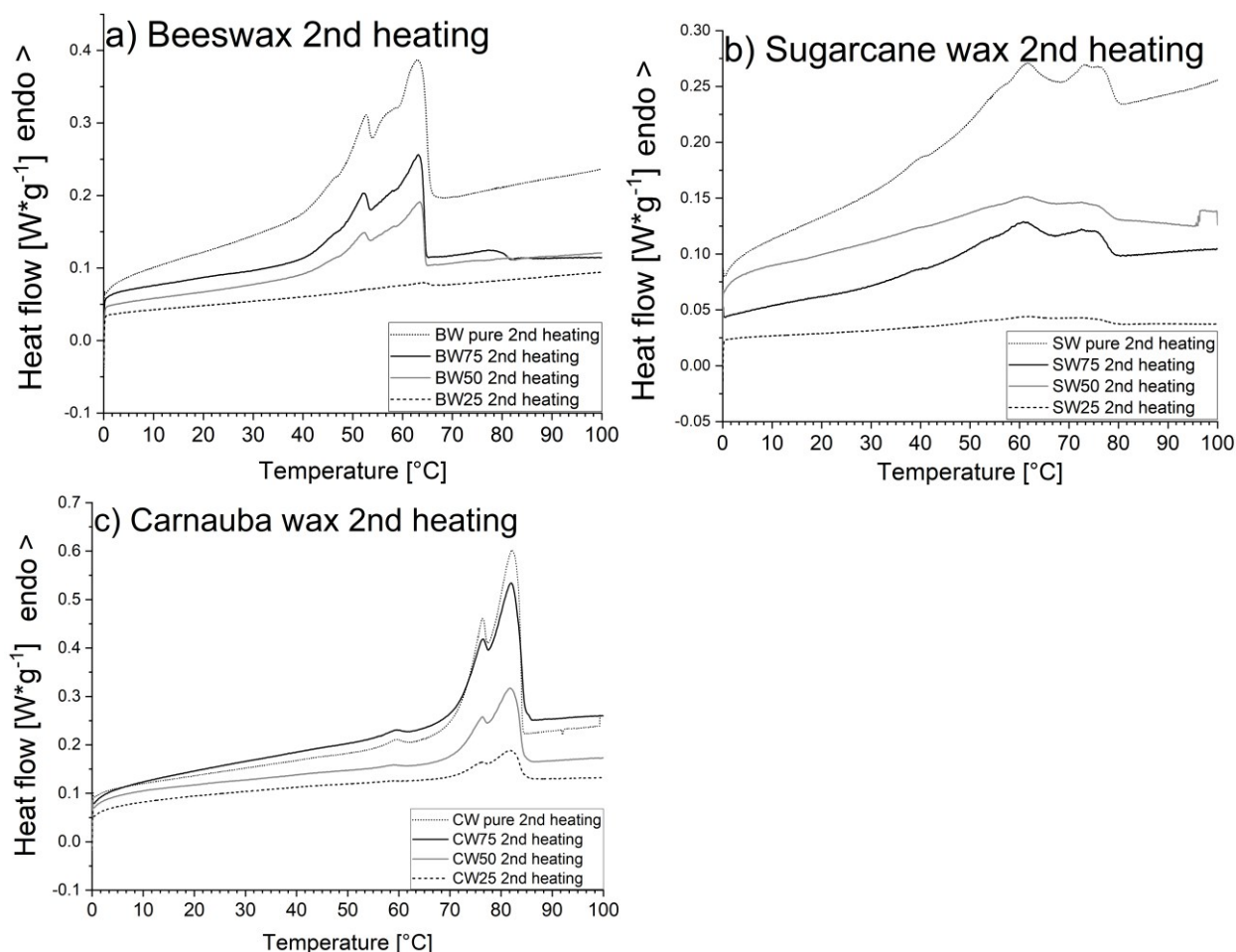


Figure 4.10: Thermogram of the 2nd heating cycle of pure wax and physical mixtures of 25:75, 50:50 and 75:50 (w/w) (SB/wax); a) beeswax, b) sugarcane wax and c) carnauba wax; heat rate 1 °C/min.

The thermograms of the second heating cycle of the physical mixtures are similar to those of the pure wax. Physical mixtures of BW and SB still had significant peaks at 52 and 62°C. As described for the first heating cycle, the additional thermal event at 78°C not found for pure BW was also visible for the second heating cycle of the physical mixtures. The peak was less clearly recognizable than it was during the first cycle (Figure 4.10a). The physical mixtures of SW displayed the same changes in the second heating as previously described for pure SW (Figure 4.10b). The SW50 sample showed an additional endothermic peak at 95 to 99°C. Since this was observed for the second heating cycle, an evaporation of water is very unlikely as this should have been removed during the first heating phase. No other sample of SW showed such an event, which led to the conclusion of this signal having been caused by the apparatus, not the material. Samples of CW also did not show new thermal events during the second heating cycle (Figure 4.10c). The main peak at 82°C also appeared sharper due to a faster decline of the heat flow between 76 and 78°C. The small endothermic peak at 60°C remained unchanged.

The analysis of the physical mixtures of wax and SB indicated that the thermal properties within the physical mixtures are comparable to the thermal properties of the pure waxes. The waxes were fully molten at the temperatures known from literature sources as well as from the conducted DSC-analysis of the pure waxes. The physical mixtures of BW revealed an additional endothermic event at 78°C, which was not found in samples of pure BW. This additional peak was also found during the cooling phase, indicating that the process is reversible. Thus, a degradation can be ruled out. The formation of a eutectic mixture of BW/SB might be a possible reason.

From the thermograms of the physical mixtures, applicable temperature ranges for melt-extrusion processes for each of the wax compositions were derived.

4.2.4 Crystalline properties

For additional information about possible interactions between SB and natural waxes, XRPD of the physical mixtures with ratios of 75:25, 50:50 and 25:75 (w/w) SB/wax was performed (**BaHav17-017**). The diffractograms are depicted in Figure 4.11. In all three binary mixtures, the

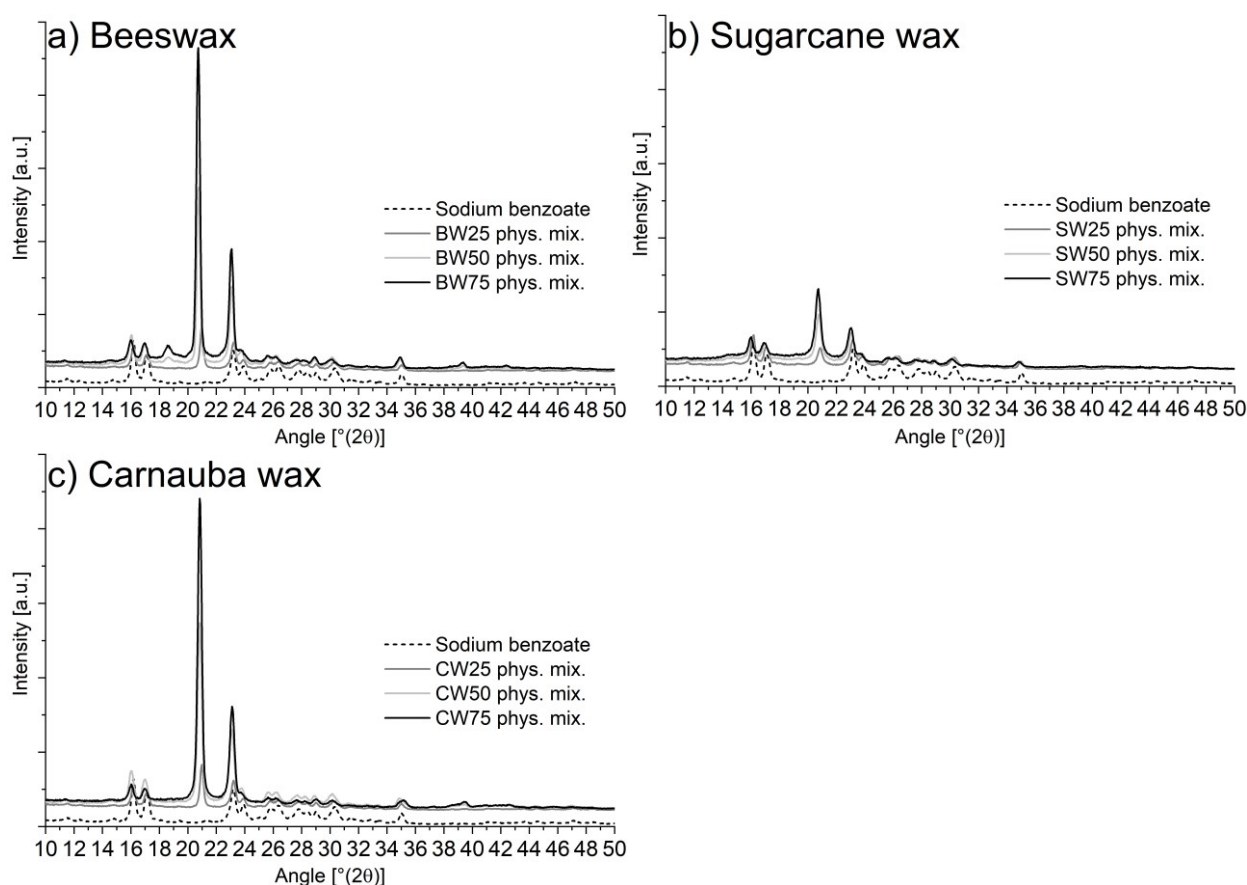


Figure 4.11: X-ray powder diffractograms of physical mixtures of wax:SB; a) beeswax, b) sugarcane wax and c) carnauba wax; BW = beeswax, SW = sugarcane wax; CW = carnauba wax; 2θ interval was used from 10 to 50 2θ .

characteristic peaks of the orthorhombic morphology of wax were observed at 21.0 2θ . The characteristic peak of wax at 23.2 overlapped with a characteristic peak of SB and could thus not be distinguished from it. The characteristic peaks of SB at 16.2 2θ and 17.1 2θ matched the physical mixture of SB/wax, as did the characteristic peaks of SB in the range of 26 2θ to 35 2θ . The

4.2 Characterization of physical mixtures

characteristic peaks of BW and CW at 29.2, 35.3 and 39.6 2θ as well as the peaks of SW at 35 and 39.4 2θ were not detected, presumably because of their low intensity.

4.2.5 Conclusion

Evaluation of the physical mixtures via DSC revealed one interaction in physical mixtures of BW/SB beyond the melting range of BW. The thermograms of physical mixtures of SW and CW did not reveal new information. The information gained from the thermograms was used to choose the process temperature for melt-extrusion processing of the natural waxes. As expected, analysis via XRPD did not reveal interactions between SB and natural waxes. In XRPD measurements, the characteristic peaks of the excipients were detected unaltered. Low-intensity peaks of wax at 29.2, 35.3 and 39.6 2θ were not detected in the physical mixture due to reduced proportion of the wax. These measurements form the reference for the analysis of extrudates manufactured via HME

4.3 Hot-melt extrusion using natural waxes

4.3.1 Introduction

In the past, drug formulations based on lipids and with improved taste characteristics were successfully developed (Krause, 2008; Michalk, 2007; Petrovick *et al.*, 2016; Vaassen *et al.*, 2012; Witzleb *et al.*, 2011). In Table 4.4, an overview of commonly used lipids in pharmaceutical industry is shown. However, these formulations were all developed using SLE.

Table 4.4: Commonly used lipids (adapted from (Walsh *et al.*, 2014), quoted from (Petrovick, 2015))

Type	Excipient name	Commercial name
Hydrogenated vegetable oil	Hydrogenated cottonseed oil	Lubritab [®]
	Hydrogenated palm oil	Dynasan [®] P60, Softisan [®] 154
Partial glycerides	Glyceryl monostearate	Inwitor [®] 191, Cutina [®] GMS or Tegin
	Glyceryl distearate	Precirol [®] ATO 5
	Glyceryl dibehenate	Compritol [®] 888 ATO
Triacylglycerides (TAG)	Glyceryl trimyristate	Dynasan [®] 114
	Glyceryl tristearate	Dynasan [®] 118
Polyoxalglycerides or macrogolglycerides	Lauryl polyoxyglycerides	Gelucire [®] 44/14
	Stearyl polyoxyglycerides	Gelucire [®] 50/13
Waxes/hard fat	Carnauba Wax	Carbowax [®]
	Beeswax	Witepsol [®] W35
	Polyethylene glycol (PEG)	Macrogol [®] 1500
	Hydrogenated coco-glycerides	Witocan [®] 42/44

Triacylglycerides and partial glycerides are obtained from vegetable oils, such as palm oil (Sambanthamurthi *et al.*, 2000). Partial glycerides compose only a small fraction of vegetable oil and are therefore derived from processing triacylglycerides to partial glycerides (Awadallak *et al.*, 2013; Zhong *et al.*, 2019), which requires the additional use of energy and chemicals and thus increases the amount of GHG emitted. Due to the high demand for this resource, mass cultivation of the African oil palm (*Elaeis guineensis* Jacq.) is performed. This results in massive deforestation which emits high amounts of CO₂ (Vijay *et al.*, 2016). SW is obtained from the sugarcane plant, which is cultivated in Brazil and India in a large scale for bioethanol production (Krendlinger *et al.*, 2015; Somerville *et al.*, 2010). Unfortunately, mass deforestation is also applied in the cultivation of SW to meet the high demands of bioethanol in Brazil (Bordonal *et al.*, 2018), thus driving its GHG-emissions. However, 50% of the bagasse, which is the source of SW, is burned to provide energy for sugarcane mills while

4.3 Hot-melt extrusion using natural waxes

the other half is stockpiled (Attard *et al.*, 2015). Upon changing the energy supply to renewable energy and using the bagasse as a source for SW, the total GHG-emission of sugarcane cultivation could be reduced and the sustainability of this production chain improved. Further advantages of the sugarcane plant are the low consumption of nitrogen as well as the improvements of water consumption necessary for cultivation of the plant (Bordonal *et al.*, 2018). A proof of concept for the use of SW as a high-quality excipient therefore would provide the opportunity to establish a sustainable excipient for the pharmaceutical industry and to contribute towards reducing the GHG-emission of the sugarcane production chain.

By processing lipids below their melting point, control of the aging of the employed triacylglycerides could be improved. During aging, triacylglycerides can transform from their unstable α -form to their stable β -form via their β' -form (Cholakova and Denkov, 2024; Larsson, 1966; Sato, 2001). This transformation can influence the dissolution profile of a formulation (Choy *et al.*, 2005). Waxes, however, show fewer polymorphic transitions (Basson and Reynhardt, 1988; 1988; Jennings and Gohla, 2000). In first experiments, temperatures below the melting point of the waxes were applied to the barrel segments of the TSE. The material began to pile up in the first barrel segment due to the sticking tendency of BW and SW. Thus, the temperature of the first barrel segment was set to 100 °C for BW and SW to prevent this. 100 °C exceeds the melting temperature of the used waxes and the extrusion process therefore constitutes HME. However, the temperature of the remaining barrel segments was set a few degrees Celsius below the melting point to obtain a solid extrudate when the material passed through the die.

4.3.2 Production of wax filaments with 50% and 75% drug load

For therapy with SB, high daily doses of the drug are required (Breitkreutz *et al.*, 2003; Nowak *et al.*, 2022). In order to achieve the best possible compliance, the drug load should be as high as possible to distribute the intake of the drug over as few units as possible. Krause *et al.* (2009) have shown that solid lipid cold extrusion and subsequent pelletization are possible for a drug-load of up to 80%. To test the feasibility of extrusion of SB with natural waxes, drug loads of 50% and 75% were evaluated. The three natural waxes BW, SW and CW were used alongside a die with a diameter of 2.0 mm. The process temperature was chosen individually for each wax and based on the data obtained from the previously described thermal analysis via DSC. The aim of these feasibility experiments was to obtain filaments of varying drug-loads. To obtain a filament which had solidified sufficiently after leaving the die, the temperatures were adapted based on the set drug load. The feed rate was kept constant at 20 g*min⁻¹ for all feasibility batches (**BaHav17-008**). The process temperature of the chosen wax as well as their corresponding ratios are depicted in Table 4.5.

Table 4.5: Process parameters of the extrusion of different ratios of SB/wax

Mixture (%; w/w)	Batch	RPM	Temperature [°C]
SB / BW 50:50*	BW50	50	50
SB / BW 75:25*	BW25	100	60
SB / SW 50:50*	SW50	75	60
SB / SW 75:25*	SW25	150	70
SB / CW 50:50**	CW50	50	80
SB / CW 75:25**	CW25	100	86

*Temperature of first barrel set to 100°C; ** Temperature the same for all barrel segments

The screw speed was originally set to 50 revolutions per minute (RPM) for each formulation but had to be adapted to guarantee a stable process with constant pressure. By applying the above-mentioned parameters, extrudates were obtained, examples of which are shown below in Figure 4.12.



Figure 4.12: pictures of wax extrudates with 50% (left side of each picture) or 75% drug-load (right side of each picture) and made from either beeswax (left), sugarcane wax (middle) or carnauba wax (right).

Each extrudate exhibited the color of the respective processed wax: white for BW, brown for SW, and yellow for CW. With increasing SB content, the extrudates became paler, as the color-providing component was displaced by the API in the formulation. Also, the extrudates exhibited more brittle properties with increasing API ratios.

4.3.3 Morphological properties

Witzleb *et al.* (2011) and Petrovick (2015) have stated that the analysis of the morphology of a lipid extrudate can give valuable insight into its properties. The SEM images (**BaHav17-008**) are shown in Figure 4.13. For the BW extrudates with 50% drug load (Figure 4.13A), no crystals were observed and they had a rough surface.

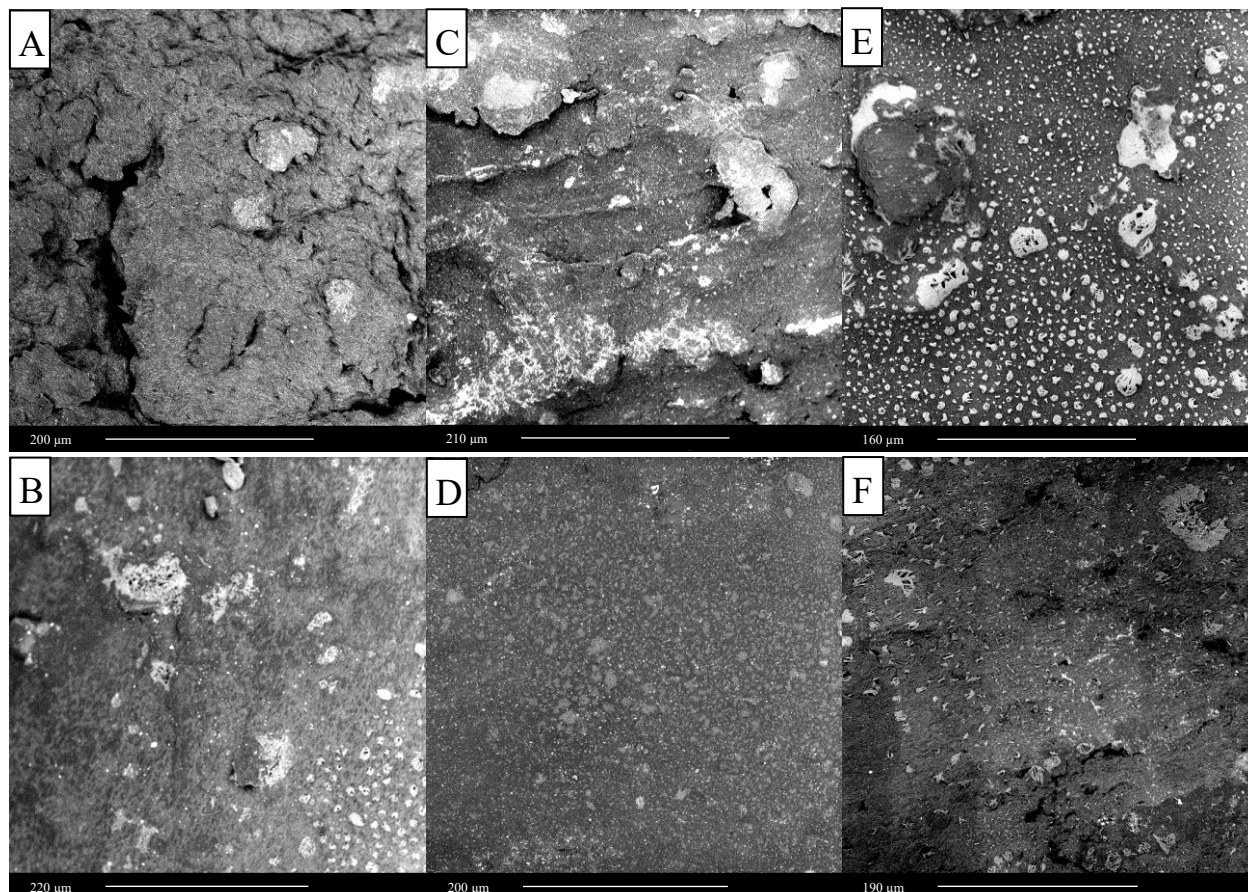


Figure 4.13: SEM images of $\varnothing = 2.0$ mm wax extrudates with 50% or 75% drug-load; A) BW 50%, B) BW 75%, C) SW 50%, D) SW 75%, E) CW 50%, F) CW 75%. The scale represents around 200 μm .

In contrast, the BW extrudates with 75% drug load (Figure 4.13B) showed a smooth surface with clearly visible crystals embedded in it. Although the proportion of SB was significantly higher in BW extrudates with 75% drug load, the difference in finding the crystals could not be explained by the mass fraction alone, especially upon comparing the images of the other extrudates as well. The SW extrudates with 50% drug load (Figure 4.13C) as well as the CW extrudates containing 50% SB (Figure 4.13E) had SB crystals exposed at their surface as well as their 75% counterpart (Figure 4.13D + F). In Figure 4.13B-F, it can be seen that the SB spheres were partially crushed into needles or collapsed under the shear forces during the extrusion process.

To gain insight into the distribution of SB inside the extrudates, a cross section of the extrudates was prepared and investigated (Figure 4.14). The cross-sections revealed a homogeneous matrix consisting of SB crystals and wax. In addition, single intact SB crystals-spheres were present. In Figure 4.14D and F, single pores were observed. At this point, it is unclear whether the pores were

created during extrusion by incorporating small amounts of air or if crystals were removed during preparation of the cross-section, leaving holes behind.

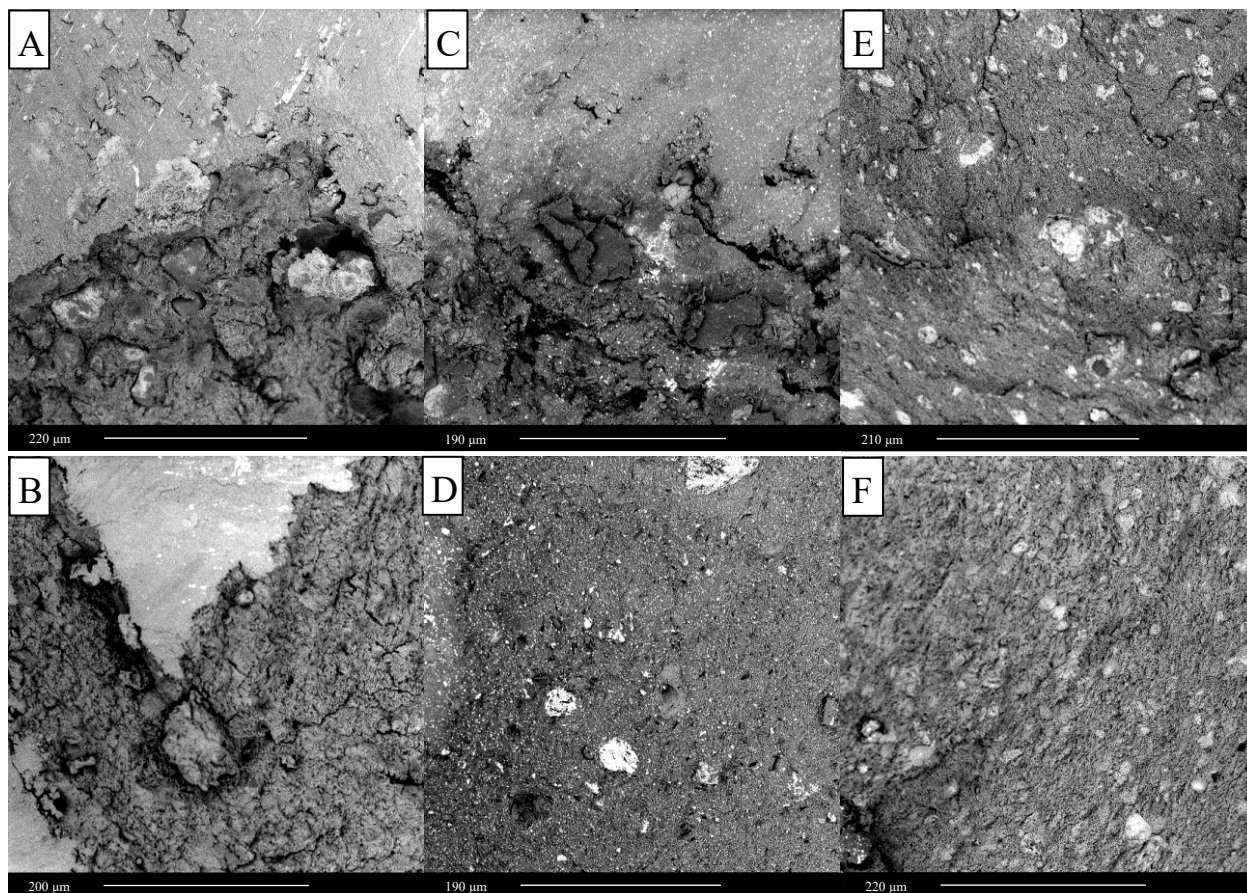


Figure 4.14: SEM images of the cross section of extrudates with 50% or 75% drug-load alongside their corresponding scale-bars; A) BW+50%, B) BW+75%, C) SW+50%, D) SW+75%, E) CW + 50%, F) CW + 75%.

4.3.4 Dissolution profiles of extrudates

Dissolution studies were performed on all extrudates. The basket apparatus was used to prevent flotation of the extrudates. The rotation speed was set to 50 rpm. The results of these dissolution studies (**BaHav17-011**) are displayed in Figure 4.15a. Different dissolution profiles were obtained depending on the processed ratios of SB/wax. Extrudates with a drug load of 75% (BW25, SW25 & CW25) released their drug content faster than those with a drug load of 50%. BW25 and CW25 extrudates showed complete release after 24 h (BW25) and after 120 min (CW25), respectively. This high API release rate can be explained through the formation of a pore network, which was described by Reitz *et al.* (2008) for similar drug formulations. Water can penetrate the extrudates through pores created by SB dissolving from their surface. Once penetrated, the water dissolves SB crystals inside the extrudates, expanding the pore network. The dissolved crystals are transported out of the pores via Fickian diffusion (Rosiaux *et al.*, 2014). SW25, however, showed an incomplete release with a reduction in the measurable amount of released SB after 24 h. The extrudates with a drug load of 75% showed a Higuchi kinetic, which is in alignment with literature sources (Dredan *et al.*, 1996). With an increased amount of wax in the extrudate, the speed of drug release was drastically reduced. BW50, SW50 and CW50 released SB significantly slower than their counterparts with 75% drug load.

4.3 Hot-melt extrusion using natural waxes

Petrovick (2015) observed similar changes in the dissolution profile by increasing the amount of lipids, describing an initial burst phase followed by a slower release phase. He concluded that the dissolution profiles of his drug formulation followed a Higuchi-kinetic. BW50, SW50 and CW50 showed a burst phase followed by a slower release phase and their dissolution profiles were thus checked for Higuchi-kinetics (Siepmann and Peppas, 2011), which could be confirmed (Figure 4.15b).

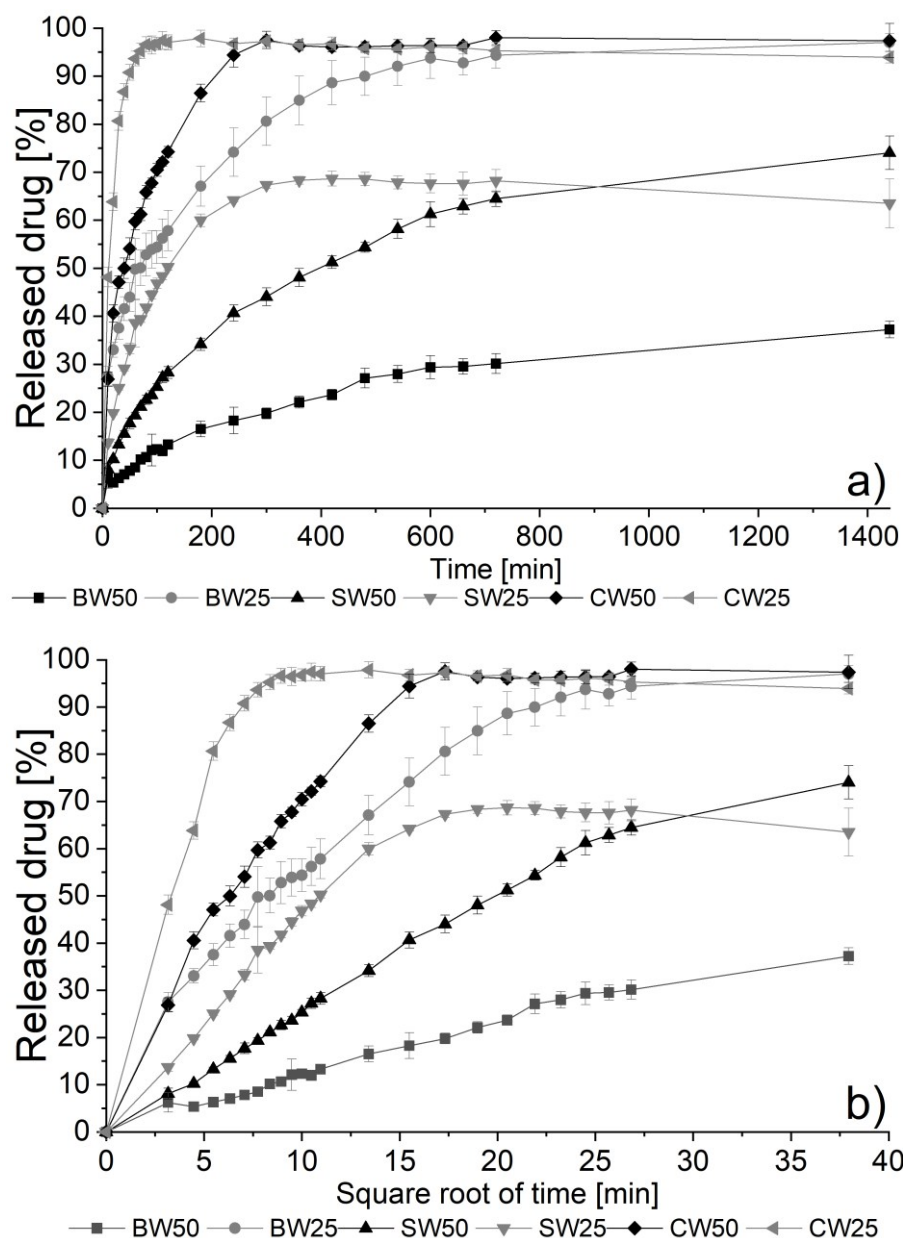


Figure 4.15: Dissolution profiles of extrudates with different drug-loads of sodium benzoate; 900 ml phosphate buffer pH 6.8, $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, basket apparatus set to 50 rpm, $\lambda = 232\text{ nm}$; $n=6$, $\bar{x} \pm \text{SD}$; BW = beeswax, SW = sugarcane wax, CW = carnauba wax; number = ratio of wax, a) normal time; b) square root of time

As the SB/wax ratio is shifted toward the water-insoluble component, fewer pores are formed through the dissolution of SB, which in turn reduces the release rate.

4.3.5 Conclusion

It was feasible to produce extrudates consisting of a wax matrix and SB via HME. By altering the amounts of wax, different dissolution profiles could be created. Extrudates containing 75% of SB as well as those containing 50% SB followed Higuchi-kinetics. These findings were in accordance with similar observations for lipid-based formulations. However, for SW25, a decreasing amount of released SB was detected after reaching a measurement peak. Since a slight decrease of SB was also detected for CW25 and CW50, possible sources of that decrease were investigated. Neither absorption to the glass or tubes, nor evaporation of the drug together with water vapor proved to be the source of the decreasing drug amount. Chemical instability was excluded due to the fact that SB is usually used as a preservative and therefore should be chemically stable for at least one day. Thus, this phenomenon remained unsolved. As a next step, the diameter of the extrudates was reduced to create the opportunity to obtain small sized pellets via an additional spheronization step. This enabled the production of a small sized multi-particular dosage form for children comparable to those using SB too (Breitkreutz *et al.*, 2003). Further, the crystalline properties were checked in order to gain a better understanding of the structure of the extrudates. The stability of the extrudates manufactured during the feasibility trials was investigated and the results compiled as described in the following chapters.

4.4 Stability of wax extrudates

4.4.1 Introduction

It is known from literature that drug formulations made from lipids can change in their crystalline structure over storage time, thus altering their surface area and consequently their dissolution profile (Choy *et al.*, 2005; Khan and Craig, 2004; Windbergs *et al.*, 2009). For BW, a similar phenomenon has been described in literature, which is called efflorescence (Bartl *et al.*, 2015; Bartl *et al.*, 2012). After solidification of molten BW, unsaturated hydrocarbons remain in a mobile state and move to the surface via diffusion. Eventually, they crystallize on the surface. A restructuring of the surface via this mechanism would alter the dissolution profile of wax-based extrudates and must therefore be investigated. Similar studies have not been performed for SW or CW, thus the potential occurrence of this mechanism should also be investigated for these waxes.

4.4.2 Thermal properties

Extrudates made from natural waxes typically show polymorphic behavior in thermal analysis. For a better understanding of the age dependency of that polymorphic behavior, the extrudates were analyzed at three different time points via DSC: right after extrusion, 10 weeks (two months) after extrusion and 14 weeks (three months) after extrusion (**BaHav19-009**). As explained in chapter 4.3.2, extrudates with high drug load have a higher significance. For that purpose, the thermograms of the extrudates with ratios of 75% and 50% wax are depicted in the annex (Figure 9.21 - Figure 9.26). These extrudates were analyzed after manufacturing and after 14 weeks (three months). Their thermograms showed the peaks, which have been described in chapter 4.2.3 and are not further discussed. The samples were stored at ambient conditions in a transparent polyethylene bag. Samples of BW25_1.0mm and BW25_0.5mm exhibited the previously described peaks characteristic for BW (Figure 4.16a + b). In addition, the presumed eutectic mixture occurred irrespective of the age of the samples. We observed in Figure 4.16 that no aging effects were visible for the samples of BW and CW in thermal properties. However, the thermograms of SW25_1.0mm and SW25_0.5mm showed a slight difference between the thermograms of freshly produced extrudates and the older samples. In the beginning of melting of freshly produced extrudates, the slope was slightly smaller than that of the samples measured after 10 and 14 weeks. The samples measured after 10 and 14 weeks showed a comparable thermogram. Thermograms of CW25_1.0mm and CW25_0.5mm revealed no difference between samples of any time point.

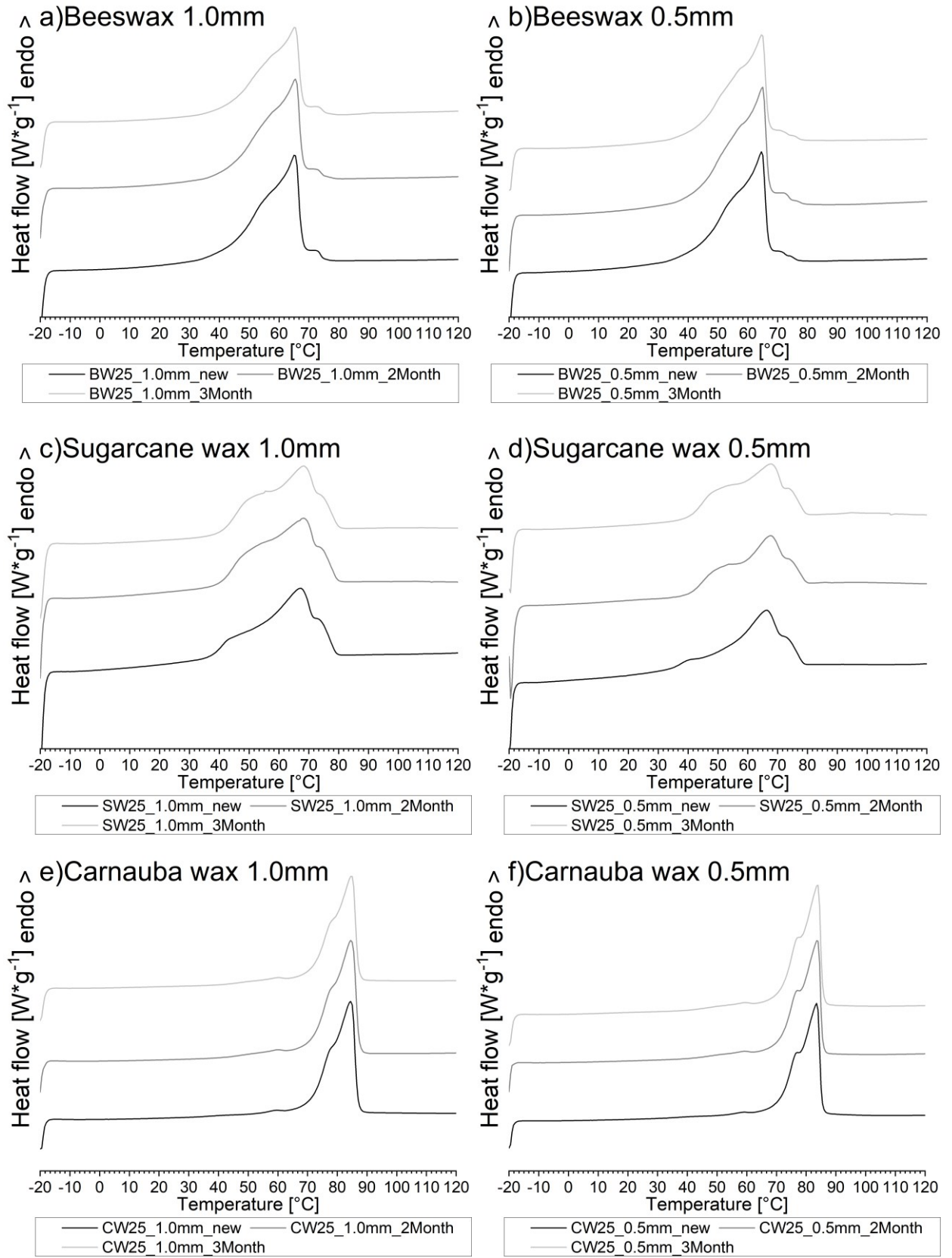


Figure 4.16: Thermograms of a) BW25_1.0mm, b) BW25_0.5mm, c) SW25_1.0mm, d) SW25_0.5mm, e) CW25_1.0mm and f) CW25_0.5mm. Heat rate $10^{\circ}\text{C}/\text{min}$.

4.4 Stability of wax extrudates

4.4.3 Crystalline properties

In addition to the thermal properties, the crystalline properties were also analyzed via XRPD (**BaHav19-017a**). The results are depicted in Figure 4.17. Samples of this experiment were 10 weeks old. Exposing the mixture of SB and wax to thermal and mechanical stress via the process of HME did not alter the diffractograms in comparison to the diffractograms of the physical mixtures (Figure 4.11). As expected, a large fraction of wax in the samples resulted in high intensities of the characteristic wax peak. At 16.2 2θ and 17.1 2θ , the characteristic peaks of SB were observed. At 21.0 2θ the characteristic peak of wax was observed. At 23.2 2θ , a peak which is measured for both wax and SB was observed. This peak had already been observed previously in the analysis of the pure materials and in the physical mixtures. Beyond 25 2θ , several small peaks attributable to SB were detected.

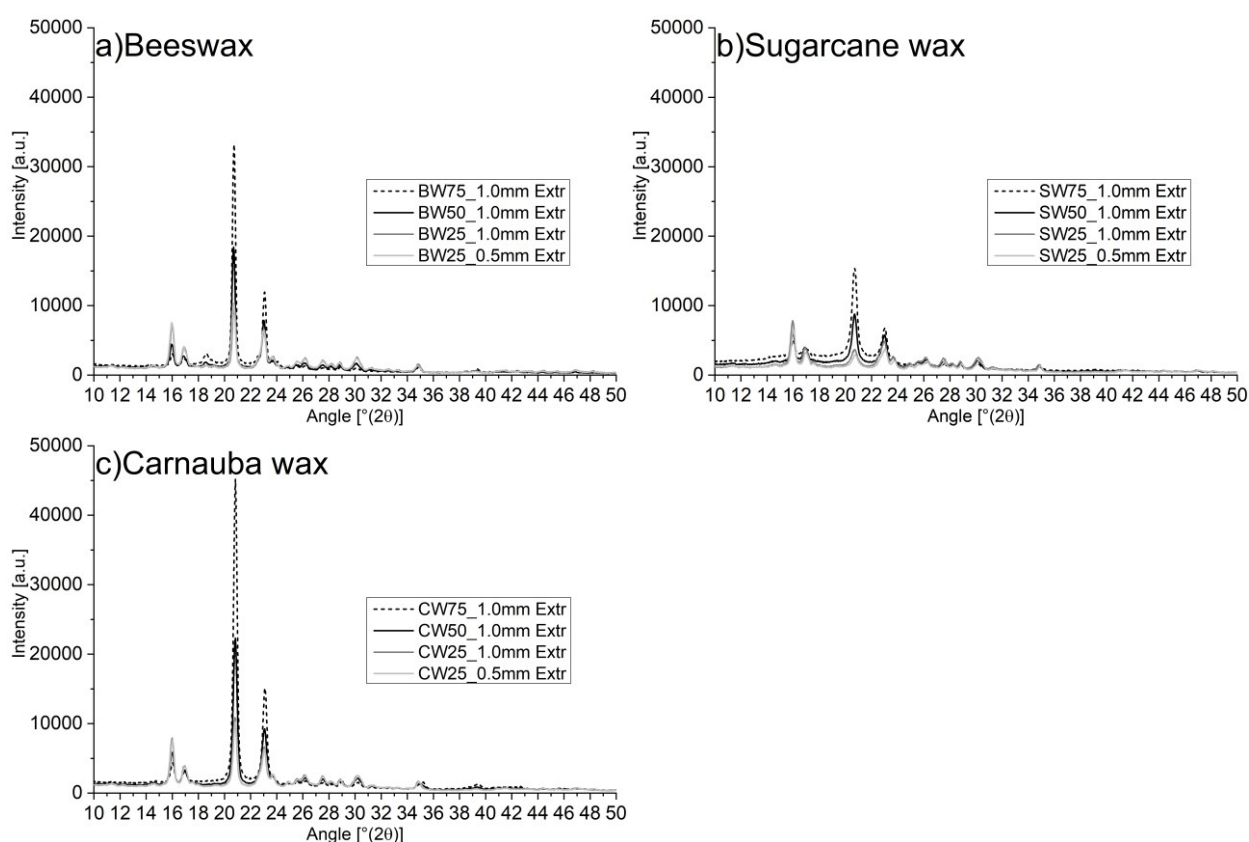


Figure 4.17: XRPD-diffractograms of SB/wax extrudates; a) BW, b) SW and c) CW; a 2θ interval was used from 10 to 50 2θ .

4.4.4 Morphological properties

The morphological properties of the wax-based extrudates were analyzed using scanning electron microscopy (**BaHav19-010**). On the surface of extrudates based on BW (Figure 4.18 A-B and Figure 4.19A-B), SB crystals were observed. In addition, the surface appeared rough and showed craters. It is noticeable that the BW50_1mm extrudates showed an especially large amount crystals on their surface (Figure 4.18B). BW extrudates with a higher SB loading showed lower amounts of SB crystals on their surface (Figure 4.19A-B). This observation corresponded to the opposite of the observations in chapter 4.3.3. In alignment with the observation of the sample BW50_1mm, the data of the release study (see Figure 4.20a) showed that these extrudates indeed released the most SB compared to the other BW extrudates. In contrast to the BW extrudates, no crystals were seen on the images of the extrudates made from SW and CW (Figure 4.18C-F and Figure 4.19C-F). Crystals were identified on the surfaces of extrudates made from these waxes in section 4.3.3. However, the extrudates analyzed in this study were extruded under different conditions. Although the temperature applied to the die was not changed in the extrusion of SW and CW compared to the extrudates on the images in chapter 4.3.3, the temperature was higher in the other barrel segments.

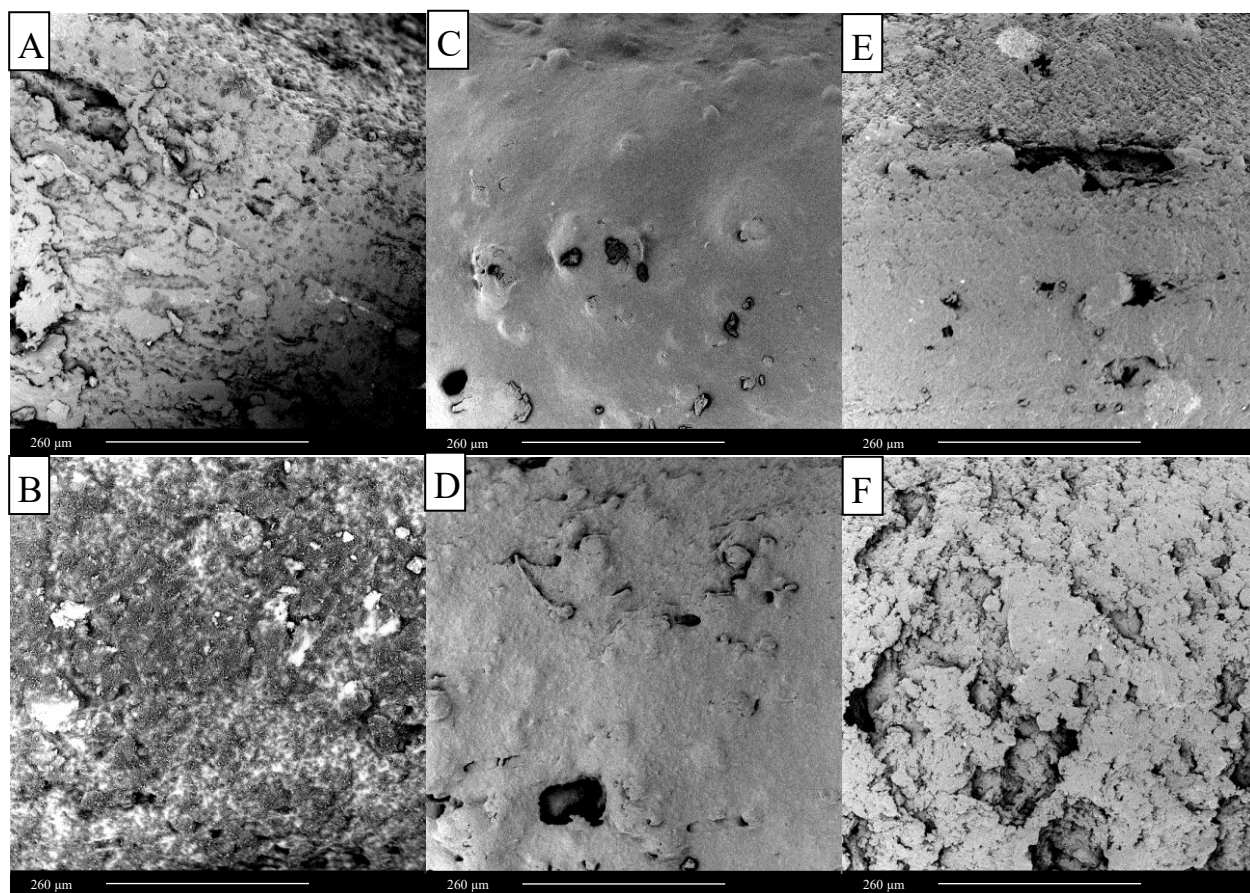


Figure 4.18: SEM images of wax-based extrudates with ratios of wax/SB of 75/25 or 50/50; A) BW75_1mm, B) BW50_1mm, C) SW75_1mm, D) SW50_1mm, E) CW75_1mm, F) CW50_1mm.; the scale represents around 260 µm.

Furthermore, a different die plate was also used. The extrudates shown in Figure 4.18A-F were produced with a die plate with 23 holes and 1.0 mm diameter while those in Figure 4.19A-F used a die of 91 holes and 0.5 mm diameter. The changed extrusion conditions might have altered the

4.4 Stability of wax extrudates

surface structure of these extrudates in comparison to extrudates based on the same material but produced under different process parameters.

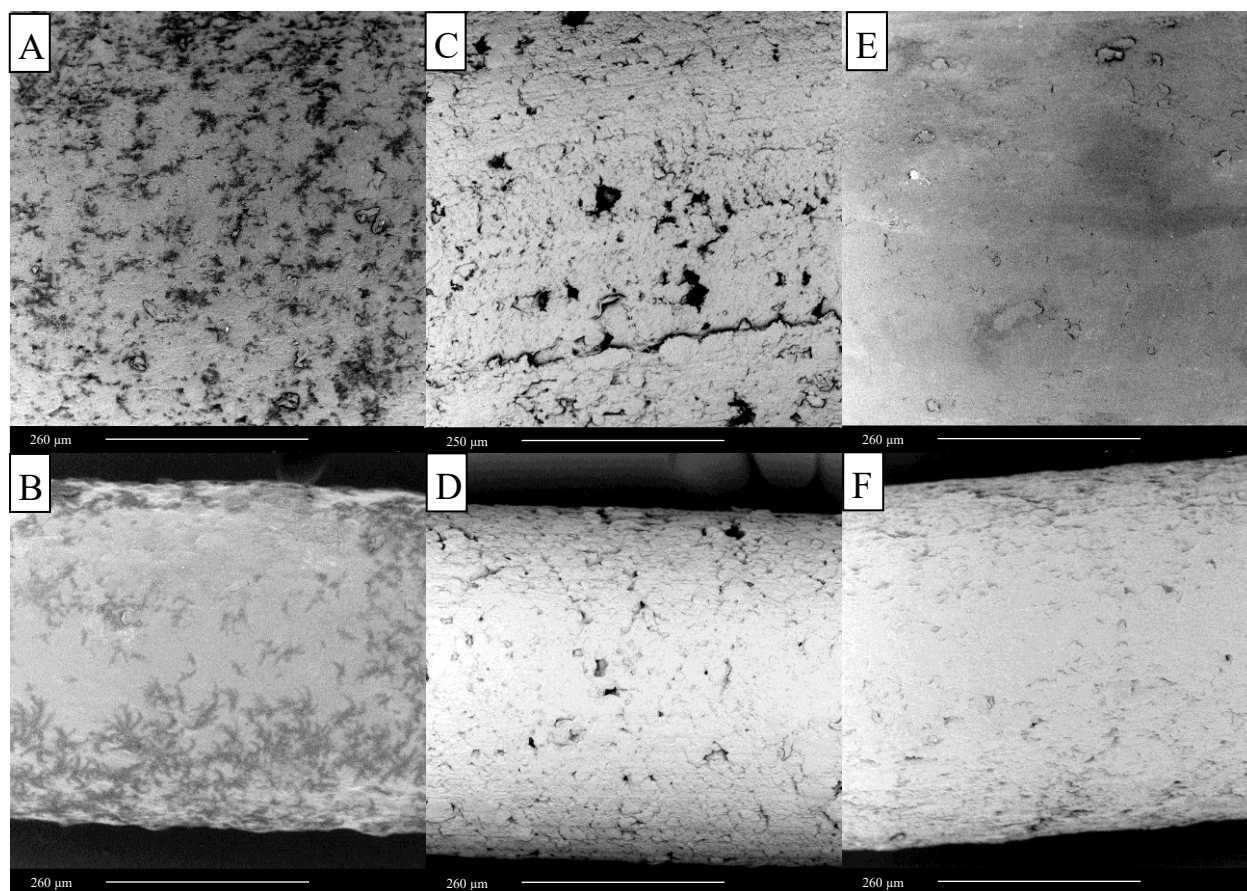


Figure 4.19 SEM images of wax-based extrudates with ratios of wax/SB of 25/75; A) BW25_1mm, B) BW25_0.5mm, C) SW25_1mm, D) SW25_0.5mm, E) CW25_1mm, F) CW25_0.5mm; the scale represents around 260 μm

4.4.5 Dissolution profiles

The influence of storage time at ambient temperatures on the dissolution profile was investigated. From previous experiments, it was known that a faster dissolution can be achieved by increasing the drug-load of the extrudates. The main focus in this work is on high drug-loaded wax-based extrudates, thus the stability of extrudates with 75% drug-load was investigated in a monthly interval. In contrast, the stability of extrudates with 50% and 25% drug load was checked only once after 3 months (**BaHav19-006**). Figure 4.20 displays the results of the freshly produced extrudates. Keeping the results from chapter 4.3.4 in mind, it was expected that the extrudates with 75% drug load and 0.5 mm diameter would show the fastest release, followed by extrudates with 75% drug load and 1.0 mm diameter, 50% drug-load and 25% drug-load. In Figure 4.20a, the results of freshly prepared BW extrudates are shown. Contrary to these expectations, BW25 extrudates with 0.5 mm as well as with 1.0 mm in diameter did not show the fastest drug-release of the three different drug loads. Instead, BW 50 showed the fastest release, followed by BW25_0.5mm, BW25_1.0mm and BW75_1.0mm. A possible explanation for that difference in comparison to the results from chapter 4.3.4 is the age of

the samples. Extrudates in the dissolution studies of chapter 4.3.4 were 6 months old while samples from this dissolution studies are only a few days old. Over the term of 6 month, the extrudates presumably underwent a transformation. Due to the phenomenon of efflorescence, the wax-matrix could have been restructured (Bartl *et al.*, 2015). This led to a reshaped surface, which potentially had new formed pores on the surface of the extrudates. The results of SW- and CW-based extrudates corresponded to expectations (Figure 4.20b+c). The fastest release was achieved for SW25_0.5mm and CW25_0.5mm followed by their counterparts with a thickness of 1.0mm. Subsequently, SW50_1.0mm and CW50_1.0mm followed. The slowest release was observed for SW75_1.0mm and CW75_1.0mm. According to the U.S. Food and Drug Administration (2018), the requirements for immediate release are met if 80% of drug content are released in 30 minutes or less. Only the CW25_0.5mm extrudates complied to this requirement. Nevertheless, CW25_1.0mm missed the threshold for immediate release very closely, releasing 77.6% within 30 minutes. Neither SW25_0.5mm nor the other SW based extrudates complied to the requirements for immediate release.

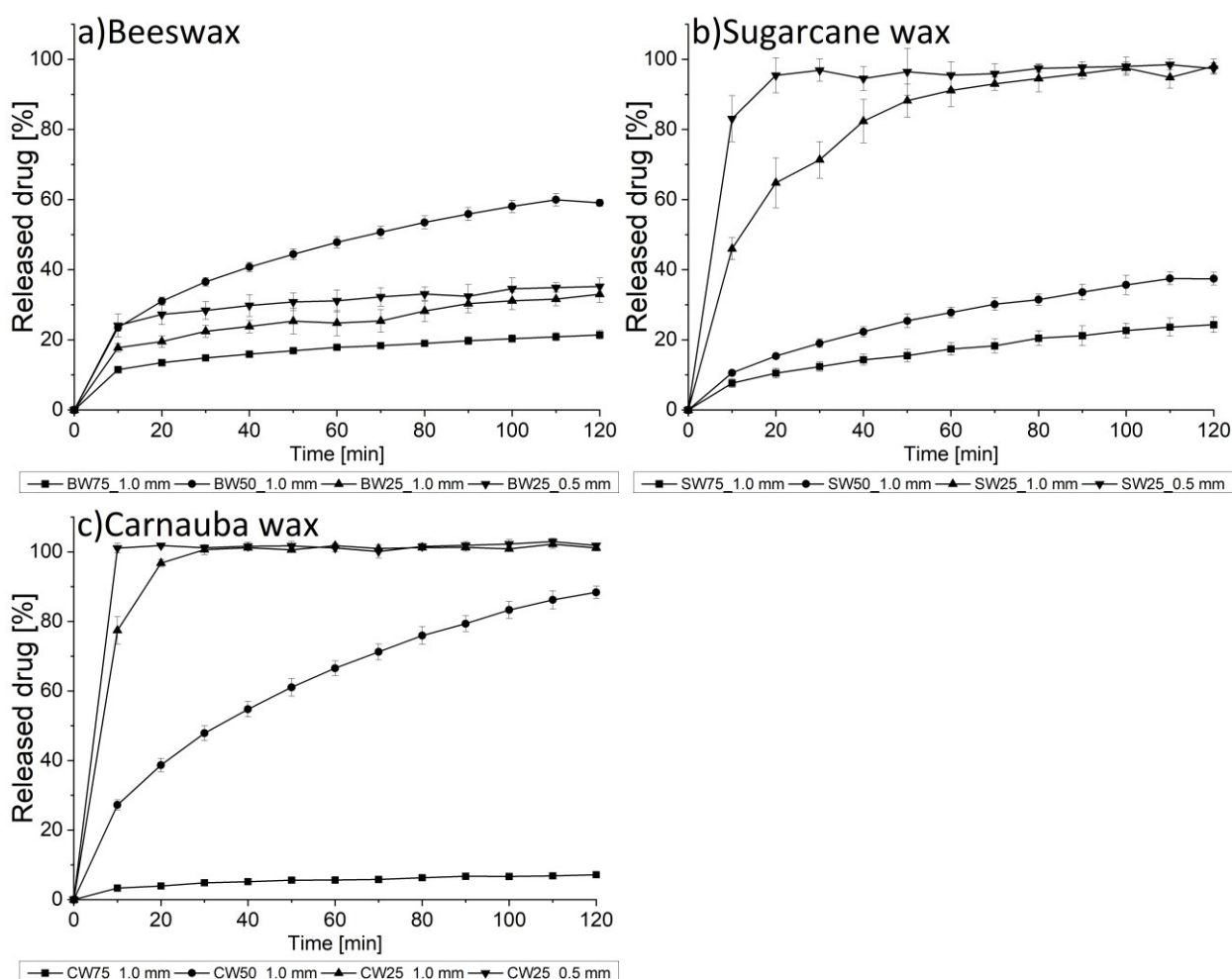


Figure 4.20: Dissolution profile of freshly produced extrudates. a) beeswax extrudates, b) sugarcane wax extrudates, c) carnauba wax extrudates.; 900 ml phosphate buffer pH 6.8, $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, basket apparatus set to 50 rpm; $n=6$, $\bar{x} \pm \text{SD}$; BW = beeswax, SW = sugarcane wax, CW = carnauba wax; number = ratio of wax.

Very interesting were the differences of the extrudates with 50% drug-load made from different waxes. BW50_1.0mm and CW50_1.0mm showed a similar amount of burst effect, but after 30 min, CW50_1.0mm had released 47 % of SB while BW50_1.0mm had only released 37 % of its drug load. After 120 min, a total release of 60 % was achieved for BW50_1.0mm and 88% for CW50_1.0mm.

4.4 Stability of wax extrudates

SW50_1.0mm showed a smaller burst followed by a slower release. According to solid lipid content calculations, 26.3 % of BW50_1.0mm and 12.2 % of SW50_1.0mm had melted at 37 °C. In comparison, CW50_1.0mm had only 7 % molten content. It is possible that the softened components sealed pores, which were created by dissolving SB. This could explain why CW released its content faster than BW and SW, but not the differences between BW50_1.0mm and SW50_1.0mm. A dissolution study with a freshly produced batch of BW25_0.5mm at two different temperatures was performed to investigate the influence of the temperature on the dissolution profile of BW extrudates. The confidence interval was calculated to show, that the results at 37°C are comparable although they were performed in two different dissolution media. The results of that calculation are shown in Table 4.6.

Table 4.6: calculation of two-sided confidence interval; n = sample size, α = level of significance, $\bar{x}$ = mean value; s = standard deviation; t = t-value; ci- = lower confidence interval; ci+ = upper confidence interval

Label	n	α	$\bar{x}$ [%]	s	t	ci-	ci+
BW25_0.5mm 22°C 5min	6	0.05	79.71	4.06	3.16	72.63	82.76
BW25_0.5mm 37°C dem. Water, 5min	3	0.05	38.37	2.03	6.21	30.01	43.76
BW25_0.5mm 37°C pH 6.8, 5min	3	0.05	29.35	0.39	6.21	27.05	30.42
BW25_0.5mm 22°C 10min	6	0.05	94.66	3.39	3.16	90.28	99.03
BW25_0.5mm 37°C dem. Water, 10min	3	0.05	43.03	2.73	6.21	33.24	52.82
BW25_0.5mm 37°C pH 6.8, 10min	3	0.05	32.71	1.46	6.21	27.48	37.95

It could be shown that the confidence interval of the two different samples at 37°C is overlapping, which means that there is no significant difference between these results. In addition, the results of the dissolution performed at 22°C shows no overlapping of its confidence interval with the samples pulled at 37°C. The results are shown in a graphical plot in Figure 4.21 (**BaHav19-029**). The temperature had a strong impact on the dissolution profile. The dissolution at 37°C is similar to the dissolution profile of BW25_0.5mm in Figure 4.20a, showing a release of 37% after 10 min. The dissolution profile recorded at 22°C looked very different. After five minutes almost 80% of SB were released from the BW-matrix.

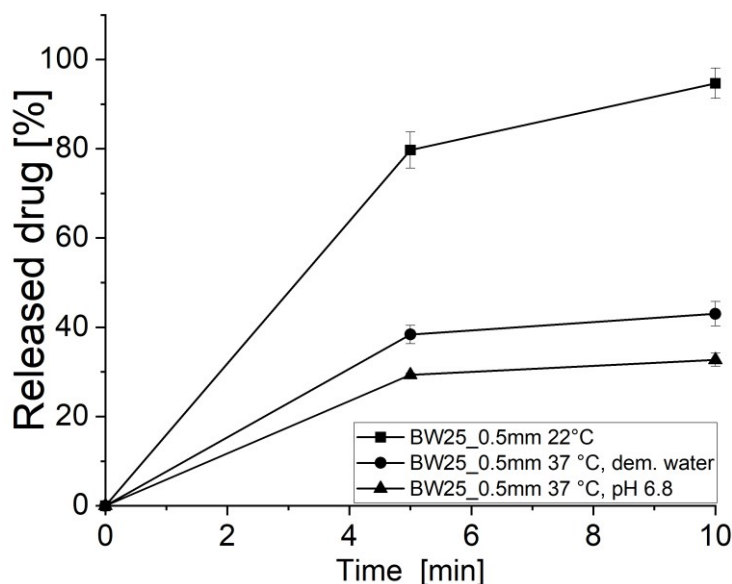


Figure 4.21: Dissolution profile of freshly produced BW25_0.5mm extrudates in 900 ml demineralized water at 22°C ($n = 6$) or 37°C in demineralized water ($n = 3$) and in 900 ml phosphate buffer pH 6.8 at 37°C, $n = 3$, basket apparatus set to 50 rpm, $\lambda = 225$ nm, $\bar{x} \pm SD$; BW = beeswax, number = ratio of wax.

After 10 minutes, 90% were released, which is very close to the amount of released SB in CW extrudates. The solid lipid content of BW at 22°C was not measurable, which indicated that no component had melted at this temperature. The monthly checked dissolution profiles of wax extrudates with 75% SB-load are shown in Figure 4.22a-f. They revealed an age dependency for extrudates made from BW or SW while extrudates made from CW remained unaltered in their dissolution profile over the time span of three months. Age-dependent alterations of the wax-matrix of BW and SW extrudates were restricted to the initially released amount of SB. Drug release of freshly produced BW extrudates and 1 month old BW extrudates differed slightly (Figure 4.22a+b). Both samples released approximately 20% SB after 10 min. After 10 min, BW25_1.0mm_1Month and BW25_0.5mm_1Month showed a higher slope in their dissolution profile, releasing 8% and 17% more SB, respectively, than the freshly produced samples. However, two months old BW extrudates showed a large increase in the initially released amount of SB after 10 min. From minute 10 to 40, the release of two months old extrudates was faster than the one-month-old or freshly produced extrudates. From minute 40 on the slope of the dissolution profile of the two months old extrudates

Table 4.7 Slopes calculated from the dissolution graphs of BW25_1.0mm and BW25_0.5mm extrudates of different age. For the calculation the period after 40 min of dissolution was observed.

Sample	Slope
BW25_1.0mm_1month	0.1431
BW25_1.0mm_2month	0.1605
BW25_1.0mm_3month	0.1613
BW25_0.5mm_1month	0.1294
BW25_0.5mm_2month	0.1543
BW25_0.5mm_3month	0.1521

was very similar to one of the one-month-old samples (Table 4.7). The comparison of slopes of BW extrudates indicates that from that moment on, the release was not affected by an age-dependent alteration of the matrix. The same observations were found for three months old BW extrudates.

4.4 Stability of wax extrudates

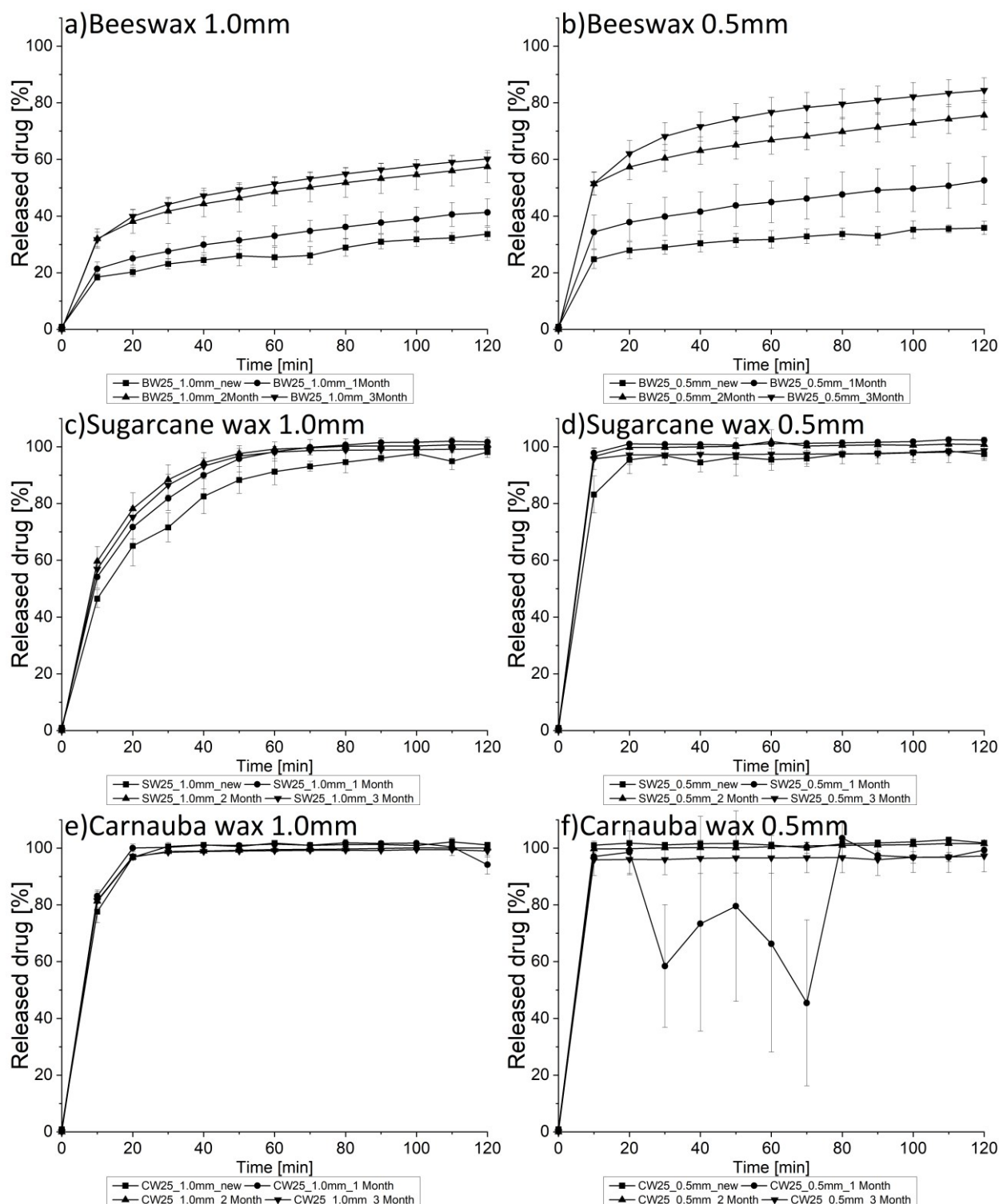


Figure 4.22: Dissolution profiles of a) BW25_1.0mm0.5mm, b) BW25_0.5mm, c) SW25_1.0mm, d) SW25_extrudates; e) CW25_1.0mm and f) CW25_0.5mm immediately after production, after 1, 2 and 3 months. 900 ml phosphate buffer pH 6.8, $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, basket apparatus set to 50 rpm; $n=6$, $\bar{x} \pm \text{SD}$; BW = beeswax, SW = sugarcane wax, CW = carnauba wax; number = ratio of wax.

The amount of initially released SB was increased in comparison to the two months old samples, followed by an increased drug release from minute 10 to minute 40. From minute 40 the dissolution profile did not show significant differences in comparison to the less old samples. The analysis of the dissolution data of SW extrudates showed similar alterations over the storage time. The amount of released SB of SW25_1.0mm within the first ten minutes was increasing successively from 45% to

62% over the term of three months (Figure 4.22c). After the initial release, the dissolution profiles of the samples were similar. An increase in the initial release was also observed for SW25_0.5mm over the storage time, but as the release was finished after ten minutes for all samples aged by at least one month, other changes cannot be observed (Figure 4.22d). The recorded dissolution profiles of samples which are one month old were almost identical for CW25_1.0mm and CW25_0.5mm. Unfortunately, the data of CW25_0.5mm_1month showed unreasonable deviations from all other recorded data. Since the first and last four data points seemed to be reasonable, an error in the sample preparation was concluded to be the source. The aging effects of three months old extrudates was not only analyzed for the ratios of 25% wax and 75% SB but also for the ratios of 75% wax and 50% wax + SB to investigate how the aging related alterations in the wax matrix influence the dissolution profile of these samples. BW75_3months showed neither an increased initial release nor a higher total release (Figure 4.23a) while BW50_3month showed an increase in initial release by releasing 50% drug load after 30 min and an increase in the total amount of released SB after 120 min by releasing 88% drug load.

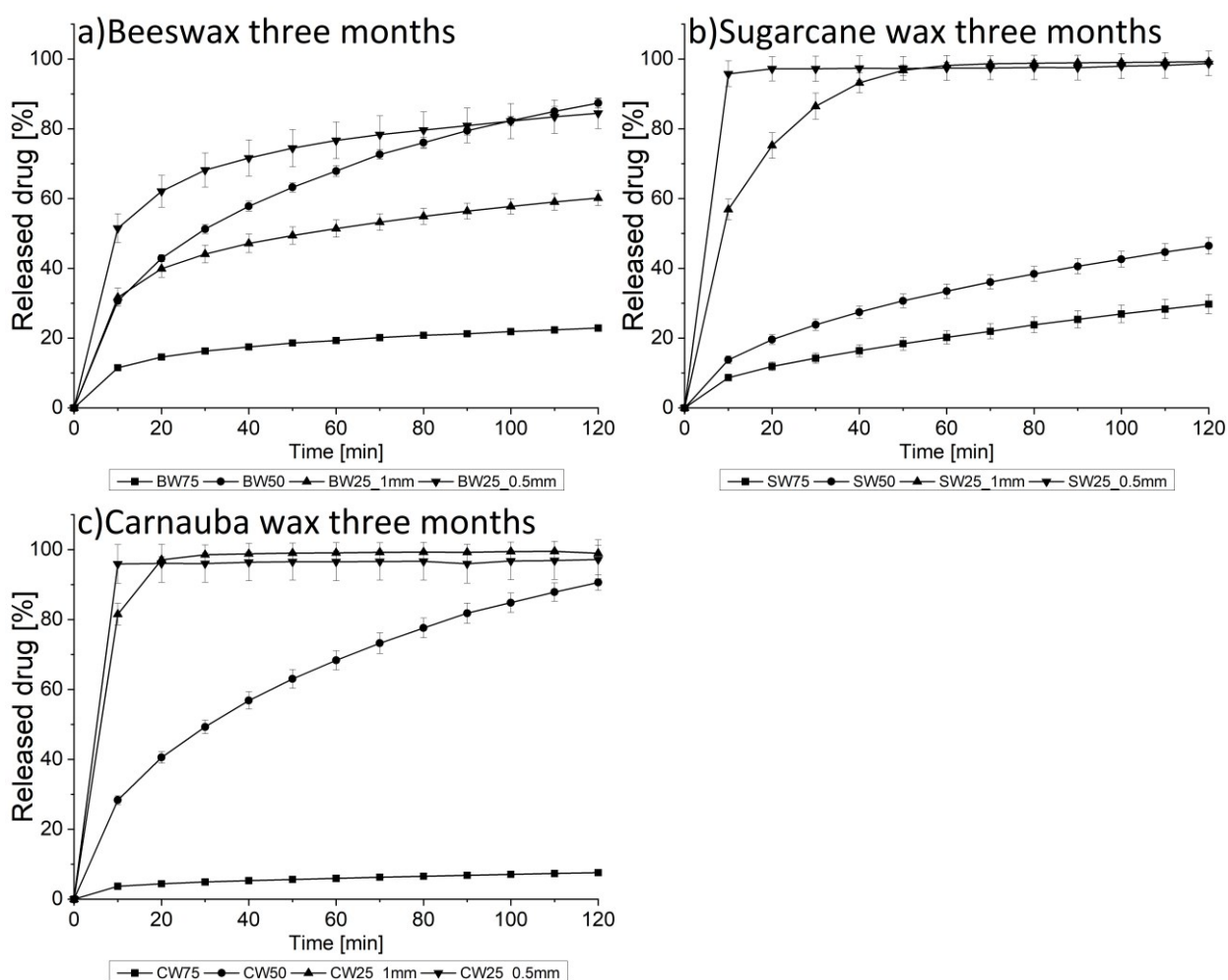


Figure 4.23: Dissolution profiles of a) BW, b) SW and c) CW extrudates and their different ratios after three months of storage at ambient conditions. 900 ml phosphate buffer pH 6.8, $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, basket apparatus set to 50 rpm; $n=6$, $\bar{x} \pm \text{SD}$; BW = beeswax, SW = sugarcane wax, CW = carnauba wax; number = ratio of wax.

A similar change was observed for SW-samples (Figure 4.23b). SW75_3month showed no increase in the initial burst but a slight increase in the total amount of released SB after 120 min. Samples of SW50_3month showed a small increase in the amount of initially released SB as well as an increase

4.4 Stability of wax extrudates

in the total amount of released SB of 10%. As previously shown Figure 4.23 for CW extrudates, the samples CW75_3month and CW50_3month were not affected by any aging phenomena. Their dissolution profiles, analyzed after 3 months, matched the dissolution profiles of the freshly prepared samples (Figure 4.23c). These observations support the hypothesis that the changes in the drug release were caused by surface restructuring in samples of BW and SW with a drug load of 75%. These samples have more SB-crystals at the surface or right underneath it. Additional cracks increase the surface and support the penetration of water into the matrix, increasing the amount of initially released SB. Samples with a lower drug load have less SB on the surface or underneath it. A restructuring would also increase the rate of released SB, as the overall pore network is increased but due to a low ratio of SB the network is not sufficiently spread over the sample to enable a high release. The particles which are located in the core of the matrix are released with the same speed as before if they are not exposed to new pores.

4.4.6 UV-Probe analysis

The standard dissolution method applied in the experiments required manual sampling (see chapter 7.2.2.10). This made it difficult to perform sampling with short time intervals. To gain detailed insight into the processes occurring within the first 10 minutes of dissolution testing, a dissolution was carried out using an UV-probe for an in-line measurement of released SB (**BaHav18-027**). This made it possible to analyze the released amount of SB every second. Figure 4.24 shows the results of the investigation via UV-probe.

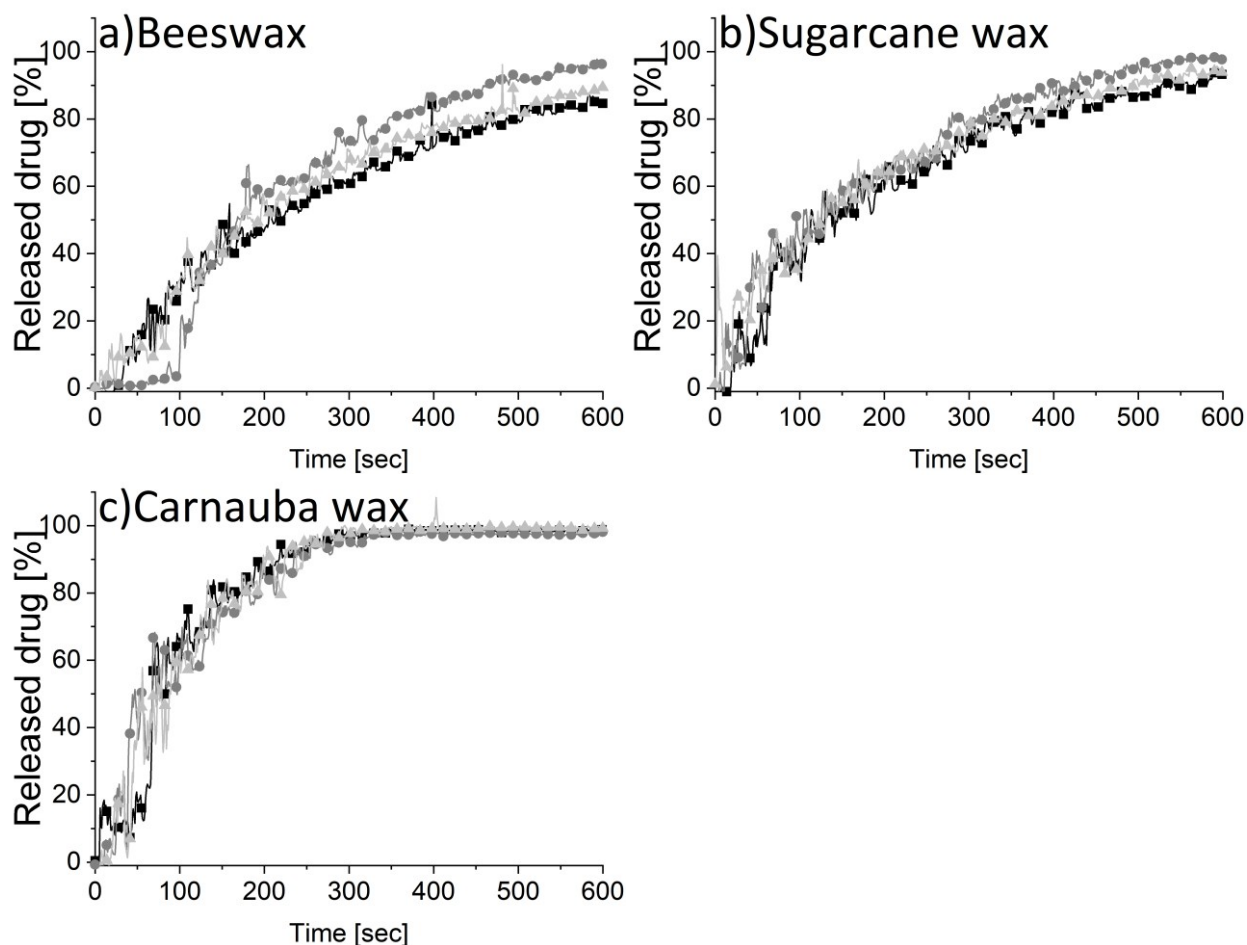


Figure 4.24: Dissolution profiles of a) BW, b) SW and c) CW extrudates. 0.5 mm in diameter, 75% drug-load, 3 months old. 900 ml phosphate buffer pH 6.8, $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, basket apparatus set to 50 rpm; $n=3$, $\bar{x} \pm \text{SD}$; for a better overview only every fifteenth data point is plotted. BW = beeswax, SW = sugarcane wax, CW = carnauba wax; number = ratio of wax.

As expected, the differences in the speed of the drug release were observed to the same extent as in the previous dissolution experiments: the release of SB was slowest from BW extrudates, followed by SW extrudates and CW extrudates. The results in Figure 4.24b + c are in good agreement with the results for SW extrudates and CW extrudates with 75% drug load and a diameter of 0.5 mm in Figure 4.23b + c. After 10 min, 100% of SB were released. The analysis with the UV-probe showed that the release of CW extrudates was completed after only 5 min. The results of the BW extrudates differed slightly from those of previous investigations. In this experiment, the extrudates released 80% of their SB after only 10 minutes. In the previous dissolution testing, it had been 60%. A possible explanation is, that the UV-probe investigation was performed on another batch of extrudates, and that there is a

4.4 Stability of wax extrudates

batch-to-batch variability, although extrudates were produced under the same set of parameters than the ones shown in Figure 4.23.

The study of the dissolution profiles via UV-probe illustrated very well that the main portion of SB is released within the first 10 minutes for all waxes. The release of SB from BW and SW extrudates was comparable, while CW showed the fastest release.

4.4.7 Morphological properties after dissolution studies

It is known from lipid-based matrices that the structure formed by the lipids is kept intact after API dissolution (Petrovick, 2015; Pinto and Silverio, 2001). After dissolution studies were performed on the BW, CW, and SW extrudates, it was noticed that the remains in the dissolution vessel were very fragile and easily destroyed when removed from the vessel. Nonetheless, a few extrudates could be removed without destroying them completely. These samples were analyzed using SEM (**BaHav19-010**). The images are depicted in Figure 4.25. As waxes are insoluble in water, a highly porous wax-structure remained in the basket of the dissolution apparatus. Due to its high porosity and low mechanical stability, the extrudates tended to collapse when force was applied. This can be observed in Figure 4.25B and C. The SW extrudate could not be removed without partially destroying its structure, leading to a deformed appearance under the microscope. The CW-based extrudate did not show as extensive destruction but it had shrunk compared to its former shape, indicating a loss in structural stability. Only the BW extrudate could be recovered largely unchanged from the basket of the release apparatus with some isolated holes being observed.

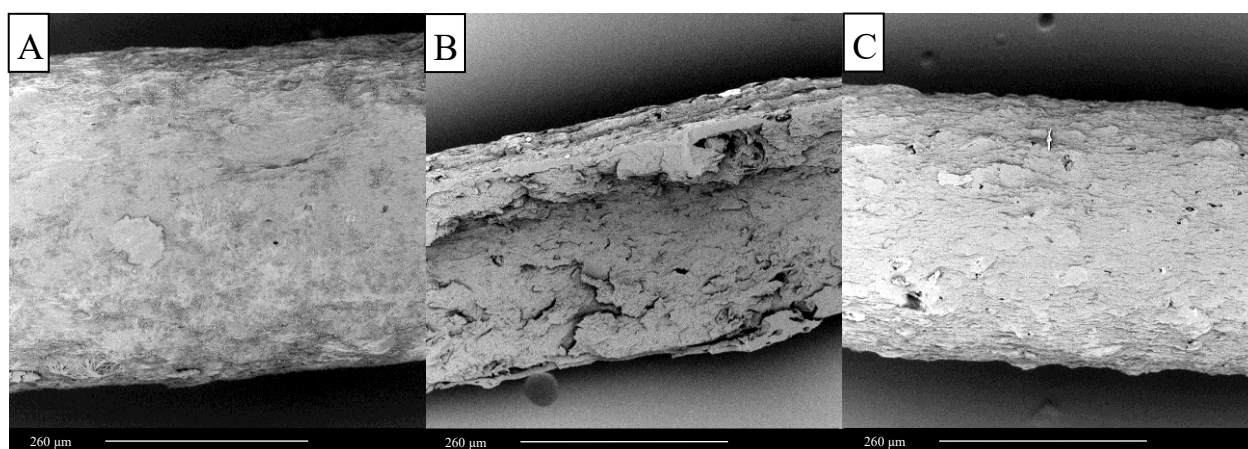


Figure 4.25: SEM images of wax-based extrudates with 75% drug load after finished dissolution studies; A) BW25_0.5mm, B) SW25_0.5mm, C) CW25_0.5mm; the scale bars represent 260 µm

The limited quantity of holes on the surface are in alignment with the observed dissolution behavior of freshly produced BW extrudates. As only 30% of its contents were released within 120 min (Figure 4.22b) only a few pores were formed over the term of the dissolution study. The samples, which were retrieved after dissolution studies of three-months old BW extrudates, showed a different picture. In Figure 4.26, post-dissolution samples of three months old wax-based extrudates are shown. In contrast to the freshly produced BW extrudate, the 3-month-old BW extrudate collapsed just as clearly after dissolution studies as the extrudates made from SW and CW. The SW and CW extrudates did not show any further changes in comparison to their freshly produced counterparts after dissolution testing. As three months old BW extrudates released up to 80% of their drug content after 120 min (Figure 4.22b), a larger proportion of the inner compartment was dissolved during the

dissolution studies. Thus, the dissolving SB increased the extrudates porosity, thus leading to the collapse of the extrudate's structure. As SW and CW extrudates already showed almost complete

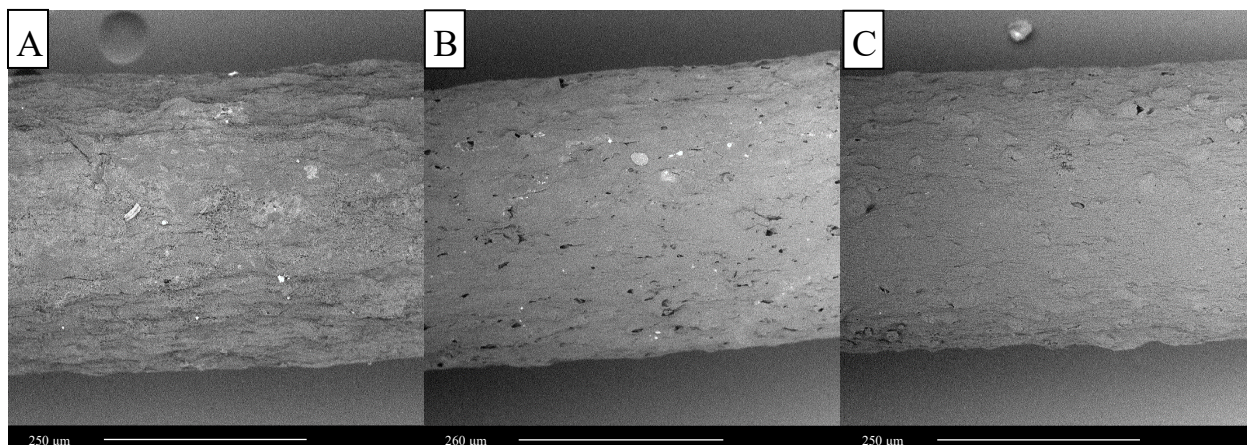


Figure 4.26: SEM images of three-months-old wax based extrudates with 75% drug load after finished dissolution studies: A) BW25_0.5mm, B) SW25_0.5mm, C) CW25_0.5mm; the scale represents around 250 μm

release of their drug content directly after manufacturing, no changes were observed in the post-dissolution samples after three months of storage under the SEM.

4.4.8 Taste masking properties

SB is used in the treatment of hyperammonemiae (Green *et al.*, 1983) and hyperglycemia (Hamosh *et al.*, 1992) of infants. Therefore, a drug formulation containing SB must have sufficient taste to ensure patient compliance. The obtained dissolution data showed a release of up to 75% SB within 180 s by CW extrudates, 60% from SW, and 50% from BW extrudates. These high amounts of released SB will most likely cause a bad taste sensation due to the bad taste properties of SB (Breitkreutz *et al.*, 2003). For better insight into the taste properties of wax-based matrices loaded with SB, a taste investigation via electronic tongue (e-tongue) was performed (**BaHav19-008**). A log-linear calibration was performed with concentrations of 10.0 g / 100 ml, 1.0 g / 100 ml, 0.1 g / 100 ml and 0.01 g / 100 ml SB by diluting 10.0 g of SB in logarithmic steps. The results of this investigation are depicted in Figure 4.27. The R^2 was determined separately for each sensor using the data measured from the SB-solutions and the obtained values are compiled in Table 4.8. All sensors showed a concentration-dependent signal for SB resulting in an electrical potential change, when the concentration of SB was increased. However, not all sensors reacted in the same way to the different SB concentrations. The sensors SB2AC0, SB2AN0 and SB2AAE did not show a log-linear correlation in the measured concentration range, which was very well shown by the low R^2 of their respective calibration graph. This weakens the significance of these. However, by plotting only the results of the concentrations of 1g SB/100 ml, 0.1 g / 100 ml and 0.01g / 100 ml their R^2 was highly improved. The sensors SB2CT0, SB2C00 and SB2AE1 showed a log-linear signal over the entire calibration range from 10.0 mg to 10.0 g/100ml with high R^2 values.

4.4 Stability of wax extrudates

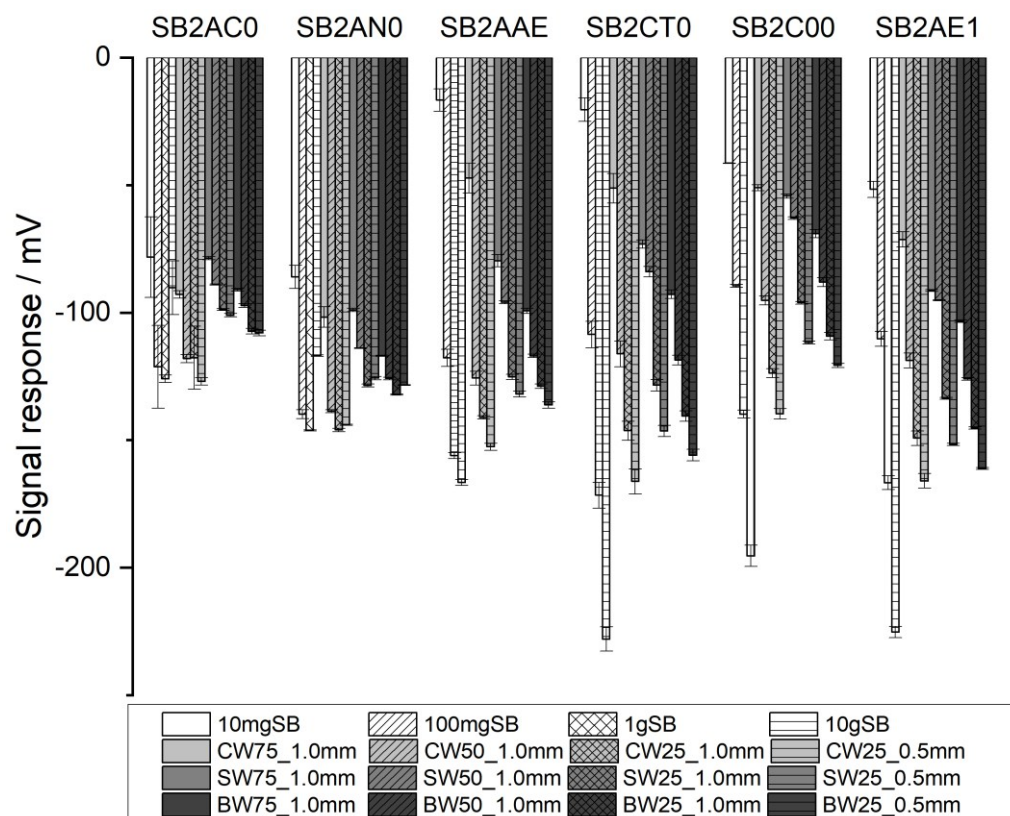


Figure 4.27: Taste investigation of two weeks old extrudates via e-tongue. BW = beeswax, SW = sugarcane wax, CW = carnauba wax; number = ratio of wax. The mass of weighed extrudates corresponds to a quantity of 1.0g pure SB, $n = 3$, $\bar{x} \pm SD$

The sensor SB2CT0 measurements displayed an R^2 of 0.9887, SB2C00 an R^2 of 0.9989, and SB2AE1 an R^2 of 0.9999, respectively. It could be well observed that with decreasing wax content, the signal for SB increased.

Table 4.8: R^2 -values determined during calibration of the taste sensors

Sensor	R^2 using four concentrations	R^2 excluding the concentration of 10g SB / 100 ml
SB2AC0	0.0505	0.8230
SB2AN0	0.2199	0.8279
SB2AAE	0.8494	0.9368
SB2CT0	0.9887	0.9909
SB2C00	0.9989	0.9998
SB2AE1	0.9999	0.9999

This corresponds to the findings of the dissolution studies, in which a larger amount of released SB was also measured for the extrudates containing a lower proportion of wax. The sensors for the sample CW25_0.5mm registered a released amount of 1.0 g SB after 2 min, corresponding to 100% release of the contained SB. The measurement results of the UV probe indicated that the release of these

samples is completed after 5 min. However, this finding is by no means contradictory, as the samples of the electronic tongue were stirred for 2 minutes and then filtered. Thus, the release time was not limited precisely to 2 minutes, as further SB release could have occurred during filtration. Additionally, the measurements with the e-tongue also showed an influence of the temperature on the release of the BW extrudates. In all of the e-tongue measurements, BW extrudates released more SB than SW extrudates. This was not observed for any of the dissolution investigations. The temperature of the "dissolution medium" in the analysis via e-tongue is at room temperature and is therefore significantly cooler than the 37°C usually applied in dissolution testing. In this study with the e-tongue, BW and SW extrudates showed a similar release rate, while CW extrudates once again released SB the fastest of the three wax types. The obtained data was used to perform a principal component analysis (PCA). The data of all 6 sensors and all samples from Figure 4.27 as well as the samples of pure wax were centered and scaled to unit variance. The data was plotted in a scatter plot as shown in Figure 4.28. The model was fitted with two principal components. The first principal component explains 92.9% of the data variation and is corresponding to the released amount of SB in each sample.

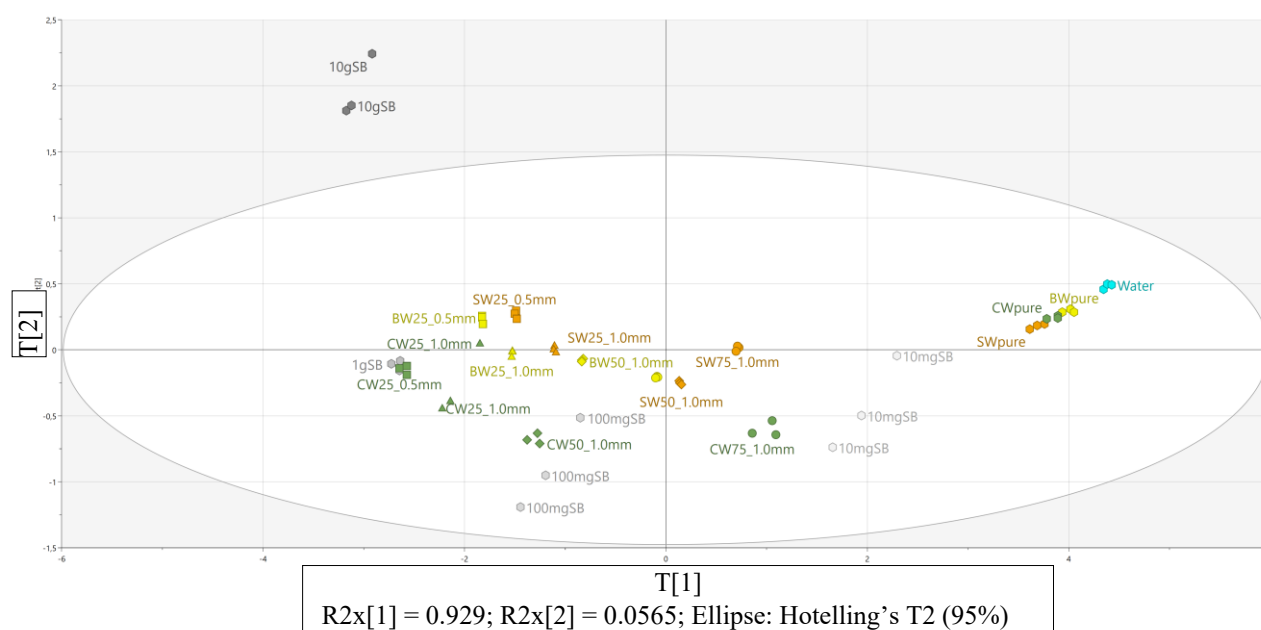


Figure 4.28: Score plot of a PCA using data of all taste sensors. Samples of extrudates made from beeswax (BW), sugarcane wax (SW) and carnauba wax (CW) or pure waxes and demineralized water (water) were analyzed. The extrudates were either made from 75%, 50% or 25% wax and had a diameter of 0.5 mm or 1.0 mm. Data was centered and scaled to unit variance. Yellow samples represent BW, brown samples SW- and green samples CW-samples. Grey samples represent SB and the blue sample is water.

The second principal component explains 5.65% of the data variation. The sample of 10 g SB is plotted outside the plot, indicating that it was identified as an outlier. This is in alignment with the observations made during the calibration measurements. The remaining samples are arranged from left to right in descending amount of measured SB-concentration. On the left, the samples of wax extrudates with a drug load of 75% and either 0.5 mm or 1.0 mm in diameter are plotted together with the sample of 1.0 g SB. On the right, the samples not containing SB at all are plotted. As the 10 g SB data point was identified as an outlier, a second PCA was conducted without including this sample. The scatter plot of the second PCA is shown in Figure 4.29. By excluding the sample of 10 g SB, the data is more spread within the scatter plot.

4.4 Stability of wax extrudates

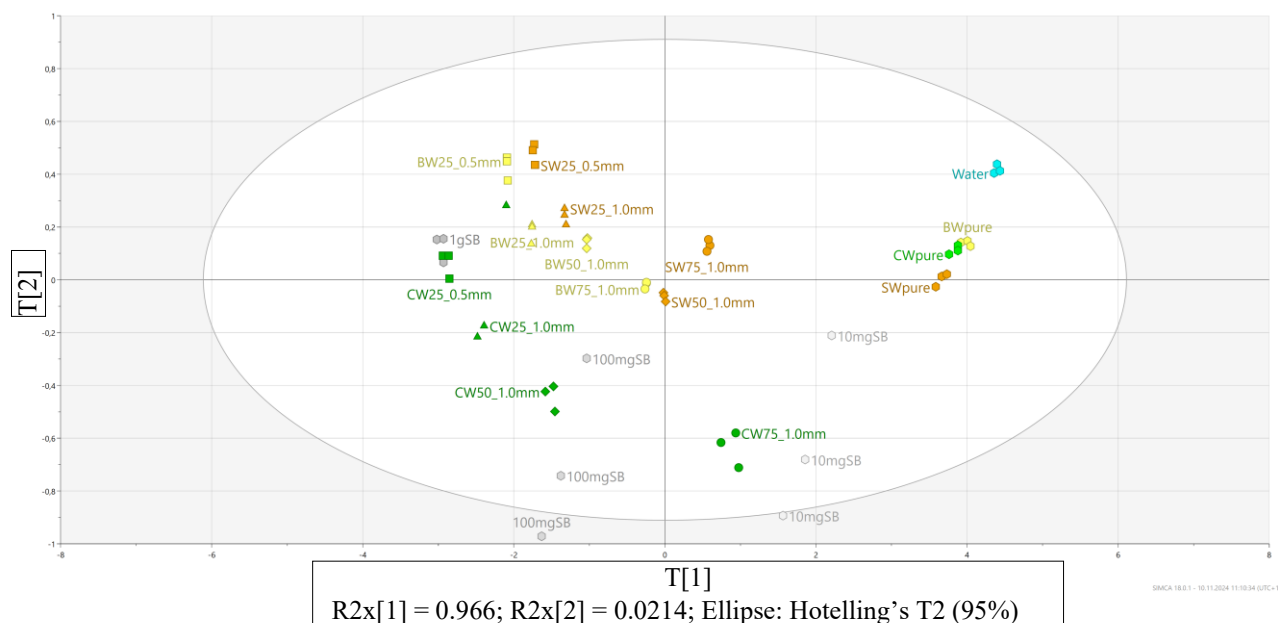


Figure 4.29: Score plot of a PCA using data of all taste sensors except of 10g SB. Samples of extrudates made from beeswax (BW), sugarcane wax (SW) and carnauba wax (CW) or pure waxes and demineralized water (water) were analyzed. The extrudates were either made from 75%, 50% or 25% wax and had a diameter of 0.5 mm or 1.0 mm. Data was centered and scaled to unit variance. Yellow samples represent BW-, brown samples SW- and green samples CW-samples. Grey samples represent SB and the blue sample is water.

Furthermore, the data variance explained by the first principal component ($R^2x[1]$) is increased to 96.6% while the variance explained by the second component ($R^2x[2]$) is decreased to 2.14%. The samples are still arranged from left to right in descending amount of measured SB-concentration. The samples of extrudates containing 75% SB are plotted close to the sample of 1g SB. The samples with a diameter of 0.5 mm and 1.0 mm are located close to each other. This was not unexpected as these samples showed a comparable dissolution graph. The samples of pure waxes are plotted very close to each other but separated from the sample of pure demineralized water indicating, that the sensors measured a slight difference in these types of samples in comparison to demineralized water. In a next step, a PCA was performed, which did not include the data of the bitter sensors, since a worse R^2 could be achieved with these during calibration. The obtained score plot is shown in Figure 4.30. It was observed that the sample of 100 mg SB is not recognized as outlier without the data of the two bitter sensors SB2AC0 and SB2AN0. The samples of wax extrudates containing 75% of SB and the sample of 1 g SB were plotted closer to each other. The variation of data explained by the first principal component was again slightly increased to 98.5% and the variation explained by the second principal component was decreased to 0.89%. Overall there was no great gain of information by excluding the two bitter sensors. However, as these sensors performed poorly in the calibration, they were not included in evaluation of other samples.



Figure 4.30: Score plot of a PCA excluding the data of sensors SB2AC0 and SB2AN0 and excluding the sample of 10 g SB. Samples of extrudates made from beeswax (BW), sugarcane wax (SW) and carnauba wax (CW) or pure waxes and demineralized water (water) were analyzed. The extrudates were either made from 75%, 50% or 25% wax and had a diameter of 0.5 mm or 1.0 mm. Data was centered and scaled to unit variance. Yellow samples represent BW-, brown samples SW- and green samples CW-samples. Grey samples represent SB and the blue sample is water.

In conclusion, the PCA did confirm the observations made in the dissolution studies. Two analytical methods showed a rapid release of SB. This means that the extrudates will most likely cause a bad taste. This property is critical for the development of a child-friendly dosage form, and should be masked to ensure compliance of the patient and ultimately therapeutic success.

4.4.9 Confocal Raman Microscope analysis

As shown in chapter 4.4.5, extrudates made from BW show an alteration of their dissolution profile over the time of storage. In order to investigate whether the structure of the surface of BW extrudates changes over time, freshly produced BW extrudates and three-month-old BW extrudates were analyzed using a confocal Raman microscope (CRM). A CRM uses the principles of Raman spectroscopy to analyze a sample. When light hits a sample, most of the light is scattered elastically. No alteration of the light's wavelength λ is observed. This phenomenon is named Rayleigh scattering. But not all photons are scattered elastically. Small portions of incoming photons are scattered inelastically, resulting in an exchange of energy between the sample and the photon and finally in a shift of the light's wavelength λ (Raman and Krishnan, 1928). If the wavelength λ of the scattered light is shorter, Anti-Stokes scattering occurs. However, if the wavelength λ is higher, Stokes scattering occurs. Based on the Stokes scattering, a CRM can be used to examine the surface of an object in regards to its composition. For this purpose, spectra of the pure substances, which are part of the composition of the sample to be examined, are first recorded with a CRM to identify possible substance-specific wavenumber maxima. If the substances to be examined show a maximum at different wavenumbers, the surface of that sample can be examined for its composition using a CRM (Windbergs *et al.*, 2010). A CRM can measure single spots of a sample in the size of 2 μm . This can be combined with the automatic measurement of a sample. A predefined area of the sample is

4.4 Stability of wax extrudates

measured with a resolution of 2 μm resulting in a Raman-map that reflects the composition of said area with a resolution of 2 μm . The spectra of pure SB and pure BW (**BaHav19-041**) are shown in Figure 4.31.

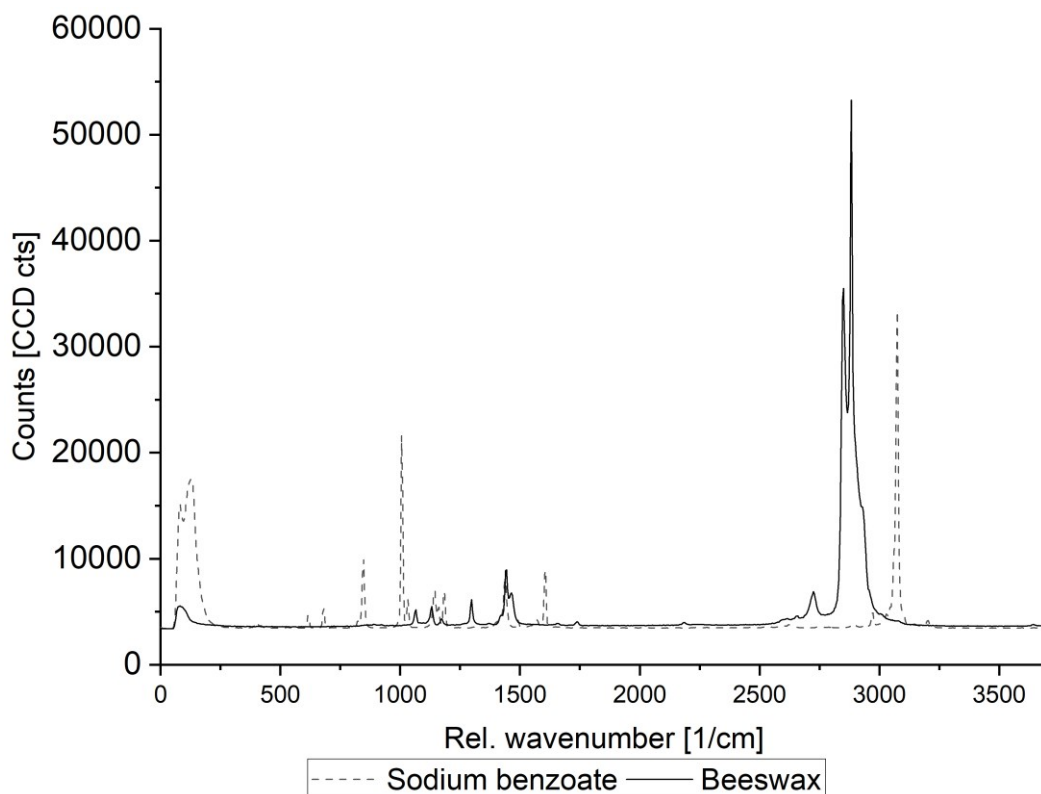


Figure 4.31: Ramanspectra of sodium benzoate and beeswax. A diode pumped solid state laser at a wavelength of 532nm was used.; integration time per spectrum 20 ms.

The two substances showed a maximum at different relative wave numbers. BW showed a maximum at a relative wavenumber of 2883. SB showed a maximum at a relative wave number of 3074. Furthermore, the two substances showed a common maximum at a relative wave number of 1439. As distinct maximums are needed for the composition determination, the maximums at the relative wavenumbers of 3074 and 2883 were used for further evaluation. An area of 550 μm times 150 μm of a 500 μm tall cross-section of a freshly produced BW extrudate with 75% drug-load (**BaHav19-028**) and a 3-month-old cross-section of the same batch were analyzed using CRM. From this scan, six different signals representing each a different ratio of SB / BW were applied to create a Raman map of the cross-section. The different ratios are plotted in Figure 4.32. In the graph, the spectra are ordered from top to bottom in ascending ratio of SB. The purple spectrum represents a spot with the most balanced ratio between BW and SB. Ascending from the purple spectrum, the proportion of SB measured increases. However, an absolute quantitative statement could not be made at this point, since no calibration was previously applied over different mixing ratios. The red spectrum represents the spectrum with the largest proportion of SB. This color coding was used to create the Raman maps of the BW25 extrudates in Figure 4.33. The Raman map of freshly produced BW extrudates showed some small red spots (Figure 4.33A). These red spots indicated a very high ratio of SB to BW in this area. This was in alignment with the observation made via SEM, in which single crystals of SB could be observed. Additionally, some yellow and green areas were observed, which also represented areas with a high amount of SB and a low amount of BW. However, the colors blue and purple dominated the overall impression of the Raman map, indicating that the outer surface of the extrudates was mainly composed of balanced mixtures of BW and SB. This observation was not unexpected, since a homogeneous extrudate should be obtained by the high mixing capacity of the extrusion process.

Additionally, this finding shows that the spherical agglomerates of SB-needle-shaped crystals are destroyed during extrusion. This was also observed using a SEM. It should be noted that an influence on the shape of the surface, which was analyzed in this experiment, via the sample preparation could not be ruled out. For sample preparation, an extrudate was cut with a scalpel. This certainly led to a change in the surface of the cross-section in the extrudate and to crystals being torn out of the surface.

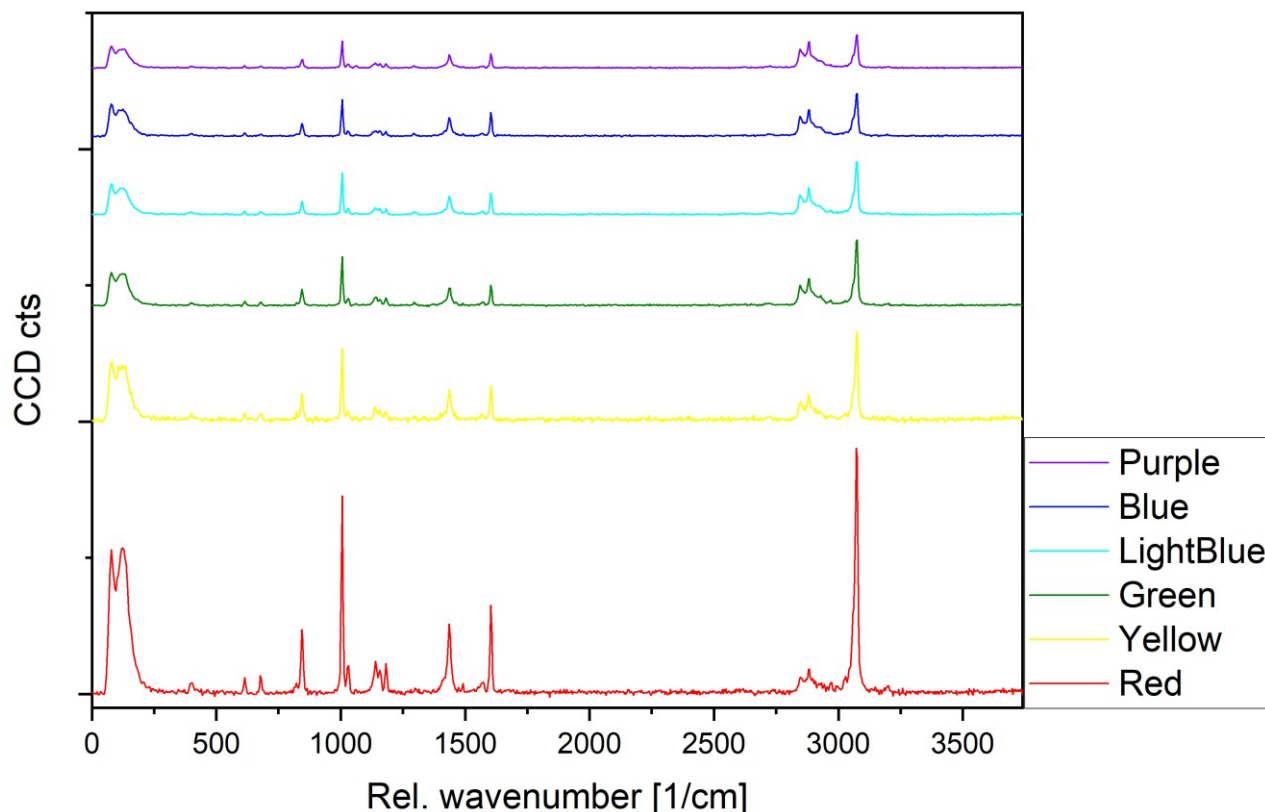


Figure 4.32: Spectra of different ratios of SB/BW recorded from the cross-section of a BW25 extrudate; the x-axis shows the relative wavenumber (in cm^{-1}), while the y-axis shows the signal intensity (in counts).

Overall, the description of the surface via the plotted Raman map was in alignment with the observations made during dissolution testing and SEM-observations. The red spots represented areas with high SB-concentrations, which were responsible for the fast drug release in the first minutes of dissolution testing. A slower drug release followed, which is enabled by small pores, which were formed by the dissolution of the low fractions of SB in the blue to purple colored areas. In comparison to the freshly manufactured extrudate, the Raman map of a three-month-old extrudate drew a different picture. In the Raman map of a three-month-old sample, the Raman map is dominated by green to yellow areas, which represent mixtures with a higher amount of SB than the purple or blue areas (see Figure 4.32). Purple colored areas, which were dominant in the Raman map of the freshly prepared sample, are greatly reduced. As before, some red spots are also observed, representing the area with the highest load of SB.

4.4 Stability of wax extrudates

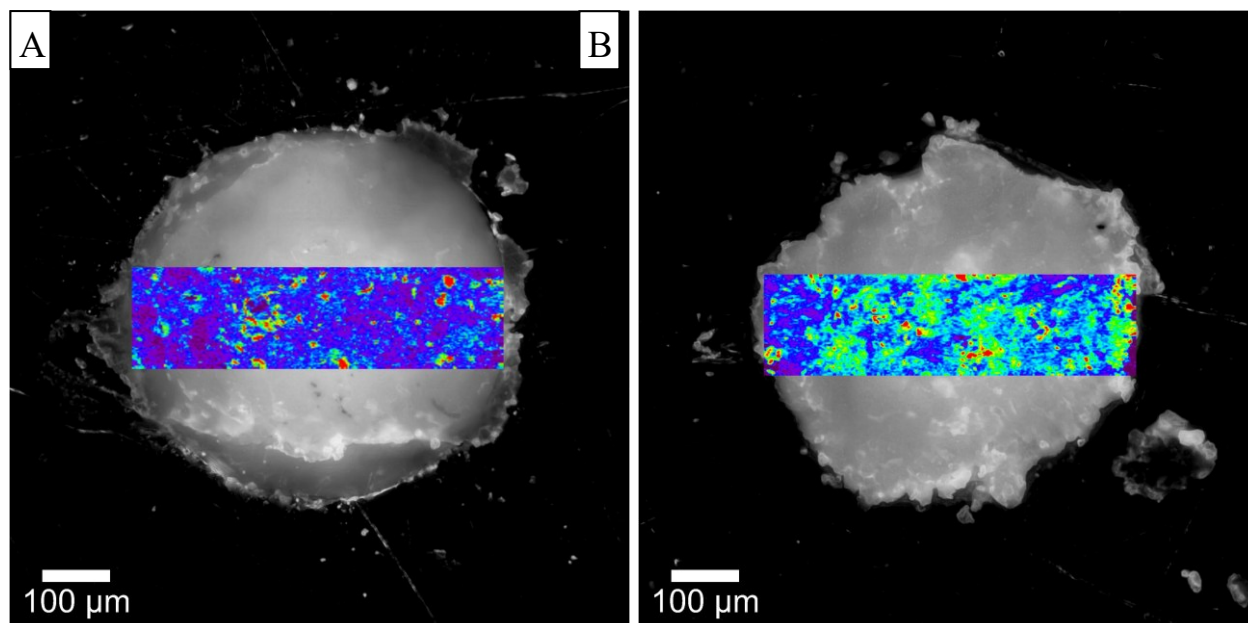


Figure 4.33: Heatmap of a freshly produced beewax extrudate with 75% drug-load and 25% beeswax (A) and a three month old sample of the same batch (B)

The observation that areas of higher SB are now more common on the surface is in agreement with the data from the dissolution studies. Within the first 10 minutes, significantly more SB was released, which can be explained by a larger proportion of SB on the surface. As a consequence of a larger amount of dissolved SB, more pores were created, which favor continued release. In any case, it should also be taken into account that the sample of the three-month-old extrudate was not the same sample that was measured after production. Although both samples come from the same batch, an examination of the exact same sample would be much more meaningful. Overall, however, the observations made during CRM are in agreement with the observations from the dissolution studies.

4.5 Impact of pore-forming agents

4.5.1 Introduction

The release of SB from freshly produced BW extrudates is insufficiently slow even for high drug loads. There are several ways to increase the dissolution rate: reducing the particle size to increase the dissolution rate, using a salt form of the drug, or using pore formers to accelerate water penetration and thus the release of the drug (Noyes and Whitney, 1897; Quodbach *et al.*, 2025; Rosiaux *et al.*, 2014). Since SB is already a salt that dissolves rapidly in water, pore formers in the form of polyethylene glycol (PEG) were processed together with the waxes in an attempt to accelerate the release (Gures *et al.*, 2012; Mikolaszek *et al.*, 2020). To enable an accurate evaluation of the pore former's impact, extrudates of SW and CW with pore formers were also produced and analyzed. PEG 6000 was selected as a pore forming agent. It starts melting at around 60°C and is solid at room temperature, enabling preparation of powder blends consisting of SB, wax and PEG 6000.

4.5.2 Thermal properties

The thermal properties of pure PEG 6000, of the physical mixtures of PEG 6000 + wax and the corresponding extrudates were investigated via DSC (**BaHav17-017**). The results of this analysis are shown in Figure 4.34. It was shown that PEG 6000 has a very distinct melting point of 61°C (Figure 4.34a). It was also observed that the peak intensity decreased and the peak width increased when the material was heated up a second time. Furthermore, the thermograms of the physical mixtures as well as of the extrudates showed the typical thermograms for the processed wax with the addition of a characteristic PEG 6000 peak. This peak protrudes from the wax thermogram at about 60°C, which corresponds to the melting temperature of PEG 6000. The thermograms of the extrudates containing PEG 6000 showed an altered thermogram in comparison to the physical mixtures. Since the extrudates represented a sample that had already been melted once in the extruder, this observation is in accordance with the observation from Figure 4.34a. It is known that PEG 6000 has stable and meta-stable crystalline forms (Craig and Newton, 1991). By heating the sample once and applying a relatively fast cooling rate of 10 K / min, a significant part of the meta-stable one- and twice-folded crystalline structure is formed, resulting in a broader melting peak of pure PEG 6000 and a melting point for PEG 6000 in combination with wax at lower temperatures. This shift is observable most clearly in the thermogram of the CW extrudate and its physical mixture Figure 4.34d, as the melting peak of the CW is clearly separated from the melting peak of PEG 6000. In the end all extrudate samples showed an increased amount of the meta stable PEG 6000 form caused by the fast cooling upon the extrudate leaving the extruder through the die. The thermograms of the waxes were not altered by the incorporation of PEG 6000 and showed their characteristic peaks which were observed in Figure 4.16.

4.5 Impact of pore-forming agents

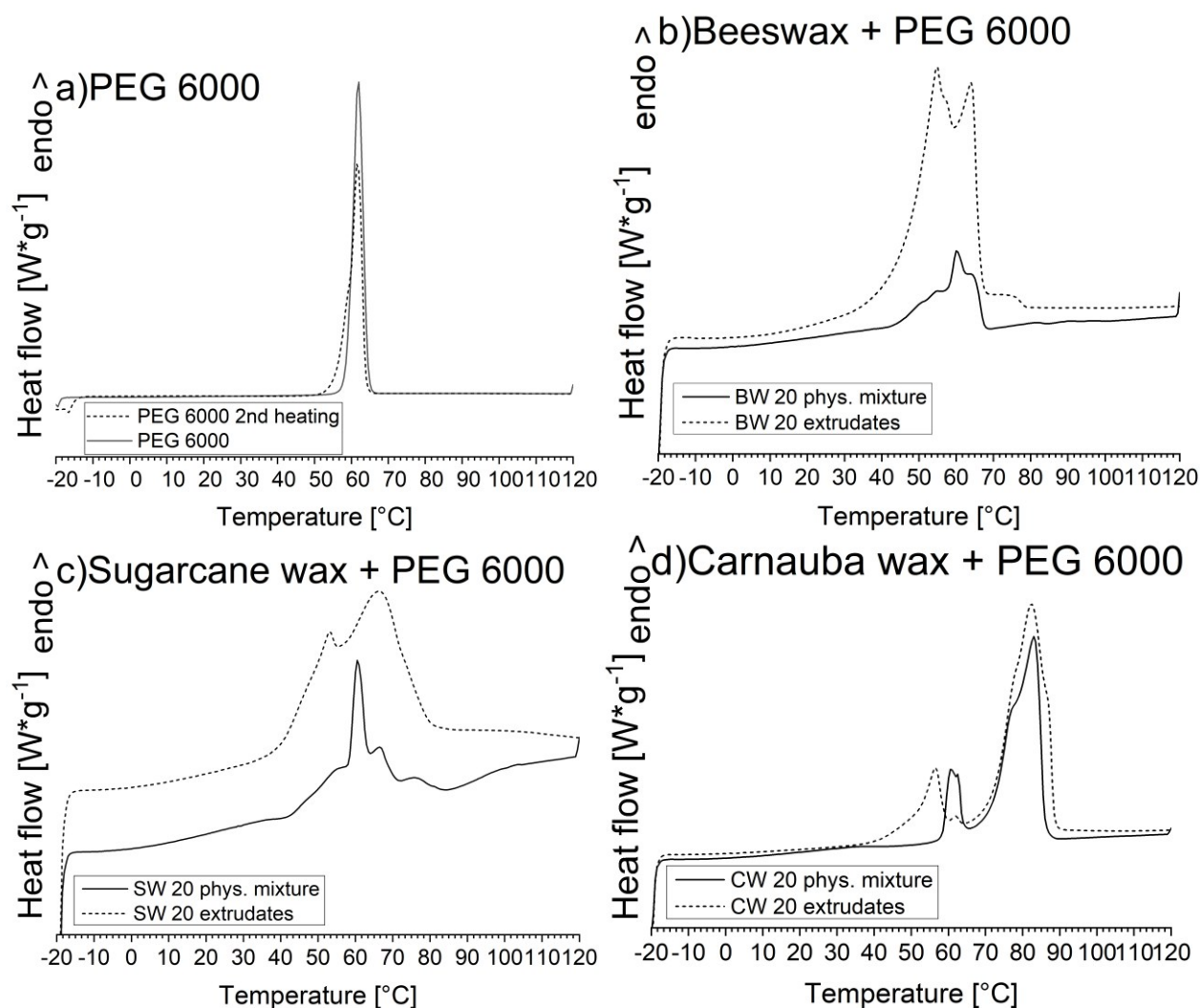


Figure 4.34: Thermograms of a) PEG 6000, b) beeswax, c) sugarcane wax and d) carnauba wax; heating rate 10°C/min; ratios of SB/PEG6000/wax of 75/5/20 were used.

4.5.3 Crystalline properties

In addition to the analysis of thermal characteristics via DSC of the combination of wax with SB and PEG 6000, XRPD-analysis was also performed (**BaHav17-017**). The results are shown in Figure 4.35. At 18.6 and 22.6 2 θ , the characteristic peaks of PEG 6000 were observed. The peak at 18.6 2 θ was also identified in the analysis of the mixture, but was small due to the low relative amount of PEG 6000 in the total composition. No other compound of the mixtures shows a signal at this angle. The second characteristic peak of the PEG 6000 could no longer be identified in a mixture with wax and SB as such, since characteristic signals of wax and SB followed immediately at slightly higher angles. Thus, the peak of PEG 6000 merges into the SB peak at 23.2 2 θ .

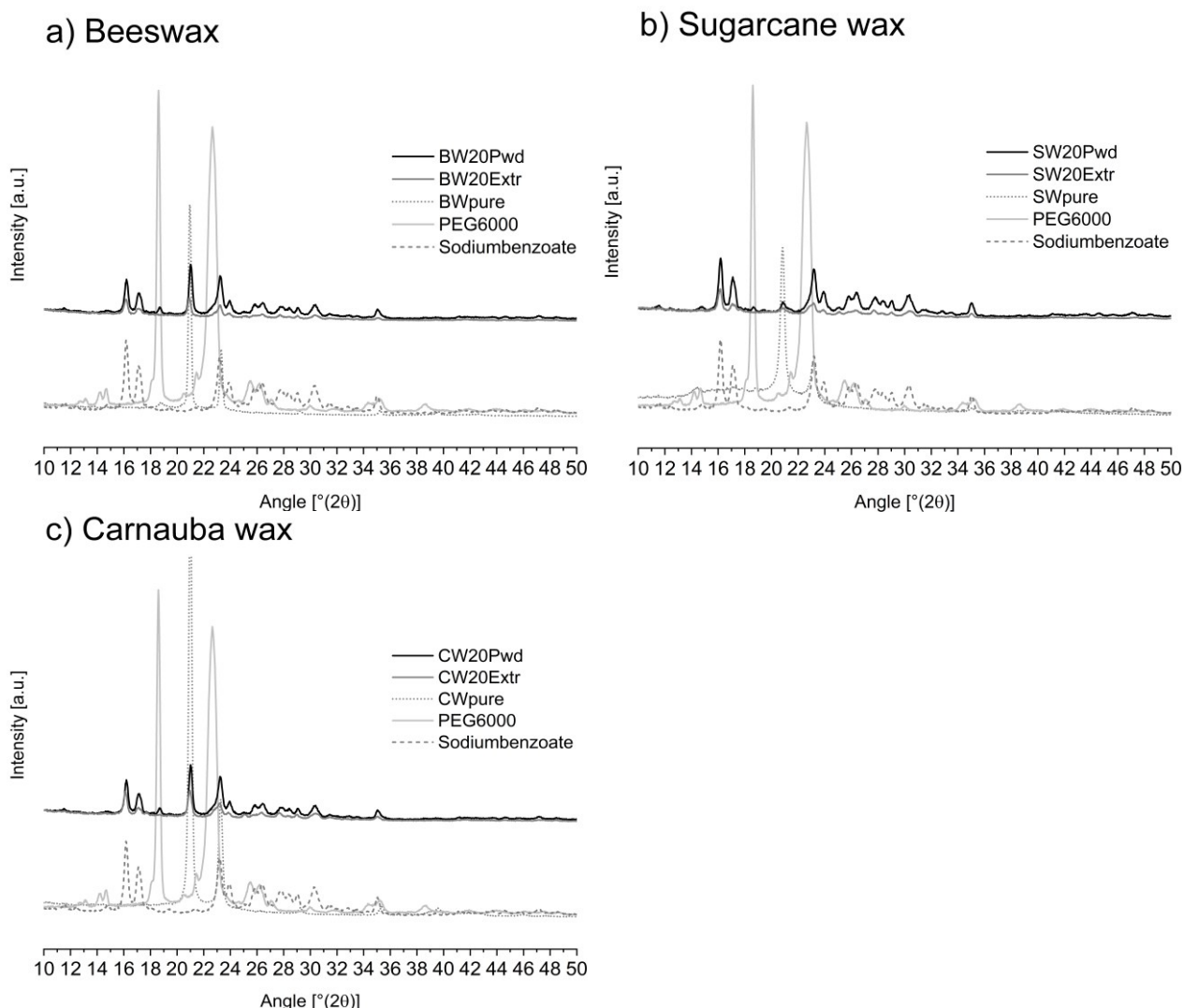


Figure 4.35: XRPD-diffractograms of physical mixtures and extrudates consisting of wax, SB and PEG 6000 and the single components.; a) BW-combination, b) SW-combination and c) CW-combination; 2θ interval was used from 10 to 50 degrees.

This is well shown in the diffractograms of all three mixtures. At angles greater than $24\ 2\theta$, the graphs are characterized solely by SB. Although PEG 6000 also occasionally shows characteristic peaks in this range, these are not visible in the analysis of the physical mixture or the extrudate due to the low proportion of PEG 6000 and high proportion of SB in the mixture. The analysis by XRPD confirmed the DSC findings: the crystalline properties of wax and SB were not affected by including PEG 6000 in the formulation.

4.5.4 Dissolution profiles

After physical characterization, the influence of PEG 6000 on the dissolution profile of the different wax-based extrudates was investigated (**BaHav17-018**). The results are shown in Figure 4.36. In previous experiments, the extrudates manufactured with CW generally released all of their drug content within 10 minutes. Thus, the addition of 5% PEG 6000 not influencing the dissolution characteristics of CW-based extrudates was well within expectation.

4.5 Impact of pore-forming agents

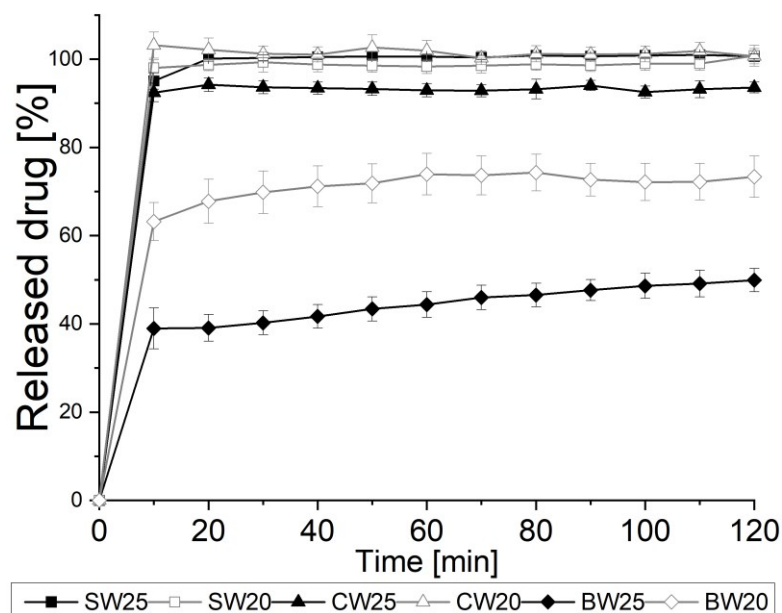


Figure 4.36: Dissolution profiles of extrudates made from 75% sodium benzoate and 25% wax only (BW25, SW25, CW25) or 75% sodium benzoate, 20% wax and 5% PEG 6000 (BW20, SW20, CW20) 12 days after manufacturing. 900 ml phosphate buffer pH 6.8, $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, basket apparatus set to 50 rpm; $n=6$, $\bar{x} \pm \text{SD}$; BW = beeswax, SW = sugarcane wax, CW = carnauba wax

SW extrudates with no additional PEG 6000 showed a surprisingly high amount of drug release in comparison to other SW-based extrudates discussed in previous chapters (see Figure 4.22d). However, these extrudates were already 12 days old, when dissolution studies were performed. As shown before, aging increases the drug release of wax-based extrudates, potentially explaining the unexpected relative quick release rates. Due to the high rates of drug release from all SW-based extrudates, no difference was observed in comparison to SW-based extrudates containing 5% PEG 6000. The most interesting results were observed for wax extrudates made from BW. Fresh BW extrudates only showed low amounts of released SB in their dissolution studies. BW25 extrudates showed a release of 40% after ten minutes and reached a total drug release of 50% over the terms of 120 minutes. The BW extrudates with 5% PEG 6000 showed a much greater drug release after ten minutes of 65%. This was also followed by a much slower release phase, ending in a total release of 75%. The general dissolution behavior was similar to that observed for the BW25 extrudate. It was interesting to see that only the initial amount of released drug was influenced by the substitution of 5% wax with 5% PEG 6000. By using a pore forming agent, the penetration of water into the wax matrix via freshly formed pores should have increased the rate of drug dissolution during the entire measurement. However, after the initial phase, BW25 and BW20 extrudates showed the same dissolution rate. As BW begins softening in the dissolution medium, this could have sealed off all pores, preventing further drug dissolution from the beeswax matrix.

4.5.5 Conclusion

The addition of 5% PEG 6000 as a replacement for the equal amount of wax accelerated the drug release from BW extrudates. CW extrudates did already show a quick release of SB in the dissolution tests and thus no further improvement was observed with the applied sampling interval. SW extrudates did not show any improvement either. The thermal or crystalline properties were not altered by the addition of PEG 6000, which would make it a usable addition in BW-based formulations since the BW formulation benefited from the addition of PEG 6000 in the dissolution

studies. SW formulations only benefitted slightly from the addition of PEG 6000 while no difference for CW formulations was observed with the addition of 5% PEG 6000.

4.6 Comparison of solid wax extrusion and hot-melt extrusion

4.6 Comparison of solid wax extrusion and hot-melt extrusion

4.6.1 Introduction

It was shown that wax-based extrudates can exhibit an alteration in their dissolution profile over the term of storage. Observations were made respectively for BW-based extrudates as well as extrudates based on SW. As previously described in chapter 2.5, it is known from lipids that characteristics like crystal habitus or dissolution behavior can be influenced by altering the temperature during extrusion process (Reitz and Kleinebudde, 2007). The processing of lipids below their melting point is called SLE and has several advantages. It enables processing at relatively low temperatures, which can be useful in the processing of temperature sensitive materials and in reducing energy consumption of manufacturing processes. The incorporation of API into a SLE-matrix can provide taste masking properties (Krause *et al.*, 2009; Michalk, 2007; Michalk *et al.*, 2008). As waxes show a broad melting range, it should be evaluated if processing of high drug-load wax mixtures at a certain temperature below the waxes melting point is feasible.

Table 4.9: Processing temperatures of wax + SB mixtures during processing of partially (pm) and fully molten (fm) wax mixtures. $\varnothing = 1.00$ mm.

<i>Mixture</i> (%; w/w)	<i>Batch</i>	<i>Temperature [°C]</i>						
		<i>Die</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>
SB / BW	BW25 fm	60	60	60	60	60	70	100
75:25								
SB / BW	BW25 pm	50	50	50	50	50	50	50
75:25								
SB / SW	SW25 fm	70	70	70	70	70	80	100
75:25								
SB / SW	SW25 pm	60	60	60	60	60	60	25
75:25								
SB / CW	CW25 fm	80	80	80	80	90	90	100
75:25								
SB / CW	CW25 pm	70	70	70	70	70	50	30
75:25								

Further it should be evaluated how it influences the properties of the obtained extrudates in comparison to extrudates obtained from a process in which the material is completely melted. The melting point of each wax was defined as the temperature at which the wax was completely melted. The applied temperatures during processing of mixtures of wax and SB are shown in

Table 4.9. For processing of partially melted waxes, a temperature 10°C below their respective melting point was chosen.

4.6.2 Thermal properties

To gain insight into the impact of the process temperature on the crystalline properties, a DSC-analysis of the extrudates was performed (**BaHav18-020**). The results are shown in Figure 4.37. In the first heating cycle of partially and fully melted wax extrudates, no differences were observed for any of the three wax combinations. For BW the maximum of the endothermic peak was observed at 65 – 66°C for both partially and fully melted extrudates. After the heat flow drops almost back to the baseline a small plateau is observed which is recorded until 75°C during the first heating cycle. After the sample was heated up a second time, the plateau had changed into a tailing of the melting curve.

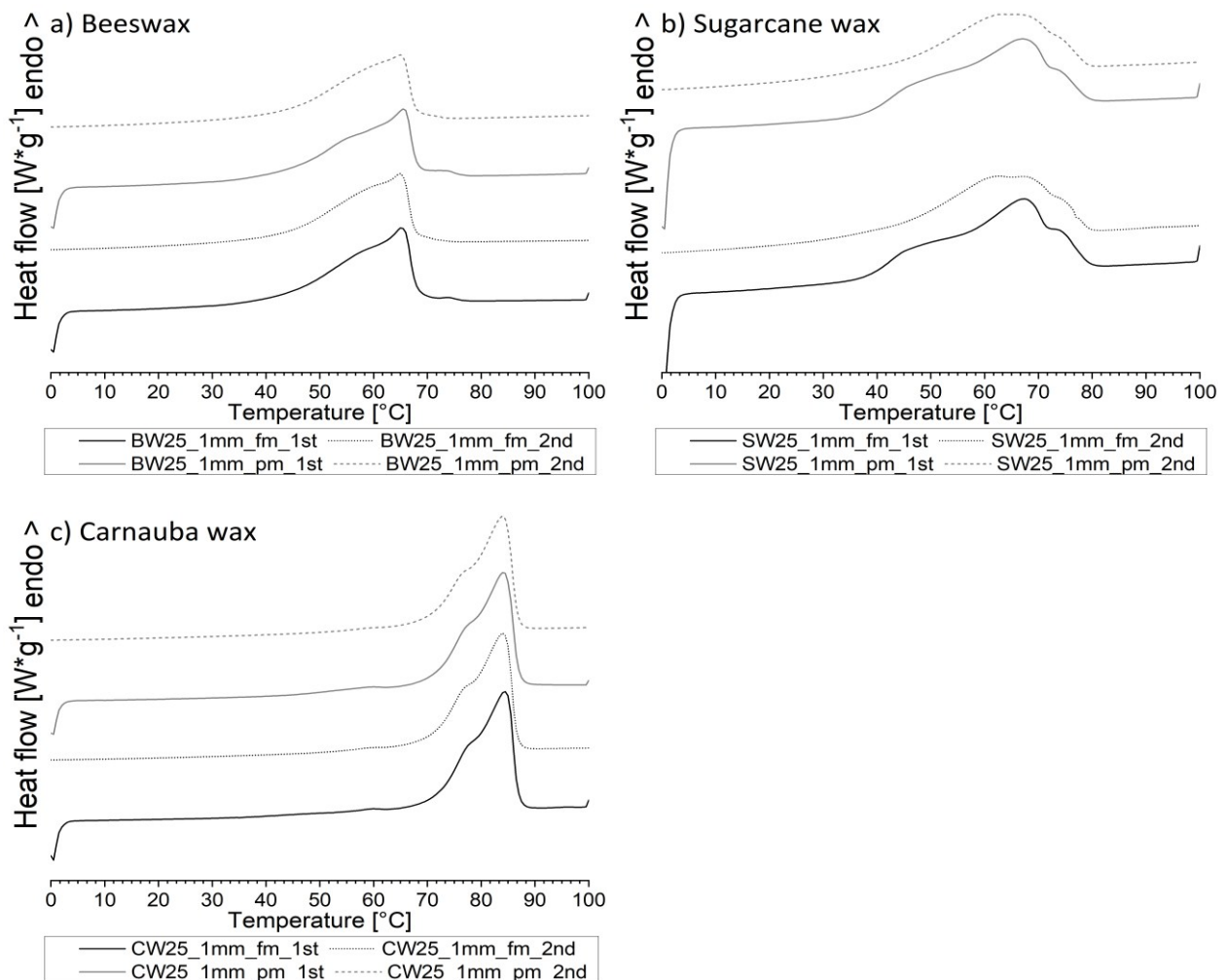


Figure 4.37: Comparison of thermograms of extrudates made from one type of wax and either fully molten (fm) or partially molten (pm) during the extrusion process; a) extrudates made from beeswax and sodium benzoate (25/75 (m/m)); b) extrudates made from sugarcane wax and sodium benzoate (25/75 (m/m)); c) extrudates made from carnauba wax and sodium benzoate (25/75 (m/m)); heating and cooling rate 10 °C/min. 1st heating from 0°C to 100°C, cooling from 100°C to -20°C, 2nd heating from -20°C to 100°C.

This has been discussed in previous chapters. The tailing of the peak might be caused by the formation of a eutectic mixture. For extrudates based on SW, no differences were observed based on the processing conditions. During the first heating cycle, the maximum peak was observed at 67°C

4.6 Comparison of solid wax extrusion and hot-melt extrusion

followed by a minor plateau at 73°C, which adds a shoulder to the decreasing part of the graph. These observations were made for both fully and partially melted SW extrudates. In the second heating cycle, the increase in heat flow was uniform, removing the aforementioned shoulder for the partially as well as the fully melted sample of SW-based extrudates. The maximum heat flow was reached at temperatures approximately 5°C lower than in the first heating cycle and was followed by the same shoulder in the decreasing part of the graph as during the first heating cycle. CW extrudates showed no alteration in their thermogram, whether they were processed fully melted or partially melted. The maximum heat flow was observed at 84°C followed and another characteristic signal was observed at 78°C. Furthermore, the cooling cycle between the two heating cycles did not affect the thermogram of the second heating cycle, indicating that CW crystallized in its stable form.

4.6.3 Crystalline properties

In addition to the analysis of the differently processed extrudates via DSC, the extrudates were also analyzed via XRPD to complement the observations made via DSC (**BaHav18-029**). The results of the XRPD-analysis are shown in Figure 4.38. At an angle of 20.9 2 θ and 23.2 2 θ , the characteristic orthorhombic peaks were detected in all samples as described in chapter 4.1.5. In the range from 25 2 θ to 35 2 θ , several small peaks connected to SB were observed. In Figure 4.38a, approximately 25 2 θ to 30 2 θ minor differences were detected in the intensity of the peaks. At 25 2 θ and 26 2 θ , the sample of fully molten BW showed a reduced intensity in comparison to the partially molten sample. No additional peaks were detected. This difference in intensity was also observed in the samples containing SW or CW. In both cases, the fully molten sample showed a reduced intensity in the range of 25 to 26 2 θ . We observed this difference between 25 2 θ to 34 2 θ for the CW sample as well. In the DSC-analysis, no changes were observed in the diffractograms of partially melted extrudates in comparison to fully melted extrudates. The analysis via XRPD also did not reveal any new crystalline structures and confirmed the observations made during DSC-analysis.

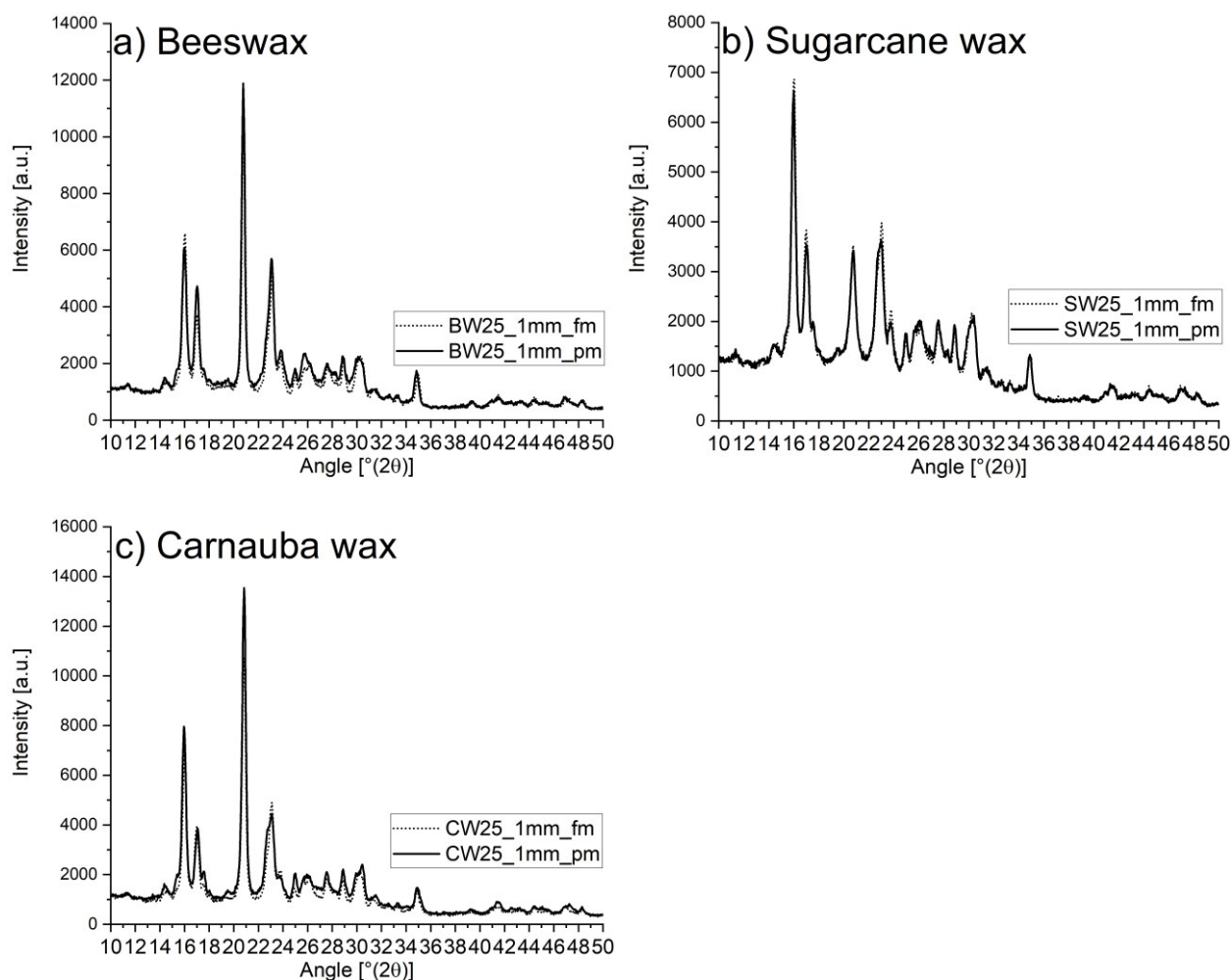


Figure 4.38: XRPD-diffractograms of extrudates consisting of wax and sodium benzoate, either fully molten (fm) or partially molten (pm) during the extrusion process; 2θ interval was used from 10 to 50 2θ . BW = Beeswax, SW = sugarcane wax; CW = Carnauba wax

4.6.4 Dissolution profiles

In addition to the characterization of the physical attributes of the extrudates, dissolution testing was performed as well (BaHav18-021). Extrudates of the same wax but either partially or fully melted were compared as shown in Figure 4.39. The data obtained was in agreement with the results of the release analysis shown in chapter 4.4.5 Dissolution profiles **Error! Reference source not found.**, Figure 4.22 for the period 0 to 120 minutes. BW extrudates released 24% (BW25_fm) and 23% (BW25_pm) SB after 120 minutes. SW extrudates released all contained SB within 120 minutes, irrespective of the process temperature used during production. After only 60 minutes, SW25_fm and SW25_pm had released 100% of the contained SB. CW extrudates again showed the fastest drug release, confirming the results of the previous dissolution experiments. Both CW25_fm and CW25_pm reached 100% release of SB after only 16 minutes. In this experimental setup, the drug release was measured over a total duration of 12 hours. As extrudates from SW and CW showed complete release after the first hour, no further information was obtained for these two types of extrudates.

4.6 Comparison of solid wax extrusion and hot-melt extrusion

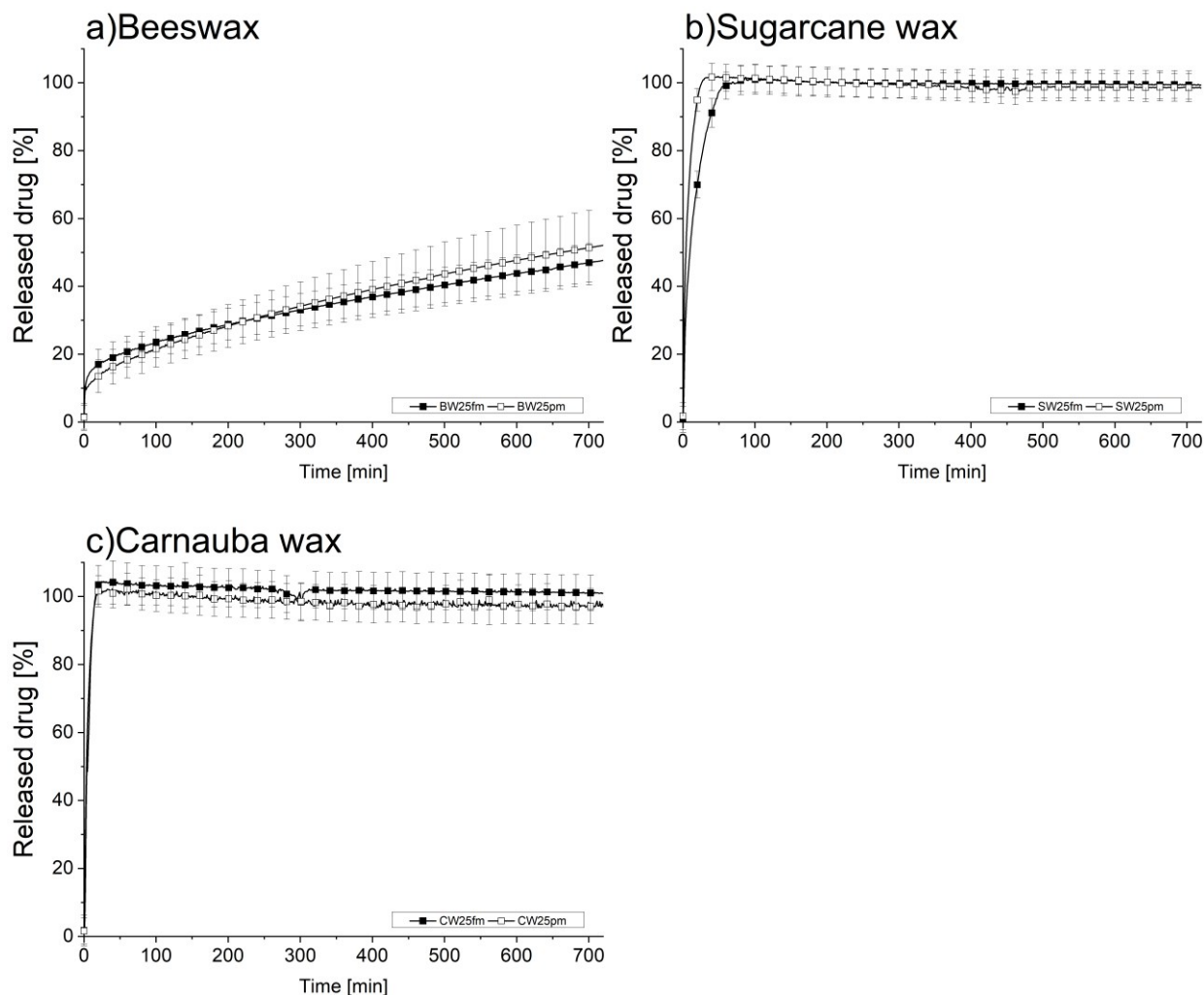


Figure 4.39: Dissolution profiles of extrudates made from 75% sodium benzoate and 25% wax only (BW25,SW25,CW25, $\varnothing = 1.00$ mm) 5 days after manufacturing. 900 ml phosphate buffer pH 6.8, $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, basket apparatus at 50 rpm, automated sampling every 60 seconds, $n=6$, $\bar{x} \pm \text{SD}$. For a better overview only every 20th data point is depicted.

The BW extrudates, on the other hand, continued to release SB over the total measurement time of 12 hours. After 12 hours, approximately 50% SB was released from the BW extrudates, reaffirming the release prolonging properties of BW.

4.6.5 Conclusion

Choosing different processing temperatures did not alter the physical properties of SW extrudates or the other two analyzed extrudates. Results obtained from DSC, XRPD, and dissolution testing showed similar results between the compared process temperatures. Based on these results, both process parameters can be chosen for manufacturing wax-based extrudates. Lower temperatures enable a reduction of the required energy consumption during manufacturing. Processing fully melted wax on the other hand reduces the risk of blocking the die as the material is able to pass the die more easily in its melted state.

4.7 Mini-tablets made from wax-based extrudates

4.7.1 Introduction

It was shown that HME as well as SLE are feasible processes for the manufacturing of SW-based extrudates containing high drug loads of SB. However, the extrudates are only an intermediate product, which need to be processed into the final dosage form. As SB is used in the treatment of newborns and infants, the final dosage form should be a multiple-unit dosage form like a granule, pellets or mini-tablets (MTs) (Krause and Breitzkreutz, 2008). MTs are tablets with a diameter of 3.0 mm or smaller (Lennartz and Mielck, 1998). Their small size makes them suitable for administering to small children and infants as they are easy to swallow even for young patients. In addition, individual dosing is possible due to their multiparticulate character. It has been shown that pediatric patients in the age range of 6 – 30 months accept MTs of up to 2 mm in diameter (Van de Vijver *et al.*, 2011). This was confirmed by Klingmann *et al.* (2013), who showed that children up to the age of 2 years accept uncoated MTs with a size of 2 mm. Furthermore, it has been shown that children between the ages of six to 23 month accept not only singular MTs, but amounts of 25 uncoated MTs (Klingmann *et al.*, 2018). This emphasizes the suitability of solid dosage forms with small particle sizes for young patients. To obtain any one of the above-mentioned dosage forms, further processing of the extrudates is required. Spheronization is the simplest next step, as it allows direct processing of the extrudates and requires no intermediate step. For tableting, however, the extrudates first require a milling step before the compression into tablets.

4.7.2 Spheronization and tableting of wax-based granules

Spheronization of lipids is a process where choosing the right temperature is essential. Too low temperatures prevent the extrudates from rounding, whereas too high temperatures cause the extrudates to stick together. (Krause *et al.*, 2009; Petrovick *et al.*, 2015; Reitz and Kleinebudde, 2009). Another key factor is the use of binary mixtures of lipids to perform a successful spheronization. Like lipids, waxes are relatively low-melting substances. Krause *et al.* (2009) spheronized extrudates made from 80% SB and 20% of a binary mixture of lipids. The lipids used by Krause *et al.* (2009), covered a melting range similar to that of the waxes used in this work. As it was not possible to determine a stable SLE process for wax-based extrudates and binary mixtures of waxes were not processed, attempts were made to spheronize extrudates consisting of 75% SB and 25% wax. Since the available DSC data showed a wide melting range of the waxes, the melting behavior of a single wax could be deemed comparable to a bi- or multi-component lipid blend. This property was hypothesized to enable spheronization despite the use of only one lipid-like component when combined with the right temperature. Spheronization was performed using an IR-lamp to heat the extrudates within the spheronizer as well as the set-up described by Petrovick *et al.* (2015). However, no satisfying results could be achieved. If the temperature was too low, the extrudates did not break and change their shape to a spherical, but were ground to dust by the friction on the wall of the spheronizer, or the material began to stick to the wall at too high a temperature. In both cases, pellets were not obtainable. Different combinations of spheronization time and temperature were tried but none of the combinations resulted in pellets. As an alternative, it was decided to grind the extrudates

4.7 Mini-tablets made from wax-based extrudates

and compress the resulting granules into mini tablets. Before tableting, the fraction smaller than 250 μm was separated by sieving. This fraction was analyzed regarding its assay to ensure that the separated fine fraction did not contain a significantly higher or lower drug content than the main fraction. This would cause an altered drug content in the main fraction used for tableting (**BaHav19-034**). The results are shown in Table 4.10. The fraction smaller than 250 μm had not a significantly

Table 4.10: Assay of fractions < 250 μm ; 1 g of each fraction was analyzed; $n=3$; $\bar{x} \pm SD$

Wax fraction < 250 μm	Assay
BW25	98.22% $\pm$ 0.32%
SW25	96.76% $\pm$ 0.61%
CW25	100.19% $\pm$ 1.79%

higher or lower drug content, thus the use of the fraction > 250 μm for tableting was considered acceptable. Tablets were made from each of the three available wax-based granules by applying either 6.7 kN or 8.7 kN of compression force. A tablet diameter of 2 mm and a tablet height of 2 mm were targeted to obtain pellet-like tablets. Tensile strength was not measured according to Ph. Eur. 2.9.8 because this test does not take the low breaking force of MTs in comparison to tablets of standard size into account. Tensile strength was evaluated using a texture analyzer (7.2.2.12 Tensile strength of mini-tablets). In Table 4.11, the tensile strength of the obtained MTs is shown (**BaHav19-031**).

Table 4.11: Tensile strength of wax-based mini-tablets. Compression force of 6.7 and 8.7 kN were used. BW= beeswax, SW= sugarcane wax, CW = carnauba wax; 25 = ratio of wax processed in original extrudate; $n=10$; mean $\pm$ SD

Sample	Tensile strength [N / mm ²]
BW25Mt6.7kN	0.83 $\pm$ 0.05
BW25Mt8.7kN	0.83 $\pm$ 0.10
SW25Mt6.7kN	0.46 $\pm$ 0.07
SW25Mt8.7kN	0.51 $\pm$ 0.09
CW25Mt6.7kN	0.53 $\pm$ 0.14
CW25Mt8.7kN	0.59 $\pm$ 0.14

The measured tensile strength differed depending on the processed wax. BW-based MTs showed a higher tensile strength than MTs made from SW granules or CW granules. There was no difference between SW-based and CW-based MTs. The use of different compression forces had no influence on the tensile strength of the BW-based mini tablets. MTs made of SW or CW showed a higher tensile strength with higher compression force. The results are consistent with the observations made during the processing of the raw materials. SW and CW are significantly more brittle than BW, with CW generally being one of the most brittle waxes (Krendlinger *et al.*, 2015). Tablets made from these two types of wax break from a measured breaking strength of 0.46 to 0.59 N/mm². Tablets made from BW, on the other hand, initially showed plastic deformation during increasing force application. As a result, the BW tablet did not break into smaller particles abruptly but was first deformed before breaking at a higher breaking force. After each measurement, residues of BW tablets had to be removed using a scraper, whereas residues of SW and CW tablets could be easily removed with a brush.

4.7.3 Dissolution studies of wax based mini tablets

Within the framework of dissolution studies, the extent to which the tableting process influences the dissolution behavior of tablets based on different waxes was investigated. The manufactured MTs have a height of approximately 2 mm and a diameter of 2 mm and thus come with an almost spherical appearance. This changes the surface available for release of SB in comparison to the cylindrical extrudates. A sphere provides less surface for release than a cylindrical shaped object and the surface area influences the dissolution speed as described in the Noyes-Whitney equation (Noyes and Whitney, 1897). The results of the performed dissolution studies are shown in Figure 4.40 (BaHav19-033). Independent of the wax used for manufacturing, the wax-based MTs show a relative SB release between 90% and 100% after 120 min. As observed in previous release studies, the BW-based dosage form had the smallest release rate of the three formulations. Only the MTs made of SW and CW met

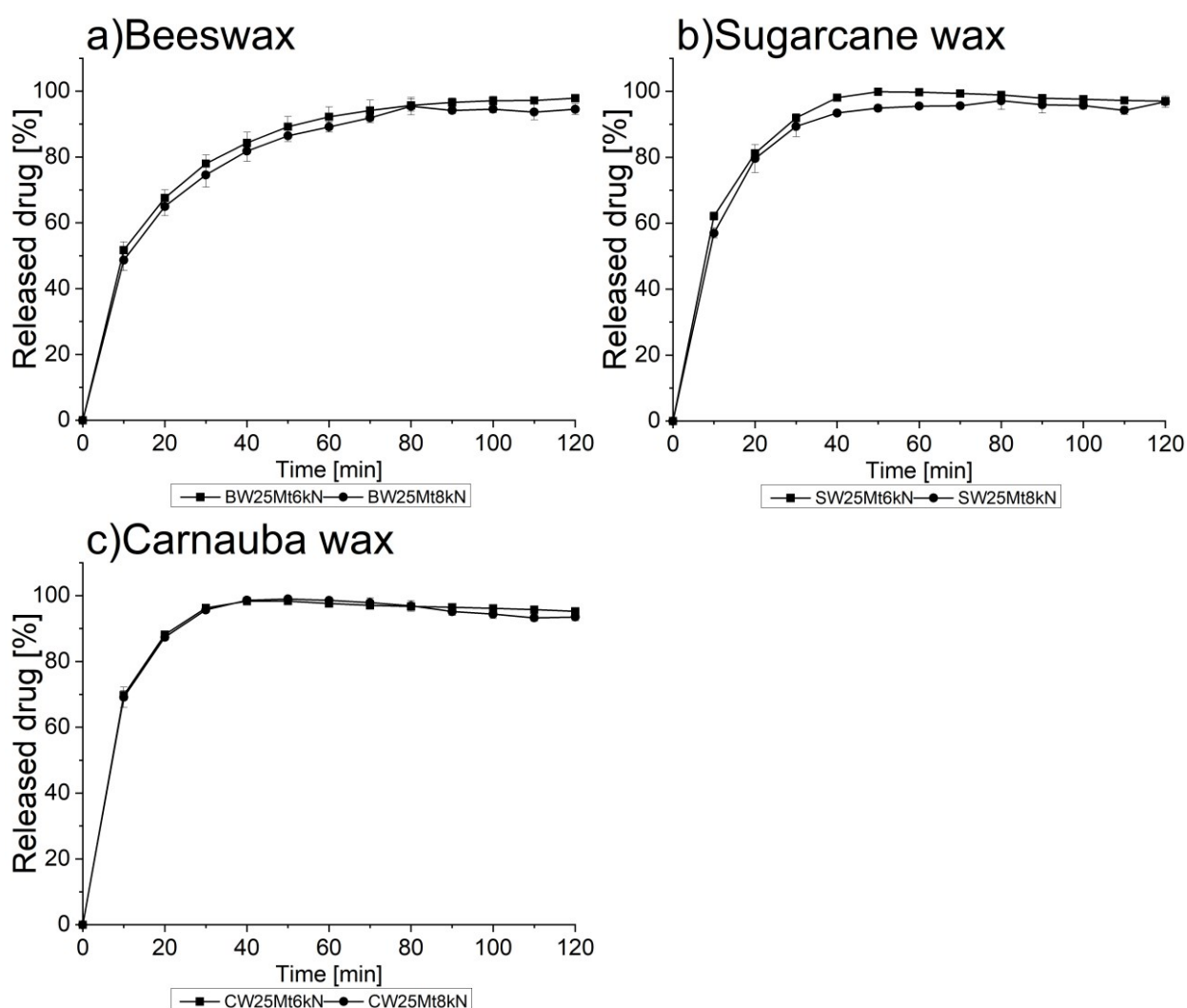


Figure 4.40: Dissolution profiles of MT made from waxes: a) BW-based; b) SW-based; c) CW-based; 900 ml phosphate buffer pH 6.8, $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, basket apparatus set to 50 rpm; $n=6$, $\bar{x} \pm \text{SD}$; BW = beeswax, SW = sugarcane wax, CW = carnauba wax; number = ratio of wax used in granule.

the FDA's immediate release criterion, as they released more than 80% within 30 minutes (U.S. Food and Drug Administration, 2018). After ten minutes, 50% of SB was released and after 120 min, the sample BW25Mt6kN had released 97.8% of its content. The sample BW25Mt8kN released slightly less with a released amount of SB of 94.5%. The MTs made of SW showed a faster release of SB in

4.7 Mini-tablets made from wax-based extrudates

comparison to the BW formulation, releasing 60% of the contained SB within 10 min. After 50 min, SW25Mt6kN had released 99.6%. However, SW25Mt8kN released SB more slowly and completed the release of SB after 80 min at 97.2% released. Also, in agreement with the results of previous studies of wax-based dosage forms, MTs based on CW showed the fastest drug release. After 10 min, 70% of SB had been released. CW25Mt6kN showed complete release after 50 min with a release of 98.4% and CW25Mt8kN completed drug dissolution after 50 min by releasing 99.0%. The MTs made of BW showed a higher release of SB in comparison to the extrudates made of BW. After 10 min, the amount of SB released from MTs was similar to the amount of SB released from the extrudates. The release from MTs over a period of 120 min showed a larger amount of released SB. However, the stability of BW extrudates had shown that the age of the BW material significantly influences the drug release performance. The MTs made from BW used in this dissolution testing were five months old, making it difficult to separate between the impact of smaller surface of the MTs and the older age of the BW-drug formulation. In addition to the dissolution studies, the assay of the MTs made at 6kN was determined (**BaHav19-032**). The results are shown in the table below.

Table 4.12: Assay of mini-tablets made from wax. Drug-load of each mini-tablet is 74.25%; $\bar{x} \pm SD$; $n=10$

Sample	Assay
BW25Mt6kN	99.01% $\pm$ 1.18%
SW25Mt6kN	97.70% $\pm$ 1.80%
CW25Mt6kN	98.43% $\pm$ 1.10%

The results of the assay confirmed that the MTs had been successfully produced with a correct drug-load.

4.7.4 E-Tongue analysis of uncoated mini-tablets

The MTs were also analyzed using the e-tongue to gain more insight into the taste properties of this drug-formulation (**BaHav19-036**). The e-tongue analysis was performed as described in chapter 4.4.8. 1.0 g SB was compared to wax-based MTs containing an equivalent amount of SB. As noticed in the previous study, the BW-formulation showed a much faster drug-release in the cooler “dissolution medium” during preparation of the e-tongue samples than during the standard tempered dissolution studies. As shown in Figure 4.41, the signal measured for samples of MTs made from BW-granules is similar to the signal of MTs made from SW or CW-granules.

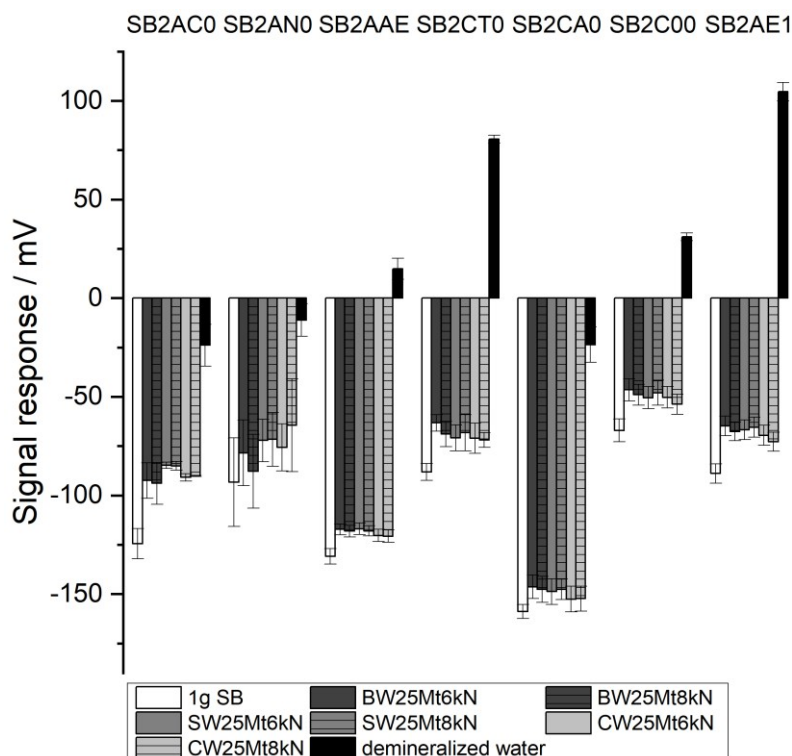


Figure 4.41: Taste investigation of MTs made from wax-based granules via e-tongue. BW = made from beeswax extrudates, SW = sugarcane wax extrudates, CW = carnauba wax extrudates; number = ratio of wax in the granule. The mass of weighed MTs corresponds to a quantity of 1.0g pure SB.

Based on the results of the dissolution studies, a much weaker signal was expected for the BW samples, since the BW samples released significantly lower amounts of SB within the first 10 minutes in the dissolution experiments than the corresponding samples consisting of SW or CW. In general, it was observed that all samples released about the same amount of SB. In addition, all samples showed a slightly weaker signal compared to the reference sample of 1.0 g SB and at the same time differ clearly from the negative sample of demineralized water. A PCA was performed with the data. The score plot of that PCA is shown in Figure 4.42. 99.5% of the data variance is explained by the first component of the model and 0.16% is explained by the second component. The sample of demineralized water was identified as an outlier because this was the only sample not containing any SB. The samples of MTs and pure 1 g SB are plotted very close to the x-axis, which means that these

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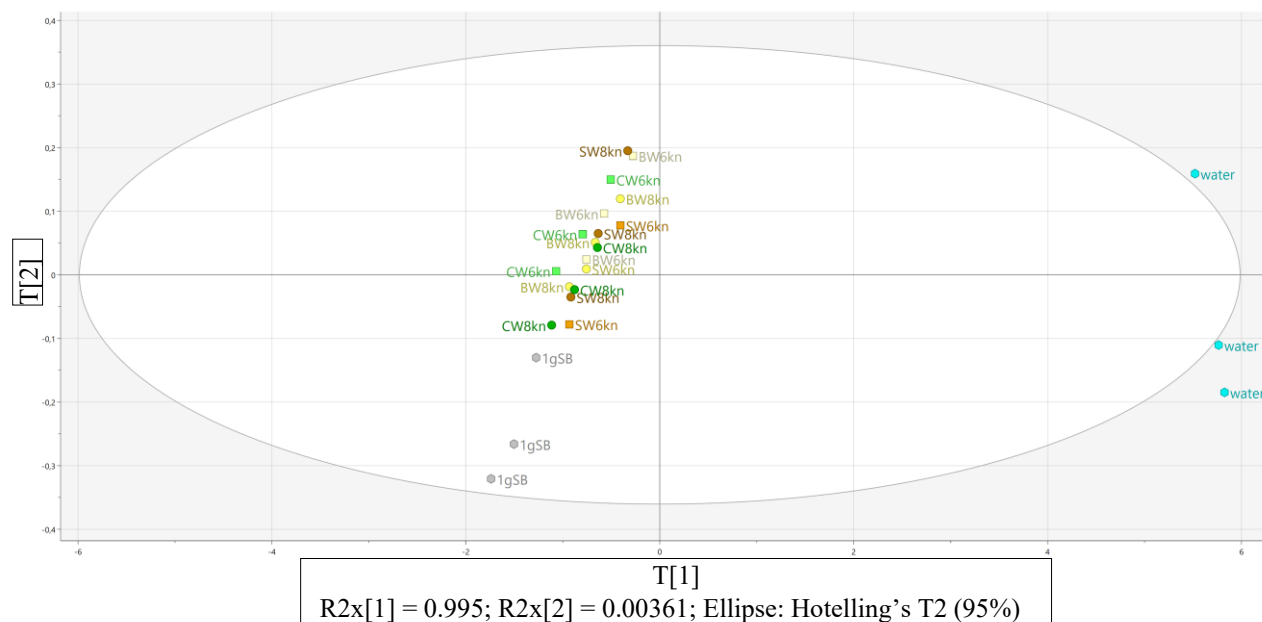


Figure 4.42: Score plot of a PCA using data of sensors SB2AAE, SB2CT0, SB2CA0, SB2C00 and SBAE1. Mini-tablets were either made from beeswax (BW), sugarcane wax (SW) or carnauba wax (CW) and made with a pressure of 6 or 8 kN. 1 g of pure SB was measured as reference as well as demineralized water.

samples are identified as samples with a comparable amount of SB according to the e-tongue. The difference of the samples on the y-axis was marginal as only a very small amount of variance of the samples was explained by the second component. The results indicate that bad taste is very likely to be experienced by the patient during intake of a typical required dose, which would be 1.6 g for a one-year-old infant. Thus, it was concluded that an additional saliva resistant coating should be applied to avoid drug release in the oral cavity.

4.7.5 Dissolution of coated mini-tablets

Based on the results of the e-tongue analysis, MTs consisting of BW, SB, and magnesium stearate (**BaHav18-041**) were coated with basic butylated methacrylate copolymer (Eudragit® E) using a fluid bed coater (**BaHav19-022**). This coating is saliva resistant, thus preventing bitter tasting SB from dissolving in the oral cavity and causing bad taste. Breitkreutz *et al.* (2003) and Krause (2008) showed that Eudragit® E can be used to create a taste masked drug-formulation for SB. Due to the small batch sizes of SW- and CW-based MTs, there were not enough MTs available to apply coating on these MTs as well. BW-MTs were available in sufficient quantity to perform a coating step. These MTs were coated successfully with Butylated Methacrylate Copolymer using a Huettlin Mycrolab Coater from Bosch. A batch size of 100 g of BW-MTs was coated with Eudragit® E 12,5. Coating was completed with a mass gain of 30% of polymer. Composition of the coating fluid is shown in Table 4.13.

Table 4.13: Composition of the coating-suspension of Eudragit® E

Component	Quantity [g]
Eudragit E 12,5	366.8
PEG 6000 33% aqueous	13.9
Talc Pharma M	22.9
Aceton / Isopropanol 40:60	330

The freshly coated MTs were analyzed in a dissolution study using demineralized water and 0.1 N hydrochloric acid (**BaHav19-019**) and compared against a dissolution study in phosphate buffer pH 6.8 (**BaHav18-042**). The dissolution results are shown in Figure 4.43.

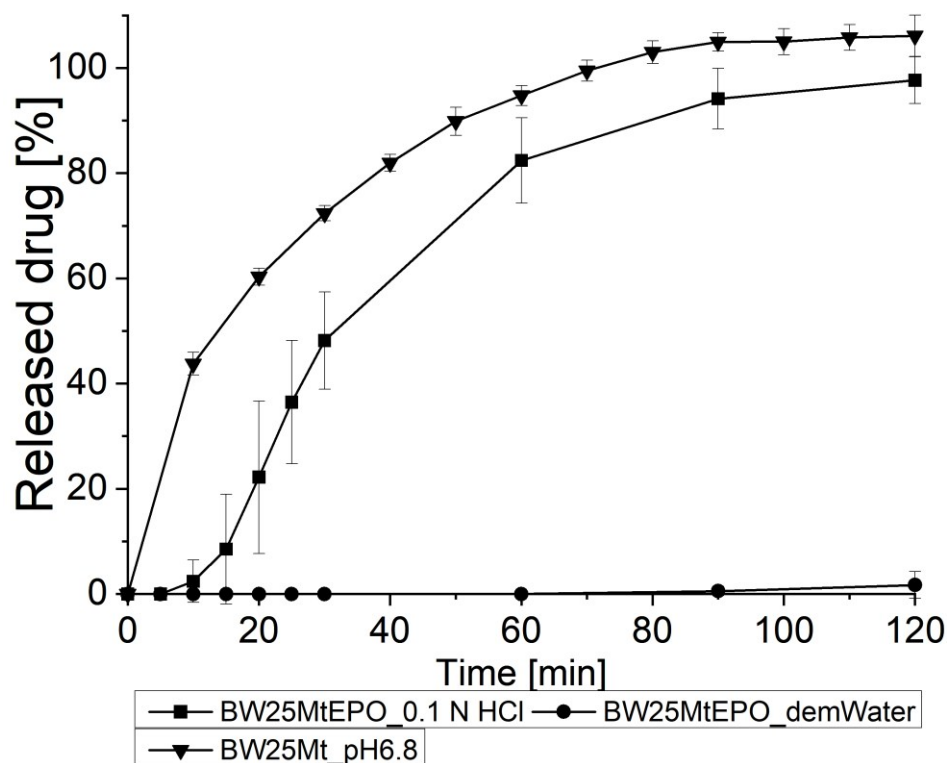


Figure 4.43: Dissolution of coated and uncoated BW-mini-tablets. 900 ml of the designated medium; $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, basket apparatus set to 50 rpm; $n=6$, mean $\pm$ SD; 3 mini-tablets per basket. BW25MtEPO_0.1 N HCl = coated mini-tablets in 0.1 N HCl as dissolution medium; BW25MtEPO_demWater = coated mini-tablets in demineralized water; BW25Mt in phosphate buffer pH 6.8. For a better comparison only data of the same time points of sample pull are shown.

When using demineralized water as dissolution medium, no SB was released from the coated MTs within the first 60 min. In the period of 60 min to 120 min only minor quantities of SB were released in the end. This indicated that the coating was intact and an untimely release of the active ingredient was prevented. When the test was performed in the acidic medium, a delay in the release of SB was observed for the very first 10 minutes. The observed delay represents the time required for dissolution of the acid-sensitive coating. The thickness of the applied coating was around $65.9\ \mu\text{m}$ to $86.8\ \mu\text{m}$, as shown in Figure 4.44. This is comparable to values found in literature (Podrekar *et al.*, 2018).

4.7 Mini-tablets made from wax-based extrudates

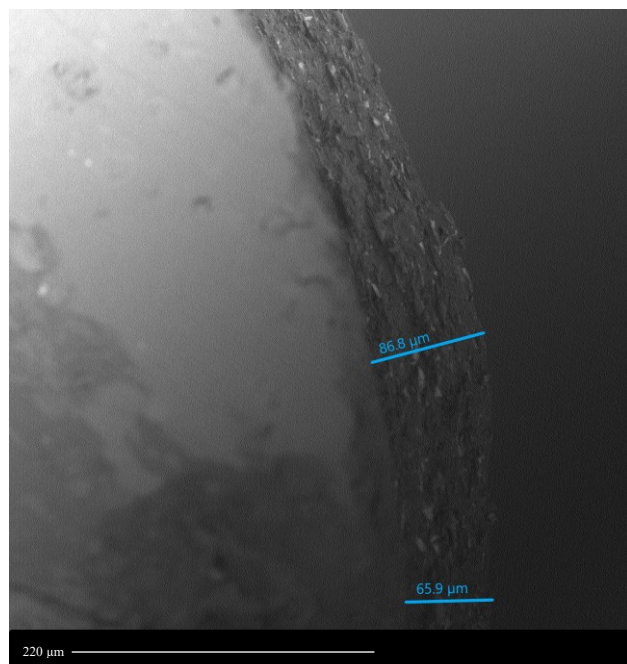


Figure 4.44: Coated BW25MtEPO; the scale represents 220 μm .

Three uncoated tablets weighed 20-21 mg, whereas three coated tablets weighed 28-31 mg, which complies to the mass gain of 30%. Due to the delay in drug release, the coated MTs, like the non-coated MTs, did not meet the FDA's criterion for immediate release drug formulations. However, it was successfully shown, that API-release was prevented in a pH-neutral medium. Additionally, the assay of the tablets was determined (**BaHav19-002** and **BaHav19-021**) and the results are shown in the table below.

Table 4.14: Assay of uncoated and coated BW25 mini-tablets; $\bar{x} \pm SD$; $n=10$.

Sample	Assay
BW25Mt	99.51% $\pm$ 4.10%
BW25MtEPO	99.99% $\pm$ 4.36%

Since both MTs were determined to have a drug-load of over 99%, the drug-load was sufficient and a complete release of SB could be expected under the correct dissolution conditions.

4.7.6 E-Tongue analysis of coated mini-tablets

The coated MTs were also analyzed with the e-tongue (**BaHav19-024**). 1 g of pure SB was dissolved in 100 ml demineralized water for use as a reference solution and compared with the corresponding amount of coated MTs containing 1 g SB. The results are shown in Figure 4.45. All sensors were able to discriminate between the analyzed samples. The strongest signal was observed for pure SB-solution followed by the second highest signal for the uncoated MTs. The signal of the sample of coated MTs was comparable to the sample of demineralized water, which represented the blind sample. These results confirmed the observations gained from Figure 4.43. MTs which had been

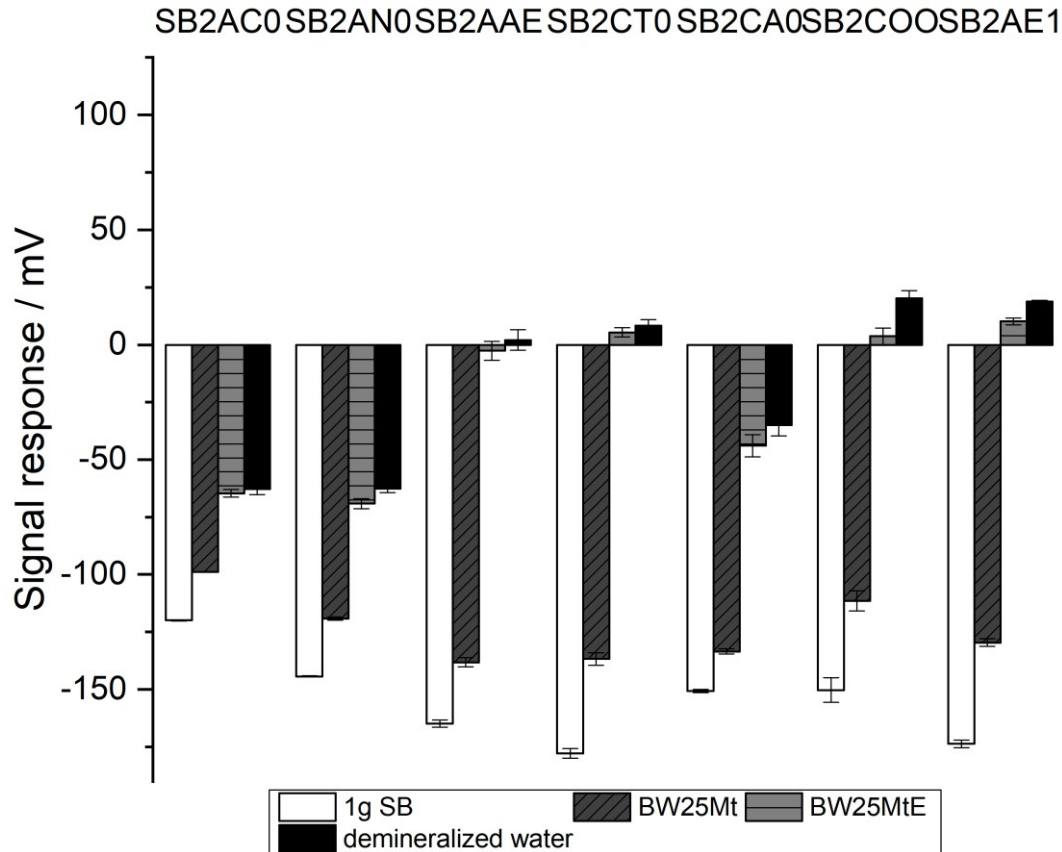


Figure 4.45: Taste investigation of coated MTs and uncoated MTs made from BW granules in comparison to pure sodium benzoate and demineralized water via e-tongue. BW25MT = made from beeswax-granules, BW25MtE = coated mini-tablet made from beeswax granules, SB 1g = solution of 1 g of sodium benzoate in 100 ml demineralized water; number = ratio of wax in the granule. The mass of weighed MTs corresponds to a quantity of 1.0g pure SB

coated with Eudragit E[®] did not release any SB in the test medium of demineralized water, which has a similar pH-value to the medium in the human oral cavity. In consequence, no bad taste should be experienced by a patient during the administration of the MT. In addition, a PCA was conducted with the e-tongue data. The score plot of that PCA is shown in Figure 4.46. 99.7% of the variance of the data were explained by the first component while only 0.2% were explained by the second component. On the left, the samples which showed the highest amount of released SB were plotted in the scatter plot. On the right side, the samples of coated MTs as well as demineralized water were plotted. This confirmed that the sample of coated MT is recognized to be equivalent to demineralized in regards to the amount of released SB.

4.7 Mini-tablets made from wax-based extrudates

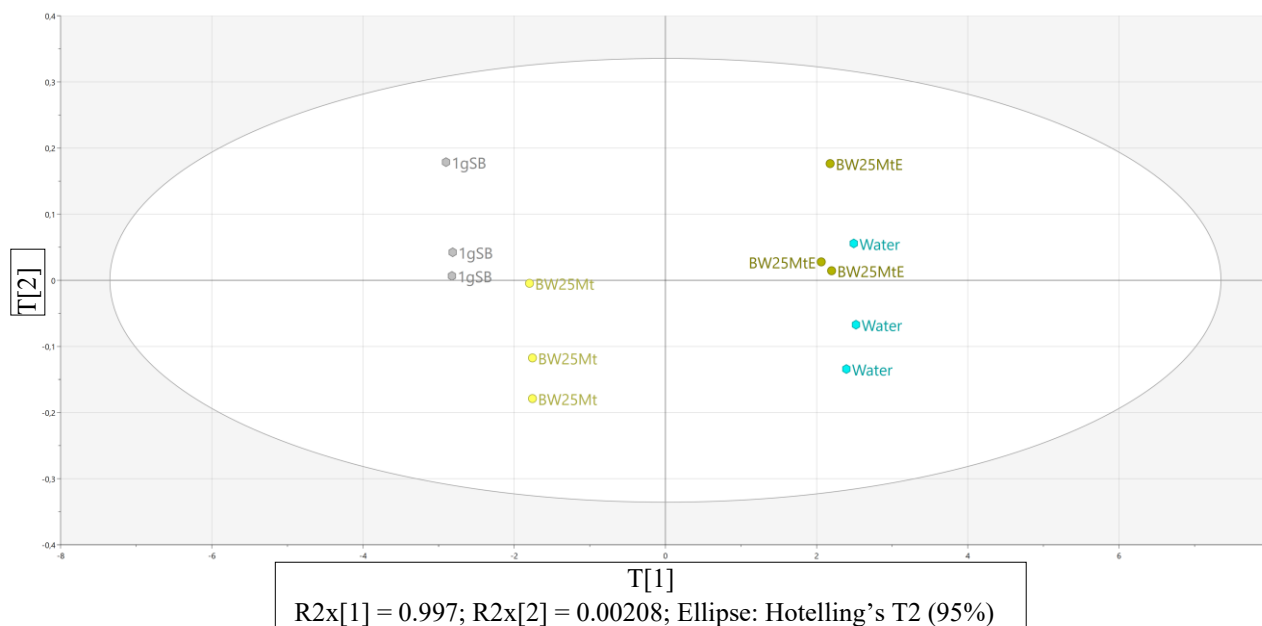


Figure 4.46: PCA using data of sensors SB2AAE, SB2CT0, SB2CA0, SB2C00 and SBAE1. Data of samples 1 g of sodium benzoate (1gSB) as well as mini-tablets made from beeswax extrudates with coating (BW25Mt), mini-tablets made from beeswax extrudates with additional Eudragit E[®] coating (BW25MtE) and demineralized water are plotted.

On the other hand, the sample of uncoated MTs showed a comparable result of released SB to the sample of pure SB with a slight difference in the total amount.

The applied coating successfully prevented the release of any SB within 60 minutes. Overall only slight amounts of SB were released over the time of 120 minutes. Uncoated MTs showed almost the same signal as a sample of the equivalent amount pure SB. In conclusion, a coating should be applied to the tablets to avoid low compliance due to a bad taste experience during the intake of the MTs.

5 Summary

In this work it was shown that extrudates with a drug-load up to 75% SB can be manufactured via HME using SW as a binder. Furthermore, it was shown that extrudates made from SW show comparable properties to extrudates with the same drug-load made from commercially available CW. Extrudates made from BW differed in their dissolution profile. Also, depending on the drug-load, the diameter and the age of the extrudates, the dissolution profile was altered. Extrudates with a higher drug-load showed a faster release of their API in comparison to extrudates with a lower fraction of API and higher wax fraction. Extrudates with the same amount of API but with a smaller diameter released their drug-content faster than those with a larger diameter. A strong age-dependency was observed for BW extrudates regarding the drug content release. This also to SW extrudates but was not observed for extrudates made from CW. Freshly produced BW extrudates with a drug-load of 75% showed a slow release which increased over the weeks of storage. In comparison, the dissolution profile of SW showed a higher amount of drug-release from day one and only a minor increase after four weeks of storage. It was shown that BW extrudates performed differently in drug-release depending on the temperature applied during dissolution testing. This temperature dependency was not shown for extrudates made from SW and CW, making them superior for the administration into the human body where the temperature of 37.5°C is the standard condition. CW extrudates showed a very fast and complete release independent of their age. In addition to the dissolution testing, a taste evaluation was conducted by analyzing samples with different drug-loads and made from different waxes with an e-tongue. These measurements aligned with the results of the dissolution studies, showing a correlation between a quick release of SB and a strong signal response in the e-tongue.

The addition of a pore-forming agent did hasten the initial dissolution profile of BW extrudates. The dissolution profile of SW extrudates, however, was only slightly accelerated. The profile of CW extrudates was not influenced as these samples already showed a very fast drug release.

The processing of SW at lower process temperatures to perform SLE was investigated. The processing at temperatures below the melting point of the wax did not alter the physical properties analyzed via XRPD and DSC. Furthermore, the dissolution profile was not altered in comparison to extrudates manufactured by completely melting the wax binder. However, it was observed that the process is more prone to extruder die blockages. Despite this disadvantage, SLE might still be worth considering due to the reduced energy consumption.

The extrudates were successfully processed into MTs with an intermediate milling step. It was shown that the MTs had a fast release of their API content, which would likely cause a bad taste experience for the patient. The intensity of the taste strongly depends on the total amount of released SB. MTs made from BW were subsequently coated with Eudragit E[®] and a comparison between coated and uncoated BW MTs was performed. Both sample types were compared regarding their dissolution profiles as well as their e-tongue signals. Both experimental setups showed that the application of a Eudragit E[®] coating prevented the release of SB at a pH comparable to that of the human oral cavity.

The above-described application shows that the resource SW, as well as the comparator materials BW and CW, which are both monographed in the European and American Pharmacopoeia, are suitable for processing into pharmaceutical dosage forms. The introduction of SW as pharmaceutical excipient would create a valuable use case for this material aside from the combustion for energy generation purposes. This would reduce carbon dioxide emissions by reducing combustion of SW and contribute to a more sustainable supply chain in the pharmaceutical industry.

6 Zusammenfassung

In dieser Arbeit wurde gezeigt, dass Extrudate mit einem Wirkstoffgehalt von bis zu 75 % Natriumbenzoat durch Schmelzextrusion mit Zuckerrohrwachs als Bindemittel hergestellt werden können. Weiterhin wurde gezeigt, dass Extrudate aus Zuckerrohrwachs vergleichbare Eigenschaften in ihrem Freisetzungsprofil aufweisen wie Extrudate mit der gleichen Wirkstoffbeladung aus Carnaubawachs. Extrudate, die aus Bienenwachs hergestellt wurden, zeigten unterschiedliche Eigenschaften in ihrem Freisetzungsprofil. In Abhängigkeit von der Wirkstoffbeladung, dem Durchmesser und dem Alter der Extrudate wurden unterschiedliche Freisetzungsprofile beobachtet. Extrudate mit einer höheren Wirkstoffbeladung zeigten eine schnellere Freisetzung ihres Wirkstoffs im Vergleich zu Extrudaten mit einem geringeren Anteil an Wirkstoff und einem höheren Wachsanteil. Extrudate mit der gleichen Menge an Wirkstoff und einem kleineren Durchmesser setzten ihren Wirkstoffgehalt schneller frei als solche mit einem größeren Durchmesser. Bei Bienenwachsextrudaten wurde eine starke Abhängigkeit von der Lagerdauer bei der Freisetzung des Wirkstoffgehalts beobachtet, die auch in geringem Maße für Zuckerrohrwachsextrudate galt und bei Extrudaten aus Carnaubawachs nicht beobachtet werden konnte. Frisch hergestellte Bienenwachsextrudate mit einer Wirkstoffbeladung von 75 % zeigten eine langsame Freisetzung, die sich aber über die Wochen der Lagerung beschleunigte. Im Vergleich dazu zeigte das Freisetzungsprofil von Zuckerrohrwachs auch nach vier Wochen Lagerung nur eine marginal schnellere Freisetzung. Es wurde gezeigt, dass Bienenwachsextrudate Unterschiede bei der Wirkstofffreisetzung in Abhängigkeit von der während des Freisetzungstests angewandten Temperatur zeigten. Diese Temperaturabhängigkeit wurde bei Extrudaten aus Zuckerrohrwachs und Carnaubawachs nicht festgestellt, so dass sie sich besser für die Verabreichung in den menschlichen Körper eignen, in dem Temperaturen von 37,5°C die Standardbedingungen sind. Carnaubawachsextrudate zeigten unabhängig von ihrem Alter eine schnelle und vollständige Freisetzung. Zusätzlich zu den Freisetzungsuntersuchungen wurde eine Analytik mit einer elektronischen Zunge durchgeführt, in derer Proben mit unterschiedlicher Wirkstoffbeladung und aus verschiedenen Wachsen analysiert wurden. Es zeigte sich, dass in Übereinstimmung mit den Ergebnissen der Freisetzungsstudien eine schnelle Freisetzung von Natriumbenzoat zu einer starken Signalantwort in der elektronischen Zunge führte. Basierend auf diesen Messungen wäre ohne weitere Maßnahmen ein negatives Geschmackserlebnis zu erwarten.

Der Zusatz eines Porenbildners verbesserte das anfängliche Freisetzungsprofil von Bienenwachsextrudaten. Das Auflösungsprofil von Zuckerrohrwachsextrudaten wurde dagegen geringfügig beschleunigt. Das Profil von Carnaubawachsextrudaten wurde nicht weiter beschleunigt, da diese bereits ohne Porenbildner eine sehr schnelle Freisetzung aufwiesen.

Weiterhin wurde die Verarbeitung von Zuckerrohrwachs bei niedrigeren Prozesstemperaturen für die Extrusion von festen Lipiden untersucht. Die Verarbeitung bei Temperaturen unterhalb des Schmelzpunkts des verwendeten Wachses veränderte die mittels XRPD oder DSC analysierten physikalischen Eigenschaften nicht. Auch das Freisetzungsprofil wurde im Vergleich zu Extrudaten, die durch Schmelzextrusion hergestellt wurden, nicht verbessert. Es wurde jedoch festgestellt, dass das Verfahren anfälliger für ein Verstopfen der Extruderdüse ist. Aufgrund des geringeren Energieverbrauchs könnte dies jedoch ein Nachteil sein, der in Kauf genommen wird.

Die Extrudate wurden nach einem Mahlschritt erfolgreich zu Minitabletten weiterverarbeitet. Es zeigte sich, dass die Mini-Tabletten eine schnelle Freisetzung des Wirkstoffs aufweisen, was mit

6. Zusammenfassung

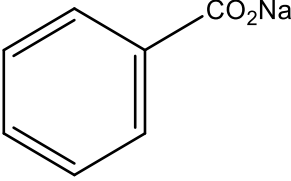
hoher Wahrscheinlichkeit ein schlechtes Geschmackserlebnis beim Patienten hervorrufen würde. Die Intensität des Signals hing stark von der Gesamtmenge des freigesetzten Natriumbenzoats ab. Minitabletten aus Bienenwachs wurden mit Eudragit E® beschichtet und es wurde ein Vergleich zwischen beschichteten und unbeschichteten Bienenwachsminitabletten durchgeführt. Verglichen wurden sowohl die Freisetzungsprofile als auch die Signale mit der Analyse mittels elektronischer Zunge. Beide Vergleiche zeigten, dass eine aufgebrauchte Eudragit E®-Beschichtung die Freisetzung von Natriumbenzoat bei einem pH-Wert verhindert, der mit dem pH-Wert in der menschlichen Mundhöhle vergleichbar ist.

Die oben beschriebene Anwendung zeigt, dass die Ressource Zuckerrohrwachs ebenso wie die Vergleichsmaterialien Bienenwachs und Carnaubawachs, die beide im europäischen und amerikanischen Arzneibuch monographiert sind, für die Verarbeitung zu pharmazeutischen Darreichungsformen geeignet sind. Die Einführung von Zuckerrohrwachs als pharmazeutischem Hilfsstoff würde die Möglichkeit bieten, neben der Verbrennung von Zuckerrohrwachs zur Energiegewinnung einen wertvollen Anwendungsfall für dieses Material zu schaffen. Dies würde die Kohlendioxidemissionen verringern und zu einer nachhaltigen Lieferkette in der pharmazeutischen Industrie beitragen.

7 Material and Methods

7.1 Materials

Table 7.1: Used API

Properties	Sodium benzoate
Molecular formula	C ₇ H ₅ NaO ₂
Molecular weight	144.1
CAS	532-32-1
Melting point	436
Chemical structure	
Solubility	Freely soluble in water, sparingly soluble in ethanol (90% V/V)
Batches	BNA00109 Microsin, Bucharest, Romania BNA00116 Microsin, Bucharest, Romania BNA00413 Microsin, Bucharest, Romania

* Ph. Eur

** GESTIS-Stoffdatenbank

Table 7.2: Used excipients for extrusion

Excipient	Commercial name	Batch	Company
Beeswax	Cera alba	16163103	Caesar & Loretz, Hilden, Germany
Sugarcane wax	DEUREX® X52G	15-250 M-102838	DEUREX, Elsteraue, Germany
Carnauba wax		P16-130	Norevo, Hamburg, Germany
Polyethylenglycol 6000	Polyglycol 6000	DEG4045255	Clariant, Frankfurt am Main, Germany

Table 7.3: Other used substances

Excipients	Commercial name	Batch	Company
Potassium dihydrogen phosphate		1664365 1850676	Fisher scientific, Leicester, United Kingdom
Di-sodium phosphate dihydrate		296245327 26.4591602	Carl Roth, Karlsruhe, Germany Th.Geyer, Renningen, Germany
Phosphoric acid 85%		1707973	Fisher scientific, Leicester, United Kingdom
Sodium dihydrogen phosphate hydrate		000787235	AppliChem, Darmstadt, Germany
Hydrochloric acid 37%		1873580	Fisher scientific, Leicester, United Kingdom
Polysorbate 20	Tween 20	17325414	Caesar & Loretz, Hilden, Germany
Acetonitril	Acetonitril UHPLC	0001565492	AppliChem, Darmstadt, Germany
Quinine hydrochloride dihydrate		6021	Buchler, Braunschweig, Germany
Potassium chloride		15D080013	VWR, Leuven, Belgium
Tartaric acid		9H008458	AppliChem, Darmstadt, Germany
Potassium hydroxide		203199720	Carl Roth Karlsruhe, Germany
Ethanol absolute		V7F440087F	Th.Geyer, Renningen, Germany
Silver chloride		50770	Sigma-Aldrich, Seelze, Germany
Polyethylene glycole 6000 33% aqueous	PEG 6000 33% aqueous	437260868	Merck, Darmstadt, Germany
Basic Butylated Metacrylate Copolymer	Eudragit E 12,5	A181021558	Evonik, Darmstadt, Germany
Talcum	Talc Pharma M	S21/18	Imerys, Paris, France

7. Material and Methods

7.2 Methods

7.2.1 Preparative Methods

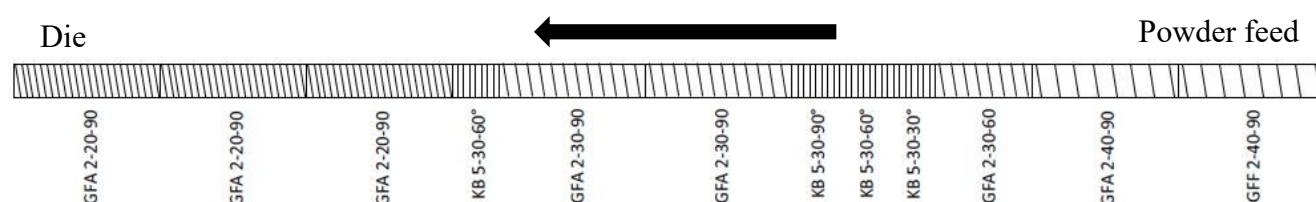
7.2.1.1 Milling

Milling of the raw wax material prior to extrusion as well as of the extrudates was performed with an ultra-centrifugal mill (ZAA200, Retsch, Haan, Germany). A sieve with a mesh size of 2.0 mm was used to mill wax plates and extrudates of experiment **BaHav18-039**. A sieve with a mesh size of 1.5 mm was used to mill extrudates of experiment **BaHav18-014b**. In every experiment the speed was kept at 6000 RPM. Before milling, all materials were cooled with liquid nitrogen.

7.2.1.2 Hot melt extrusion

Hot melt extrusion was performed using a co-rotating TSE (MICRO 27GL-28D, Leistritz, Nürnberg, Germany). The barrel consisted of 6 segments and a die, which can each be heated individually. The material was fed into the barrel via a gravimetric powder feeder (K-SFS-24/6, K-Tron, Niederlenz, Switzerland). All experiments were performed using the screw-configuration recommended by the supplier (Figure 7.1). GFF and GFA label conveying elements, KB references kneading blocks.

Figure 7.1 Hot melt screw-configuration recommended by the company Leistritz



Blending of mixtures of milled wax material and sodium benzoate was performed for 15 min with a turbula blender (Willy A. Bachofen, Niederau-Heldenbergen, Germany) in experiment **BaHav17-008**. For all further extrusion experiments, a laboratory mixer (LM20, R. B. Bohle, Ennigerloh, Germany) was used for blending at a speed of 25 rpm for a duration of 20 minutes. To allow for additional cooling time before storage, the extrudates were transferred onto a conveyor belt (Brabender, Duisburg, Germany).



Figure 7.2: Twin-screw extruder Leistritz MICRO 27GL-28D (left) and the assembled twin-screw in the hot-melt configuration.

The temperature of the barrel, the material feed rate and the diameter and number of the dies were varied between different experiments and are shown in the tables below (Table 7.4 -Table 7.10).

Table 7.4: Process parameters of extrusion experiment BaHav17-008

<i>Mixture [%; w/w]</i>	<i>Batch</i>	<i>Speed [rpm]</i>	<i>Temperature [°C]</i>
SB / BW 50:50*	BW50	50	50
SB / BW 75:25*	BW25	100	60
SB / SW 50:50*	SW50	75	60
SB / SW 75:25*	SW25	150	70
SB / CW 50:50**	CW50	50	80
SB / CW 75:25**	CW25	100	86

*Temperature of first barrel set to 100°C; ** Temperature kept consistent over all barrel segments

The feed rate was kept constant at 20 g/min and a die plate with three dies with a diameter of 2.00 mm and a length of 5.0 mm were used.

Table 7.5: Process parameters of extrusion experiment BaHav17-017

<i>Mixture [%; w/w]</i>	<i>Batch</i>	<i>Speed [rpm]</i>	<i>Temperature [°C]</i>						
			<i>Die</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>
SB / BW / PEG 6000 75:20:5	BW20	17	64	58	60	62	63	64	100
SB / BW / PEG 6000 75:25:0	BW25	25	64	60	62	64	65	66	100
SB / SW / PEG 6000 75:20:5	SW20	20	74	70	70	72	73	74	100
SB / SW / PEG 6000 75:25:0	SW25	25	74	68	70	72	73	74	100
SB / CW / PEG 6000 75:20:5	CW20	20	84	85	100	100	100	100	100
SB / CW / PEG 6000 75:25:0	CW25	20	84	85	100	100	100	100	100

The feed rate was kept constant at 10 g/min and a die plate with 91 dies with a diameter of 0.5 mm and a length of 1.35 mm were used.

7. Material and Methods

Table 7.6: Process parameter of extrusion experiment BaHav18-014b

Mixture [%; w/w]	Batch	Speed [rpm]	Temperature [°C]						
			Die	1	2	3	4	5	6
SB / BW 75:25	BW25	50	60	60	60	60	60	70	100
SB / SW 75:25	SW25	50	70	70	70	70	70	80	100
SB / CW 75:25	CW25	50	80	80	80	80	80	90	100

Feed rate was kept constant at 30 g / min and a die plate with 91 dies with a diameter of 0.5 mm and a length of 1.35 mm were used.

Table 7.7: Process parameters of extrusion experiment BaHav18-020

Mixture [%; w/w]	Batch	feed rate [g/min]	Speed [rpm]	Temperature [°C]						
				Die	1	2	3	4	5	6
SB / BW 75:25	BW25 fm	10	200	60	60	60	60	60	70	100
SB / BW 75:25	BW25 pm	20	70	50	50	50	50	50	50	50
SB / SW 75:25	SW25 fm	20	100	70	70	70	70	70	80	100
SB / SW 75:25	SW25 pm	20	200	60	60	60	60	60	60	25
SB / CW 75:25	CW25 fm	20	100	80	80	80	80	90	90	100
SB / CW 75:25	CW25 pm	20	200	70	70	70	70	70	50	30

A die plate with one die with a diameter of 1.0 mm and a length of 5.00 mm was used.

Table 7.8: Process parameter of extrusion experiment BaHav18-039

Mixture [%; w/w]	Batch	feed rate [g/min]	Speed [rpm]	Temperature [°C]						
				Die	1	2	3	4	5	6
SB / BW 75:25	BW25	30	50	60	60	60	60	60	70	100
SB / SW 75:25	SW25	30	50	70	70	70	70	70	80	100
SB / CW 75:25	CW25	30	50	80	80	80	80	80	90	100

Physical mixtures were blended for 20 min in a LM20 blender (LM20, LBB Bohle Maschinen + Verfahren GmbH, Ennigerloh, Germany). A die plate with 91 dies with a diameter of 0.5 mm and a length of 1.35 mm were used. The feed rate was set to 30 g/min and a speed of 50 rpm was used for all experiments.

Table 7.9 Process parameters of extrusion experiment BaHav19-005

Mixture [%; w/w]	Batch	Temperature [°C]							
		Die	1	2	3	4	5	6	
SB / BW 25:75	BW75_1.0mm	50	50	50	50	50	60	80	
SB / BW 50:50	BW50_1.0mm	60	60	60	60	60	60	80	
SB / BW 75:25	BW25_1.0mm	60	60	60	60	60	70	100	
SB / BW 75:25	BW25_0.5mm	60	60	60	60	60	70	100	
SB / SW 25:75	SW75_1.0mm	55	55	55	55	55	55	80	
SB / SW 50:50	SW50_1.0mm	60	60	60	60	60	60	80	
SB / SW 75:25	SW25_1.0mm	60	60	60	60	60	60	80	
SB / SW 75:25	SW25_0.5mm	70	70	70	70	70	70	100	
SB / CW 25:75	CW75_1.0mm	70	70	70	70	70	70	90	
SB / CW 50:50	CW50_1.0mm	70	70	70	70	70	70	90	
SB / CW 75:25	CW25_1.0mm	80	80	80	80	80	80	90	
SB / CW 75:25	CW25_0.5mm	80	80	80	80	80	90	90	

A die plate with 23 dies with a diameter of 1.0 mm and a length of 2.5 mm and a die plate with 91 dies with a diameter of 0.5 mm and a length of 1.35 mm were used. Feed rate was set to 30 g / min and 50 rpm were used for all experiments.

7. Material and Methods

Table 7.10 Process parameters of extrusion experiment BaHav19-028

Mixture (%; w/w)	Batch	Temperature [°C]							
		Die	1	2	3	4	5	6	
SB / BW 75:25	BW25_0.5mm	60	60	60	60	60	70	100	

A die plate with 91 dies with a diameter of 0.5 mm and a length of 1.35 mm were used. Feed rate was set to 30 g / min and 50 rpm was used for that experiment.

7.2.1.3 Spheronization

Spheronization (**BaHav19-015**) was performed with spheronizer RM-300 (Schlüter, Neustadt, Germany). A cross-hatched rotor plate of 300 mm in diameter was installed. For heating of extrudates an infrared-lamp (IR-Spot, Optron, Garbsen, Germany) or the water jacket of the spheronizer was used. Preliminary experiments were conducted using the following parameters: 600 seconds or 900 seconds of spheronization time. Spheronization speed was 1000 rpm. The IR-lamp was used at 70, 80 or 90%. 50 g of extrudates made of 25% BW, SW or CW and 75% SB with a diameter of either 0.5 mm or 1.0 mm (from experiment **BaHav19-005**) were used. As these preliminary experiments did not show promising results, the spheronization process was not further pursued.

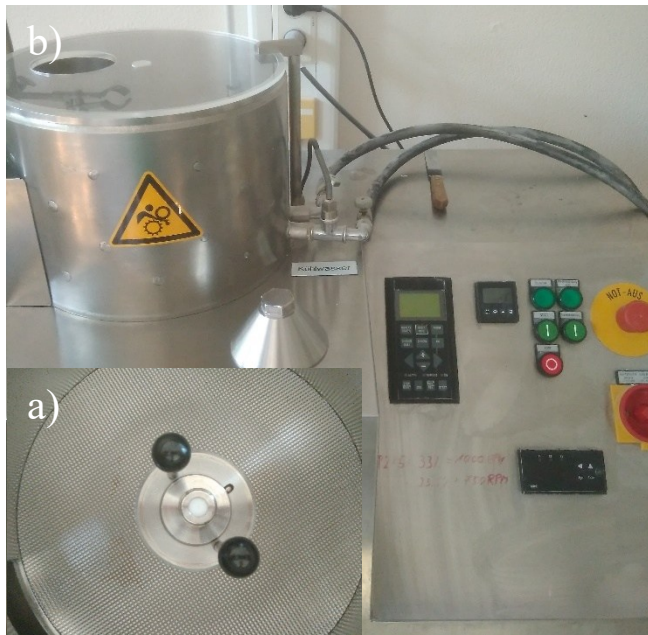


Figure 7.3: Rotor plate (a) and spheronizer RM-300 (b)

7.2.1.4 Tableting of mini-tablets

Before tableting, the fines were removed with a sieve of 250 μm mesh size. 1% magnesium stearate was added and mixed in a turbula blender (T2F, W.A. Bachofen, Muttensz, Switzerland) for 2 min.

The MTs of experiment **BaHav18-041** were produced by compressing granules of experiment **BaHav18-040** with a rotary die press (Pressima, IMA-Kilian, Cologne, Germany). 19-tips 2 mm bi-concave punches (Ritter Pharma-Technik, Stapelfeld, Germany) were used at a speed of 10 rpm with a compression force of 7-8 kN. A tablet height of 2 mm was targeted to obtain spherical like tablets.



Figure 7.4: Rotary tableting machine Pressima by IMA-Kilian

MTs for experiment **BaHav19-030** were produced with milled extrudates from experiment **BaHav19-005** on a uniaxial compaction simulator (Styl'One Evolution, Medelpharm, Beynost, France). The same punches as mentioned before were used at compression forces of 6 and 8 kN. A tablet height of 2 mm was targeted to obtain approximately spherical tablets.

7.2.1.5 Film-coating of mini-tablets

Coating (experiment **BaHav19-022**) was performed at the site of Evonik using a fluidized bed coater (Huettlin Mycrolab, Bosch, Stuttgart, Germany). The bottom spray method was used with a two-fluid nozzle and an inner nozzle diameter of 0.8 mm. 100.0 g of BW25Mt (**BaHav18-041**) were coated with 489.1 g of an organic coating suspension containing 49.99% Eudragit E 12.5, 1.9% macrogol 6000, 3.13% talcum and 44.98% of a mixture of acetone / isopropyl alcohol 40:60 (w/w). The coating was finished after 156 min at a coating weight gain of 48.9% . In Table 7.11, the corresponding process parameters are shown.

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Table 7.11: Process parameters of coating experiment

	Spray rate [g/min*kg ⁻¹]	Inlet air volume [m ³ /h]	Inlet air temperature [°C]	Exhaust air temperature [°C]
Pre-heating	-	20.0	34.1	22.5
Coating	31.4	20.0	36.0	25.0
Drying	-	23.1	35.0	25.6

The MTs were dedusted using an air jet sieve (200 LS-N, Hosokawa Alpine AG, Augsburg, Germany) with an under-pressure of 500 Pa for 3 min before the coating process.

7.2.2 Analytical methods

7.2.2.1 Differential scanning calorimetry

For insight into the crystalline properties of the used unprocessed and processed excipients, DSC-analysis was performed. Samples of approximately 5 mg were analyzed with a DSC1 (Mettler Toledo, Columbus, U.S.). Samples of experiment **BaHav17-017** were heated from -20°C to 120°C with a heating rate of 10°C/min, cooled down to -20°C with -20°C / min and then heated a second time with 10°C / min up to 120°C. In experiment **BaHav19-009**, samples were heated once from -20°C to 120°C with 10°C / min. For experiment **BaHav18-020** the samples were heated from 0°C to 100°C with 10°C / min, cooled with -10°C / min to -20°C and then heated up a second time to 100°C with 10°C / min. In experiment **BaHav19-040**, samples were heated from 0°C up to 100°C with a heat rate of 1°C / min, cooled down back to 0°C with the same rate and heated up a second time to 100°C with 1°C / min. All samples were measured twice to verify reproducibility. To improve comparability of the different measurements within a single graph, only data points recorded at x.0 °C and x.5 °C were included in the thermograms.

7.2.2.2 Solid lipid content calculation

The solid lipid content of waxes was analyzed using the data obtained from DSC (**BaHav19-009**) measurements. The AUC at a temperature of interest and the AUC after complete melting of the material were used in combination with the following equation to calculate the solid lipid content:

$$SFC = \frac{\int_{T_0}^{T_f} m dT}{\int_{T_0}^{T_f} m dT} * 100 \quad \text{Equation 7.1}$$

7.2.2.3 X-ray powder diffraction

Investigations of the crystalline properties of excipients and extrudates were conducted via x-ray powder diffractometry (X'Pert Pro MPD, PANalytical, Almelo, Netherlands). The measurement is based on the Bragg-Brentano principle, which means that source and detector adjust their position automatically. The sample holder is located centrally in between the radiation source and the detector, spinning around its own axis horizontally. A copper anode with a K α -radiation of $\lambda = 1.5406 \text{ \AA}$ was

used as source. All measurements were conducted in transmission mode in the range of $10\ 2\theta$ to $50\ 2\theta$ with an increment of $0.03\ 2\theta$. XRPD was performed in the following experiments: **BaHav17-017**, **BaHav18-029** and **BaHav19-017a**

7.2.2.4 Scanning electron microscope

The surface structure of extrudates and MTs before and after dissolution testing was investigated using a SEM (Phenom® G2 PRO Desktop SEM, Phenom World, Eindhoven, Netherlands). Small pieces of a sample were placed onto a sample-holder with an adhesive surface. The samples were gold sputter coated with a coating thickness of 12 nm at 5 mA in a MSC1T (LOT-Quantum Design, Darmstadt, Germany) and then analyzed in the SEM. The following experiments were conducted using the SEM: **BaHav17-008**, **BaHav19-010**. The samples of **BaHav19-020** were analyzed without prior gold sputter coating.

7.2.2.5 Flowing properties of physical mixtures

The bulk and tapped densities of all used excipients (**BaHav19-017b**) and their prepared mixtures (**BaHav19-027**) were determined according to European Pharmacopoeia (European Pharmacopoeia, 2023). A tapped density tester (STAV II, J. Engelsmann, Ludwigshafen am Rhein, Germany) was used. A sample mass of 100.0 g was transferred carefully into a 250 ml volumetric cylinder to measure bulk density. In case the bulk density was too low and 100.0 g could not fit into the 250 ml cylinder, the sample mass was reduced to 60% to 100% of the available volume. Tap density was measured via tapping the cylinder 10 (V_{10}), 500 (V_{500}) and 1250 (V_{1250}) times and recording the volume after each tapping increment. Tapping was continued in 1250-tapping steps if the difference in the last two recorded volumes exceeded 2 ml.

The Hausner-Factor and compressibility-index were calculated using the determined bulk- and tap-volumes and the equations below.

$$\text{Compressibility} - \text{index} = \frac{100(V_0 - V_f)}{V_0} \quad \text{Equation 7.2}$$

V_0 = bulk volume

V_f = final tap volume

$$\text{Hausner} - \text{factor} = \frac{V_0}{V_f} \quad \text{Equation 7.3}$$

7.2.2.6 Laser diffraction

Measuring the particle size and particle size distribution of excipients (**BaHav19-004b**) was performed via laser diffractometry (Mastersizer 3000, Malvern Instruments, United Kingdom). A dispersion pressure of 3.0 bar was used in a dry-dispersion unit. Particle size calculations were performed using Mie-theory. The calculation of the span was performed using the following equation (Heng *et al.*, 2001):

$$\text{span} = \frac{d_{90} - d_{10}}{d_{50}} \quad \text{Equation 7.4}$$

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Repeatability according to ISO 13320:2009 was determined by calculating the CV applying the following equation:

$$CV \text{ in } \% = \frac{SD}{\bar{x}} \times 100 \quad \text{Equation 7.5}$$

SD = standard deviation

$\bar{x}$ = mean value

7.2.2.7 Helium pycnometrie

The particle density (**BaHav18-008**) of the waxes and sodium benzoate was measured via helium pycnometry (AccuPyc, Micromeritics, Norcross, USA). A chamber with the size of 3.5 cm³ was filled to two thirds with the respective sample. Each sample was measured in triplicate. The value of each measurement was calculated as mean value from a quintuplicate determined by the apparatus during one measurement. All measurements were conducted at 25.0°C ± 0.1°C.

7.2.2.8 Taste evaluation

7.2.2.8.1 Measuring method

Taste evaluation was performed using an e-tongue (TS-5000z, Insent, Atsugi, Japan), which was equipped with taste sensors for bitterness, umami, saltiness, sourness and acidic bitterness (SB2AC0, SB2AAE, SB2CT0, SB2CA0, SB2C00 and SB2AE1, TecLabS, Essen, Germany). The sensors and a reference electrode were filled with an inner solution consisting of a saturated silver chloride solution containing 3.33 M potassium chloride.



Figure 7.5: E-tongue TS-5000z by Insent. Equipped with sensors by TecLabs

Before each measurement, the sensors were conditioned in a solution containing 30 mM potassium chloride and 0.3 mM tartaric acid for two days. The compositions of the sensor membranes are shown in Table 7.12. All cleaning solutions and the change of membrane potent caused by adsorption (CPA) solution contained 30 mM KCl and 0.3 mM tartaric acid. The positively charged conditioning

solution contained 100 mM potassium chloride and 10 mM potassium hydroxide dissolved in 30% ethanol (m/m). The negatively charged conditioning solution consisted of 100 mM hydrochloric acid dissolved in 30% ethanol. Each sample was measured five times. The following measuring routine was used: sensor dipping 90 s in cleaning solution 1, followed by immersion for 120 s each in cleaning solution 2 and cleaning solution 3. Afterwards, a sensor check was performed in the conditioning solution for 20 s. Then, the sample was measured for 30 s followed by 3 s in cleaning solution 4 and 5 each before the change of membrane potential caused by adsorption (CPA) values were measured in the CPA solution for 30 s.

Table 7.12 Compositions of taste sensors modified from Y. Kobayashi et al

Taste sensor	Artificial lipid	Plasticizer
Umami sensor AAE	Phosphoric acid di(2-ethylhexyl) ester Trioctylmethylammonium chloride	Diethyl phenylphosphonate
Saltiness Sensor CT0	Tetradodecylammonium bromide 1-Hexadecanol	Diethyl phenylphosphonate
Sourness sensor CA0	Phosphoric acid di(2-ethylhexyl) ester Trioctylmethylammonium chloride	Diethyl phenylphosphonate
Bitterness sensor C00 (for acidic bitter materials)	Tetradodecylammonium bromide	2-Nitrophenyl octyl ether
Astringency sensor AE1	Tetradodecylammonium bromide	Diethyl phenylphosphonate

7.2.2.8.2 Sample preparation for taste evaluation

Samples of extrudates in e-tongue experiment **BaHav19-008** were prepared as follows: a mass of the sample corresponding to a drug-load of 1.0 g sodium benzoate was weighed. The samples were then stirred for two minutes in 100 ml demineralized water, filtered and transferred into a receptacle for measurement with the e-tongue. Extrudate samples were taken from experiment **BaHav19-005**.

Samples of sodium benzoate in e-tongue experiment **BaHav19-008** were prepared by consecutively diluting a stock solution of 10.0 g SB in 100 ml demineralized water in logarithmic steps until solutions with concentrations of 10.0g /100 ml, 1.0 g / 100 ml, 100 mg / 100 ml and 10 mg / 100 ml were obtained.

The samples measured in experiment **BaHav19-014** were prepared as follows: 2.0 g of pure wax or 1.0 g of sodium benzoate were weighed. Each sample was then stirred for two min in 100 ml demineralized water, filtrated and transferred into a receptacle for measurement with the e-tongue. Extrudate samples were taken from experiment **BaHav19-005**.

Samples of MTs in experiment **BaHav19-024** and **-036** had been prepared as follows: a mass of the sample, which complied to 1.0 g of SB drug-load was weighed. The samples were then stirred for two minutes in 100 ml demineralized water, filtrated and transferred into a receptacle for

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measurement with the e-tongue. MTs in experiment **BaHav19-024** were taken from the experiments **BaHav18-041** and **BaHav19-022**. MTs in experiment **BaHav19-036** were taken from experiment **BaHav19-030**.

Table 7.13: Samples and their mass for taste evaluation of experiment BaHav19-008

Sample	Mass /100 ml
BW75_1.0mm	4.0 g
BW50_1.0mm	2.0 g
BW25_1.0mm	1.35 g
BW25_0.5mm	1.35 g
SW75_1.0mm	4.0 g
SW50_1.0mm	2.0 g
SW25_1.0mm	1.35 g
SW25_0.5mm	1.35 g
CW75_1.0mm	4.0 g
CW50_1.0mm	2.0 g
CW25_1.0mm	1.35 g
CW25_0.5mm	1.35 g
10 mg Sodium benzoate	10.0 mg
100 mg Sodium benzoate	100.0 mg
1.0 g Sodium benzoate	1.0 g
10.0 g Sodium benzoate	10.0 g

7.2.2.8.3 Data evaluation of taste assessment

The test results were expressed as the voltage change in mV exhibited by each sensor. A total of five measurement cycles were performed for each sample. The first two measurement cycles were discarded to avoid unstable data, while the other three were averaged. To eliminate external influences, a 0.5 mm quinine hydrochloride solution was measured as external standard. The value measured for quinine hydrochloride was subtracted from each value in order to exclude external factors such as evaporation over the term of measurement. All values represent the arithmetic mean of a triplicate issued in mV. Graphics and data evaluation were either done using OriginPro2020 (OriginLab Corporation, Northampton, United States) or using multivariate data analysis. For

multivariate data analysis, SIMCA® v.18.0.1 (Sartorius AG, Goettingen, Germany) was used. The data was pre-treated by mean centering and scaling to unit variance.

Table 7.14: Samples and their composition for taste evaluation of experiments BaHav19-024 & -036

Experiment	Sample	Mass
BaHav19-036	1g SB	1.0 g sodium benzoate
	BW25Mt6kN	1.35 g beeswax mini-tablets pressed at 6 kN
	BW25Mt8kN	1.35 g beeswax mini-tablets pressed at 8 kN
	SW25Mt6kN	1.35 g sugarcane wax mini-tablets pressed at 6 kN
	SW25Mt8kN	1.35 g sugarcane wax mini-tablets pressed at 8 kN
	CW25Mt6kN	1.35 g carnauba wax mini-tablets pressed at 6 kN
	CW25Mt8kN	1.35 g carnauba wax mini-tablets pressed at 8 kN
BaHav19-024	1g SB	1.0 g sodium benzoate
	BW25Mt	1.35 g beeswax mini-tablets
	BW25MtE	2.1 g beeswax mini-tablets with Eudragit® E coating

7.2.2.9 Assay

7.2.2.9.1 Analytical methods

Several different methods were used for assay analysis. An overview over the applied methods of the specific experiments can be seen in Table 7.15.

Table 7.15: Overview of applied methods for assay analysis

Method	Experiment
Method D	BaHav19-002
	BaHav19-021
Method E	BaHav19-032
	BaHav19-034

BaHav19-002: A single MT (**BaHav18-041**) was transferred into a flask with phosphate buffer pH 6.8 and then disintegrated by using an ultra-turrax (IKA-Werke, Staufen im Breisgau, Germany). The flask was then filled up to a total volume of 1000.0 ml phosphate buffer and a sample was drawn from the flask. The drawn sample was filtered with a 25 mm polypropylene membrane filter with a

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pore size of 0.45 μm (VWR, Langenfeld, Germany). **Method D:** Assay of samples was determined via high performance liquid chromatography (HPLC) (HPLC Elite LaChrom, VWR, Darmstadt, Germany) The system consisted of a L2130 pump, a L2200 auto-sampler, a 2300 oven and a L2400 UV-detector. Analysis was performed using a RP18 column (Symmetry Shield, WATER, Eschborn, Germany) 250 mm x 4.6 mm, 5 μm size of packing. Following parameters were used for analysis: flowrate 1.0 ml / min of acetonitrile / phosphate buffer pH 2.9-3.0 50:50 (v/v), detection at 225 nm, column temperature of 30°C and an injection volume of 20 μl . For calibration concentrations of 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.014, 0.016, 0.018, 0.02, 0.022 and 0.024 mg / ml were prepared by diluting a stock solution containing 200.2 mg SB in 1000.0 ml in phosphate buffer pH 6.8 in volumetric aliquots. All samples were measured in triplicate.

BaHav19-021: A single MT was transferred with demineralized water into a flask and then destroyed by using an ultra-turrax (IKA-Werke, Staufen im Breisgau, Germany). The flask was filled up to 1000.0 ml with demineralized water and a sample was drawn from the flask. It was filtered with a 25 mm polypropylene membrane filter with a pore size of 0.45 μm (VWR, Langenfeld, Germany). **Method D:** Assay of samples was determined via HPLC (HPLC Elite LaChrom, VWR, Darmstadt, Germany) The system consisted of a L2130 pump, a L2200 auto-sampler, a 2300 oven and a L2400 UV-detector. Analysis was performed using a RP18 column (Symmetry Shield, WATER, Eschborn, Germany) 250 mm x 4.6 mm, 5 μm size of packing. Following parameters were used for analysis: flowrate 1.0 ml / min of acetonitrile / phosphate buffer pH 2.9-3.0 50:50 (v/v), detection at 225 nm, column temperature of 30°C and an injection volume of 20 μl . For calibration concentrations of 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.014, 0.016, 0.018, 0.02, 0.022 and 0.024 mg / ml were prepared by diluting a stock solution containing 200 mg SB in 1000.0 ml in demineralized water in volumetric aliquots.

BaHav19-032 A single MT was transferred with demineralized water into a flask and then destroyed by using an ultra-turrax (IKA-Werke, Staufen im Breisgau, Germany). The flask was filled up to 1000.0 ml and a sample was drawn from the flask. It was filtered with a 25 mm polypropylene membrane filter with a pore size of 0.45 μm (VWR, Langenfeld, Germany). **Method E:** Content of samples was determined via HPLC (HPLC 1260 Infinity, Agilent, Santa Clara, United States). The system consisted of a G1312B bin pump, a G1329B auto-sampler, a G1316A oven and a G4212B diodearray-detector (DAD). For analysis the same column like in method D was used. Following parameters were used: flowrate 1.0ml / min of acetonitrile / phosphate buffer pH 2.9 60:40 (v/v), detection at 225nm, column temperature of 30°C and an injection volume of 20 μl . For calibration concentrations of 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.014, 0.016, 0.018, 0.02, 0.022 and 0.024 mg / ml were prepared by diluting a stock solution containing 200.2 mg SB in 1000.0 ml in phosphatebuffer pH 6.8. All samples were measured in triplicate.

BaHav19-034. Approximately 1 g of each fraction < 250 μm of the milled extrudates of BaHav18-014b was transferred with demineralized water into a flask. The flask was filled up to 1000.0 ml with demineralized water and a sample was drawn from the flask. It was filtered with a 25 mm polypropylene membrane filter with a pore size of 0.45 μm (VWR, Langenfeld, Germany) Samples were diluted 1:100 with demineralized water and analyzed in triplicates. **Method E:** Content of samples was determined via HPLC (HPLC 1260 Infinity, Agilent, Santa Clara, United States). The system consisted of a G1312B bin pump, a G1329B auto-sampler, a G1316A oven and a G4212B diodearray-detector (DAD). For analysis the same column like in method D was used. Following parameters were used: flowrate 1.0ml / min of acetonitrile / phosphate buffer pH 2.9 60:40 (v/v),

detection at 225nm, column temperature of 30°C and an injection volume of 20 µl. For calibration concentrations of 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.014, 0.016, 0.018, 0.02, 0.022 and 0.024 mg / ml were prepared by diluting a stock solution containing 200.2 mg in 1000.0 ml in phosphate buffer pH 6.8. All samples were measured in triplicate.

7.2.2.10 Dissolution testing

All dissolution tests were performed at $37.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, 50 rpm using the basket apparatus (USP 1). If the temperature was varied from 37°C it is explained below. Other parameters were varied for different experiments and are explained below. Phosphatebuffer pH 6.8 was made as follows: 17.0 g of potassium-dihydrogen-phosphate and 22.13g of Di-sodiumhydrogenphosphate were dissolved in a 5.0L volumetric flask. Before filling up to 5.0L 24 drops of phosphoric acid 85% were added.

All HPLC-vials containing samples were prepared as follows: From each collected dissolution sample 3 ml were pulled with a syringe and pushed into an HPLC vial through a 25 mm polypropylene membrane filter with a pore size of 0.45 µm (VWR, Langenfeld, Germany). HPLC-vials were analyzed in triplicate

BaHav17-011: dissolution testing was performed with an Erweka DT 700 (Heusenstamm, Germany) in 900 ml phosphate buffer pH 6.8 for 24 h. Samples of 5.0 ml were taken every 10 min (0-120min), every 1 h (120-700min) and once after 24 h had passed. Removed medium was refilled. n = 6. Samples were analysed applying the following method. **Method A:** Content of samples was measured at-line with an UV/Vis-spectrometer (UV-1800, Shimadzu, Kyoto, Japan) at 232 nm. For calibration a stock solution with 20.47 mg sodium benzoate in 100.0 ml phosphate buffer pH 6.8 was prepared. Volumetric aliquots were diluted separately with 100.0 ml phosphate buffer pH 6.8 to obtain the following concentrations: 0.002, 0.004, 0.006, 0.010 and 0.016 and 0.020 mg / ml. All samples were measured in triplicates.

BaHav17-018: dissolution was performed with a Erweka DT 700 (Heusenstamm, Germany) in 900 ml phosphate buffer pH 6.8 for 120 min. Samples of 10.0 ml were taken every 10 min and removed medium was replaced with fresh buffer. n = 6. Samples were analysed applying the following method. **Method A:** Content of samples was measured at-line with an UV/Vis-spectrometer (UV-1800, Shimadzu, Kyoto, Japan) at 232 nm. For calibration a stock solution with 20.47 mg sodium benzoate in 100.0 ml phosphate buffer pH 6.8 was prepared. Volumetric aliquots were diluted separately with 100.0 ml phosphate buffer pH 6.8 to obtain the following concentrations: 0.002, 0.004, 0.006, 0.010 and 0.016 and 0.020 mg / ml. All samples were measured in triplicates.

BaHav18-021: dissolution testing was performed with an Erweka DT 700 (Heusenstamm, Germany) in 900 ml phosphate buffer pH 6.8 + 0.001% Polysorbate 20 for 12 h. n = 6. Samples were measured applying the following method. **Method B:** Content of samples was measured in-line with an UV/Vis-spectrometer (Lambda 40, Perkin Elmer, Waltham, United States) at 232 nm every 60 seconds. For calibration a stock solution with 200.36 mg sodium benzoate in 1000.0 ml phosphate buffer pH 6.8 was prepared. Volumetric aliquots were diluted separately with 100.0 ml phosphate buffer pH 6.8 to obtain the following concentrations: 0.002, 0.004, 0.006, 0.008, 0.010, 0.012, 0.014, 0.016, 0.018 and 0.020mg / ml. All samples were measured in triplicates

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BaHav18-027: dissolution testing was performed with an IKA Eurostar 20 digital (IKA-Werke, Staufen im Breisgau, Germany) in 900 ml of phosphate buffer pH 6.8 for 10 min. $n = 3$. Samples were analyzed applying the following method. **Method C:** In-line UV-analysis of dissolution was performed using an UV-probe (T 300 dip probe, 5mm tip, USB 4000 detector, DH 2000 BAL light source, Ocean Optics, Dunedin, USA). Measurement was performed at 232 nm. Data was read out with the software “Spectra suite” (Ocean Optics, Dunedin, USA). Integration time was set to 70 ms. A mean value calculated over one second was used for analysis. For calibration a stock solution with 200.0 mg sodium benzoate in 1000.0 ml phosphate buffer pH 6.8 was prepared. Volumetric aliquots were diluted separately with 100.0 ml phosphate buffer pH 6.8 to obtain the following concentrations: 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.014, 0.016, 0.018 and 0.02 mg / ml. All samples were measured in triplicates. The probe was placed one centimeter away from the wall of the vessel and 2 cm beneath the surface of the buffer

BaHav18-042: dissolution testing was performed with an Erweka DT 700 (Heusenstamm, Germany) in 900 ml phosphate buffer pH 6.8 + 0.001% Polysorbate 20 for 24h. Samples of 5.0 ml were taken every 10 min (0-120 min), at 300 min and at 1440 min. Removed medium was replaced with fresh buffer. $n = 6$. Samples were measured applying the following method. **Method D:** Assay of samples was determined via HPLC (HPLC Elite LaChrom, VWR, Darmstadt, Germany) The system consisted of a L2130 pump, a L2200 auto-sampler, a 2300 oven and a L2400 UV-detector. Analysis was performed using a RP18 column (Symmetry Shield, WATER, Eschborn, Germany) 250 mm x 4.6 mm, 5 μ m size of packing. Following parameters were used for analysis: flowrate 1.0 ml / min of acetonitrile / phosphate buffer pH 2.9-3.0 50:50 (v/v), detection at 225 nm, column temperature of 30°C and an injection volume of 20 μ l. For calibration concentrations of 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.014, 0.016, 0.018, 0.02 mg / ml were prepared by diluting a stock solution containing 200.1 mg in 1000.0 ml in phosphate buffer pH 6.8 in volumetric aliquots.

BaHav19-006: Dissolution was performed with a RW20 DZM and a IKA Eurostar 20 digital (IKA-Werke, Staufen im Breisgau, Germany) in 900 ml phosphate buffer pH 6.8 for 120 min. Samples of 5.0 ml were taken every 10min and removed medium was replaced with fresh buffer. $n = 6$. Samples were analyzed applying the following method. **Method D:** Content of samples was determined via HPLC (HPLC Elite LaChrom, VWR, Darmstadt, Germany) The system consisted of a L2130 pump, a L2200 auto-sampler, a 2300 oven and a L2400 UV-detector. Analysis was performed using a RP18 column (Symmetry Shield, WATER, Eschborn, Germany) 250 mm x 4.6 mm, 5 μ m size of packing. Following parameters were used for analysis: flowrate 1.0 ml / min of acetonitrile / phosphate buffer pH 2.9-3.0 50:50 (v/v), detection at 225 nm, column temperature of 30°C and an injection volume of 20 μ l. For calibration concentrations of 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.014, 0.016, 0.018, 0.02, 0.022 and 0.024 mg / ml were prepared by diluting a stock solution containing 200.1 mg in 1000.0 ml phosphate buffer pH 6.8 in volumetric aliquots.

BaHav19-033: Dissolution was performed with a RW20 DZM and a IKA Eurostar 20 digital (IKA-Werke, Staufen im Breisgau, Germany) in 900 ml phosphate buffer pH 6.8 for 120 min. Samples of 5.0 ml (BW25MT6.7kn sample) or 2.0 ml (all remaining samples) were taken every 10 min and removed medium was replaced with fresh buffer. $n = 6$. Samples were analysed applying the following method. **Method E:** Content of samples was determined via HPLC (HPLC 1260 Infinity, Agilent, Santa Clara, United States). The system consisted of a G1312B bin pump, a G1329B auto-sampler, a G1316A oven and a G4212B diodenarray-detector (DAD). Analysis was performed using a RP18 column (Symmetry Shield, WATER, Eschborn, Germany) 250 mm x 4.6 mm, 5 μ m size of

packing. Following parameters were used: flowrate 1.0 ml / min of acetonitrile / phosphate buffer pH 2.9 60:40 (v/v), detection at 225 nm, column temperature of 30°C and an injection volume of 20 µl. For calibration concentrations of 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.014, 0.016, 0.018, 0.02, 0.022 and 0.024 mg / ml were prepared by diluting a stock solution containing 200.2 mg in 1000.0 ml in phosphate buffer pH 6.8 in volumetric aliquots.

BaHav19-019: Dissolution was performed with a RW20 DZM and a IKA Eurostar 20 digital (IKA-Werke, Staufen im Breisgau, Germany) in either 900 ml demineralized water or 0.1 N hydrochloric acid for 120 min. Samples were taken every 5 min from minute 0 -30 and every 30 min from minute 30 to 120 and removed medium was replaced with fresh medium. n = 6. Samples were analyzed applying the following method. **Method D:** Content of samples was determined via HPLC (HPLC Elite LaChrom, VWR, Darmstadt, Germany) The system consisted of a L2130 pump, a L2200 auto-sampler, a 2300 oven and a L2400 UV-detector. Analysis was performed using a RP18 column (Symmetry Shield, WATER, Eschborn, Germany) 250 mm x 4.6 mm, 5 µm size of packing. Following parameters were used for analysis: flowrate 1.0 ml / min of acetonitrile / phosphate buffer pH 2.9-3.0 50:50 (v/v), detection at 225 nm, column temperature of 30°C and an injection volume of 20 µl. For calibration concentrations of 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.014, 0.016, 0.018, 0.02, 0.022 and 0.024 mg / ml were prepared by diluting a stock solution containing 200.1 mg in 1000.0 ml in phosphate buffer pH 6.8 in volumetric aliquots. A second stock solution was prepared the same way but using demineralized water as medium instead of phosphate buffer pH 6.8

BaHav19-029: Dissolution of extrudates from experiment **BaHav19-028** was performed with a RW20 DZM and a IKA Eurostar 20 digital (IKA-Werke, Staufen im Breisgau, Germany) in either 900 ml demineralized water at 22 °C ± 0.5 °C (n = 6) or in 900 ml demineralized water at 37°C ± 0.5°C (n = 3) or in phosphate buffer pH 6.8 at 37°C ± 0.5°C (n = 3). Time of dissolution testing was 10 minutes. Samples were analyzed applying the following method. **Method D:** Content of samples was determined via HPLC (HPLC Elite LaChrom, VWR, Darmstadt, Germany) The system consisted of a L2130 pump, a L2200 auto-sampler, a 2300 oven and a L2400 UV-detector. Analysis was performed using a RP18 column (Symmetry Shield, WATER, Eschborn, Germany) 250 mm x 4.6 mm, 5 µm size of packing. Following parameters were used for analysis: flowrate 1.0 ml / min of acetonitrile / phosphate buffer pH 2.9-3.0 50:50 (v/v), detection at 225 nm, column temperature of 30 °C and an injection volume of 20 µl. For calibration concentrations of 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.014, 0.016, 0.018, 0.02, 0.022 and 0.024 mg / ml were prepared by diluting a stock solution containing 200.2 mg in 1000.0 ml in phosphate buffer pH 6.8 in volumetric aliquots.

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7.2.2.11 Calculation of confidence interval

The confidence interval was calculated using the following equation.

$$\bar{x} \pm t_{f, 1-\alpha/2} \cdot \frac{s}{\sqrt{n}} \text{ Equation 7.6}$$

$\bar{x}$ = mean value

n = sample size

t = t-value

α = level of significance

f = degree of freedom

s = standard deviation

7.2.2.12 Tensile strength of mini-tablets

The tensile strength was measured with a texture analyser (TA.XTplus, Stable Micro Systems, Goldaming, United Kingdom) (**BaHav19-031**). A flat punch of 5 mm diameter and a selected pre-speed of 0.1 mm/s was used. Tensile strength was calculated according to Fell and Newton (Fell and Newton, 1970) and measured on the MTs from experiment **BaHav19-030**.

7.2.2.13 Raman-microscopy

The distribution of SB-molecules within a BW-matrix was analyzed by placing a 500 μm tall cross-section of a BW extrudate (**BaHav19-028**) into a 500 μm drill hole and scanning an area of 550 μm times 150 μm of this cross section using an alpha300 R confocal Raman microscope (**BaHav19-041**). An integration time of 20 ms per spectrum was used and a spatial resolution of 2 μm . A fiber-coupled single-mode diode laser with 532 nm excitation wavelength was used for the measurement, applying 10 mW of laser power to the sample. A Zeiss EC Epiplan-Neofluar Dic 50x / 0.8 NA microscope objective as well as a WITec UHTS 300 spectrometer and an Andor iDus Deep Depletion CCD detector were used. For spectral dispersion of the Raman scattered light, a reflection grating with 600 lines/mm was used. The measurement was performed with an average spectral resolution of 3.8 cm^{-1} /pixel. For the reference spectra, pure sodium benzoate and BW were measured separately.

8 References

Administration, U. S. F. a. D.; (2019); CFR 21 Part 184 - Direct food substances affirmed as generally recognized as safe - subpart B, Sec. 184.1973 Beeswax (yellow or white); USA; U.S. department of health and human services

Aitken-Nichol, C.; Zhang, F. and McGinity, J. W.; Hot melt extrusion of acrylic films; *Pharmaceutical Research*; 13 (1996); pp. 804–808.

Akombatwa, N.; Muungo, L. T.; Nyirenda, J.; Muwowo, S.; Chichonyi, A. K.; Mukosha, M. and Mwila, C.; Formulation and assessment of the efficacy and stability of an ointment containing ocimum americanum L. extract; *Clinical Complementary Medicine and Pharmacology*; 3 (2023); pp. 11.

AlphaMOS; Technical note T-SAS-04; (2004); pp.

Alzahrani, A.; Nyavanandi, D.; Mandati, P.; Youssef, A. A. A.; Narala, S.; Bandari, S. and Repka, M.; A systematic and robust assessment of hot-melt extrusion-based amorphous solid dispersions: theoretical prediction to practical implementation; *Int J Pharm*; 624 (2022); pp. 121951.

Anastas, P. T. and Warner, J. C.; Green chemistry: theory and practice; Oxford university press; (1998).

Applegarth, D. A. and Toone, J. R.; Nonketotic hyperglycinemia (glycine encephalopathy): laboratory diagnosis; *Molecular Genetics and Metabolism*; 74 (2001); pp. 139–146.

Arhewoh, M. I.; Eraga, S. O.; Avbunudiogba, J. A.; Fregene, Q. and Okah, P.; Preparation and evaluation of matrix tablet of metronidazole using carnauba wax; *British Journal of Pharmaceutical Research*; 8 (2015); pp.

Arrhenius, S. and Holden, E. S.; On the influence of carbonic acid in the air upon the temperature of the earth; *Publications of the Astronomical Society of the Pacific*; 9 (1897); pp. 14–24.

Arruzazabala, M. L.; Molina, V.; Mas, R.; Fernandez, L.; Carbajal, D.; Valdes, S. and Castano, G.; Antiplatelet effects of policosanol (20 and 40 mg/day) in healthy volunteers and dyslipidaemic patients; *Clinical and Experimental Pharmacology and Physiology*; 29 (2002); pp. 891–897.

Ashour, E. A.; Majumdar, S.; Alsheteli, A.; Alshehri, S.; Alsulays, B.; Feng, X.; Gryczke, A.; Kolter, K.; Langley, N. and Repka, M. A.; Hot melt extrusion as an approach to improve solubility, permeability and oral absorption of a psychoactive natural product, piperine; *Journal of Pharmacy and Pharmacology*; 68 (2016); pp. 989–998.

Asikin, Y.; Takahashi, M.; Hirose, N.; Hou, D. X.; Takara, K. and Wada, K.; Wax, policosanol, and long-chain aldehydes of different sugarcane (*Saccharum officinarum* L.) cultivars; *European Journal of Lipid Science and Technology*; 114 (2012); pp. 583–591.

Attama, A. A. and Muller-Goymann, C. C.; Effect of beeswax modification on the lipid matrix and solid lipid nanoparticle crystallinity; *Colloids and Surfaces A: Physicochemical and Engineering Aspects*; 315 (2008); pp. 189–195.

8. References

- Attard, T. M.; McElroy, C. R.; Rezende, C. A.; Polikarpov, I.; Clark, J. H. and Hunt, A. J.; Sugarcane waste as a valuable source of lipophilic molecules; *Industrial Crops and Products*; 76 (2015); pp. 95–103.
- Awadallak, J. A.; Voll, F.; Ribas, M. C.; da Silva, C.; Cardozo, L. and da Silva, E. A.; Enzymatic catalyzed palm oil hydrolysis under ultrasound irradiation: Diacylglycerol synthesis; *Ultrasonics Sonochemistry*; 20 (2013); pp. 1002–1007.
- Bagde, A.; Patel, K.; Kutlehria, S.; Chowdhury, N. and Singh, M.; Formulation of topical ibuprofen solid lipid nanoparticle (SLN) gel using hot melt extrusion technique (HME) and determining its anti-inflammatory strength; *Drug Delivery and Translational Research*; 9 (2019); pp. 816–827.
- Balducci, A. G.; Colombo, G.; Corace, G.; Cavallari, C.; Rodriguez, L.; Buttini, F.; Colombo, P. and Rossi, A.; Layered lipid microcapsules for mesalazine delayed-release in children; *International Journal of Pharmaceutics*; 421 (2011); pp. 293–300.
- Baranello, G.; Alfei, E.; Martinelli, D.; Rizzetto, M.; Cazzaniga, F.; Dionisi-Vici, C.; Gellera, C. and Castellotti, B.; Hyperargininemia: 7-month follow-up under sodium benzoate therapy in an italian child presenting progressive spastic paraparesis, cognitive decline, and novel mutation in gene; *Pediatric Neurology*; 51 (2014); pp. 430–433.
- Bartl, B.; Kobera, L.; Drabkova, K.; Durovic, M. and Brus, J.; 'Wax bloom' on beeswax cultural heritage objects: exploring the causes of the phenomenon; *Magnetic Resonance in Chemistry*; 53 (2015); pp. 509–513.
- Bartl, B.; Trejbal, J.; Durovic, M.; Vasickova, S. and Valterova, I.; Analysis of efflorescence on surface of beeswax seals; *Journal of Cultural Heritage*; 13 (2012); pp. 275–284.
- Basson, I. and Reynhardt, E. C.; An investigation of the structures and molecular-dynamics of natural waxes .1. Beeswax; *Journal of Physics D: Applied Physics*; 21 (1988); pp. 1421–1428.
- Basson, I. and Reynhardt, E. C.; An Investigation of the structures and molecular dynamics of natural waxes .2. Carnauba wax; *Journal of Physics D: Applied Physics*; 21 (1988); pp. 1429–1433.
- Batshaw, M. L.; MacArthur, R. B. and Tuchman, M.; Alternative pathway therapy for urea cycle disorders: Twenty years later; *Journal of Pediatrics*; 138 (2001); pp. S46–S54.
- Baumer-Harrison, C.; Raymond, M. A.; Myers, T. A.; Sussman, K. M.; Rynberg, S. T.; Ugartechea, A. P.; Lauterbach, D.; Mast, T. G. and Breza, J. M.; Optogenetic stimulation of type I GAD65 cells in taste buds activates gustatory neurons and drives appetitive licking behavior in sodium-depleted mice; *Journal of Neuroscience*; 40 (2020); pp. 7795–7810.
- Belkhir, L. and Elmeligi, A.; Carbon footprint of the global pharmaceutical industry and relative impact of its major players; *Journal of Cleaner Production*; 214 (2019); pp. 185–194.
- Bertoni, S.; Passerini, N. and Albertini, B.; Liquid lipids act as polymorphic modifiers of tristearin-based formulations produced by melting technologies; *Pharmaceutics*; 13 (2021); pp. 1089.
- Bhagurkar, A. M.; Repka, M. A. and Murthy, S. N.; A novel approach for the development of a nanostructured lipid carrier formulation by hot-melt extrusion technology; *J Pharm Sci*; 106 (2017); pp. 1085–1091.

- Bhardwaj, R. and Blanchard, J.; In vitro evaluation of poly(D,L-lactide-co-glycolide) polymer-based implants containing the alpha-melanocyte stimulating hormone analog, melanotan-I; *Journal of Controlled Release*; 45 (1997); pp. 49–55.
- Bogdanov, S.; (2016); Beeswax: history, uses and trade; in: *Online Beeswax book: Chapter 2*; 2; bee-hexagon.net - Bee Product Science:
- Boneh, A.; Degani, Y. and Harari, M.; Prognostic clues and outcome of early treatment of nonketotic hyperglycinemia; *Pediatric Neurology*; 15 (1996); pp. 137–141.
- Bordonal, R. D.; Carvalho, J. L. N.; Lal, R.; de Figueiredo, E. B.; de Oliveira, B. G. and La Scala, N.; Sustainability of sugarcane production in brazil. a review; *Agronomy for Sustainable Development*; 38 (2018); pp. 13.
- Breitkreutz, J. and Boos, J.; Paediatric and geriatric drug delivery; *Expert Opinion on Drug Delivery*; 4 (2007); pp. 37–45.
- Breitkreutz, J.; Bornhoft, M.; Woll, F. and Kleinebudde, P.; Pediatric drug formulations of sodium benzoate: I. coated granules with a hydrophilic binder; *European Journal of Pharmaceutics and Biopharmaceutics*; 56 (2003); pp. 247–253.
- Breitkreutz, J.; El-Saleh, F.; Kiera, C.; Kleinebudde, P. and Wiedey, W.; Pediatric drug formulations of sodium benzoate: II. coated granules with a lipophilic binder; *European Journal of Pharmaceutics and Biopharmaceutics*; 56 (2003); pp. 255–260.
- Breitkreutz, J.; Wessel, T. and Boos, J.; Dosage forms for per oral administration to children.; *Paediatric Perinatal Drug Therapy*; 3 (1999); pp. 25–33.
- Buckton, G.; (2018); Solid-state properties; in: Aulton, M. E. and Taylor, K. M. G. (Eds.) *Aulton's Pharmaceutics: The Design and Manufacture of Medicines*; Edinburgh, Churchill Livingstone - Elsevier: 128–139
- Butterhof, C.; Milius, W. and Breu, J.; Co-crystallisation of benzoic acid with sodium benzoate: the significance of stoichiometry; *Crystengcomm*; 14 (2012); pp. 3945–3950.
- Callendar, G. S.; The artificial production of carbon dioxide and its influence on temperature; *Quarterly Journal of the Royal Meteorological Society*; 64 (1938); pp. 223–240.
- Cassier, P. and Lensky, Y.; Ultrastructure of the wax gland complex and secretion of beeswax in the worker honey-bee *apis-mellifera* L; *Apidologie*; 26 (1995); pp. 17–26.
- Chaudhari, N.; Pereira, E. and Roper, S. D.; Taste receptors for umami: the case for multiple receptors; *American Journal of Clinical Nutrition*; 90 (2009); pp. 738s–742s.
- Chauhan, A. and Chauhan, P.; Powder XRD technique and its applications in science and technology; *Journal of Analytical & Bioanalytical Techniques*; 5 (2014); pp. 1–5.
- Chemical Abstract Service *Beeswax*;15.Dec.2024;
https://commonchemistry.cas.org/detail?cas_rn=8012-89-3&search=beeswax
- Chemical Abstract Service *Carnauba wax*;15.Dec.2024;
https://commonchemistry.cas.org/detail?cas_rn=8015-86-9&search=Carnauba wax

8. References

- Chinnadurai, C.; (2017); Potential Health Benefits of Sugarcane; in: Mohan, C. (Ed.) *Sugarcane Biotechnology: Challenges and Prospects*; 1; - Springer: 1–2
- Cholakova, D. and Denkov, N.; Polymorphic phase transitions in triglycerides and their mixtures studied by SAXS/WAXS techniques: In bulk and in emulsions; *Advances in Colloid and Interface Science*; 323 (2024); pp. 103071.
- Choy, Y. W.; Khan, N. and Yuen, K. H.; Significance of lipid matrix aging on in vitro release and in vivo bioavailability; *International Journal of Pharmarmaceutics*; 299 (2005); pp. 55–64.
- Cordeiro, A. P.; Feuser, P. E.; Araújo, P. H. H.; dos Santos, D. C.; Ourique, F.; Hübner, L. J.; Pedrosa, R. C. and Sayer, C.; Doxorubicin and 4-nitrochalcone loaded in beeswax-based nanostructured lipid carriers: In vitro antitumoral screening and evaluation of synergistic effect on HepG-2 cells; *International Journal of Pharmaceutics*; 666 (2024); pp. 124788.
- Coughlin, C. R.; Swanson, M. A.; Kronquist, K.; Acquaviva, C.; Hutchin, T.; Rodríguez-Pomba, P.; Väisänen, M. L.; Spector, E.; Creadon-Swindell, G.; Brás-Goldberg, A. M.; Rahikkala, E.; Moilanen, J. S.; Mahieu, V.; Matthijs, G.; Bravo-Alonso, I.; Pérez-Cerdá, C.; Ugarte, M.; Vianey-Saban, C.; Scharer, G. H. and Van Hove, J. L. K.; The genetic basis of classic nonketotic hyperglycinemia due to mutations in GLDC and AMT; *Genetics in Medicine*; 19 (2017); pp. 104–111.
- Craig, D. Q. M. and Newton, J. M.; Characterization of Polyethylene Glycols Using Differential Scanning Calorimetry; *International Journal of Pharmaceutics*; 74 (1991); pp. 33–41.
- Crowley, M. M.; Zhang, F.; Koleng, J. J. and McGinity, J. W.; Stability of polyethylene oxide in matrix tablets prepared by hot-melt extrusion; *Biomaterials*; 23 (2002); pp. 4241–4248.
- Crowley, M. M.; Zhang, F.; Repka, M. A.; Thumma, S.; Upadhye, S. B.; Battu, S. K.; McGinity, J. W. and Martin, C.; Pharmaceutical applications of hot-melt extrusion: Part I; *Drug Development and Industrial Pharmacy*; 33 (2007); pp. 909–926.
- de Boer, G. B. J.; de Weerd, C.; Thoenes, D. and Goossens, H. W. J.; Laser diffraction spectrometry: fraunhofer diffraction versus mie scattering; *Particle & Particle Systems Characterization*; 4 (1987); pp. 14–19.
- De Oliveira, M. E. D.; Vaughan, B. E. and Rykiel, E. J.; Ethanol as fuels: energy, carbon dioxide balances, and ecological footprint; *Bioscience*; 55 (2005); pp. 593–602.
- Deshpande, A. H. and Wasule, D. D.; Formulation and evaluation of wax matrix fast dissolving mini-tablets of montelukast sodium; *Asian Journal of Pharmaceutics*; 11 (2017); pp. S107–S112.
- DEUREX (2018); *DEUREX X 52 Sugarcane wax*; Safety data sheet; <https://www.deurex.de/wachse/zuckerrohrwachse/>
- Deutsche Gesellschaft für Fettwissenschaft (1974); *Deutsche Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten und verwandten Stoffen*;
- Diepeveen, J.; Moerdijk-Poortvliet, T. C. W. and van der Leij, F. R.; Molecular insights into human taste perception and umami tastants: A review; *Journal of Food Science*; 87 (2022); pp. 1449–1465.
- Dredan, J.; Antal, I. and Racz, I.; Evaluation of mathematical models describing drug release from lipophilic matrices; *International Journal of Pharmaceutics*; 145 (1996); pp. 61–64.

- European Commission; (2019); *The European Green Deal*; Brussels; European Union
- European Commission (2020); *Green Deal: Commission adopts new chemicals strategy towards a toxic-free environment*; 29.Sep.2024;
https://ec.europa.eu/commission/presscorner/detail/en/ip_20_1839
- European Commission; (2020); *A new circular economy action plan*; Brussel; European Commission
- European Food Safety Authority; Beeswax (E 901) as a glazing agent and as carrier for flavours; *The EFSA Journal*; 615 (2007); pp. 1–28.
- European Food Safety Authority; Scientific opinion on the re-evaluation of candelilla wax (E 902) as a food additive; *The EFSA Journal*; 10 (2012); pp.
- European Food Safety Authority; Scientific opinion on the re-evaluation of carnauba wax (E903) as a food additive; *The EFSA Journal*; 10 (2012); pp. 2880–2903.
- European Medicines Agency; (2006); *Reflection paper: formulations of choice for the paediatric population*; EMA/CHMP/PEG/194810/2005; Committee for Medicinal Products for Human Use (CHMP)
- European Medicines Agency; (2013); *Guideline on pharmaceutical development of medicines for paediatric use*; EMA/CHMP/QWP/805880/2012 Rev.2.; Committee for Medicinal Products for Human Use (CHMP) and Paediatric Committee (PDCO)
- European Medicines Agency; (2018); *ICH E11(R1) guideline on clinical investigation of medicinal products in the pediatric population*; EMA/CPMP/ICH/2711/1999; Products, C. f. H. M.
- European Parliament and the Council; (2006); *Paediatric Regulation (EC) No 1901/2006 of the European Parliament and of the Council of [12] December 2006, on medicinal products for paediatric use and amending Regulation (EEC) No 1768/92, Directive 2001/20/EC, Directive 2001/83/EC and Regulation (EC) No 726/2004*; Official Journal of the European Union L 378/1;
- European Pharmacopoeia; E.P., (2023); *European Pharmacopoeia: 2.5.6 Saponification number*; Strasbourg, France; Europe, C. o.
- European Pharmacopoeia; E.P., (2023); *European Pharmacopoeia: 2.9.36 Powder flow*; Strasbourg, France; Council of Europe
- European Pharmacopoeia; E.P. (2023); *European Pharmacopoeia: Bienenwachs*; Strasbourg, France; Council of Europe
- European Pharmacopoeia; E.P. (2023); *European Pharmacopoeia: Carnaubawachs*; Strasbourg, France; Council of Europe
- Fahr, A.; (2018); 3.2 Pharmaceutical solids; in: Fahr, A. (Ed.) *Voigt's Pharmaceutical Technology; 3. Physical and Physicochemical Principles of Drug Formulation*; Germany - Wiley: 44–55
- Fahr, A.; (2018); 8.6 Test methods for powders and granules; in: Fahr, A. (Ed.) *Voigt's Pharmaceutical Technology; 8. Powders and Granules*; Germany - Wiley: 335–337

8. References

- Fahr, A.; (2018); 9.2 Excipients in tablet manufacturing; in: Fahr, A. (Ed.) *Voigt's Pharmaceutical Technology*; 9. Tablets; Germany - Wiley: 347–350
- Fietz, A. (2020); *Sugarcane field*; 11.Aug.2024; <https://pixabay.com/de/photos/zuckerrohr-kulturpflanzen-ackerland-5525004/>
- Flaherty, B.; Characterisation of waxes by differential scanning calorimetry; *Journal of Applied Chemistry and Biotechnology*; 21 (1971); pp. 144–148.
- Flusser, H.; Korman, S. H.; Sato, K.; Matsubara, Y.; Galil, A. and Kure, S.; Mild glycine encephalopathy (NKH) in a large kindred due to a silent exonic GLDC splice mutation; *Neurology*; 64 (2005); pp. 1426–1430.
- Follonier, N.; Doelker, E. and Cole, E. T.; Evaluation of hot-melt extrusion as a new technique for the production of polymer-based pellets for sustained-release capsules containing high loadings of freely soluble drugs; *Drug Development and Industrial Pharmacy*; 20 (1994); pp. 1323–1339.
- Follonier, N.; Doelker, E. and Cole, E. T.; Various ways of modulating the release of diltiazem hydrochloride from hot-melt extruded sustained-release pellets prepared using polymeric materials; *Journal of Controlled Release*; 36 (1995); pp. 243–250.
- Forster, A.; Hempenstall, J. and Rades, T.; Characterization of glass solutions of poorly water-soluble drugs produced by melt extrusion with hydrophilic amorphous polymers; *Journal of Pharmacy and Pharmacology*; 53 (2001); pp. 303–315.
- Fratini, F.; Cilia, G.; Turchi, B. and Felicioli, A.; Beeswax: a minireview of its antimicrobial activity and its application in medicine; *Asian Pacific Journal of Tropical Medicine*; 9 (2016); pp. 817–821.
- Gaillard, Y.; Mija, A.; Burr, A.; Darque-Ceretti, E.; Felder, E. and Sbirrazzuoli, N.; Green material composites from renewable resources: polymorphic transitions and phase diagram of beeswax/rosin resin; *Thermochimica Acta*; 521 (2011); pp. 90–97.
- Ghanbari, E.; Picken, S. J. and van Esch, J. H.; Analysis of differential scanning calorimetry (DSC): determining the transition temperatures, and enthalpy and heat capacity changes in multicomponent systems by analytical model fitting; *Journal of Thermal Analysis and Calorimetry*; 148 (2023); pp. 12393–12409.
- Gielen, D.; Boshell, F.; Saygin, D.; Bazilian, M. D.; Wagner, N. and Gorini, R.; The role of renewable energy in the global energy transformation; *Energy Strategy Reviews*; 24 (2019); pp. 38–50.
- Gowda, D. V.; Hemant, K. S. Y.; Moin, A. and ShivaKumar, H. G.; Preparation and characterization of sugar cane wax microspheres containing indomethacin; *Tropical Journal of Pharmaceutical Research*; 11 (2012); pp. 177–183.
- Green, T. P.; Marchessault, R. P. and Freese, D. K.; Disposition of sodium benzoate in newborn infants with hyperammonemia; *The Journal of Pediatrics*; 102 (1983); pp. 785–790.
- Gures, S.; Siepmann, F.; Siepmann, J. and Kleinebudde, P.; Drug release from extruded solid lipid matrices: theoretical predictions and independent experiments; *European Journal of Pharmaceutics and Biopharmaceutics*; 80 (2012); pp. 122–129.

- Habashy, R.; Khoder, M.; Zhang, S.; Pereira, B.; Bohus, M.; Tzu-Wen Wang, J.; Isreb, A. and Alhnan, M. A.; An innovative wax-based enteric coating for pharmaceutical and nutraceutical oral products; *Int J Pharm*; 591 (2020); pp. 119935.
- Haddad, L.; Trad, S.; Charafeddine, L. and Karam, P. E.; Seizure control in glycine encephalopathy using the ketamine-dextromethorphan-sodium benzoate triple therapy; *Epilepsy & Behavior Reports*; 31 (2025); pp. 100797.
- Hamosh, A.; Maher, J. F.; Bellus, G. A.; Rasmussen, S. A. and Johnston, M. V.; Long-term use of high-dose benzoate and dextromethorphan for the treatment of nonketotic hyperglycinemia; *The Journal of Pediatrics*; 132 (1998); pp. 709–713.
- Hamosh, A.; McDonald, J. W.; Valle, D.; Francomano, C. A.; Niedermeyer, E. and Johnston, M. V.; Dextromethorphan and high-dose benzoate therapy for nonketotic hyperglycinemia in an infant; *The Journal of Pediatrics*; 121 (1992); pp. 131–135.
- Heng, P. W. S.; Chan, L. W.; Easterbrook, M. G. and Li, X. M.; Investigation of the influence of mean HPMC particle size and number of polymer particles on the release of aspirin from swellable hydrophilic matrix tablets; *Journal of Controlled Release*; 76 (2001); pp. 39–49.
- Hennermann, J. B.; Barufe, J. M. and Mönch, E.; Klinische Variabilität bei Kindern mit Nichtketotischer Hyperglycinämie - Eine Verlaufsstudie von 36 Patienten; *Monatsschrift Kinderheilkunde*; 150 (2002); pp.
- Hepburn, H. R.; Bernard, R. T. F.; Davidson, B. C.; Muller, W. J.; Lloyd, P.; Kurstjens, S. P. and Vincent, S. L.; Synthesis and secretion of beeswax in honeybees; *Apidologie*; 22 (1991); pp. 21–36.
- Hoover-Fong, J. E.; Shah, S.; Van Hove, J. L. K.; Applegarth, D.; Toone, J. and Hamosh, A.; Natural history of nonketotic hyperglycinemia in 65 patients; *Neurology*; 63 (2004); pp. 1847–1853.
- Hutchinson, J. M.; Ruddy, M. and Wilson, M. R.; Differential scanning calorimetry of polymer glasses: corrections for thermal lag; *Polymer*; 29 (1988); pp. 152–159.
- Insent (2024); *Measurement of taste information*; 07.Sep.2024; <https://www.insentjp.com/>
- Intergovernmental Panel on Climate Change (2022); *Climate change 2022: impacts, adaptation and vulnerability. Contribution of working group II to the sixth assessment report of the intergovernmental panel on climate change*;
- Intergovernmental Panel on Climate Change (2024); *History of the IPCC*; 08.Sep.2024; <https://www.ipcc.ch/about/history/>
- Intergovernmental Panel on Climate Change (2024); *Preparing reports*; <https://www.ipcc.ch/about/preparingreports/>
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; International Council for Harmonisation (2017); *ICH Harmonised Guideline - addendum to ICH E11: clinical investigation of medicinal products in the pediatric population*;
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; International Council for Harmonisation (2019); *Biopharmaceutics classification system-based biowaivers*;

8. References

- International Organization for Standardization; (2009); *Particle size analysis - laser diffraction methods*; (ISO Standard No. 13320:2009); <https://www.iso.org/obp/ui/#iso:std:iso:13320:ed-1:v2:en>
- Ishizaka, T.; Miyanaga, Y.; Mukai, M.; Asaka, K.; Nakai, Y.; Tsuji, E. and Uchida, T.; Bitterness evaluation of medicines for pediatric use by a taste sensor; *Chemical & Pharmaceutical Bulletin*; 52 (2004); pp. 943–948.
- Jenning, V. and Gohla, S.; Comparison of wax and glyceride solid lipid nanoparticles (SLN (R)); *International Journal of Pharmaceutics*; 196 (2000); pp. 219–222.
- Kameda, T. and Tamada, Y.; Variable-temperature C-13 solid-state NMR study of the molecular structure of honeybee wax and silk; *International Journal of Biological Macromolecules*; 44 (2009); pp. 64–69.
- Kanno, J.; Hutchin, T.; Kamada, F.; Narisawa, A.; Aoki, Y.; Matsubara, Y. and Kure, S.; Genomic deletion within GLDC is a major cause of non-ketotic hyperglycinaemia; *Journal of Medical Genetics*; 44 (2007); pp.
- Kar, S.; Sanderson, H.; Roy, K.; Benfenati, E. and Leszczynski, J.; Green chemistry in the synthesis of pharmaceuticals; *Chemical Reviews*; 122 (2022); pp. 3637–3710.
- Keeling, C. D.; The concentration and isotopic abundances of carbon dioxide in the atmosphere; *Tellus*; 12 (1960); pp. 200–203.
- Kennedy, J. P. and Niebergall, P. J.; Evaluation of extended-release applications for solid dispersion hot-melt fluid bed coatings utilizing hydrophobic coating agents; *Pharmaceutical Development and Technology*; 3 (1998); pp. 95–101.
- Khan, N. and Craig, D. Q. M.; Role of blooming in determining the storage stability of lipid-based dosage forms; *Journal of Pharmaceutical Sciences*; 93 (2004); pp. 2962–2971.
- Kleinebudde, P. and Lindner, H.; Experiments with an Instrumented twin-screw extruder using a single-step granulation extrusion process; *International Journal of Pharmaceutics*; 94 (1993); pp. 49–58.
- Klingmann, V.; Acceptability of Mini-Tablets in Young Children: Results from Three Prospective Cross-over Studies; *AAPS PharmSciTech*; 18 (2017); pp. 263–266.
- Klingmann, V.; Linderskamp, H.; Meissner, T.; Mayatepek, E.; Moeltner, A.; Breitkreutz, J. and Bosse, H. M.; Acceptability of Multiple Uncoated Minitablets in Infants and Toddlers: A Randomized Controlled Trial; *The Journal of Pediatrics*; 201 (2018); pp. 202–207 e201.
- Klingmann, V.; Pohly, C. E.; Meissner, T.; Mayatepek, E.; Moltner, A.; Flunkert, K.; Breitkreutz, J. and Bosse, H. M.; Acceptability of an orodispersible film compared to syrup in neonates and infants: a randomized controlled trial; *European Journal of Pharmaceutics and Biopharmaceutics*; 151 (2020); pp. 239–245.
- Klingmann, V.; Spomer, N.; Lerch, C.; Stoltenberg, I.; Fromke, C.; Bosse, H. M.; Breitkreutz, J. and Meissner, T.; Favorable acceptance of mini-tablets compared with syrup: a randomized controlled trial in infants and preschool children; *Journal of Pediatrics*; 163 (2013); pp. 1728–U1283.

- Klingmann, V.; Vallet, T.; Munch, J.; Stegemann, R.; Wolters, L.; Bosse, H. M. and Ruiz, F.; Dosage forms suitability in pediatrics: acceptability of analgesics and antipyretics in a German hospital; *Pharmaceutics*; 14 (2022); pp.
- Kluk, A.; Sznitowska, M.; Brandt, A.; Sznurkowska, K.; Plata-Nazar, K.; Mysliwiec, M.; Kaminska, B. and Kotłowska, H.; Can preschool-aged children swallow several minitablets at a time? Results from a clinical pilot study; *International Journal of Pharmaceutics*; 485 (2015); pp. 1–6.
- Kobayashi, Y.; Habara, M.; Ikezaki, H.; Chen, R. G.; Naito, Y. and Toko, K.; Advanced taste Sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores; *Sensors*; 10 (2010); pp. 3411–3443.
- Kotel'nikova, E. N.; Platonova, N. V. and Filatov, S. K.; Identification of biogenic paraffins and their thermal phase transitions; *Geology of Ore Deposits*; 49 (2007); pp. 697–709.
- Koyata, H. and Hiraga, K.; The glycine cleavage system: structure of a cDNA encoding human H-protein, and partial characterization of its gene in patients with hyperglycinemias; *American Journal of Human Genetics*; 48 (1991); pp. 351–361.
- Krause, J. (2008); *Novel paediatric formulations for the drug sodium benzoate*; Promotionsarbeit; Heinrich-Heine-Universität Düsseldorf; Düsseldorf.
- Krause, J. and Breitzkreutz, J.; Improving drug delivery in paediatric medicine; *International Journal of Pharmaceutical Medicine*; 22 (1) (2008); pp. 41–50.
- Krause, J.; Thommes, M. and Breitzkreutz, J.; Immediate release pellets with lipid binders obtained by solvent-free cold extrusion; *European Journal of Pharmaceutics and Biopharmaceutics*; 71 (2009); pp. 138–144.
- Krendlinger, E.; Wolfmeier, U.; Schmidt, H.; Heinrichs, F.-L.; Michalczyk, G.; Payer, W.; Dietsche, W.; Boehlke, K.; Hohner, G. and Wildgruber, J.; (2015); Waxes; in: *Ullmann's Encyclopedia of Industrial Chemistry*; Weinheim - Wiley-VCH: 60
- Kubota, K. and Ishizaki, T.; Dose-dependent pharmacokinetics of benzoic-acid following oral-administration of sodium benzoate to humans; *European Journal of Clinical Pharmacology*; 41 (1991); pp. 363–368.
- Larsson, K.; Classification of glyceride crystal forms; *Acta Chemica Scandinavica*; 20 (1966); pp. 2255–2260.
- Legin, A.; Rudnitskaya, A.; Clapham, D.; Seleznev, B.; Lord, K. and Vlasov, Y.; Electronic tongue for pharmaceutical analytics: quantification of tastes and masking effects; *Analytical and Bioanalytical Chemistry*; 380 (2004); pp. 36–45.
- Leister, D.; Geilen, T. and Geissler, T.; (2012); Twin-screw extruders for pharmaceutical hot-melt extrusion: technology, techniques and practices.; in: *Hot-Melt Extrusion Pharmaceutical Applications*; 2; - John Wiley & Sons, Ltd: 23–42
- Lennartz, P. and Mielck, J. B.; Minitabletting: improving the compactability of paracetamol powder mixtures; *International Journal of Pharmaceutics*; 173 (1998); pp. 75–85.

8. References

- Liem, D. G. and Mennella, J. A.; Sweet and sour preferences during childhood: role of early experiences; *Developmental Psychobiology*; 41 (2002); pp. 388–395.
- Madsen, K. (2020); *Honeybees + honeycomb*; 11.Aug.2024; <https://pixabay.com/de/photos/bier-honig-insekt-honigbienen-5433299/>
- Malaquias, L. F. B.; Sa-Barreto, L. C. L.; Freire, D. O.; Silva, I. C. R.; Karan, K.; Durig, T.; Lima, E. M.; Marreto, R. N.; Gelfuso, G. M.; Gratieri, T. and Cunha-Filho, M.; Taste masking and rheology improvement of drug complexed with beta-cyclodextrin and hydroxypropyl-beta-cyclodextrin by hot-melt extrusion; *Carbohydr Polym*; 185 (2018); pp. 19–26.
- Malik, A.; Lenzen, M.; McAlister, S. and McGain, F.; The carbon footprint of australian health care; *Lancet Planetary Health*; 2 (2018); pp. E27–E35.
- Marreto, R. N.; Cardoso, G.; Dos Santos Souza, B.; Martin-Pastor, M.; Cunha-Filho, M.; Taveira, S. F.; Concheiro, A. and Alvarez-Lorenzo, C.; Hot melt-extrusion improves the properties of cyclodextrin-based poly(pseudo)rotaxanes for transdermal formulation; *International Journal of Pharmaceutics*; 586 (2020); pp. 119510.
- Meadows, D. H.; Meadows, D. L.; Randers, J. and Behrens |||, W. W.; Limits to Growth; Potomac Associates - Universe Books; New York; (1972).
- Medeiros, T. S.; Moreira, L.; Oliveira, T. M. T.; Melo, D. F.; Azevedo, E. P.; Gadelha, A. E. G.; Fook, M. V. L.; Oshiro-Junior, J. A. and Damasceno, B.; Bemotrizinol-loaded carnauba wax-based nanostructured lipid carriers for sunscreen: optimization, characterization, and in vitro evaluation; *AAPS PharmSciTech*; 21 (2020); pp. 288.
- Mendonsa, N. S.; Murthy, S. N.; Hashemnejad, S. M.; Kundu, S.; Zhang, F. and Repka, M. A.; Development of poloxamer gel formulations via hot-melt extrusion technology; *International Journal of Pharmaceutics*; 537 (2018); pp. 122–131.
- Merck Millipore (2006); *Natriumbenzoat EMPROVE® ESSENTIAL*; Safety data sheet; http://www.merckmillipore.com/DE/de/product/Sodium-benzoate,MDA_CHEM-106290
- Michalk, A. (2007); *Geschmacksmaskierung durch Festfett-Extrusion*; Doctorate Thesis; Heinrich-Heine-University Düsseldorf; Düsseldorf.
- Michalk, A.; Kanikanti, V.-R.; Hamann, H.-J. and Kleinebudde, P.; Controlled release of active as a consequence of the die diameter in solid lipid extrusion; *Journal of Controlled Release*; 132 (2008); pp. 35–41.
- Mikolaszek, B.; Kazlauskas, J.; Larsson, A. and Sznitowska, M.; Controlled drug release by the pore structure in polydimethylsiloxane transdermal patches; *Polymers (Basel)*; 12 (2020); pp.
- Miura, H.; Kusakabe, Y.; Sugiyama, C.; Kawamatsu, M.; Ninomiya, Y.; Motoyama, J. and Hino, A.; Shh and Ptc are associated with taste bud maintenance in the adult mouse; *Mechanisms of Development*; 106 (2001); pp. 143–145.
- Miyagawa, Y.; Okabe, T.; Yamaguchi, Y.; Miyajima, M.; Sato, H. and Sunada, H.; Controlled-release of diclofenac sodium from wax matrix granule; *International Journal of Pharmaceutics*; 138 (1996); pp. 215–224.

- Nart, V.; Beringhs, A. O.; Franca, M. T.; de Espindola, B.; Pezzini, B. R. and Stulzer, H. K.; Carnauba wax as a promising excipient in melt granulation targeting the preparation of mini-tablets for sustained release of highly soluble drugs; *Mater Sci Eng C Mater Biol Appl*; 70 (2017); pp. 250–257.
- Nassogne, M. C.; Heron, B.; Touati, G.; Rabier, D. and Saudubray, J. M.; Urea cycle defects: management and outcome; *Journal of Inherited Metabolic Disease*; 28 (2005); pp. 407–414.
- Noa, M. and Mas, R.; Protective effect of policosanol on atherosclerotic plaque on aortas in monkeys; *Archives of Medical Research*; 36 (2005); pp. 441–447.
- Nowak, M.; Chuchra, P. and Paprocka, J.; Nonketotic hyperglycinemia: insight into current therapies; *Journal of Clinical Medicine*; 11 (2022); pp. 3027.
- Noyes, A. A. and Whitney, W. R.; The rate of solution of solid substances in their own solution; *Journal of the American Chemical Society*; 19 (1897); pp. 930–934.
- Nuissier, G.; Bourgeois, P.; Grignon-Dubois, M.; Pardon, P. and Lescure, M. H.; Composition of sugarcane waxes in rum factory wastes; *Phytochemistry*; 61 (2002); pp. 721–726.
- Ohya, Y.; Ochi, N.; Mizutani, N.; Hayakawa, C. and Watanabe, K.; Nonketotic hyperglycinemia: treatment with NMDA antagonist and consideration of neuropathogenesis; *Pediatric Neurology*; 7 (1991); pp. 65–68.
- Origen Chemicals (2024); *Natural sugarcane wax*; 28 July; <https://www.origenchem.com/product-range/sugar-cane-wax/>
- Park, J.; Kelly, M. A.; Kang, J. S. X.; Seemakurti, S. S.; Ramirez, J. L.; Hatzell, M. C.; Sievers, C. and Bommarius, A. S.; Production of active pharmaceutical ingredients (APIs) from lignin-derived phenol and catechol; *Green Chemistry*; 23 (2021); pp. 7488–7498.
- Parviz, S. and Hooshyar, D.; Neonatal nonketotic hyperglycinemia: a severe case with prenatal indicators and comprehensive review of recognition and management; *Clin Case Rep*; 13 (2025); pp. e70035.
- Petrovick, G. F. (2015); *Orodispersible tablets containing taste-masked lipid pellets with metformin hydrochloride for use by elderly patients*; Doctorate Thesis; Heinrich-Heine-University Düsseldorf; Düsseldorf.
- Petrovick, G. F. and Breitzkreutz, J.; Spheronization of solid lipid extrudates: Elucidation of spheroid formation mechanism; *European Journal of Pharmaceutics and Biopharmaceutics*; 125 (2018); pp. 148–158.
- Petrovick, G. F.; Breitzkreutz, J. and Pein-Hackelbusch, M.; Taste-masking properties of solid lipid based micropellets obtained by cold extrusion-spheronization; *International Journal of Pharmaceutics*; 506 (2016); pp. 361–370.
- Petrovick, G. F.; Kleinebudde, P. and Breitzkreutz, J.; Orodispersible tablets containing taste-masked solid lipid pellets with metformin hydrochloride: Influence of process parameters on tablet properties; *European Journal of Pharmaceutics and Biopharmaceutics*; 122 (2018); pp. 137–145.

8. References

- Petrovick, G. F.; Pein, M.; Thommes, M. and Breitzkreutz, J.; Spheronization of solid lipid extrudates: A novel approach on controlling critical process parameters; *European Journal of Pharmaceutics and Biopharmaceutics*; 92 (2015); pp. 15–21.
- Piek, T.; Synthesis of wax in the honeybee (*apis-mellifera* L); *Journal of Insect Physiology*; 10 (1964); pp. 563–572.
- Pinto, J. F. and Silverio, N. P.; Assessment of the extrudability of three different mixtures of saturated polyglycolysed glycerides by determination of the "specific work of extrusion" and by capillary rheometry; *Pharmaceutical Development and Technology*; 6 (2001); pp. 117–128.
- Podrekar, G.; Kitak, D.; Mehle, A.; Lavric, Z.; Likar, B.; Tomazevic, D. and Dreu, R.; In-line film coating thickness estimation of minitabets in a fluid-bed coating equipment; *AAPS PharmSciTech*; 19 (2018); pp. 3440–3453.
- Prabhu, S.; Ortega, M. and Ma, C.; Novel lipid-based formulations enhancing the in vitro dissolution and permeability characteristics of a poorly water-soluble model drug, piroxicam; *International Journal of Pharmaceutics*; 301 (2005); pp. 209–216.
- Quodbach, J.; Preis, E.; Karkossa, F.; Winck, J.; Finke, J. H. and Steiner, D.; Novel strategies for the formulation of poorly water-soluble drug substances by different physical modification strategies with a focus on peroral applications; *Pharmaceuticals (Basel)*; 18 (2025); pp.
- Qureshi, I. A.; Letarte, J.; Ouellet, R.; Batshaw, M. L. and Brusilow, S.; Treatment of hyperargininemia with sodium benzoate and arginine-restricted diet; *The Journal of Pediatrics*; 104 (1984); pp. 473–476.
- Racz, I.; Dredan, J.; Antal, I. and Gondar, E.; Comparative evaluation of microcapsules prepared by fluidization atomization and melt coating process; *Drug Development and Industrial Pharmacy*; 23 (1997); pp. 583–587.
- Raman, C. and Krishnan, K.; A new type of secondary radiation; *Nature*; 121 (1928); pp. 501–502.
- Randak, C.; Roschinger, W.; Rolinski, B.; Hadorn, H. B.; Applegarth, D. A. and Roscher, A. A.; Three siblings with nonketotic hyperglycinaemia, mildly elevated plasma homocysteine concentrations and moderate methylmalonic aciduria; *Journal of Inherited Metabolic Disease*; 23 (2000); pp. 520–522.
- Reitz, C. and Kleinebudde, P.; Influence of thermal and thermo-mechanical treatment; *Journal of Thermal Analysis and Calorimetry*; 89 (2007); pp. 669–673.
- Reitz, C. and Kleinebudde, P.; Spheronization of solid lipid extrudates; *Powder Technology*; 189 (2009); pp. 238–244.
- Reitz, C.; Strachan, C. and Kleinebudde, P.; Solid lipid extrudates as sustained-release matrices: The effect of surface structure on drug release properties; *European Journal of Pharmaceutical Sciences*; 35 (2008); pp. 335–343.
- Repka, M. A.; Gerding, T. G.; Repka, S. L. and McGinity, J. W.; Influence of plasticizers and drugs on the physical-mechanical properties of hydroxypropylcellulose films prepared by hot melt extrusion; *Drug Development and Industrial Pharmacy*; 25 (1999); pp. 625–633.

- Revelle, R. and Suess, H. E.; Carbon dioxide exchange between atmosphere and ocean and the question of an increase of atmospheric Co₂ during the past decades; *Tellus*; 9 (1957); pp. 18–27.
- Ritter, B.; Schulte, J.; Schulte, E. and Thier, H. P.; Detection of coating waxes on apples by differential scanning calorimetry; *European Food Research and Technology*; 212 (2001); pp. 603–607.
- Rocha, J. C. B.; Lopes, J. D.; Mascarenhas, M. C. N.; Arellano, D. B.; Guerreiro, L. M. R. and da Cunha, R. L.; Thermal and rheological properties of organogels formed by sugarcane or candelilla wax in soybean oil; *Food Research International*; 50 (2013); pp. 318–323.
- Roschangar, F.; Sheldon, R. A. and Senanayake, C. H.; Overcoming barriers to green chemistry in the pharmaceutical industry - the green aspiration level™ concept; *Green Chemistry*; 17 (2015); pp. 752–768.
- Rosenberg, J. and Breitenbach, J. (2002); *Use of lipids as adjuvants in the production of solid medicinal forms by the melt extrusion process*; U.S.
- Rosiaux, Y.; Jannin, V.; Hughes, S. and Marchaud, D.; Solid lipid excipients - matrix agents for sustained drug delivery; *Journal of Controlled Release*; 188 (2014); pp. 18–30.
- Sadrieh, N.; Brower, J.; Yu, L.; Doub, W.; Straughn, A.; Machado, S.; Pelsor, F.; Saint Martin, E.; Moore, T.; Reepmeyer, J.; Toler, D.; Nguyenpho, A.; Roberts, R.; Schuirmann, D. J.; Nasr, M. and Buhse, L.; Stability, dose uniformity, and palatability of three counterterrorism drugs - human subject and electronic tongue studies; *Pharmaceutical Research*; 22 (2005); pp. 1747–1756.
- Sam, A. P.; Controlled release contraceptive devices - a status-report; *Journal of Controlled Release*; 22 (1992); pp. 35–46.
- Sambanthamurthi, R.; Sundram, K. and Tan, Y.; Chemistry and biochemistry of palm oil; *Progress in Lipid Research*; 39 (2000); pp. 507–558.
- Sandu, C. and Singh, R. K.; Physical transformations in differential scanning calorimetry; *Thermochimica Acta*; 132 (1988); pp. 89–99.
- Sato, K.; Crystallization behaviour of fats and lipids - a review; *Chemical Engineering Science*; 56 (2001); pp. 2255–2265.
- Sawant, K. P.; Fule, R.; Maniruzzaman, M. and Amin, P. D.; Extended release delivery system of metoprolol succinate using hot-melt extrusion: effect of release modifier on methacrylic acid copolymer; *Drug Delivery and Translational Research*; 8 (2018); pp. 1679–1693.
- Schutgens, R. B.; Ket, J. L.; Hayasaka, K. and Tada, K.; Non-ketotic hyperglycinaemia due to a deficiency of T-protein in the glycine cleavage system in liver and brain; *Journal of Inherited Metabolic Disease*; 9 (1986); pp. 208–214.
- Shamshad, R.; Ali, S. N.; Gul, S.; Ahmed, S. S.; Chandio, A. D.; Aziz, A.; Daniel, P. and Channa, I. A.; Sugarcane wax-based solid lipid nanoparticles as an atorvastatin carrier: characterization and in vivo evaluation of antihyperlipidemic activity in rats; *Bionanoscience*; 15 (2025); pp.
- Siepmann, J. and Peppas, N. A.; Higuchi equation: derivation, applications, use and misuse; *International Journal of Pharmaceutics*; 418 (2011); pp. 6–12.

8. References

- Singh, D. K.; Li, L. and Porter, T. D.; Policosanol inhibits cholesterol synthesis in hepatoma cells by activation of AMP-kinase; *The Journal of Pharmacology Experimental Therapeutics*; 318 (2006); pp. 1020–1026.
- Snehavardhan, P.; Lal, B. B.; Sood, V.; Khanna, R. and Alam, S.; Efficacy and safety of sodium benzoate in the management of hyperammonemia in decompensated chronic liver disease of the childhood-a double-blind randomized controlled trial; *Journal of Pediatric Gastroenterology and Nutrition*; 70 (2020); pp. 165–170.
- Somerville, C.; Youngs, H.; Taylor, C.; Davis, S. C. and Long, S. P.; Feedstocks for lignocellulosic biofuels; *Science*; 329 (2010); pp. 790–792.
- Sood, S.; Methven, L. and Cheng, Q. F.; Role of taste receptors in salty taste perception of minerals and amino acids and developments in salt reduction strategies: A review; *Critical Reviews in Food Science and Nutrition*; 65 (2025); pp. 3444–3458.
- Souza, C.; de Freitas, L. A. P. and Campos, P. M. B. G. M.; Topical formulation containing beeswax-based nanoparticles improved in vivo skin barrier function; *AAPS PharmSciTech*; 18 (2017); pp. 2505–2516.
- Staniforth, J. and Taylor, K. M. G.; (2018); Particle size analysis; in: Aulton, M. E. and Taylor, K. M. G. (Eds.) *Aulton's Pharmaceutics: The Design and Manufacture of Medicines.*; London, UK, Churchill Livingstone - Elsevier: 140–157
- Stark, H.; Graßmann, S. and Reichert, U.; Struktur, Funktion und potentielle therapeutische Bedeutung von NMDA-Rezeptoren; *Pharmazie in unserer Zeit*; 29 (2000); pp. 159–166.
- Stoltenberg, I. and Breitzkreutz, J.; Orally disintegrating mini-tablets (ODMTs)--a novel solid oral dosage form for paediatric use; *European Journal of Pharmaceutics and Biopharmaceutics*; 78 (2011); pp. 462–469.
- Sugao, H.; Yamazaki, S.; Shiozawa, H. and Yano, K.; Taste masking of bitter drug powder without loss of bioavailability by heat treatment of wax-coated microparticles; *Journal of Pharmaceutical Sciences*; 87 (1998); pp. 96–100.
- Sutananta, W.; Craig, D. Q. M. and Newton, J. M.; The effects of aging on the thermal-behavior and mechanical-properties of pharmaceutical glycerides; *International Journal of Pharmaceutics*; 111 (1994); pp. 51–62.
- Tacarijus (2007); *Carnauba palm*; 11.Aug.2024;
<https://commons.wikimedia.org/wiki/File:Carnauba.jpg>
- Tada, K. and Hayasaka, K.; Non-ketotic hyperglycinaemia: clinical and biochemical aspects; *European Journal of Pediatrics*; 146 (1987); pp. 221–227.
- Thomson, S. A.; Tuleu, C.; Wong, I. C.; Keady, S.; Pitt, K. G. and Sutcliffe, A. G.; Minitablets: new modality to deliver medicines to preschool-aged children; *Pediatrics*; 123 (2009); pp. e235–238.
- Toko, K.; Electronic sensing of tastes; *Electroanalysis*; 10 (1998); pp. 657–669.

- Toone, J. R.; Applegarth, D. A.; Levy, H. L.; Coulter-Mackie, M. B. and Lee, G.; Molecular genetic and potential biochemical characteristics of patients with T-protein deficiency as a cause of glycine encephalopathy (NKH); *Molecular Genetics and Metabolism*; 79 (2003); pp. 272–280.
- Tulloch, A. P.; Analysis of whole beeswax by gas liquid chromatography; *Journal of the American Oil Chemists Society*; 49 (1972); pp. 609–610.
- Tulloch, A. P.; Comparison of some commercial waxes by gas liquid chromatography; *Journal of the American Oil Chemists Society*; 50 (1973); pp. 367–371.
- Tyndall, J.; XXIII. On the absorption and radiation of heat by gases and vapours, and on the physical connexion of radiation, absorption, and conduction.—The bakerian lecture; *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science*; 22 (1861); pp. 169–194.
- U.S. Food and Drug Administration; Center for Drug Evaluation and Research (CDER) (2018); *Dissolution testing and acceptance criteria for immediate-release solid oral dosage form drug products containing high solubility drug substances*; Services, U. S. D. o. H. a. H.
- U.S. Food and Drug Administration; (2019); CFR 21 Part 184 - Direct food substances affirmed as generally recognized as safe - subpart B, Sec. 184.1978 Carnauba wax; USA; U.S. department of health and human services
- Uhumwangho, M. U.; Okor, R. S. and Adogah, J. T.; Potential of carnauba wax in ameliorating brittle fracture during tableting; *Pakistan Journal of Pharmaceutical Sciences*; 22 (2009); pp. 58–61.
- United Nations (1993); *Report of the United Nations conference on environment and development*; New York
- United Nations (1997); *Kyoto protocol to the United Nations framework convention on climate change*; Kyoto
- United Nations, Paris agreement, in: Paris Climate Change Conference, United Nations Framework Convention on Climate Change, Paris, 2015, pp. 60.
- United Nations Environment Programme (2000); *Climate Change Information Sheet 17*; 08.Sep.2024; <https://unfccc.int/cop3/fccc/climate/fact17.htm>
- United States Pharmacopeia; United States Pharmacopeial Convention (2024); *United States Pharmacopeia - National Formulary: Carnauba Wax*; Convention, U. S. P.
- Vaassen, J.; Bartscher, K. and Breitschütz, J.; Taste masked lipid pellets with enhanced release of hydrophobic active ingredient; *International Journal of Pharmaceutics*; 429 (2012); pp. 99–103.
- Van de Vijver, E.; Desager, K.; Mulberg, A. E.; Staelens, S.; Verkade, H. J.; Bodewes, F. A. J. A.; Malfroot, A.; Hauser, B.; Sinaasappel, M.; Van Biervliet, S.; Behm, M.; Pelckmans, P.; Callens, D. and Veereman-Wauters, G.; Treatment of infants and toddlers with cystic fibrosis-related pancreatic insufficiency and fat malabsorption with pancrelipase MT; *Journal of Pediatric Gastroenterology and Nutrition*; 53 (2011); pp. 61–64.
- van der Werf, A.; Rovithi, M.; Langius, J. A. E.; de van der Schueren, M. A. E. and Verheul, H. M. W.; Insight in taste alterations during treatment with protein kinase inhibitors; *European Journal of Cancer*; 86 (2017); pp. 125–134.

8. References

- Van Hove, J. L. K.; Kishnani, P.; Muenzer, J.; Wenstrup, R. J.; Summar, M. L.; Brummond, M. R.; Lachiewicz, A. M.; Millington, D. S. and Kahler, S. G.; Benzoate therapy and carnitine deficiency in non-ketotic hyperglycinemia; *American Journal of Medical Genetics*; 59 (1995); pp. 444–453.
- van Hove, J. L. K.; Vande Kerckhove, K.; Hennermann, J. B.; Mahieu, V.; Declercq, P.; Mertens, S.; De Becker, M.; Kishnani, P. S. and Jaeken, J.; Benzoate treatment and the glycine index in nonketotic hyperglycinaemia; *Journal of Inherited Metabolic Disease*; 28 (2005); pp. 651–663.
- Vijay, V.; Pimm, S. L.; Jenkins, C. N. and Smith, S. J.; The Impacts of Oil Palm on Recent Deforestation and Biodiversity Loss; *PLoS One*; 11 (2016); pp. e0159668.
- Vollrath, M.; Engert, J. and Winter, G.; New insights into process understanding of solid lipid extrusion (SLE) of extruded lipid implants for sustained protein delivery; *European Journal of Pharmaceutics and Biopharmaceutics*; 130 (2018); pp. 11–21.
- Walsh, J.; Cram, A.; Woertz, K.; Breitreutz, J.; Winzenburg, G.; Turner, R. and Tuleu, C.; Playing hide and seek with poorly tasting paediatric medicines: Do not forget the excipients; *Advanced Drug Delivery Reviews*; 73 (2014); pp. 14–33.
- Wang, H.; Dumpa, N.; Bandari, S.; Durig, T. and Repka, M. A.; Fabrication of taste-masked donut-shaped tablets via fused filament fabrication 3D printing paired with hot-melt extrusion techniques; *AAPS PharmSciTech*; 21 (2020); pp. 243.
- Wilson, C. E.; Lasher, R. S.; Yang, R.; Dzowo, Y.; Kinnamon, J. C. and Finger, T. E.; Taste bud connectome: implications for taste information processing; *Journal of Neuroscience*; 42 (2022); pp. 804–816.
- Wilson, C. E.; Vandenbeuch, A. and Kinnamon, S. C.; Physiological and behavioral responses to optogenetic stimulation of PKD2L1(+) type III taste cells; *eNeuro*; 6 (2019); pp.
- Windbergs, M.; Haaser, M.; McGoverin, C. M.; Gordon, K. C.; Kleinebudde, P. and Strachan, C. J.; Investigating the relationship between drug distribution in solid lipid matrices and dissolution behaviour using raman spectroscopy and mapping; *Journal of Pharmaceutical Sciences*; 99 (2010); pp. 1464–1475.
- Windbergs, M.; Strachan, C. J. and Kleinebudde, P.; Understanding the solid-state behaviour of triglyceride solid lipid extrudates and its influence on dissolution; *European Journal of Pharmaceutics and Biopharmaceutics*; 71 (2009); pp. 80–87.
- Witzleb, R.; Kanikanti, V.-R.; Hamann, H.-J. and Kleinebudde, P.; Solid lipid extrusion with small die diameters – Electrostatic charging, taste masking and continuous production; *European Journal of Pharmaceutics and Biopharmaceutics*; 77 (2011); pp. 170–177.
- World Commission on Environment and Development (1987); *The Brundtland report: 'Our common future'*; London
- World Health Organisation (2008); *"Make medicines child size". progress reports. reports by the secretariat*; https://apps.who.int/gb/ebwha/pdf_files/EB124/B124_33Add1-en.pdf
- Xu, C. P.; Nasrollahzadeh, M.; Selva, M.; Issaabadi, Z. and Luque, R.; Waste-to-wealth: biowaste valorization into valuable bio(nano)materials; *Chemical Society Reviews*; 48 (2019); pp. 4791–4822.

- Xu, R. M.; Ma, B. B.; Yang, Y. Y.; Dong, X. C.; Li, J. K.; Xu, X. and Fang, Y.; Proteome-metabolome profiling of wax gland complex reveals functional changes in honeybee, *Apis mellifera* L.; *Isience*; 27 (2024); pp.
- Yeung, V. W. and Wong, I. C. K.; When do children convert from liquid antiretroviral to solid formulations?; *Pharmacy World & Science*; 27 (2005); pp. 399–402.
- Yoshida, R. and Ninomiya, Y.; Mechanisms and functions of sweet reception in oral and extraoral organs; *International Journal of Molecular Sciences*; 25 (2024); pp. 7398.
- Zhang, F. and McGinity, J. W.; Properties of sustained-release tablets prepared by hot-melt extrusion; *Pharmaceutical Development and Technology*; 4 (1999); pp. 241–250.
- Zhang, Y. F.; Hoon, M. A.; Chandrashekar, J.; Mueller, K. L.; Cook, B.; Wu, D. Q.; Zuker, C. S. and Ryba, N. J. P.; Coding of sweet, bitter, and umami tastes: Different receptor cells sharing similar signaling pathways; *Cell*; 112 (2003); pp. 293–301.
- Zhao, Y. G.; Zhang, M.; Devahastin, S. and Liu, Y. P.; Progresses on processing methods of umami substances: A review; *Trends in Food Science & Technology*; 93 (2019); pp. 125–135.
- Zhong, N. J.; Chen, W. L.; Liu, L. Y. and Chen, H. X.; Immobilization of lipase onto the organic functionalized SBA-15: Their enzymatic properties and glycerolysis efficiencies for diacylglycerols production; *Food Chemistry*; 271 (2019); pp. 739–746.
- Ziegler, I. and Jacobs, I. (2008); *Coated pellets*; U.S. Patent 8,147,874B2.

9 Annex

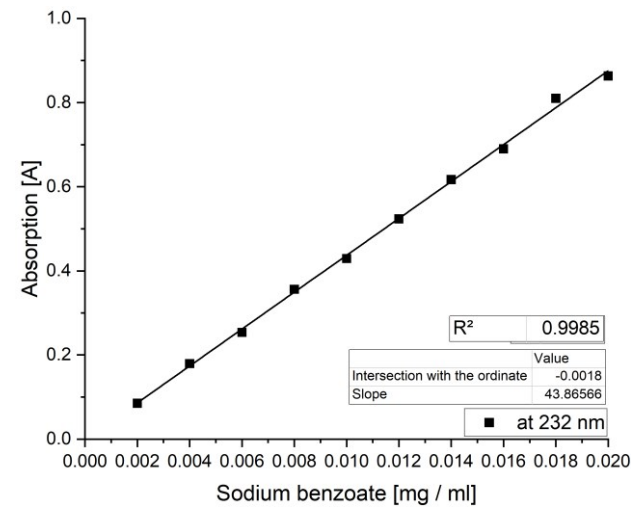


Figure 9.1: Calibration curve of experiment BaHav18-021 in phosphate buffer pH6.8

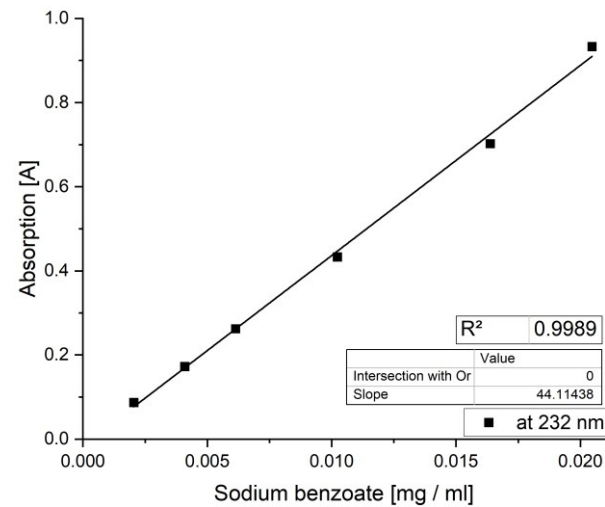


Figure 9.2: Calibration graph of experiment BaHav17-011, BaHav17-018 in phosphate buffer pH6.8

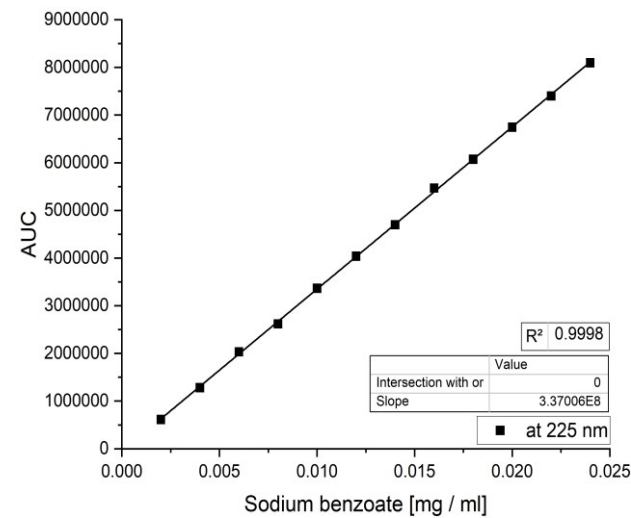


Figure 9.3: Calibration graph of experiment BaHav19-002, BaHav19-006 fresh and 1 month old in phosphate buffer pH6.8

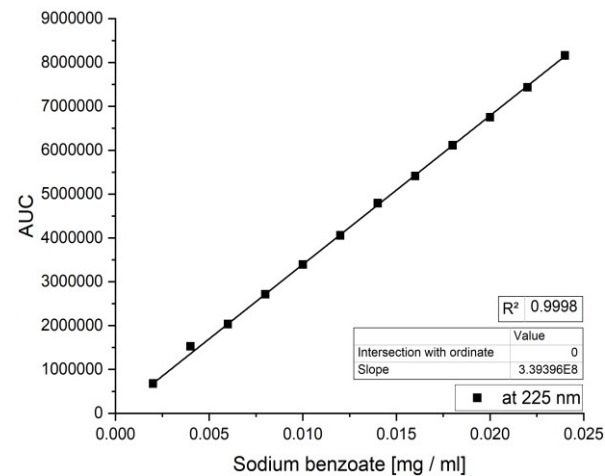


Figure 9.4: Calibration graph of experiment BaHav19-006 2month and 3 month old, BaHav19-019 (0.1N HCL) and BaHav19-029 measured in phosphate buffer pH 6.8

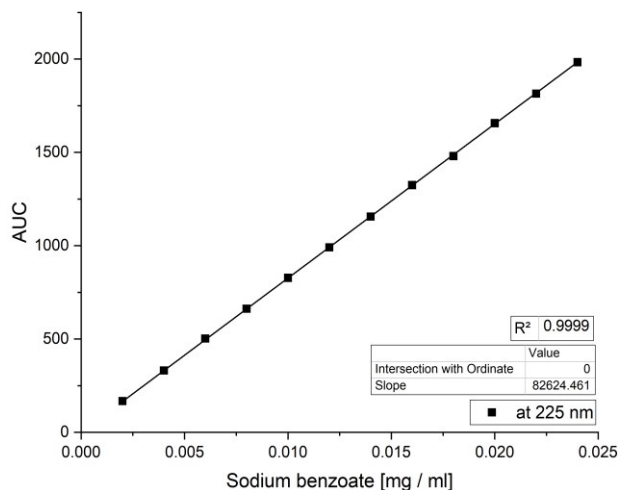


Figure 9.5: Calibration graph of experiment BaHav19-032, -033 and -034 measured in phosphatebuffer pH 6.8

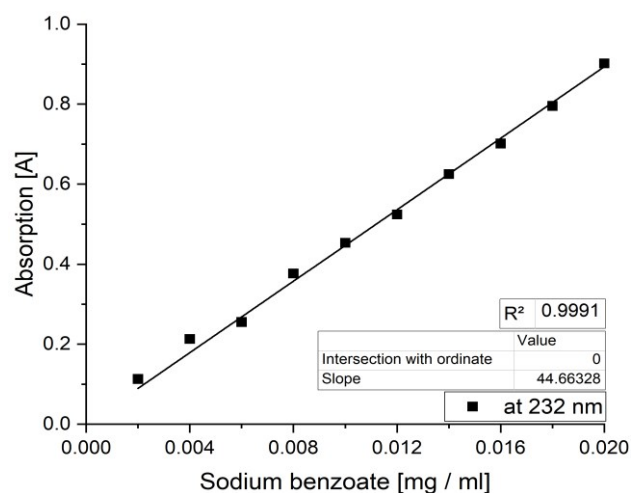


Figure 9.6: Calibration graph of experiment BaHav18-027

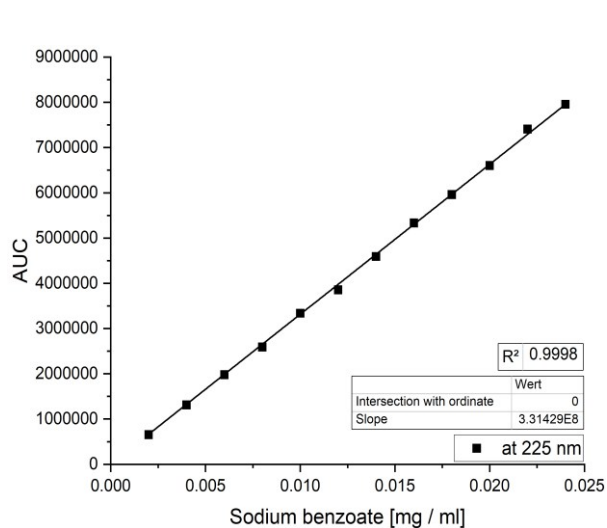


Figure 9.7: Calibration graph of experiment BaHav19-019, BaHav19-021 and BaHav19-029 measured in demineralized water

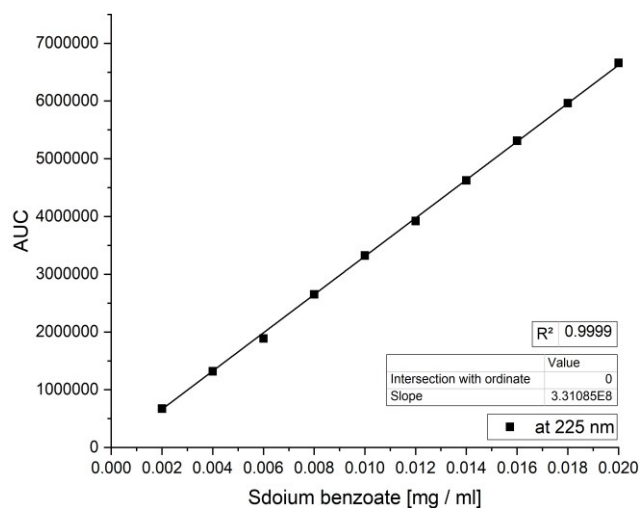


Figure 9.8: Calibration graph of experiment BaHav18-042 measured in phosphate buffer pH 6.8

9. Annex

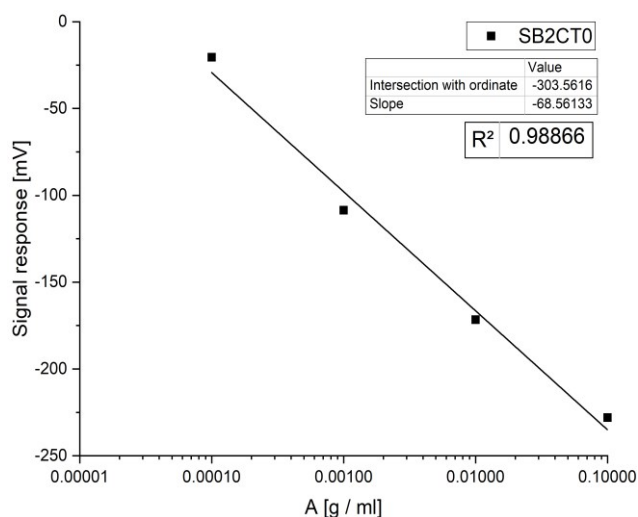


Figure 9.9: Calibration of sensor SB2CT0 using 4 different sodium benzoate solutions. BaHav19-008

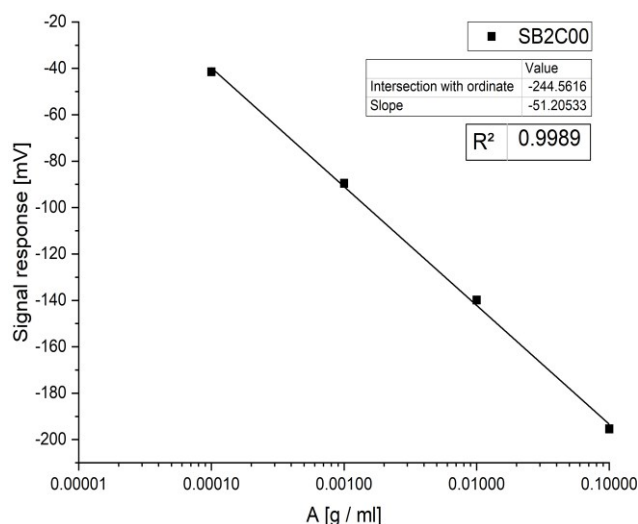


Figure 9.10: Calibration of sensor SB2C00 using 4 different sodium benzoate solutions. BaHav19-008

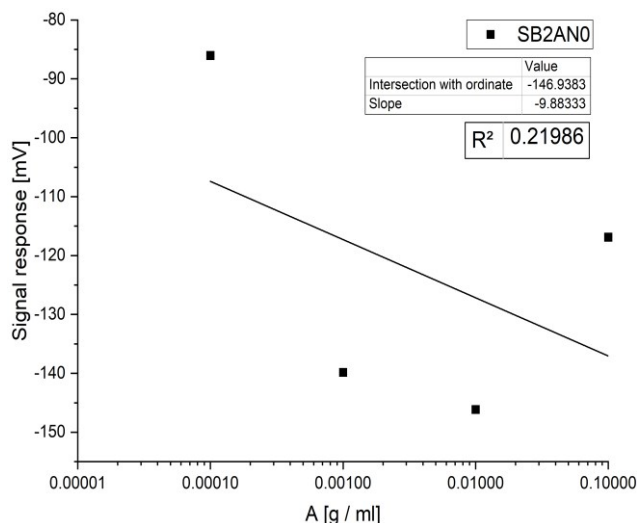


Figure 9.11: Calibration of sensor SB2AN0 using 4 different sodium benzoate solutions. BaHav19-008

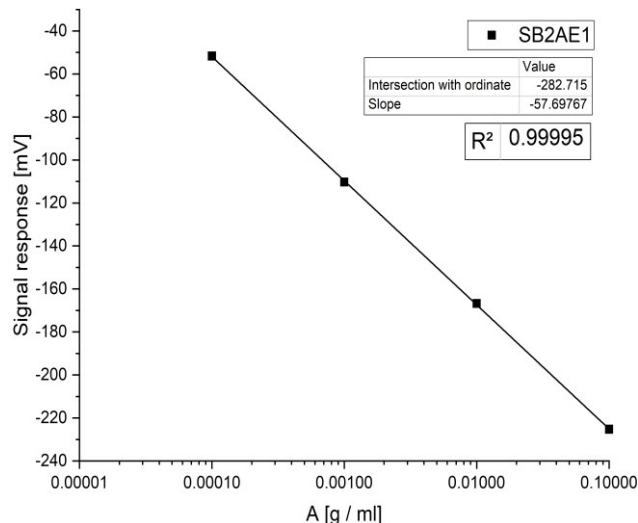


Figure 9.12: Calibration of sensor SB2AE1 using 4 different sodium benzoate solutions. BaHav19-008

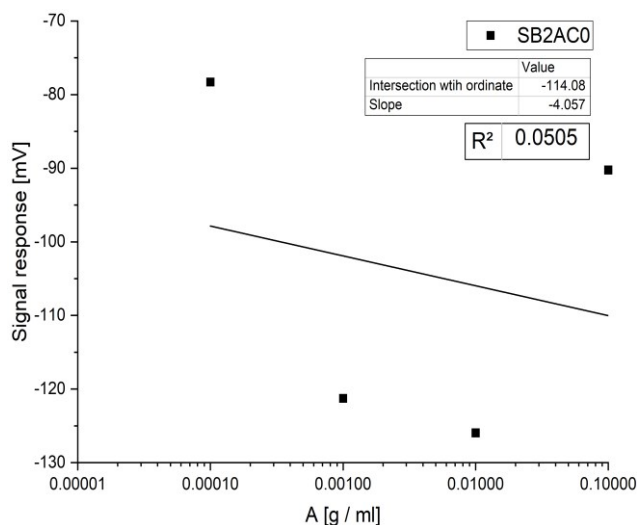


Figure 9.13: Calibration of sensor SB2AC0 using 4 different sodium benzoate solutions. BaHav19-008

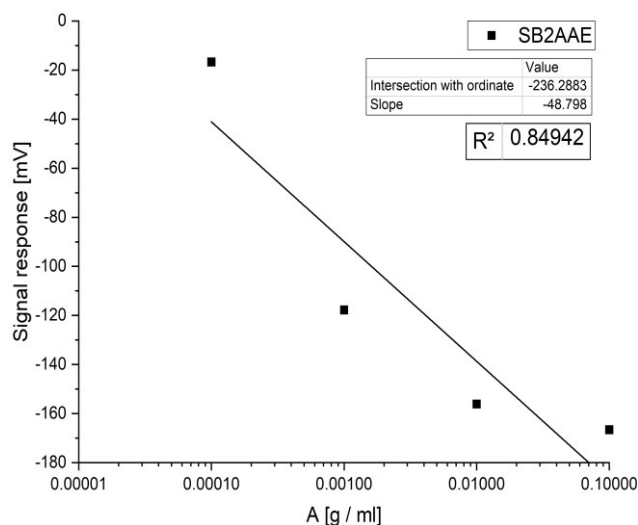


Figure 9.14: Calibration of sensor SB2AAE using 4 different sodium benzoate solutions. BaHav19-008

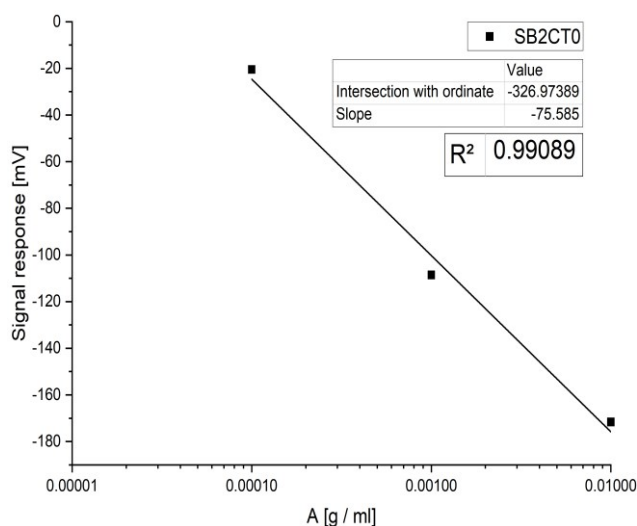


Figure 9.15: Calibration of sensor SB2CT0 using 3 different sodium benzoate solutions. BaHav19-008

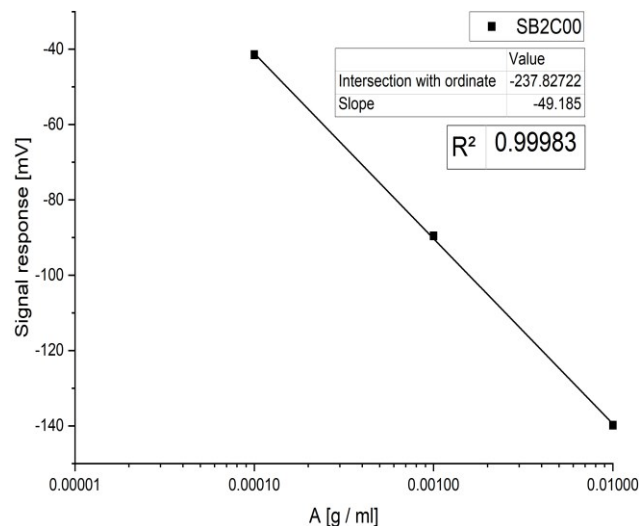


Figure 9.16: Calibration of sensor SB2C00 using 3 different sodium benzoate solutions. BaHav19-008

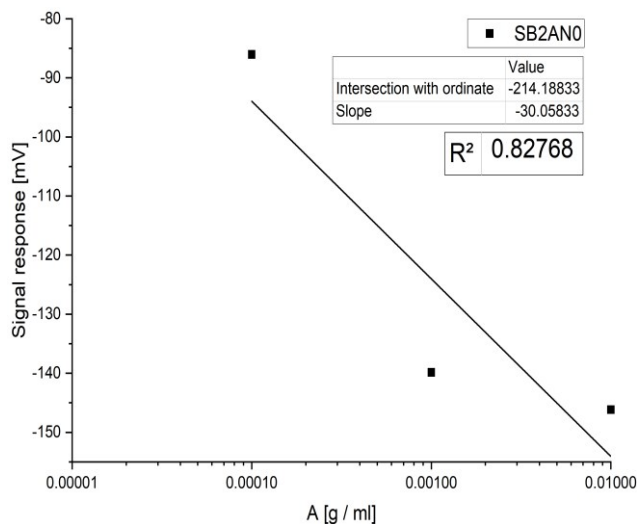


Figure 9.17: Calibration of sensor SB2AN0 using 3 different sodium benzoate solutions. BaHav19-008

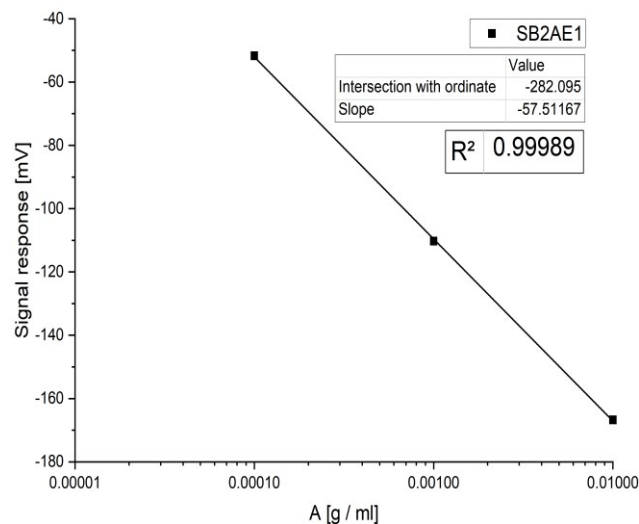


Figure 9.18: Calibration of sensor SB2AE1 using 3 different sodium benzoate solutions. BaHav19-008

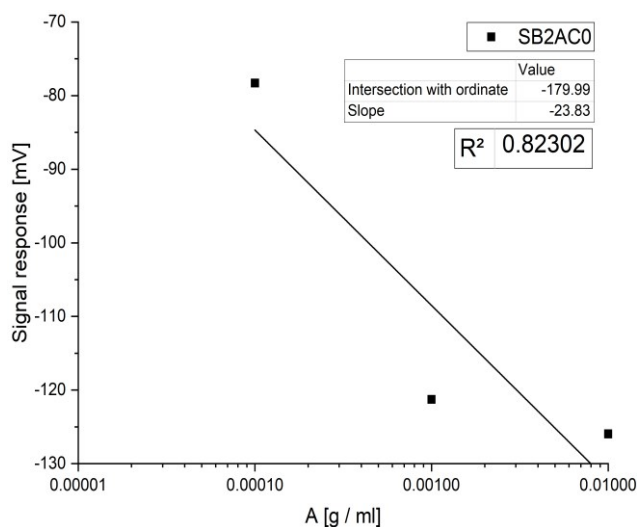


Figure 9.19: Calibration of sensor SB2AC0 using 3 different sodium benzoate solutions. BaHav19-008

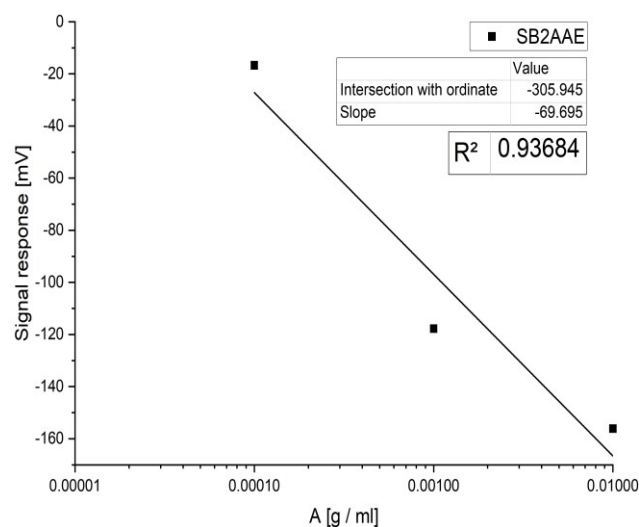


Figure 9.20: Calibration of sensor SB2AAE using 4 different sodium benzoate solutions. BaHav19-008

9. Annex

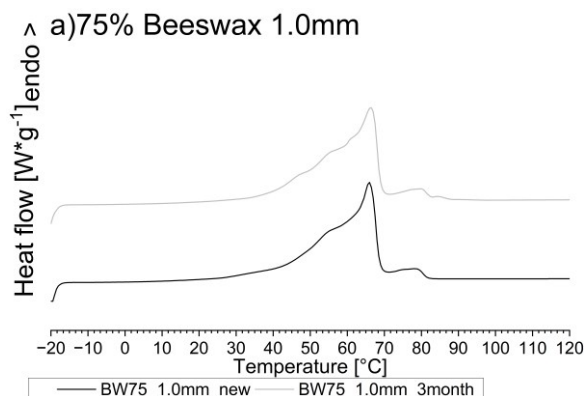


Figure 9.21: Thermograms of a) BW75_1.0mm, Heat rate 10°C/min. Experiment BaHav19-009

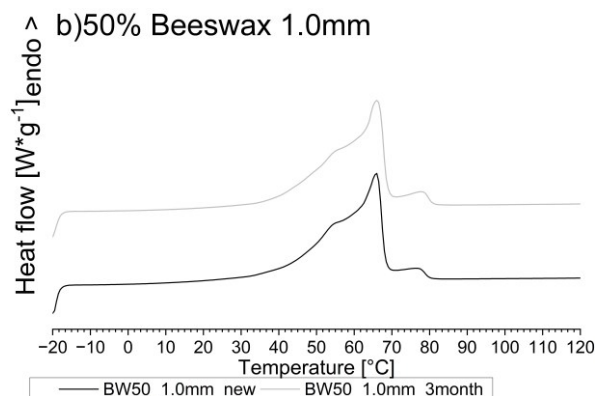


Figure 9.22: Thermograms of b) BW50_1.0mm, Heat rate 10°C/min. Experiment BaHav19-009

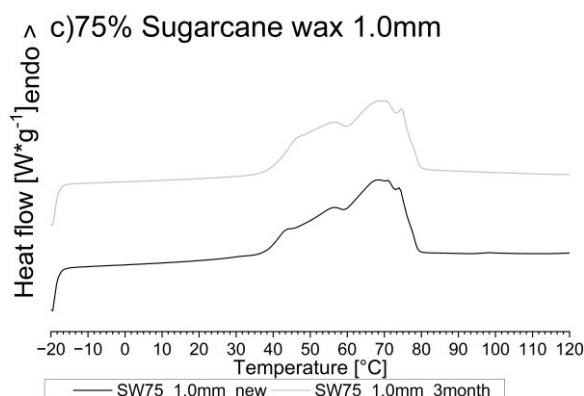


Figure 9.23: Thermograms of c) SW75_1.0mm, Heat rate 10°C/min. Experiment BaHav19-009

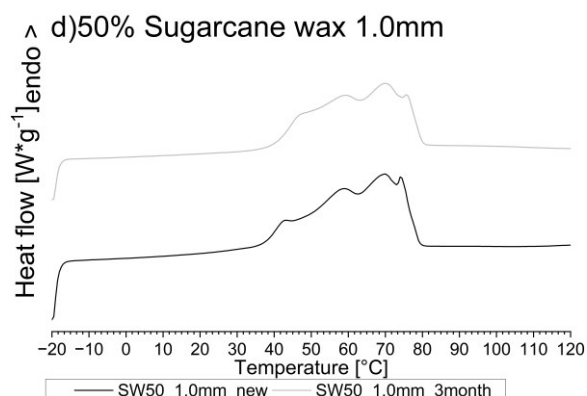


Figure 9.24: Thermograms of d) SW50_1.0mm, Heat rate 10°C/min. Experiment BaHav19-009

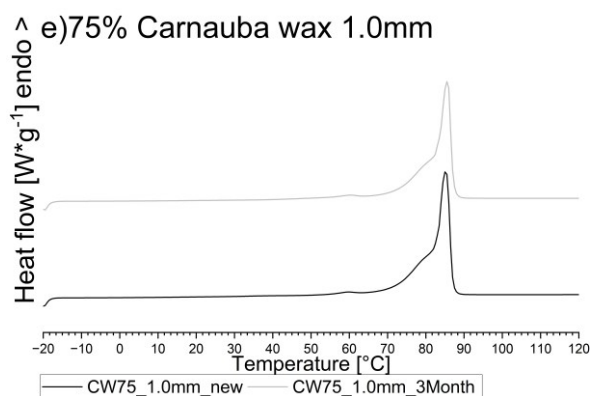


Figure 9.25: Thermograms of e) CW75_1.0mm, Heat rate 10°C/min. Experiment BaHav19-009

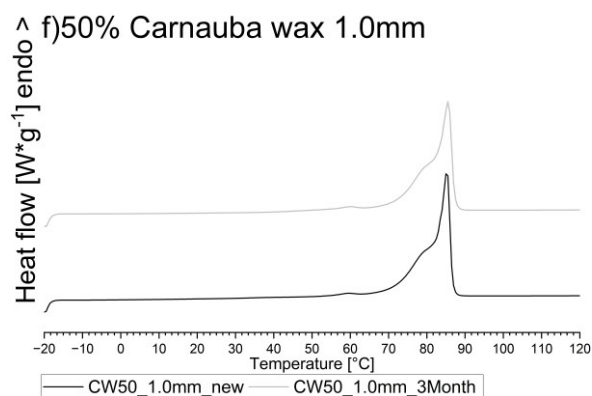


Figure 9.26: Thermograms of f) CW50_1.0mm. Heat rate 10°C/min. Experiment BaHav19-009

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11 Eidestattliche Erklärung

Ich versichere an Eides Statt, dass die vorliegende Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zu Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist. Die vorliegende Dissertation wurde bisher bei keiner anderen Fakultät vorgelegt.

Bastian Hahn

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